Fine Needle Aspiration Cytology of the Breast

Atlas of Cyto-Histologic Correlates

Gary Tse
Puay-Hoon Tan
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Editors

Second Edition

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1 Anatomy and Physiology of the Breast

Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

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 largely 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.

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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, 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 an onset of cyclical estrogen and progesterone secretions that initiate 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 hormones. Lobular growth and development continue from puberty until adulthood and are further enhanced during pregnancy and lactation.

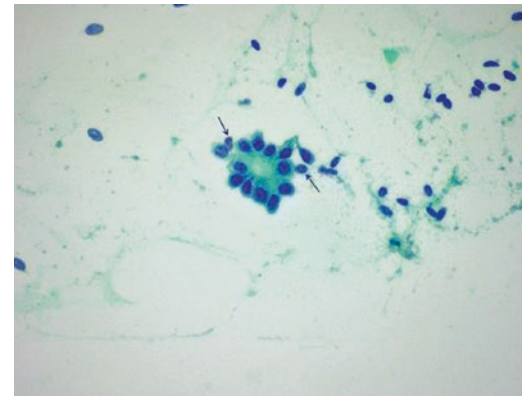

Fig. 1.1 Normal breast acinus comprising a wreath of luminal epithelial cells and occasional myoepithelial cells (*arrows*). Scattered bipolar naked nuclei are seen in the background

1.2.3 Menstrual Cycle

During the menstrual cycle, physiologic variations 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 midphase 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. 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 a 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 the 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 receptors 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 occasionally be overinterpreted as atypical, suspi-

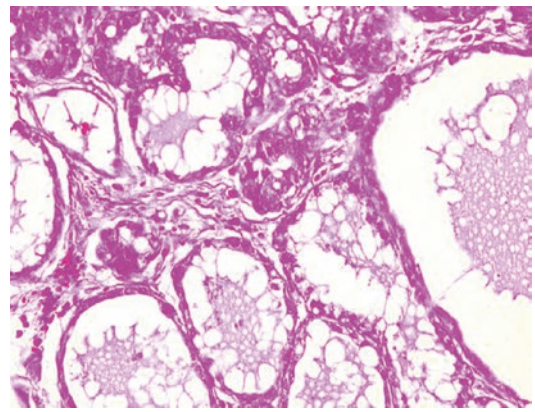

Fig. 1.2 Lactational changes. Corresponding histology shows dilated acini lined by cells with variably sized nuclei, containing luminal pink secretions

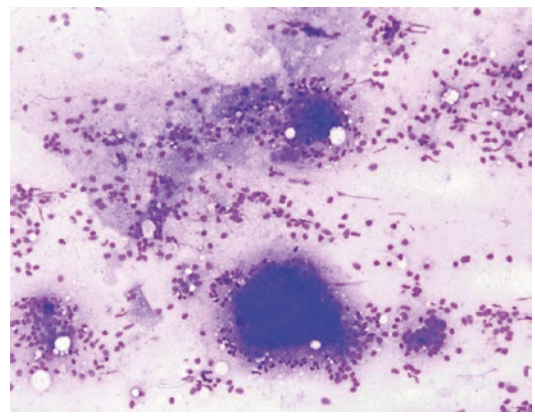

Fig. 1.3 Lactational changes. The smear shows cohesive epithelial clusters with many dispersed naked nuclei in a lipoproteinaceous background

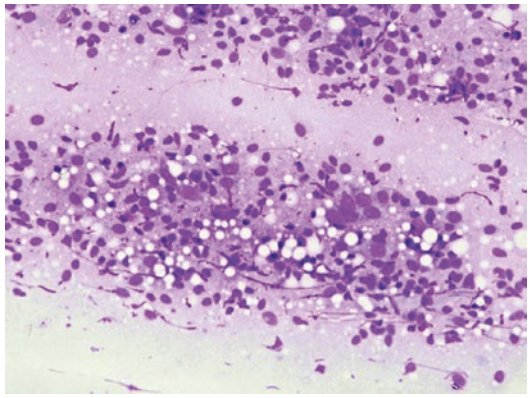

Fig. 1.4 Lactational changes. Higher magnification of the dispersed cells which show slight nuclear enlargement and variability in size, with discernible nucleoli. Cytoplasm is indistinct, and the lipoproteinaceous background incorporates some debris

cious, 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 decrease. 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 the 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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2 Basic Histopathology of Breast Lesions

Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

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 about 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 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 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 a 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. A 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 of acute inflammation or abscess of the breast. Rarely patients with breast cancer may present with enlarged axillary lymph nodes in the absence of a clinicoradiologically apparent primary breast tumor; such cases are referred to as an "occult pri-

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mary" 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 during the postpartum period, when the lactating breast tissue is swollen, and sometimes the ducts are obstructed, with inspissation of the secretion. In addition, breast-feeding may cause trauma and cracks to the nipple, resulting in ascending infection of commensals originating either 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 exudates; at the same time, inflammatory reaction will result in granulation tissue formation and fibrotic tissue around the inflammatory 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 contents into the breast parenchyma will incite an 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; non-caseating 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 a 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 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. On ultrasound, acute fat necrosis shows 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

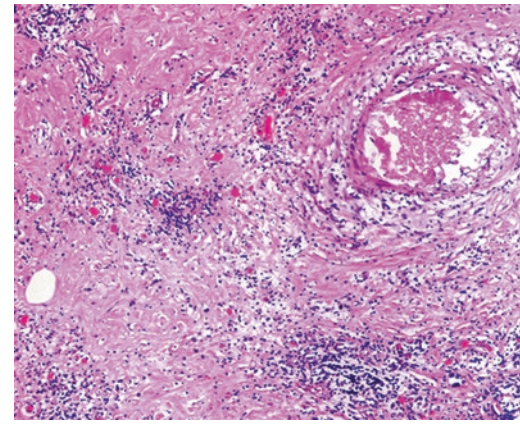

Fig. 2.1 Breast tissue with chronic inflammatory cells within a congested fibrous stromal background. There is also an accumulation of debris and inflammatory cells within a ductal lumen that is mostly denuded of visible lining epithelium

can be indistinguishable from carcinoma. Lipid cysts on mammography are diagnostic of fat necrosis. Similar effects can be encountered in cases of breast augmentation, as some augmented materials may penetrate the breast parenchyma, eliciting a similar inflammatory response. 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 including smears, cultures, and molecular studies 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. 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 the 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). Cystic neutrophilic granulomatous mastitis is another inflammatory condition in which non-caseating granulomas can be seen with mixed inflammatory cells, associated with cystic spaces that can sometimes reveal Gram-positive bacterial organisms, usually *Corynebacterium kroppenstedtii* (Fig. 2.3).

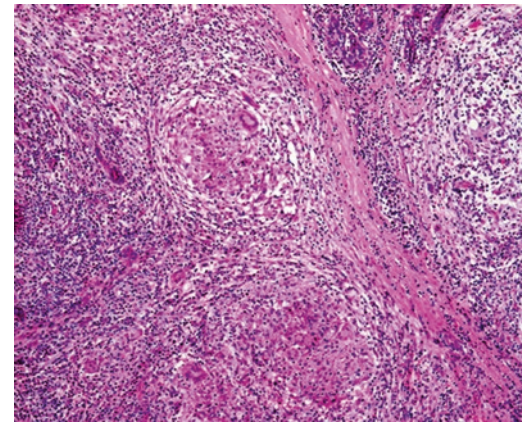

Fig. 2.2 Granulomatous mastitis showing well-formed granulomas composed of epithelioid histiocytes and occasional Langhans giant cells. No caseation necrosis is seen. A lymphocytic infiltrate is noted in the background

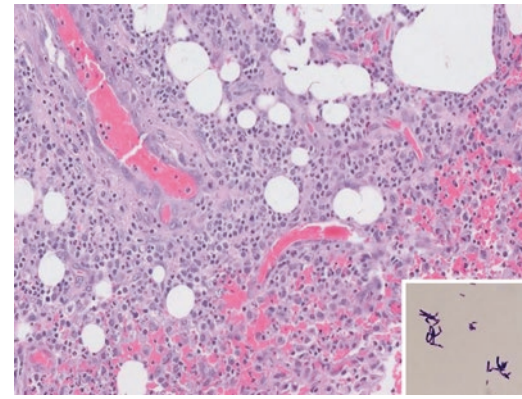

Fig. 2.3 Cystic neutrophilic granulomatous mastitis shows inflamed hemorrhagic granulation tissue in the abscess wall. There are empty spaces within which Gram-positive bacterial organisms are found (*inset*) (Tan and Sahin 2017)

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 no symptoms to mastalgia 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 for-

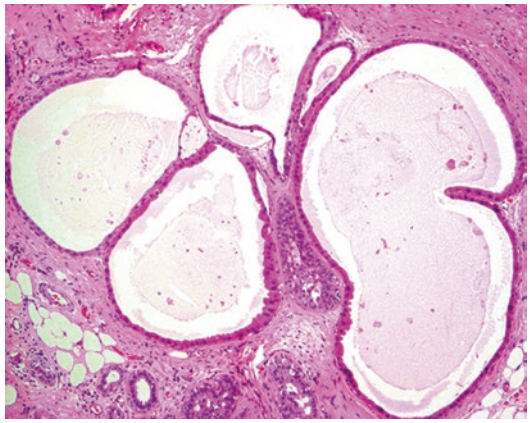

Fig. 2.4 Fibrocystic changes with apocrine cyst formation and mild usual ductal hyperplasia in adjacent ducts. An intact myoepithelial cell layer can be discerned

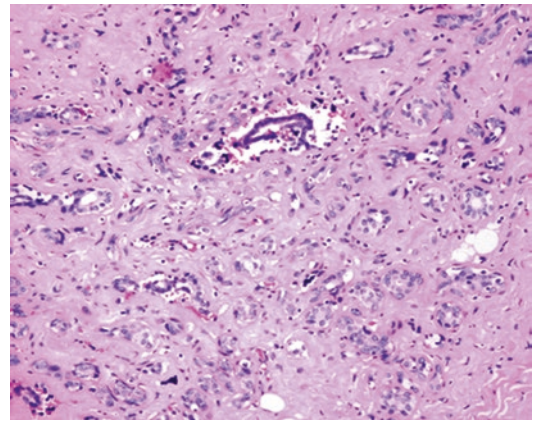

Fig. 2.5 Sclerosing adenosis with fibrosis of the intralobular stroma resulting in compression of the epithelial structures to give a pseudoinfiltrative growth pattern

mation, inflammatory changes, and fibrosis (Fig. 2.4). 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 degrees of epithelial hyperplasia may be present, from mild to moderate and florid usual ductal hyperplasia, as well as columnar cell changes; the latter is often associated with calcifications. As fibrocystic changes are very common, they represent the majority of breast lesions undergoing 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. 7.

Sclerosing adenosis is a form of breast proliferation. This is an important entity to recognize, as clinically symptomatic lesions can be characterized by irregular hard masses fixed to adjacent structures. By imaging, it can show significant architectural distortion, rendering this indistinguishable from carcinoma. Histologically, there are bilayered tubules featuring preserved myoepithelial cells disposed within a densely fibrotic stroma. At low magnification, a lobular configuration can be appreciated. At times, the dense fibrosis or sclerosis causes architectural distortion and compression of these tubular structures, giving rise to a pseudoinfiltrative pattern (Fig. 2.5). Careful histologic examination to establish the benign nature of the epithelial cells with the presence of an intact myoepithelial cell layer and a retained lobular architecture, along with the judicious use of immunohistochemistry for identification of preserved myoepithelial cells, helps to establish the diagnosis.

Other changes include columnar cell lesions, microglandular adenosis, apocrine adenosis, and nodular adenosis. The term "blunt duct adenosis" which was used previously to describe columnar cell lesions is not recommended by the 2019 WHO classification of breast tumors.

Fibroadenomas are the most common benign breast tumor, presenting as solitary painless, mobile, and well-defined nodules. Multiple lesions can occur. Use of the immunosuppressant cyclosporine in transplant patients has resulted in an increased risk of fibroadenoma development. Macroscopically, the 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 resulting from compression of the ductal elements by the proliferating stromal component into slit-like spaces (Fig. 2.6). These patterns have little prognostic

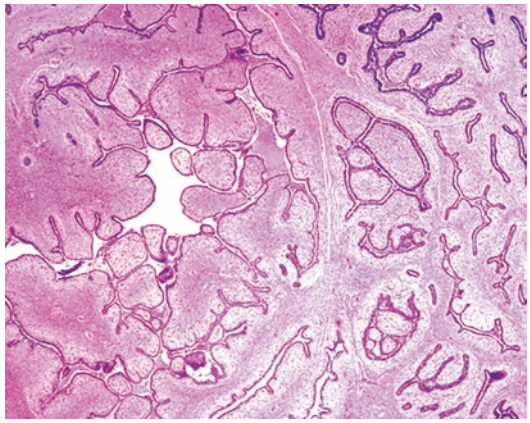

Fig. 2.6 Fibroadenoma showing proliferation and expansion of the stroma that is of usually low cellularity, and the ductal element sometimes forms an intracanalicular pattern with a "leaflike" pattern, though this architecture is neither exaggerated nor accompanied by stromal hypercellularity

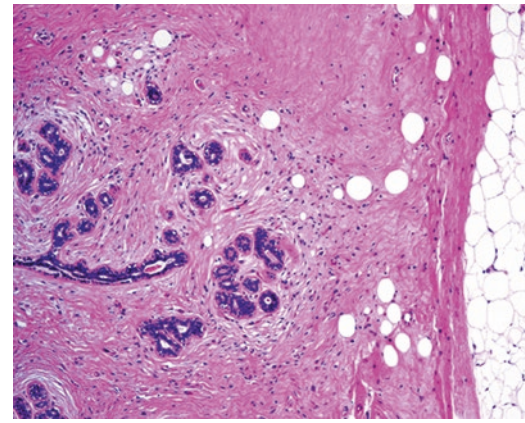

Fig. 2.7 Hamartoma showing a rounded border, with adipocytes within a fibrotic stroma. There is also intralobular fibrosis

significance apart from the more frequent association of *MED12* mutations in intracanalicular fibroadenomas. Occasionally stromal giant cells, myxoid changes, dystrophic calcifications, and mesenchymal metaplasias 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 reveals a slightly higher (1.6×) cancer risk compared to the usual fibroadenomas, though this increased risk is related to the associated epithelial proliferative changes rather than the fibroadenoma per se. 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), well described as a "breast within a breast" histology. This is a benign tumor and rarely recurs (Fig. 2.7).

Diabetic mastopathy is an inflammatory disorder of the breast characterized by a perilobular and perivascular lymphocytic infiltrate. It usually 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 and collagenous, with plump epithelioid fibroblasts. In the stroma, keloidal-type fibrosis is seen.

Phyllodes tumor is an 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 bilaterality are rare. Imaging often shows 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 appearance with curved spaces resembling leaves or leaf buds. There may be hemorrhage or necrosis. Microscopically, phyllodes tumor shows a prominent exaggerated intracanalicular growth pattern with leaflike pat-

terns projecting into irregularly dilated 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 often shows geographic variation within the lesion. These stromal cells are bland looking, with scanty mitotic figures (Fig. 2.8a, b) in the benign form of the tumor. Within the tumor, stromal areas of low cellularity, hyalinization, or myxoid changes are commonly 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 phyllodes tumors are benign. The clinical outcome of phyllodes tumor is dependent on the histologic grade which is categorized into benign, borderline, or malignant forms. Benign phyllodes tumor may rarely recur but does not metastasize. Malignant phyllodes tumors including those of borderline or frank malignancy may both recur or metastasize, more commonly with the latter group (Tse and Tan 2005; Lerwill et al. 2022).

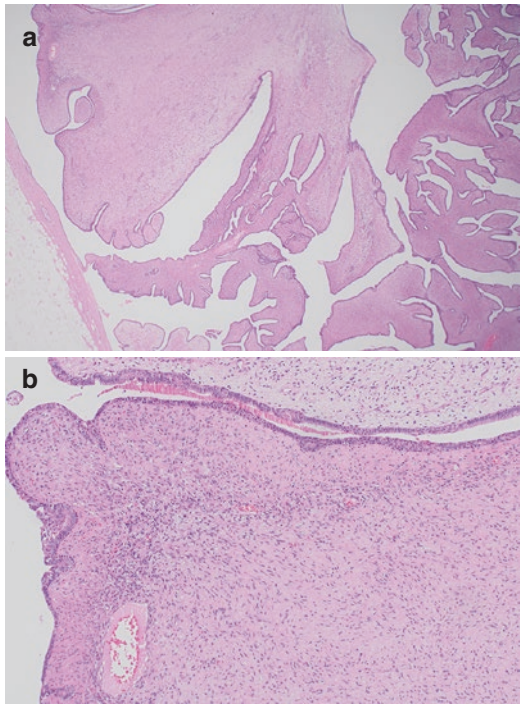

Fig. 2.8 (**a**) Benign phyllodes tumor showing a fronded appearance, with variable stromal cellularity, and a benign epithelial lining on the surface. (**b**) Benign phyllodes tumor at higher magnification showing areas of stromal expansion with moderately cellular stroma

Papillomas can be divided into solitary or multiple. Solitary papilloma is usually located beneath the nipple, whereas 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, with the fibrovascular cores covered by epithelial cells, together with an intervening layer of myoepithelial cells separating the luminal epithelium from the stromal tissue (Fig. 2.9a). The epithelial cells are benign; 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. 2.9b). The fibrovascular cores may also show sclerotic changes resulting in compression and entrapment of the benign glandular component to yield a pseudoinvasive pattern. Demonstration of a layer of residual myoepithelial cells as well as the absence of cellular atypia of these entrapped epithelial cells helps to differentiate this phenomenon from malignancy (Mulligan and O'Malley 2007). On the whole, papillomas are benign but are associated with a slightly increased risk for cancer, more for multiple papillomas than for solitary papilloma (Lewis et al. 2006).

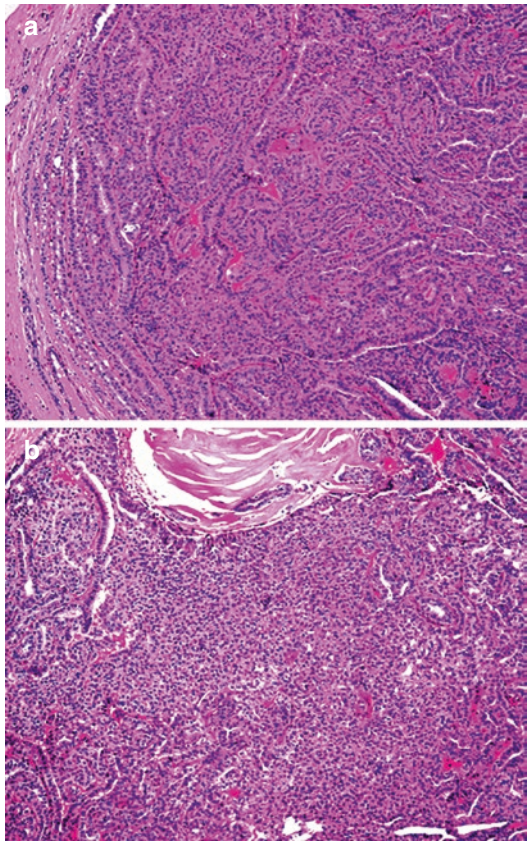

Fig. 2.9 (**a**) A benign papilloma showing a network of fibrovascular cores lined on the outside by benign ductal epithelial cells. The lesion is rounded and is present within a large ductal space. (**b**) A benign papilloma with a solid area of epithelial hyperplasia. The hyperplastic epithelium shows spindled nuclei with nuclear streaming, a feature characteristic of florid epithelial hyperplasia

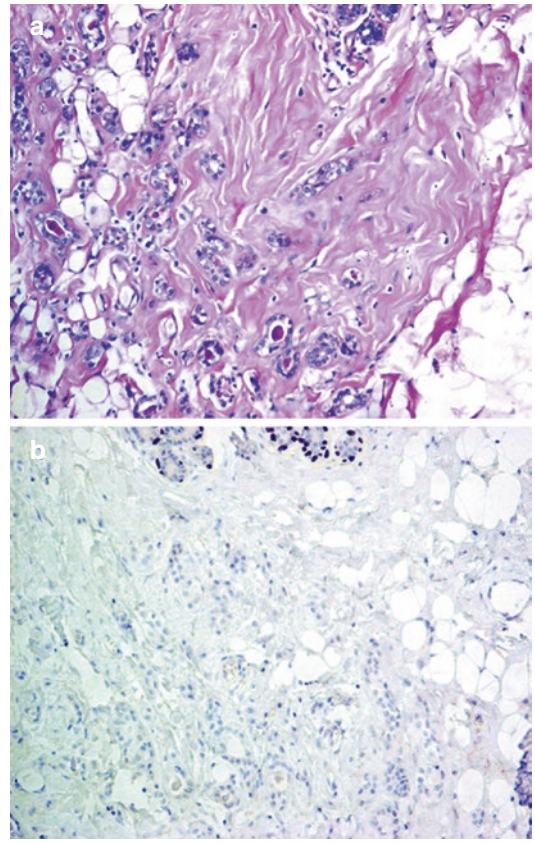

Fig. 2.10 (**a**) Microglandular adenosis showing permeation of the breast stromal tissue by small tubules formed by bland-looking epithelial cells with rounded lumens. Eosinophilic material may be seen within the lumens. (**b**) Immunohistochemical staining of microglandular adenosis for the myoepithelial marker p63 shows a lack of myoepithelial lining around the tubules, in comparison with terminal duct lobular units containing an intact layer of myoepithelial cells

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. 2.10a, b). Within the lesion, 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.

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

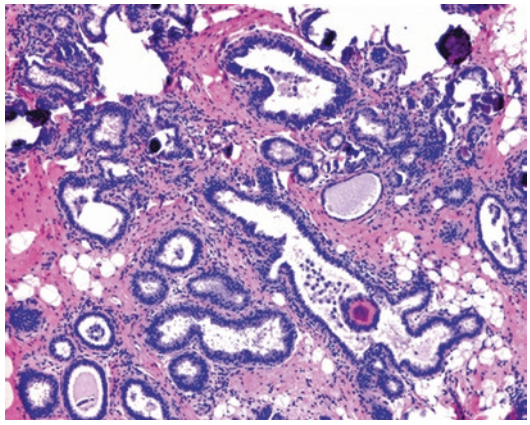

Fig. 2.11 Columnar cell changes showing dilatation of the acini with the cells demonstrating columnar morphology, with apical cytoplasmic snouts and the presence of flocculent material and calcifications within the lumens

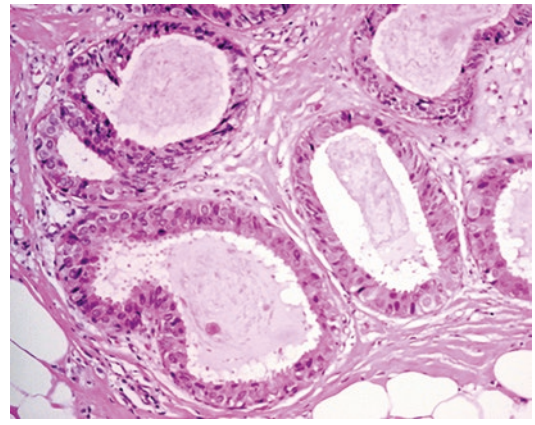

Fig. 2.12 Flat epithelial atypia showing dilated acini containing secretory material. The spaces are lined by one to two layers of columnar epithelial cells with apical snouts and monomorphic nuclei. The nuclei are similar in appearance to those of ADH or low-grade DCIS

lumens, and these are frequently associated with calcifications (Fig. 2.11). 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. 2.12). Studies suggest that 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 or usual ductal 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. Usual hyperplasia is described with ductal terminology (intraduct hyperplasia, hyperplasia of usual type, or usual duct hyperplasia), whereas for atypical epithelial hyperplasia, both ductal and lobular morphologies are described (atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH)).

Usual ductal hyperplasia may be of variable extent, and although the morphology can be somewhat similar to low-grade DCIS, such a concept of progression is not generally accepted.

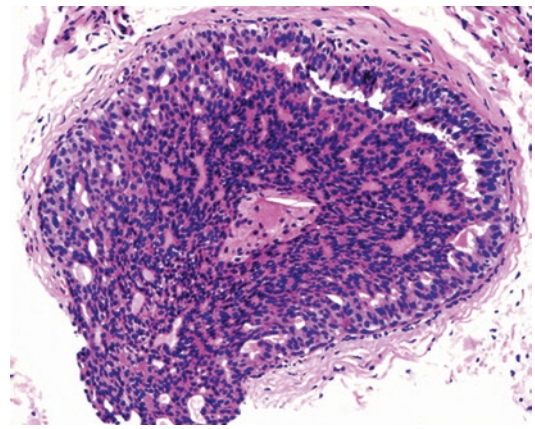

Fig. 2.13 Florid epithelial hyperplasia showing distension of the ductal space by an epithelial proliferation, with the epithelial cells showing nuclear streaming and forming irregular and slit-like secondary lumens

The changes in usual ductal hyperplasia range from mild epithelial hyperplasia, in which the number of epithelial cell layers increased from two to four, to florid epithelial hyperplasia, in which there are solid epithelial clusters within and obliterating ductal lumens, with the formation of slit-like irregular peripherally located secondary lumens. The epithelial cells show mostly oval nuclei with irregular and streaming arrangements (Fig. 2.13). Apocrine metaplasia may be present.

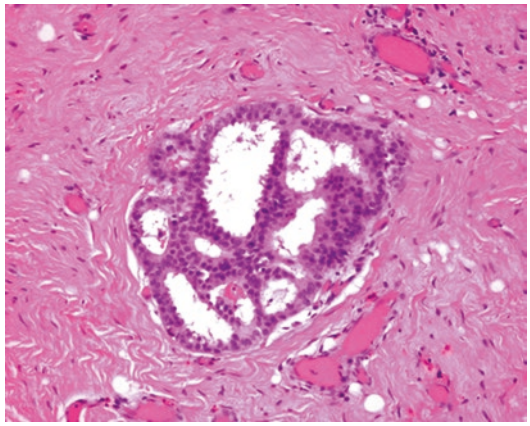

Fig. 2.14 Atypical ductal hyperplasia showing proliferation of a monomorphic population of epithelial cells forming a cribriform structure. The cellular monotony and architectural pattern are similar to those seen in low-grade DCIS

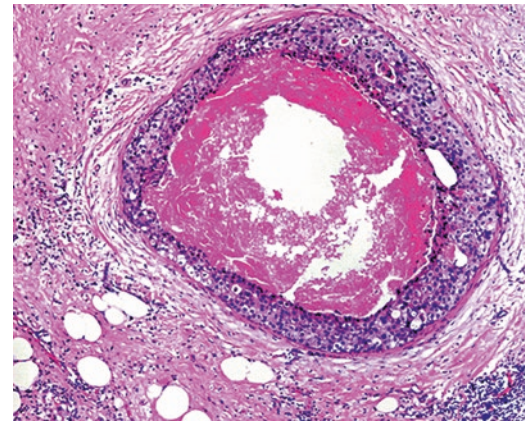

Fig. 2.15 High-grade ductal carcinoma in situ with highly pleomorphic tumor cells present within and distending the duct space, associated with central comedo necrosis

ADH, when properly defined using stringent criteria (Page and Rogers 1992), carries a significant 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. 2.14). Where the cutoff is drawn continues to be debated although the 2019 WHO consensus is to use 2 mm as the threshold (Simpson et al. 2012; WHO 2019).

2.5 Malignant Breast Tumors

2.5.1 Carcinoma in Situ

With 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 as it does not have a consistent association with biological behavior. Current classifications/grading always use nuclear grade as one of the defining features of 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. 2.15). The ducts may be so distended that aggregation of these ducts with accompanying stromal fibrosis 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 of DCIS 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. 2.16). The solid pattern is often observed with high-nuclear grade lesions. Secretions may be seen within the ductal lumen and should not be confused with necrosis which is often accompanied by karyorrhexis, necrotic cells, and nuclear debris. Calcifications, if present, are

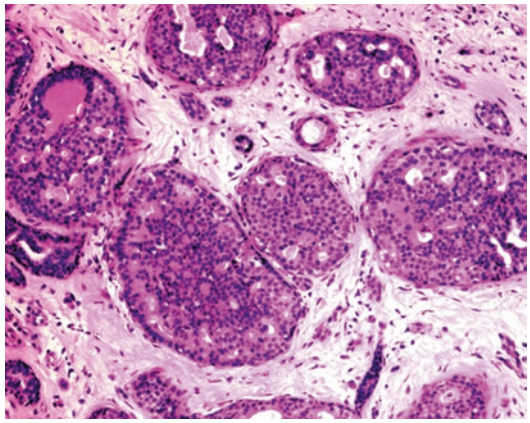

Fig. 2.16 Low-grade ductal carcinoma in situ with monotonous tumor cells distending ductal spaces and forming cribriform structures with geometric lumens

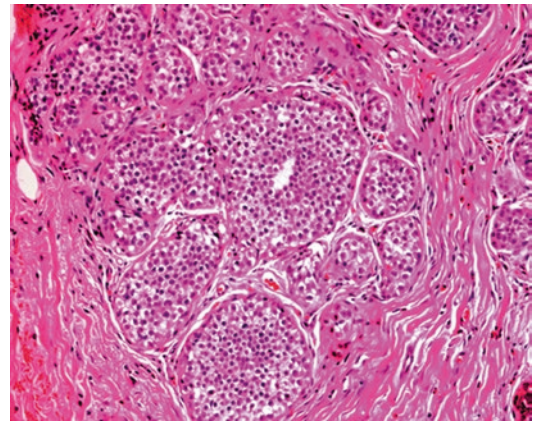

Fig. 2.17 Lobular neoplasia (lobular carcinoma in situ) showing distension of the acini by a uniform population of small round cells with bland nuclear features. The growth pattern is solid

associated with the secretions and are usually smaller, rounded, and psammomatous in low-grade DCIS, whereas calcifications seen in high-grade DCIS with necrosis are often amorphous and granular, superimposed on the necrotic material. Intermediate-grade DCIS usually shows features in between high- and low-grade lesions.

Lobular carcinoma in situ (LCIS) is grouped with ALH under the umbrella term of lobular neoplasia. In both cases, the lobular architecture is essentially preserved, but individual acini are enlarged, distended, and distorted with obliterated lumens in LCIS. 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. 2.17). Calcifications are much less frequent than ductal lesions. ALH and LCIS exist along a continuum and differentiating features are based on severity of changes, 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 overlying elaborate fibrovascular cores. Qualification of the in situ and/or invasive nature of the tumor is required. Papilloma with DCIS and encapsulated papillary carcinoma are typical examples of papillary lesions with malignancy, though in the former, the malignant lesion is DCIS which may not be architecturally papillary; and the underlying papillary process is a benign papilloma upon which DCIS occurs. Papilloma with DCIS is diagnosed when there is a focus of atypical epithelial proliferation fulfilling criteria for DCIS, occurring 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. 2.18) (Page et al. 1996). The differentiation between papilloma with ADH or papilloma with DCIS is arbitrary, with a threshold size criterion of 3 mm being used according to WHO 2019. If the atypical focus is 3 mm or more, the lesion should be termed as papilloma with DCIS, and when the focus is less than 3 mm, it should be termed papilloma with ADH (WHO 2019).

Encapsulated papillary carcinoma is uncommon, usually occurring in older women, and may present as a breast mass. Microscopically, it is usually a solitary expansile papillary neoplasm, but may also exhibit cribriform or micropapillary

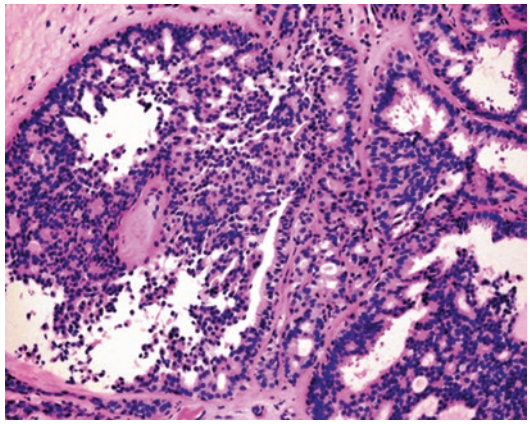

Fig. 2.18 Papilloma involved by atypical ductal hyperplasia. There are areas of atypical ductal hyperplasia separating the papillary fibrovascular stromal tissue cores. The atypical ductal hyperplasia shows cribriform morphology with uniform cells forming geometric structures

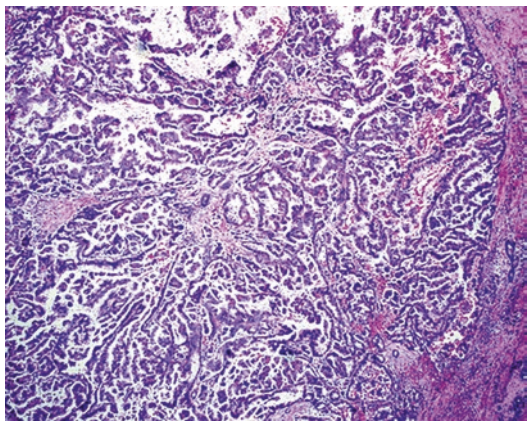

Fig. 2.19 Encapsulated papillary carcinoma showing delicate fibrovascular cores within a rounded tumor, with the absence of an outer myoepithelial cell layer

patterns as minor components. The characteristic feature of this tumor is the absence of a complete layer of myoepithelial cells on its external contour and the delicate nature of the papillary fronds (Collins et al. 2006) (Fig. 2.19). Encapsulated papillary carcinoma has a good prognosis, having better outcomes than mixed encapsulated 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. The WHO 2019 classification recognizes a high-grade form of encapsulated papillary carcinoma which is believed to behave like invasive disease and may require more aggressive treatment.

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 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, stromal desmoplasia, or inflammation. It is usually firm, fibrotic, or stellate. The histomorphology of the tumor is 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 disclosing highly pleomorphic tumor morphology, with the tumor cells arranged in solid sheets and groups, showing brisk mitotic activity and abundant tumor necrosis (Fig. 2.20). IDCs are graded based on three microscopic features, namely, the degree of

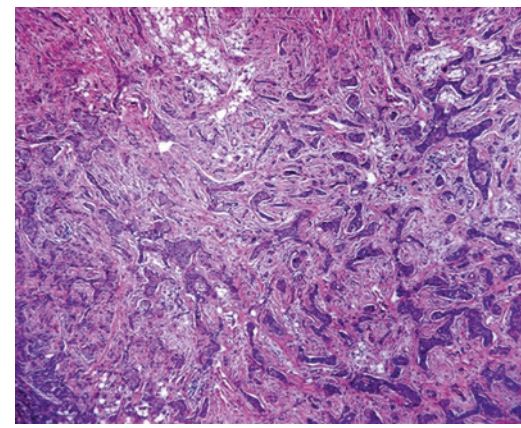

Fig. 2.20 Infiltrating duct carcinoma showing irregular groups of malignant cells invading a desmoplastic stroma. The normal ductal-lobular architecture is obliterated

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 evaluated in terms of variation in nuclear size, regularity of the nuclear border, hyperchromasia, and nucleolar prominence. Mitoses are counted per 10 high-power microscopic fields, taking into account the size of the microscopic high-power field, with a recommendation by the fifth WHO tumor classification series to apply a per area calculation for mitoses. A combination score on these three components reflects tumor grade and informs prognosis. Hormone receptor assessment is also mandatory in invasive cancer 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 a better prognosis and are highly responsive to hormonal therapy, whereas tumors expressing HER2/neu have a poor prognosis but may respond to specific anti-HER2/neu targeted therapy.

Invasive lobular carcinoma represents 5–10% of invasive breast tumors. It is usually seen as an irregular thickening and with a lower density on mammography and is less likely to be associated with calcifications. The tumor can be relatively small and possesses poorly defined edges. The tumor cells are small and visually subtle, containing small amounts of cytoplasm. Often, the tumor cells form cords or are arranged in a single file pattern (Fig. 2.21). The tumor cells usually show loss of cytoplasmic membranous E-cadherin protein, 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.

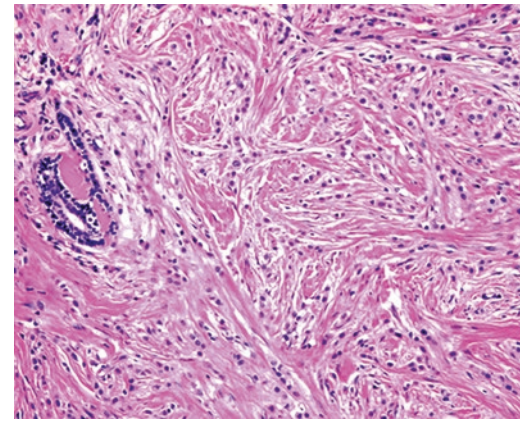

Fig. 2.21 Infiltrating lobular carcinoma showing poorly cohesive small round tumor cells arranged in single files within a fibrotic stroma

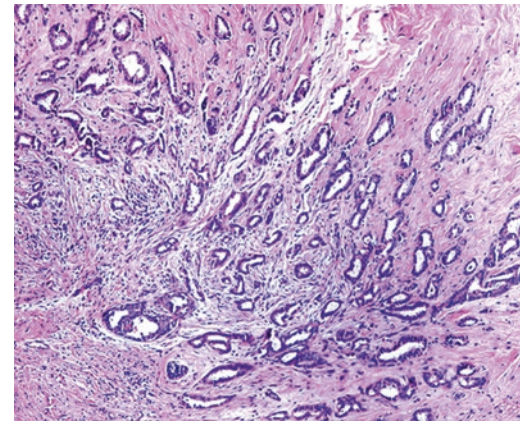

Fig. 2.22 Tubular carcinoma showing bland-looking tumor cells arranged in tubules that possess widely patent lumens. Myoepithelial cells are absent, and the malignant tubules are arranged in an irregular stellate pattern. The intervening stroma is densely fibrotic

Tubular carcinoma is a rare breast tumor, accounting for less than 3% of all tumor types. Most of these tumors are detected via the screening program with a characteristic mammographic appearance of an irregular spiculated mass which may or may not be accompanied by calcifications. The tumor is usually small and composed of angulated tubules embedded in a desmoplastic stroma (Fig. 2.22). This is one of the well-differ-

entiated 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, and may present at the clinic with a palpable mass. The tumor is usually circumscribed and can be associated with radiologic microcalcifications. Grossly, a glistening gelatinous appearance is appreciated. Microscopically, the tumor cells are usually in tubules, clusters, and small sheets floating in pools of mucin. They have a characteristic pale to eosinophilic cytoplasm with low nuclear and mitotic grade. The amount of tumor cells suspended within extracellular mucin varies, and hypercellular tumors may sometimes show neuroendocrine differentiation (Fig. 2.23). Furthermore, neuroendocrine differentiation in mucinous carcinoma is associated with favorable histologic and immunohistochemical parameters. The presence of malignant cells is a prerequisite in the diagnosis in order to distinguish it from benign mucocele-like lesions. Generally, mucinous carcinoma confers good prognosis, mostly diagnosed as grade I or II with a 5-year survival of 90%.

Carcinoma with medullary features, a term used in the 2012 WHO breast tumor classification, is now regarded by the WHO 2019 blue book as part of the spectrum of invasive breast carcinoma, no special type, with prominent tumor infiltrating lymphocytes (TILs). It often 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 infiltrate in the perimeter (Fig. 2.24). Morphological appearance of the cells is high grade, usually with more than 20 mitotic figures per 10 high-power fields. This histologic type is also seen among patients with BRCA1 mutation-related tumors. However, not all BRCA1-related tumors possess prominent TILs. 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 refers to breast carcinomas with mixed epithelial and mesenchy-

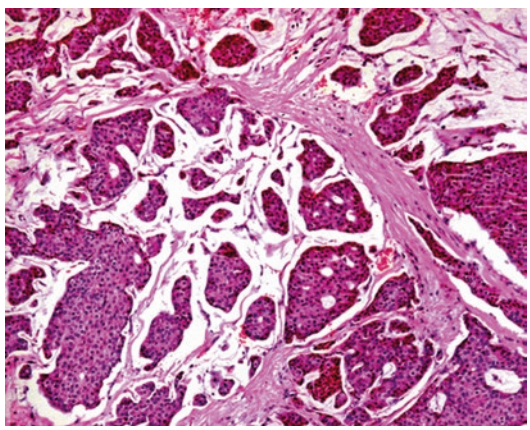

Fig. 2.23 Mucinous carcinoma showing large sheets of low-grade malignant cells present within pools of accumulated extracellular mucinous material

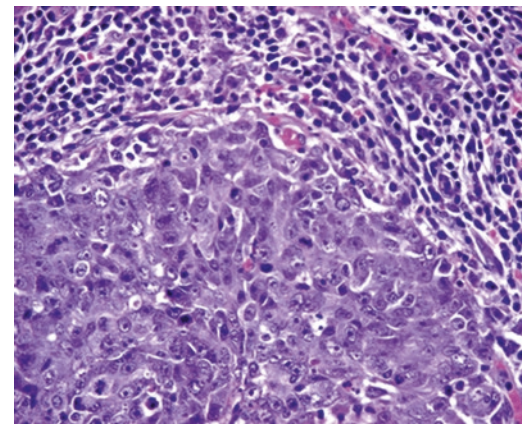

Fig. 2.24 Carcinoma with prominent TILs, previously referred to as carcinoma with medullary features, showing a rounded margin (not shown) and an intense lymphocytic infiltrate at the periphery. The tumor cells form a sheetlike pattern with high degree of nuclear pleomorphism and brisk mitotic activity

mal appearances, including those that display immunohistochemical evidence of epithelial differentiation despite a predominantly mesenchymal morphology. 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 surfaces. Malignant squamous cells and glands are often the histological components encountered mixed with spindle cells (Fig. 2.25). They are further morphologically categorized based on the predominance of the epithelial and/or mesenchymal cells. Generally, the prognosis is poor, showing an increased incidence of recurrence and metastasis.

Invasive micropapillary carcinoma accounts for approximately 2% of all invasive breast carcinomas and is associated with a poorer prognosis. The tumor cells are seen as tubular nests surrounded by clear spaces which may reflect an "inside-out" pattern of lumen formation, though some 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. 2.26). 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. 2.27). 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 similar to that of invasive

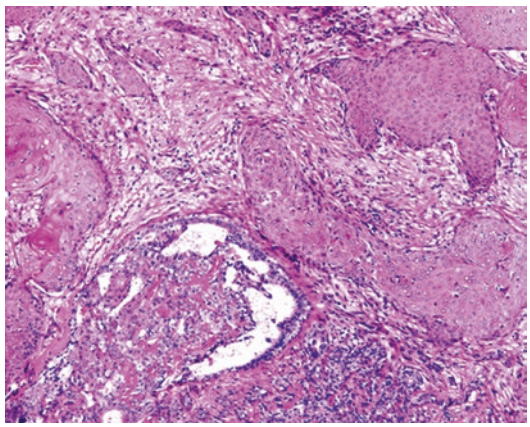

Fig. 2.25 Metaplastic carcinoma showing mixed squamous cell carcinoma and ductal carcinoma components. The intervening areas show malignant spindle cell proliferation

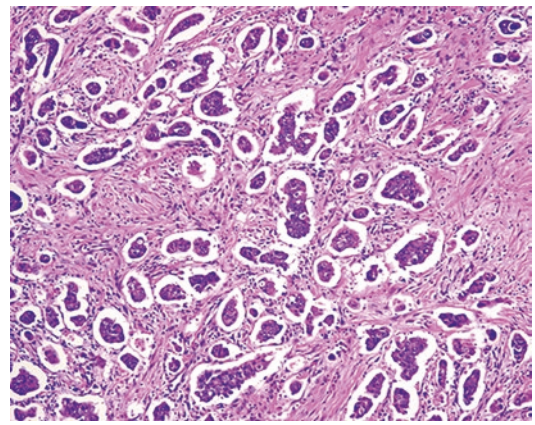

Fig. 2.26 Invasive micropapillary carcinoma showing rounded groups of tumor cells with a peripheral clear rim

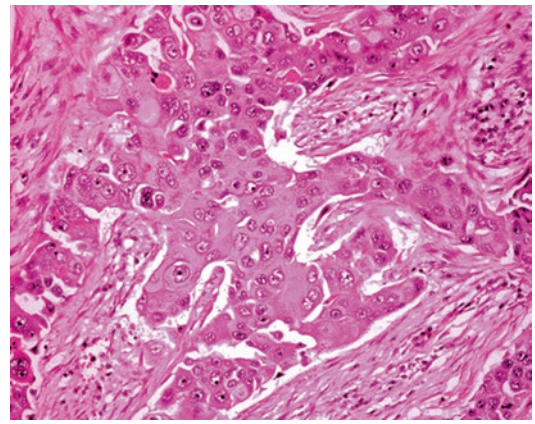

Fig. 2.27 Apocrine carcinoma showing tumor cells with the characteristic appearance of abundant eosinophilic and granular cytoplasm

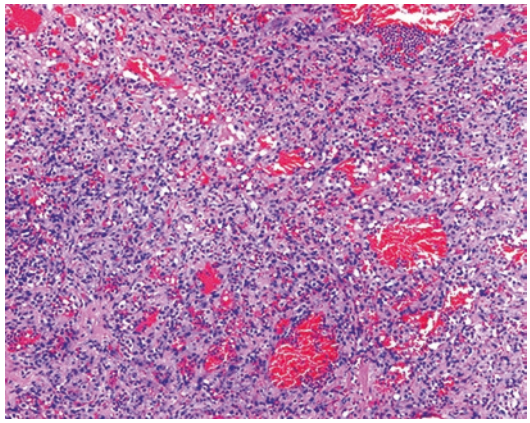

Fig. 2.28 Angiosarcoma showing malignant spindle cells with moderate nuclear pleomorphism forming vascular channels of varying sizes and shapes. Some of these lumens are blood filled

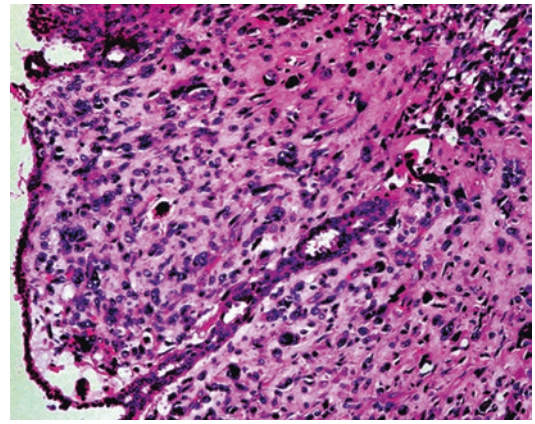

Fig. 2.29 Malignant phyllodes tumor showing highly pleomorphic malignant stromal cells, with bizarre cells adjacent to benign ductal epithelium. Atypical mitotic figures are seen within the malignant stromal cells

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. It is not a morphology tumor type. This tumor mimics an infection and may appear to transiently respond to medications; thus, clinical caution and a high index of suspicion are important. The prognosis is usually poor with tendency for dermal lymphatic permeation.

Angiosarcoma of the breast is a tumor arising from the blood vessels and is grossly seen as a poorly defined mass with hemorrhage. When an older 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 and atypia are seen. Necrosis and hemorrhage are common in aggressive tumors (Fig. 2.28).

Malignant phyllodes tumor is distinguished from the benign tumor based on microscopic features of the stromal element. Stromal hypercellularity, overgrowth, atypia, and mitotic activity of 10 or more per 10 high-power fields are the features seen in malignant phyllodes which will also show an infiltrative margin (Fig. 2.29). Management is challenging, with many surgeons and patients opting for 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 can reveal a peculiar architecture that is different from the common breast histologic types. The common metastatic tumors in the breast are lung carcinoma and malignant melanoma. The tumor cells are usually high-grade or anaplastic, making it more difficult to decipher the origin. Presence of an in situ carcinoma supports a primary breast tumor, but its absence will 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. 2.30a, b).

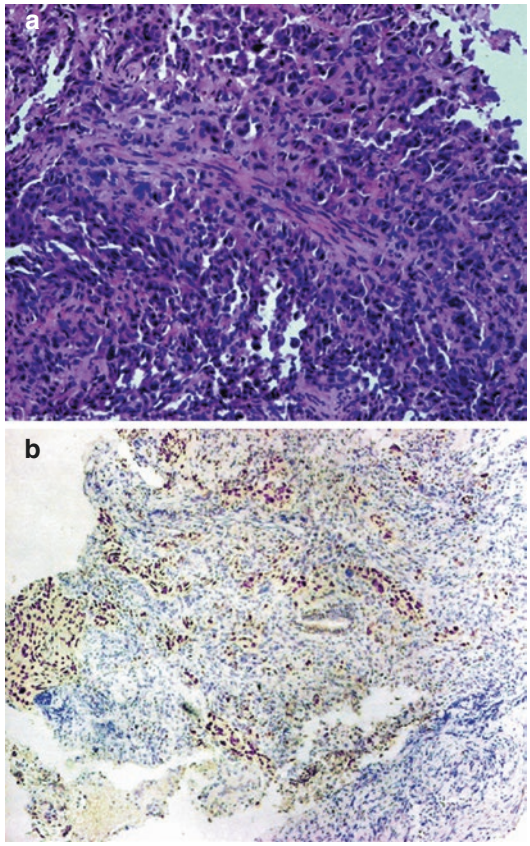

Fig. 2.30 (**a**) High-grade malignant cells presenting as a tumor nodule in the breast. The tumor cells show hyperchromatic nuclei and moderate amounts of cytoplasm. (**b**) The same tumor showed positivity for TTF1, confirming a metastasis from a lung primary

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Aspiration Techniques

3

Daniel Gomes Pinto, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

3.1 Introduction

We live in the era of minimally invasive medicine, often achieved through the use of complex and costly equipment and procedures. Fine needle aspiration cytology (FNAC) runs opposite to this trend, managing to be both accessible and minimally invasive (De Rosa et al. 2020). It requires little equipment and can be performed quickly. It is also safe, causing minimal or no complications for the patient, with little discomfort. Furthermore, when employed correctly, FNAC provides a high sensitivity and specificity in the diagnosis of breast malignancies.

This technique is usually done as an outpatient procedure and enables rapid on-site evaluation (ROSE) for sample adequacy, reducing the need for hospital beds and repeat procedures. This allows for resources to be diverted to other areas, which may be particularly important in low-resource settings and in pandemic contexts, such as that which arose in 2020 due to COVID-19 (Pinto and Schmitt 2020).

It is important to stress, however, that both the aspiration technique and the correct interpretation of cytology slides require practice and skill. Practices unfamiliar with the technique should start with low volumes, progressing safely but surely. Of note, the interpretation of breast cytology was recently made more accessible by the release of The Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology (Field et al. 2020), which defines diagnostic categories with well-defined diagnostic criteria and risks of malignancy.

3.2 Role of Breast FNAC in the Clinical Practice

In the breast, FNAC should be used as a component of the so-called triple test, which is the assessment of suspect lesions integrating clinical,

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imaging, and morphological data. In this context, core needle biopsies (CNB) should only be used in a subset of cases with discordant findings in the triple test.

It is true that CNB have replaced FNACs in most practices in the developed world. This is because they are perceived to enable a more reliable and definitive diagnosis and also provide adequate material for ancillary testing, which is mandatory in the context of breast cancer. This perception is not entirely accurate; however, great strides have been made in improving the diagnostic yield of FNACs of the breast, and in using the material thus obtained for ancillary studies. Therefore, it is no surprise that not only do FNACs remain very important in low-resource settings but are also part of the daily life in certain large, specialized, practices in the developed world (De Rosa et al. 2020). Furthermore, CNB are more expensive, cause more discomfort, and have a higher potential for complications.

The advantages of FNAC are:

- Greater mobility of the needle during aspiration, allowing an increased area of sampling (vs. CNB, which obtain tissue in only one plane).
- Greater sensitivity of the physical nature of the lesion through palpation, and therefore better needle localization.
- Better evaluation of the texture of the lesion during the aspiration, which may hint at the diagnosis and helps to determine if additional needle passes are needed. For example, gritty, rubbery, or "fatty" resistance feelings suggest the possibilities of carcinoma, fibroadenoma, and fat necrosis, respectively.
- Allows for ROSE, that is, 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 lowering turnaround times and enabling a faster diagnosis.
- Lower cost, discomfort, and complications when compared to CNB.

On the other hand, disadvantages of FNAC include:

- Little experience among pathologists with performing FNAC and in the interpretation of the corresponding slides. In many centers, pathologists are not trained adequately to perform FNAC or in reading cytological slides, particularly in the context of breast lesions and as a result are more comfortable with histology. This is probably one of the main reasons why FNACs have been largely replaced by CNB when cost is no objection.
- Perceived limitations in the available material for ancillary studies and research endeavors. Cytology samples are thought of as inadequate for these ends, particularly for performing immunohistochemistry, which is mandatory for theranostic purposes. However, as demonstrated in other chapters of this book, most ancillary techniques can be performed in cytological material with results comparable to histology, particularly if cell blocks are used.
- Possible low cellular yield in some lesions, namely carcinomas with a highly desmoplastic stroma.
- Difficulty in classifying proliferative lesions that have a degree of atypia but lack unequivocal features of malignancy. Recent research suggests this may be solvable using immunohistochemistry such as 34βE12, however (Hoshikawa et al. 2016).
- Difficulty in distinguishing in situ versus invasive carcinomas. This may be a significant drawback, particularly in the context of neoadjuvant chemotherapy or sentinel lymph node biopsies. However, many of these cases can be resolved by using the triple assessment (Kocjan 2006; Kocjan et al. 2006, 2008). Furthermore, p63 has been successfully used for this purpose in several publications, alone or in combination with a high-molecular weight cytokeratin. These results have not been validated in large, prospective series,

however (Harton et al. 2007; Aiad et al. 2011; Tanaka et al. 2016).

- 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. This is particularly important in the context of the screening of breast cancer using mammography, which often detects malignancies through microcalcifications.

These perceived drawbacks and the ease of use of CNB have led to a decline in FNAC of the breast, particularly in the evaluation of primary breast lesions. As we have seen, this is not totally warranted. And particularly so in the setting of metastatic disease. Due to their minimally invasive nature, FNACs allow for a quick confirmation or exclusion of disease progression and also enable the gathering of material for repeat testing of biomarkers, as currently recommended by clinical guidelines. They may be used for the confirmation of multicentric disease and for the aspiration of regional lymph nodes, enabling staging of a patient's disease and planning of suitable therapy (Francis et al. 2019). As previously stated, FNAC can be performed quickly and in an outpatient setting. When healthcare resources are otherwise constrained (such as in the COVID-19 pandemic) or limited, the advantages of FNAC may outweigh the disadvantages (Pinto and Schmitt 2020).

FNAC or CNB are complementary, and both techniques should be available. The choice ought to be made based on the particular circumstances and a conjunction of clinical, radiologic, and pathologic findings.

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 as an outpatient service in the clinic. Patients are usually booked in advance, by letter of referral or by telephone. The staff and equipment requirements are minimal and include an assistant (particularly important when performing image-guided FNAC), an examination couch, a writing desk, a work surface, an examination tray (containing instruments), and good lighting. The work surface should ideally have access to running water (i.e., a sink). Consumables should be placed on the work surface, perhaps inside a tray and including: cotton pads/gauze and an antiseptic solution for skin antisepsis before puncture; gloves for hygiene and protection; a syringe, needle, and a syringe holder for the aspiration procedure; glass slides for smear preparation; liquid fixatives, preferentially 95 or 100% alcohol or formaldehyde, for cell block preparation; a small bandage, for post-procedure hemostasis; and a disposal boxes for needles and another for material contaminated with biological fluids (Fig. 3.1).

By placing these materials inside a small box, one can arrange a simple kit enabling the performance of FNACs at any location, be it the radiologist's room, a clinician's office, or an internment ward. If ROSE is to be done, a microscope and quick staining kit should also be available.

Needles are a crucial element of the FNAC procedure and should be chosen with care. In terms of diameter, experienced cytopathologists advocate for the use of needles with 23G or smaller, although others use needles up to

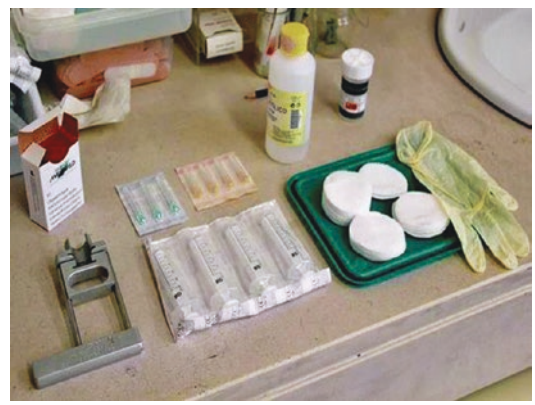

Fig. 3.1 Basic equipment for performing breast FNAC

27G. There are advantages to a smaller diameter: they result in less pain for the patient and in lower rates of complications (such as hemorrhage). As for length, it depends on the target lesion. Most breast lesions can be reached with a 25–30 mm needle.

Syringes are also an integral part of the procedure. Transparent plastic, disposable, sterile syringes are preferred. Most pathologists and clinicians use 10 ml syringes for FNAC, but larger 20 ml ones may also be employed and are particularly useful in the context of large cystic lesions. It is important to remember, however, that in most cases larger syringes will not provide larger or better samples: on the contrary—since the pressure of the vacuum is larger, these may result in more hemorrhage and a higher number of inadequate samples, particularly if the target lesions are highly vascularized.

Finally, the syringe holder. This device allows for suction and release of the syringe to be accomplished with one hand, freeing the other to locate and stabilize palpable lesions. Several types are available, including those made of metal or plastic. The authors prefer those made of metal, which have a higher durability while still weighing below 200 g. As a last piece of advice, the pistol-grip syringe holder allows for the creation of large vacuums, which, as we have discussed, may result in the aspiration of blood and little else. A good quality aspiration sample should fill just the needle hub and not the syringe.

3.3.2 Lesion Localization

Palpable and non-palpable breast lesions can be submitted to the FNAC procedure. Breast aspiration should preferably be guided by imaging, but it is largely accepted that palpable lesions can be sampled using only palpation as a guide.

3.3.2.1 Palpation

The localization of a nodule may be obvious on simple observation, and a thorough palpation of the breast is not necessary for every procedure. However, when the lesion is not easily identified, a good clinical examination of the breast should be performed. In this situation, it is best to examine the patient in the supine position, forearm up and underneath the head, palpating the breast systematically, nipple to axillary tail in a circular fashion. 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. Aspiration may be done in the supine position. However, this can at times prove difficult, due to the fact that the breast will flatten out moving the lesion closer to the chest wall and increasing the risk of pneumothorax. The risk can be mitigated by using a tangential approach, but this is not ideal, since it may lower the diagnostic yield.

Thus, it is usually better to ask the patient to stand upright after the lesion has been located and identified. In the upright position, a mass within the breast will become suspended by gravity and more tethered to the surrounding connective tissue. This added immobilization of the mass enhances material acquisition and moves the nodule away from the chest wall, decreasing the risk of pneumothorax (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. However, given that the breast is largely made of adipose tissue, lesions contained within are usually mobile, which may hinder adequate sampling. Consequently, it is crucial to immobilize the mass so that when a needle is introduced, a coring action is achieved. This can be done by placing the fingers on each side of the mass, stretching the skin, and flattening the breast. When possible the fingers should be placed along the axis of greatest mobility. This procedure also enables almost any mass to be reached with a 30-mm needle. Sometimes, because of the tumor's depth within the breast, the large size of the breast, and/or the infiltrative nature of the mass, a lesion will lack definition. On palpation, only a vague firmness will be appreciated, usually felt over a large portion of the breast. In this context, aspirations may yield little significant tissue, even when multiple passes are performed. This is typically due to an erroneous evaluation

on the part of the operator: since the mass is deep seated, its firmness may be mistaken for the chest wall, leading to superficial aspiration attempts in fear of risking a pneumothorax.

An additional difficulty may arise in this context, if there is a tendency to approach lesions with the needle positioned tangentially to the skin, rather than with a near vertical or perpendicular approach. This tangential positioning may be more comfortable for the aspirator but it is not optimal for small deep-seated nodules. 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, even if it feels uncomfortable to some aspirators. The best approach to fix the nodule appropriately is to use the middle and index fingers for immobilization, instead of the commonly used thumb and index finger (Staerkel and Sneige 2006).

3.3.2.2 Ultrasound-Guided FNAC

Nowadays, ultrasound is frequently used to guide breast FNAC, even if lesions are palpable. This technology enables the cytopathologist to visualize the lesion and choose the right area for aspiration (for example, solid areas, instead of cystic or necrotic areas—Liao et al. 2004). There are two methods which can be used for guidance, depending on the site of the lesion: in the first one the transducer probe is used to locate the lesion and place it in the middle of the ultrasound field. The operator then makes a mark using the transducer to pressure the underlying skin, circumscribing the puncture site; this is followed by passing the needle through the skin and advancing it slowly into the lesion, in a way that the transducer probe can be placed perpendicular to the needle, guiding its movements. In the second method, the FNAC procedure is guided step by step so that the ultrasound field encompasses the entire path of the needle, from the moment it pierces the skin and until it is removed. The transducer probe is used to locate the lesion in one of the edges of the ultrasound field; the aspirator then passes the needle through the skin at the edge of the probe and in parallel to it. 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 FNAC of the breast (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, which enables better cutting and coring of the mass. Once the lesions are immobilized, as described above, the needle, with a syringe and holder attached is inserted into the mass. Then, the syringe plunger is pulled back, creating negative pressure, as the needle is moved back and forth in a rocking motion. 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,

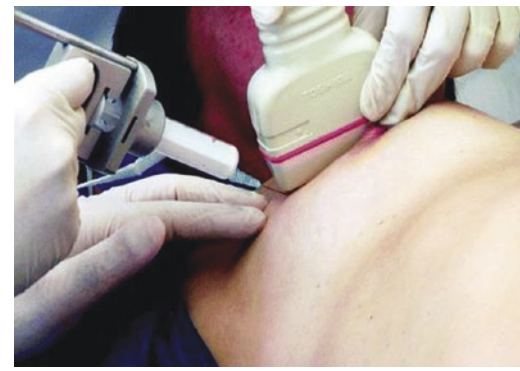

Fig. 3.2 Ultrasound-guided breast FNAC. The transducer probe locates the lesion in one of the edges of the ultrasound field; the aspirator passes the needle through the skin, in parallel with the transducer probe at the edge where the lesion is located in

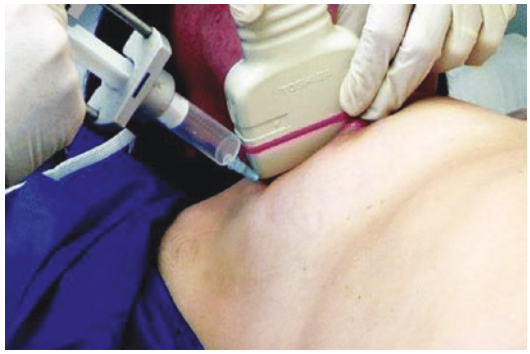

Fig. 3.3 Applying suction while moving the needle helps to pull cells into the needle. In general, needle movements that are more frequent, longer in length, and kept within the tumor during the entire aspiration yield more tissue. A blood-tinged specimen will appear in the hub of the needle. Suction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

and kept within the tumor during the 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 should be made over a 10–20 s period. A blood-tinged specimen will appear in the hub of the needle. Suction should then be released, and the needle is withdrawn. Although little bleeding occurs in fine needle aspirations, it is best to avoid all bleeding, as it can decrease cellular yield and lesion demarcation. Therefore, after withdrawing the needle, a gauze pad should immediately be applied over the puncture site and pressure applied, for at least 1–3 mins (a longer time should be applied in individuals with an easy bruising history or for those currently taking blood-thinning agents).

After removing the needle from the patient, the syringe should be detached from the needle. The plunger should be pulled back, filling the syringe with air. Leaving it in this position, the needle should be reattached. The aspirator then places the needle close to a glass slide, almost touching, and uses the plunger to expel a drop of the sample acquired onto the slide, repeating the procedure in as many slides as necessary until there is no material left. The drops are then smeared, fixed in 95% ethanol or air-dried, and then stained for interpretation.

The procedure outlined above is the most frequently used and is valid for both palpable and non-palpable lesions. There is an alternate technique, however, which is worthy of mentioning, the so-called capillary method. Most steps are similar to what was previously described, but neither a syringe nor a syringe holder is used. Instead, the needle is held directly by the aspirator by gripping the hub. This allows for a heightened appreciation of tissue density and an overall greater sensitivity to movements. Virtually no bleeding occurs; since there is no suction, frequently no material will be seen in the needle hub and the aspiration has to be voluntarily stopped after 15–20 seconds without visual feedback. After the needle is withdrawn, an air-filled syringe is attached, and material is expelled onto slides as previously described. This method may result in low cellular yields in fibrotic and sparsely cellular lesions. If the lesion is a cyst, rapid leakage of fluid from the end of the needle due to pressure may happen. An empty recipient should be kept at hand for this eventuality.

Usually, a single aspiration is sufficient for diagnosis. However, additional aspirations are recommended in certain circumstances that the aspirator should be aware of (Arkoumani and Wells 2006):

- Lesions that are fibrous in nature; can be felt through palpation and appreciated in ultrasound; a fibrous stroma may represent a desmoplastic carcinoma which will tendentially have a low cell yield.
- Small lesions, due to the risk of missing the target.
- A specimen that clots immediately when placed on a glass slide, since this is 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.
- If the aspirate is yellow and slightly oily in appearance; this indicates that the specimen is primarily composed of adipose tissue frequently with little or no epithelium; this may be acceptable in some contexts, however,

namely if one is relatively confident that a nonmalignant process is being evaluated—that is, a nodule with low clinical and imaging suspicion, which is soft on palpation and offers little to no resistance on needle penetration.

- Breast cysts: these require complete drainage and the drained area should be reevaluated by palpation or imaging for any residual solid mass, which, if present, will in turn require a separate needle aspiration.

3.3.4 Preparation of Smears

Glass slides should be clean and ready to use. They should be labeled using a pencil at the frosted end. The labeling can include the patient's name (initials), identification number, or site of aspiration. It is 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). 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. Cells should be spread over the slide surface by gentle pressure so that they are not crushed. If the smears are not interpretable, then a reliable diagnosis cannot be made, no matter how adequate the aspirated specimen is or how much experience the cytopathologist has. Keep in mind that an FNAC smear is not a blood cells analysis smear and one does not need the thinnest smear, but one 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 methods (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 droplet is placed near the slide label. This specimen slide is then held by the physician's dominant hand in a vertical position. Another slide is used as a spreader, held by the contralateral hand, placed perpendicularly over the other slide, at an angle, so that its superior edge is poised above the specimen droplet. Then, smoothly, the spreader slide is lowered until it touches the specimen slide, homogenizing the droplet, and, while applying a constant and gentle pressure, a downward motion is performed, 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, occupying the smallest area of the slide possible.

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

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 45° 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,

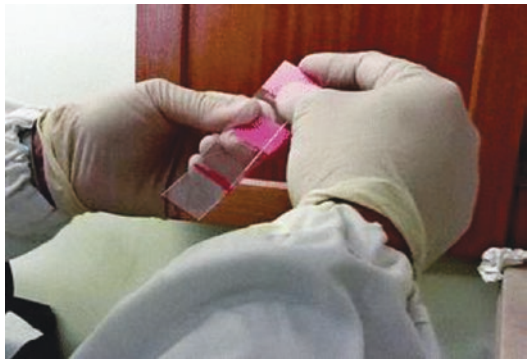

Fig. 3.5 Preparation of smears using the two-step method. Observe the concentration of the material in the middle of the slide that will be smeared according to the one-step technique

which is turned to one side in order to drain the excess fluid. 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 as 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 better fixation of the slide.

Another technique should be kept in mind. In certain circumstances, it may be necessary to prepare more than one smear from a single droplet of material. This may happen for different reasons, for example, to divide material across multiple slides for ancillary tests or when too much liquid is placed on a single slide accidentally. In the first case, the entire sample should be expelled onto a single glass slide. The slides are then held as described at the beginning of the one-step method. One of the corners furthest away from the frosted end of the spreader slide is quickly brought in contact with the sample, carrying a part of it. The spreader slide can then be turned around, and the opposite corner touched to the slide as well. The corners closest to the frosted end may also be used in a similar fashion. This can be done at least four times with the same specimen slide. Each portion of the original sample in the spreader slide is then smeared onto a new slide, and in the end this slide is used to smear the original sample, following the one-step method.

Other than smearing, samples may be directly expelled to a vial for several purposes. This may be particularly useful in liquid or bloody specimens. The vial may be filled with saline or a fixative, such as formaldehyde or ethanol. The former, enables a sample to be sent for flow cytometry, for example, while the latter two enable the preparation of cell blocks and liquid-based cytology slides. Cell blocks are particularly useful for immunohistochemistry.

In the authors' experience, processing of aspirated materials by liquid-based technique should only be used in specific situations, such as those mentioned above. For routine morphological observation, it is preferable to prepare smears, given their simplicity and better preservation of architectural features and extracellular matrix.

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 stains in FNAC, which are complementary: 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 this method. Some cytopathologists prefer to stain FNAC slides using hematoxylin and eosin (H&E) as in histology, mainly due to better familiarity with this stain.

The smears prepared after the aspiration procedure can be either intentionally air-dried for Romanowsky staining or immediately fixated 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 one with which the cytopathologist is most familiar with, but each method has its advantages, and they work 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 carcinomas.

On the other hand, nuclear details are not the strength of the Romanowsky method. Nuclei not only look larger using these stains, but they also lack chromatin and membrane contour details. For this reason, a Papanicolaou stain should 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 thus 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 and provide additional information, they are recommended to be used preferentially in cytological specimens.

3.3.6 Reporting of the Results

Reporting the results is one of the most important parts of the FNAC procedure. The cytopathologist should have in mind the possible consequences of the report and be cautious when making a suspicious or inconclusive diagnosis. Comments and explanatory notes should be used judiciously and only if they are thought to be helpful to the clinicians in making a therapeutic decision. Negative results should always be correlated with clinical and imaging findings in the context of the triple test. If the aspirated lesion is suspicious for malignancy in the clinical or imaging setting, a negative result in the FNAC specimen may not rule out malignancy, and an alternative method, such as CNB or surgical excision should be performed in order to obtain a reliable diagnosis.

The authors recommend the use of the Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology, which divides breast FNAC diagnoses into five categories, with well-defined risks of malignancy: insufficient for diagnosis, benign, atypical, suspicious for malignancy and malignant. The risks of malignancy and reproducibility of criteria for each of these categories have been validated in the literature (Montezuma et al. 2019).

Using these categories, communication with clinicians is improved, enabling better patient management. Additionally, the Yokohama system provides a framework for standardized reporting, while at the same time being adaptable to different realities. Namely, the system states that all reports should include not only the relevant category (in full, never as a code), but also a clear cytological description, including cellularity and presence or absence of key diagnostic features. A concise comment or conclusion should also be included, providing a diagnosis that is as specific as possible, or, in alternative, a list of most likely differential diagnoses.

When appropriate, synoptic reports may be used, providing a checklist for the cytopathologist, and streamlining communication with clinicians.

Based on the recommendations of this classification system, a report for breast FNAC should be structured as a report in surgical pathology, providing all the clinical information, including specimen type and localization technique, followed by a microscopic description and the diagnostic conclusions, including the final diagnosis

and category in full. An additional comment may be added, including recommendations to correlate the cytology with clinical and imaging findings and/or 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. Cytopathologists should pay particular attention to these:

- Patient age. Different pathologies have different prevalences across the age spectrum. For example, advancing age increases suspicion for carcinoma and decreases the likelihood of fibroadenoma.
- Lesion location. Different lesions arise in different areas of the breast. For example, 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 neoplasms 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 triple test—Kocjan et al. 2008; Arkoumani and Wells 2006). 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 incisional or 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/or pneumothorax. The most likely complication is soft tissue bleeding with a resulting hematoma; however, this can be avoided if, after aspiration, firm pressure is applied directly over the puncture site. Infection is uncommon but when present is of little consequence and may 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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4 Liquid-Based Cytology and Cell Block in Breast Lesions

Daniel Gomes Pinto, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

4.1 Liquid-Based Cytology

Liquid-based cytology (LBC) is a method of retrieving and processing cytologic material for assessment. First developed for cervical cytology screening in an effort to reduce inadequacy rates, the use of LBC has been extended to all areas of cytology, including FNAC, and today is a mainstay in many practices worldwide. 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. The final result is a slide with all cells concentrated in a smaller area when compared to smears, with comparatively less overlapping and background debris, which can enhance and facilitate interpretation. These monolayered preparations can also be subjected to adjunctive studies like immunohistochemistry, usually with good results (Gerhard and Schmitt 2014).

There are many ways of achieving monolayered cell preparations, most commonly through the use of automated platforms like ThinPrep and SurePath. Alternative manual techniques are also available, which are less costly and can produce similar results (Wauters et al. 2009). These may be particularly important in the setting of practices in low-income countries.

Overall, the literature reports on overlapping sensitivities and specificities between conventional smears and LBC in the context of breast FNAC, showing that this preparation type is adequate for diagnosis (Gerhard and Schmitt 2014). The importance of familiarizing oneself with the sample type cannot be overstated, however, with one publication showing an improvement of 8% in overall sensitivity after training (Feoli et al. 2013). Interestingly, and highlighting the potential of LBC in the setting of the breast, one study found that monolayered preparations 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).

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4.1.1 Advantages

LBC provides adequate cellularity for evaluation of breast lesions, and seems mostly equivalent to smears in this regard (Ryu et al. 2013). However, as was described by Wauters et al. (2009) in LBC cells are concentrated on a specific area on a single slide, resulting in higher cellularity per field, as compared to conventional smears, in which the cells are dispersed over multiple slides. Furthermore, nuclear detail has been reported to be enhanced compared to smears (Gerhard and Schmitt 2014).

LBC also allows for the direct fixation of cells, eliminating air-drying and smearing artifacts, as well as avoiding interfering background material.

ThinPrep smears are described to be consistently devoid of obscuring elements, with adequately preserved and dispersed cells, and this is also true for breast FNAC samples (Gerhard and Schmitt 2014). The background is cleaner. 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 with good or very good concordance with tissue (Tripathy et al. 2018).

4.1.2 Disadvantages

There are some limitations that need to be noted in the interpretation of LBC smears, however, 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. A more pronounced three-dimensional arrangement of clusters has also been reported.

Cells tend to be more rounded and smaller, with nuclear features which may be less obvious, or more subtle in certain entities. Prominent nucleoli are to be expected, and hyperchromasia may be seen in both benign and malignant lesions of the breast (Ryu et al. 2013). There is also an alteration in the amount and quality of background material. In particular for the breast, the number of myoepithelial cells is reduced (Feoli et al. 2013). 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 fibroadenomas or mucinous carcinoma, for which background elements are essential for a correct diagnosis. Diminished amounts or altered quality of background, along with other changes induced by LBC, may hamper the diagnosis, particularly if the pathologist has little or no experience with LBC in the setting of breast pathology.

Epithelial fragmentation may lead to difficulties in some entities, namely in papillary lesions, where the defining architecture may be difficult to recognize; cellular discohesion can simulate malignant dispersion in several lesions.

Most of these problems may be mitigated or nullified with adequate training and experience in the interpretation of LBC slides (Feoli et al. 2013).

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).

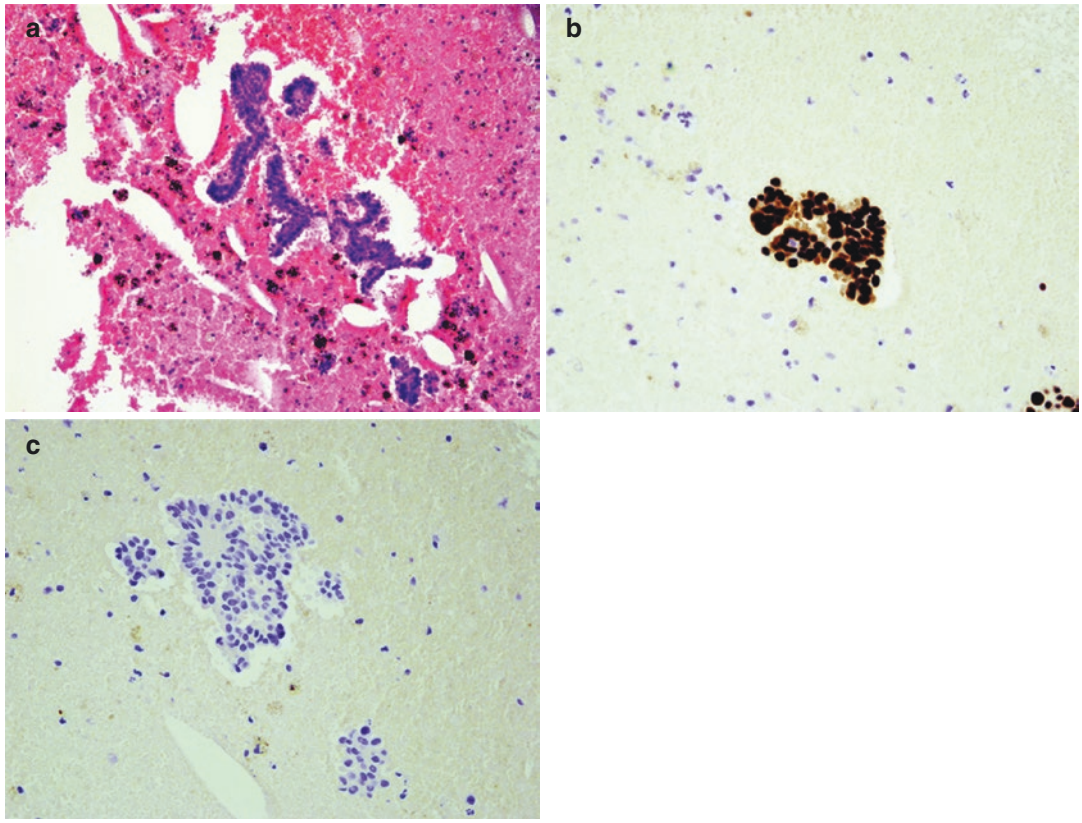

Fig. 4.1 (**a–c**) Papillary carcinoma. (**a**) Cell block shows papillary fragments within a bloody background. (**b**) Immunohistochemistry on cell block shows positive nuclear staining for estrogen receptors. (**c**) Immunohistochemistry on cell block shows no staining for CK14

There are many methods that have been described which can produce optimal cell blocks for subsequent histologic examination. Some of these are proprietary and require the use of specialized equipment, which can be costly.

Laboratories should use the one they are most familiar with and comfortable with. If cost is of importance, several simple methods have been described which can be performed in-house for a low cost (Krogerus and Kholová 2018). Of note, we have reported on 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).

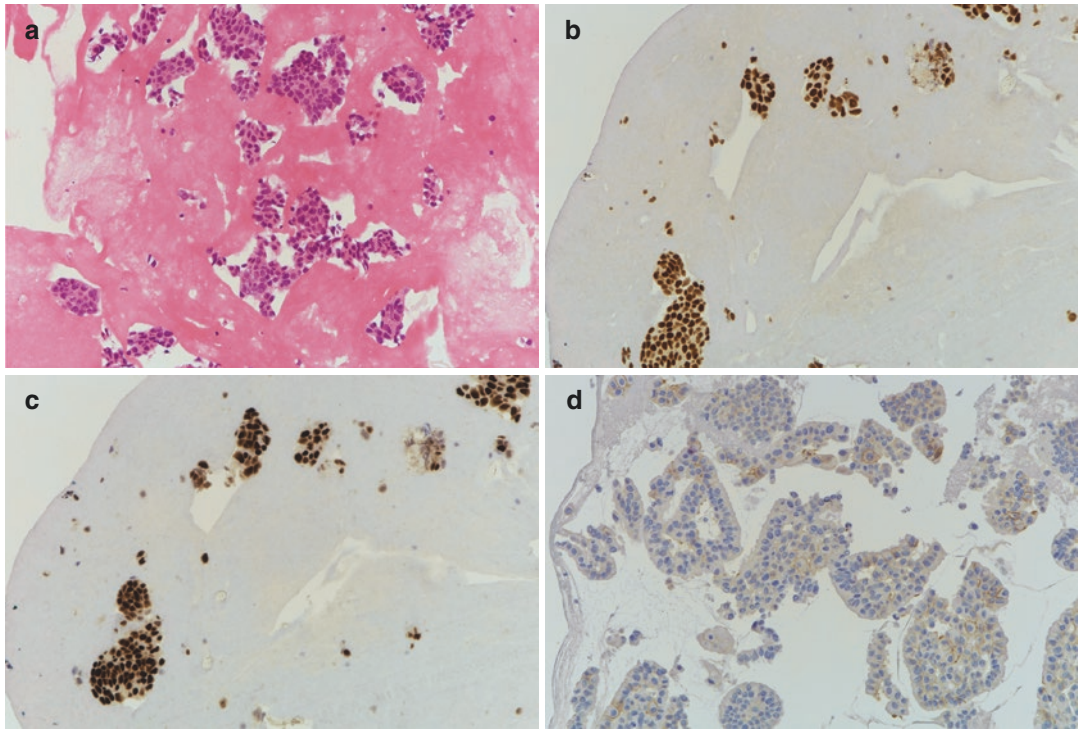

Fig. 4.2 Breast ductal carcinoma. (**a**) Cell block shows small clusters of malignant cells (H&E). (**b–d**) Cell block immunohistochemistry of breast ductal carcinoma. (**b**) Estrogen receptor (ER) positivity. (**c**) Progesterone receptor (PR) positivity. (**d**) HER2 immunohistochemistry shows 1+ staining along cytoplasmic membranes of some tumor cells, indicating a negative result

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 ourselves with any pitfalls encountered.

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5 Breast Fine Needle Aspiration Cytology: Introduction to the Yokohama Classification

Elena F. Brachtel, Fernando Schmitt, Gary Tse, and Puay-Hoon Tan

5.1 Insufficient Category

5.1.1 Definition

An insufficient or unsatisfactory breast cytology sample consists of smears or liquid-based cytology that are "too sparsely cellular or too poorly smeared or fixed to allow a cytomorphological diagnosis" (Field et al. 2019).

5.1.2 Risk of Malignancy (ROM)

An average ROM of 2.6–4.8% is provided but rates reported in the literature can vary widely (Hoda and Brachtel 2019; Montezuma et al. 2019; Wong et al. 2019).

5.1.3 Discussion

This definition is primarily technical and applied to a cytology sample that is insufficient in quality and/or quantity; the cells are too few or damaged because of smearing artifact or fixation, such as too slow or incomplete air-drying of air-dried smears, or involuntary air-drying in ethanol fixed samples (Fig. 5.1).

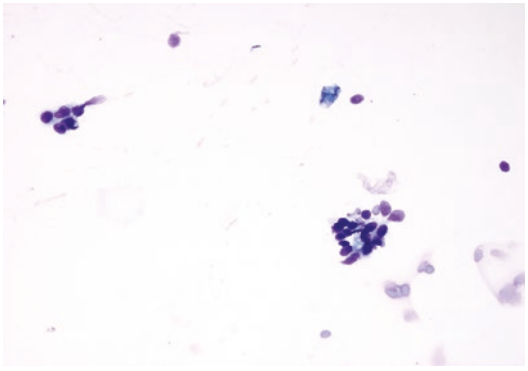

Fig. 5.1 insufficient sample: Giemsa stained air-dried smear with a few small groups of ductal-type epithelial cells and possible bipolar nuclei. Too few cells

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The insufficient rate depends on the skill of the practitioner to perform an aspiration, the technical skills to prepare smears and handle the cellular material appropriately, and of the lesion itself.

Rapid onsite evaluation (ROSE) provides immediate feedback to the aspirator and cytology staff and can help to reduce the insufficient rate (Wong et al. 2019).

The term "non-diagnostic" is a correlative statement and not preferred here (a sample can be technically perfect but still not diagnostic if the imaging or clinical impression does not match).

Even an insufficient breast cytology sample can sometimes represent a valuable assessment for a clinician, for example, if the question is cancer recurrence or scar tissue.

5.2 Benign Category

5.2.1 Definition

The benign category is applicable for "cases that have unequivocally benign cytological features, which may or may not be diagnostic of a specific benign lesion" (Field et al. 2019).

5.2.2 Risk of Malignancy (ROM)

ROM 1.4–2.3%, reported as generally under 5% (Hoda and Brachtel 2019; Montezuma et al. 2019; Wong et al. 2019).

5.2.3 Discussion

A benign breast lesion with a concordant triple test, that is matching clinical and radiographic features has a very low false negative rate.

In the context of a general clinic setting where the majority of breast lesions are benign, breast cytology can be used to rapidly diagnose and reassure patients (Smith et al. 2012).

Although a certain number of ductal epithelial groups are usually required, cellularity and content depend on the lesion: a fibroadenoma typically yields large groups of epithelial sheets and can be remarkably cellular, fibrocystic changes show ductal epithelial groups of various sizes. Generally present are the small oval nuclei of myoepithelial cells (also referred to as bipolar or stripped nuclei because of their lack of cytoplasm) which are scattered in the background of a smear or attached to the ductal epithelial groups. A lipoma, fat necrosis, abscess, or an intramammary lymph node on the other hand would not

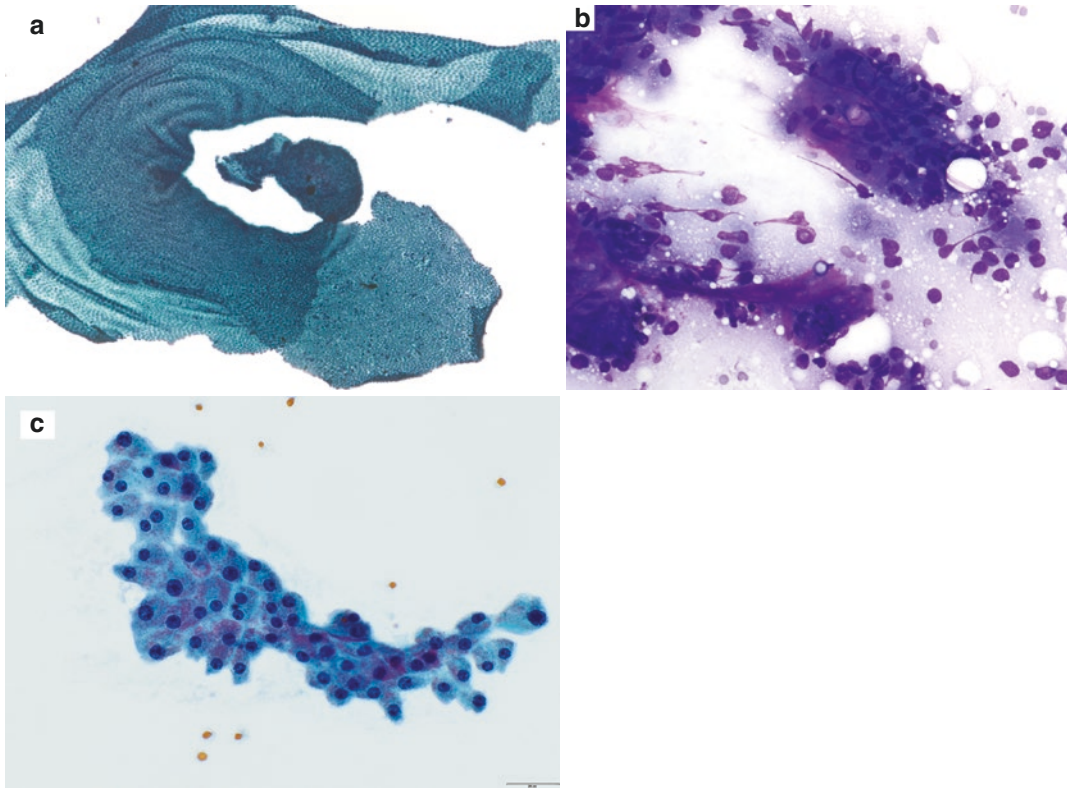

Fig. 5.2 (**a–c**) benign: (**a**) Papanicolaou stained smear shows a large flat sheet of benign ductal cells with a sprinkling of myoepithelial cells in a fibroadenoma. (**b**) Giemsa stained air-dried smear with slightly discohesive groups of ductal epithelial cells, myoepithelial cells, and vacuolated material in the background in a breastfeeding patient consistent with lactational changes. (**c**) High-power view of a benign flat sheet of apocrine cells with round nuclei with minimal nuclear size variability, abundant granular cytoplasm and low nuclear:cytoplasmic ratio from an apocrine cyst

yield ductal epithelial cells. Cyst contents typically show cyst debris with foam cells, histiocytes, and a variable number of ductal cells (Fig. 5.2a–c).

5.3 Atypical Category

5.3.1 Definition

"Cytological features seen predominantly in benign processes or lesions, but with the addition of some features that are uncommon in benign lesions, and which may be seen in malignant lesions" (Field et al. 2019). An abnormality is seen and follow-up with repeat sampling is usually indicated.

5.3.2 Risk of Malignancy (ROM)

ROM in the literature ranges between 22 and 39%, and recent studies applying the Yokohama system were reported between 13 and 15.7% (Hoda and Brachtel 2019; Montezuma et al. 2019).

5.3.3 Discussion

Once technical factors are excluded that make cells look abnormal, for example, unwanted air-drying artifacts, it is usually high cellularity, three-dimensionality, or subtle nuclear changes on the cytology preparations that elicit a diagnosis. It is acknowledged that atypical breast cytology

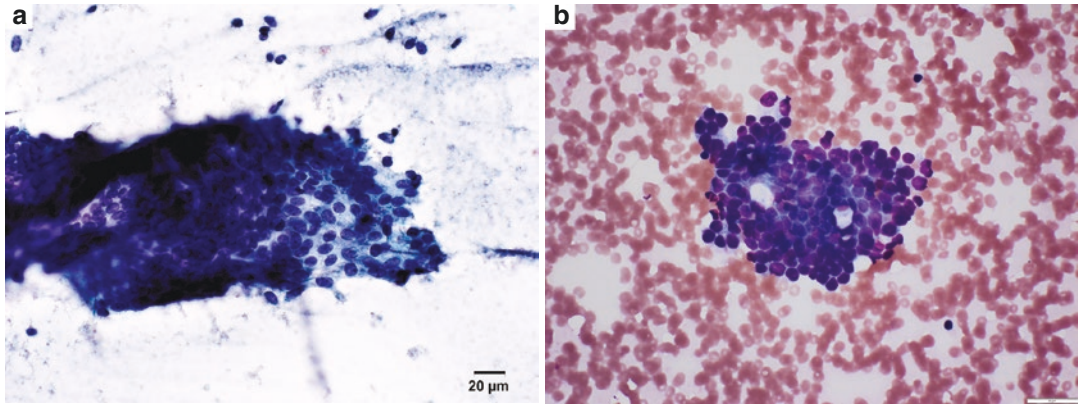

Fig. 5.3 (**a**, **b**) atypical: (**a**) Papanicolaou stained smear shows a ductal group with enlarged and crowded nuclei; histology showed a benign, sclerosing papilloma. (**b**) Giemsa stained smear shows a group composed of relatively small atypical ductal cells with slightly irregular chromatin and increased nuclear:cytoplasmic ratio; histology showed lobular carcinoma

diagnosis tends to show low interobserver agreement (Layfield et al. 2020; Marabi et al. 2021).

Atypical is when we think it is benign, but some features are unusual; therefore, this is a diagnosis to be confirmed. Papillary lesions or radial scars with a proliferative pattern can produce atypical cytology, also spindle cell lesions such as fibromatosis or inflammatory fibrous nodules with a differential diagnosis of phyllodes tumor.

Lobular-type epithelial cells are typically small, loosely cohesive, and may be sparse; the histological correlates include the range of lobular neoplasia (classified as benign) to invasive lobular carcinoma which may be associated with sparsely cellular fine needle aspirates (Fig. 5.3a, b).

5.4 Suspicious Category

5.4.1 Definition

"…some cytomorphological features, which are usually found in malignant lesions, but with insufficient malignant features, either in number or quality, to make a definitive diagnosis of malignancy" (Field et al. 2019).

5.4.2 Risk of Malignancy (ROM)

In the literature, the ROM was found to be at least 85%, in recent studies applying the Yokohama system the rate was 85–97% (Hoda and Brachtel 2019; Montezuma et al. 2019; Wong et al. 2019).

5.4.3 Discussion

The suspicious category cases present some cytomorphological features, which are usually found in malignant lesions, but with insufficient malignant features, either in number or quality, to make a definitive diagnosis of malignancy (Field et al. 2019). If there is any doubt, a diagnosis of "suspicious for malignancy" is preferred. This includes a comment type of malignancy is considered—suspicious for carcinoma, for example. Most follow-up—but not all—reveals malignancy in subsequent sampling. Diagnoses, where a definitive malignant diagnosis may be difficult, are well-differentiated mammary carcinomas with few atypical single cells and subtle nuclear irregularities. Ductal carcinoma in situ contains myoepithelial cells and can be mass forming

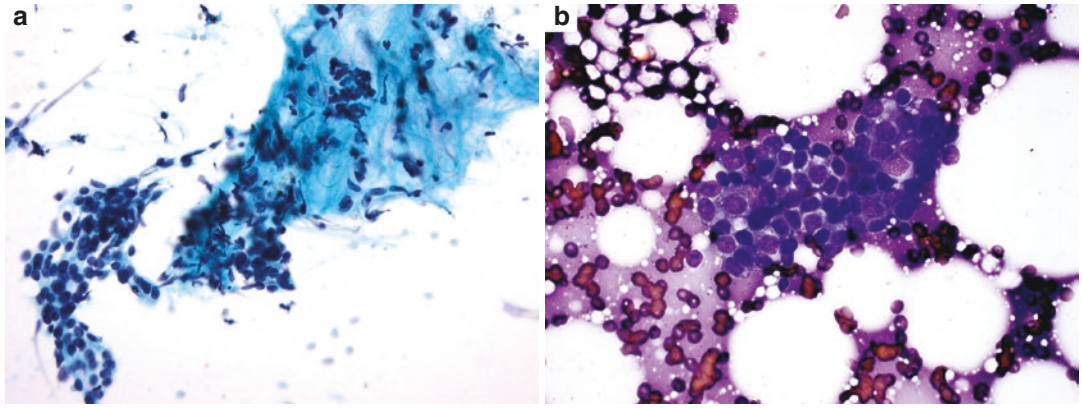

Fig. 5.4 (**a**, **b**) suspicious: (**a**) Papanicolaou stained smear with an irregular crowded group composed of atypical ductal cells with hyperchromatic chromatin and possible infiltrating into a crushed stromal fragment, histology showed well-differentiated invasive carcinoma. (**b**) Giemsa stained smear shows ductal group with enlarged, slightly irregular nuclei; histology invasive carcinoma

for which a cytologically "suspicious" diagnosis is preferred. Rare causes for a suspicious call on breast cytology have been radial scars which can also produce suspicious clinical findings (Dong et al. 2016) (Fig. 5.4 a, b).

5.5 Malignant Category

5.5.1 Definition

"A malignant cytological diagnosis is an unequivocal statement that the material is malignant, and the type of malignancy identified should be stated whenever possible" (Field et al. 2019).

5.5.2 Risk of Malignancy (ROM)

Based on recent studies using the Yokohama System diagnostic categories and literature review, ROM is 99–100% (Hoda and Brachtel 2019; Montezuma et al. 2019; Wong et al. 2019).

5.5.3 Discussion

The cytomorphological findings must be unequivocal in terms of quality and quantity. Not all samples will be vastly hypercellular as the one shown here but no compromise should be made if there is any doubt that the lesion is malignant (Dong et al. 2016).

The majority of breast cancers are invasive carcinoma of no special type (NST), moderately or poorly differentiated (Fig. 5.5a–c).

Controversy exists as to the nature of high-grade ductal carcinoma in situ (DCIS) on cytological preparations. Many breast cancers show an invasive component with admixed DCIS. Since the treatment is centered on surgery, the diagnosis of "malignant" for high-grade DCIS is appropriate. For questions of presurgical chemotherapy, ancillary techniques or further biopsy may be required to ensure the invasive nature of the cancer cells.

Invasive lobular carcinomas can show a spectrum of cellularity and nuclear pleomorphism,

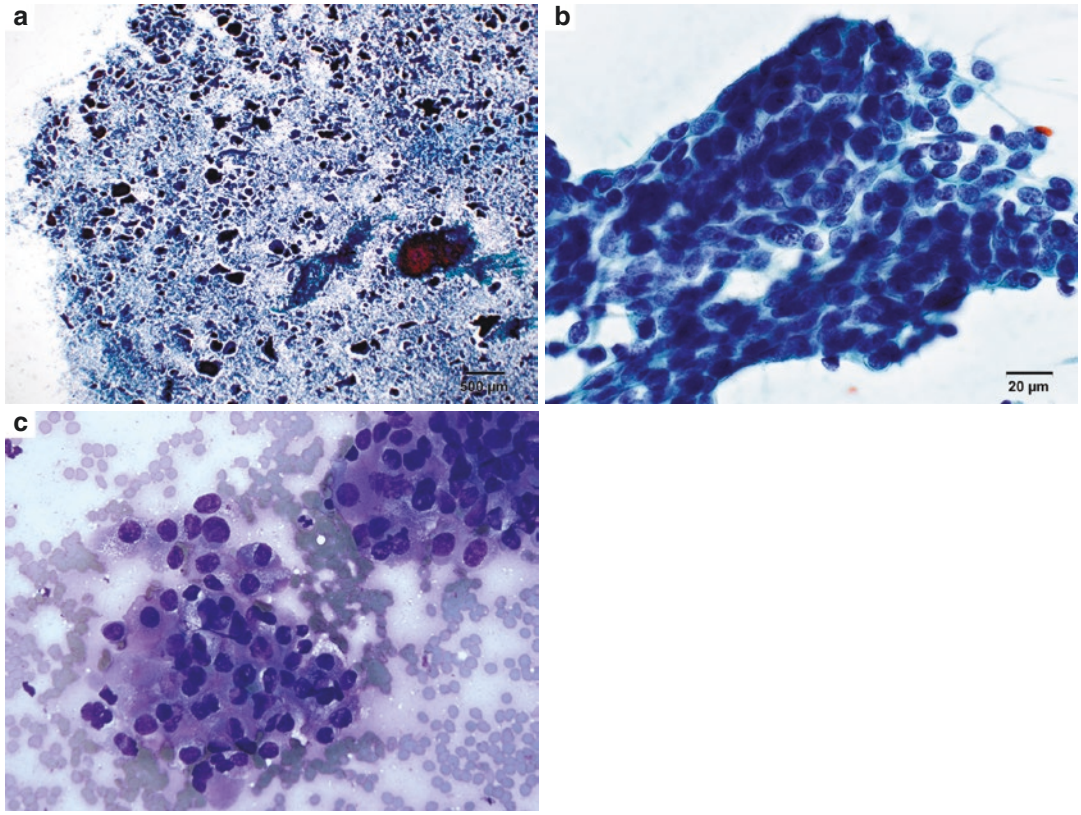

Fig. 5.5 (**a–c**) malignant: (**a**) Overview of Papanicolaou stained smear shows a markedly hypercellular sample with large irregular groups and single cells. (**b**) Large groups showing irregular pleomorphic tumor cells. (**c**) Giemsa stained aspirate showing tumor cells, singly and in groups, with irregular and enlarged nuclei

and a definitive diagnosis of malignancy may not be possible on a cytology preparation.

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Inflammatory Lesions of the Breast

6

Yunbi Ni, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

6.1 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.

6.2 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 breastfeeding, 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. 6.3). 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 abscesses are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Bacteroides* species.

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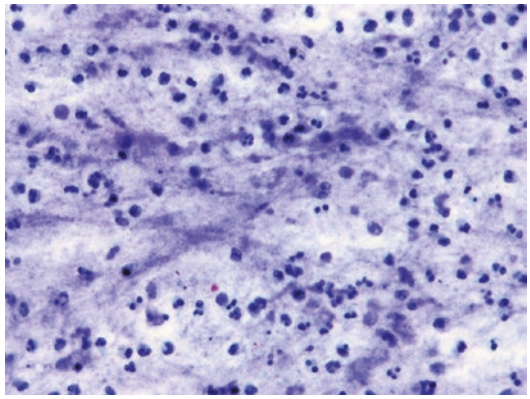

Fig. 6.1 Acute mastitis. Cellular smear showing predominantly neutrophils and fibrin (Pap stain)

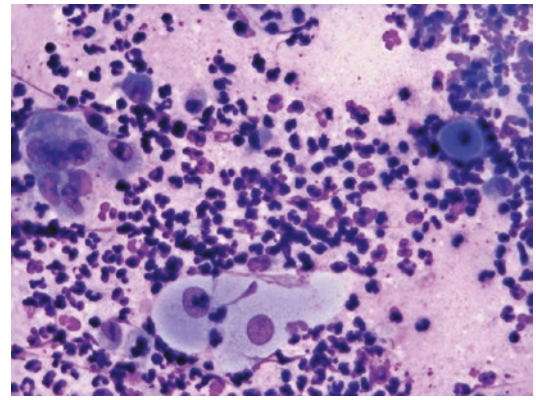

Fig. 6.2 Subareolar abscess. The smear shows the presence of inflammatory cells with multinucleated giant cells and metaplastic squamous cells (MGG stain)

During FNAC, the material aspirated is purulent. The smears are cellular, with predominantly neutrophils and fibrin (Fig. 6.1). 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).

6.3 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. It is not clear whether the lesion regresses after quitting smoking.

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. 6.2). The presence of reactive nuclear atypia in the epithelial cells can be confused with malignancy by the unwary.

6.4 Duct Ectasia

Duct ectasia is an inflammatory disorder that affects women aged between 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, FNAC is essential to rule out the possibility of malignancy. Duct ectasia affects the larger breast ducts and is

characterized by duct dilatation with periductal 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. The aspirate consists of cheesy-like thick fluid, which is composed of amorphous material, cellular debris, plasma cells, neutrophils and macrophages, and ductal cells with reactive atypia may be present.

6.5 Chronic Granulomatous Mastitis

This group can be subdivided into two: those related to specific infections, like tuberculosis, fungi, syphilis or corynebacteria, and those that are non-infectious, including involvement by systemic non-infectious granulomatous diseases, such as sarcoidosis and granulomatosis polyangiitis, and 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. 6.3a, 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. 6.4). 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. 6.5).

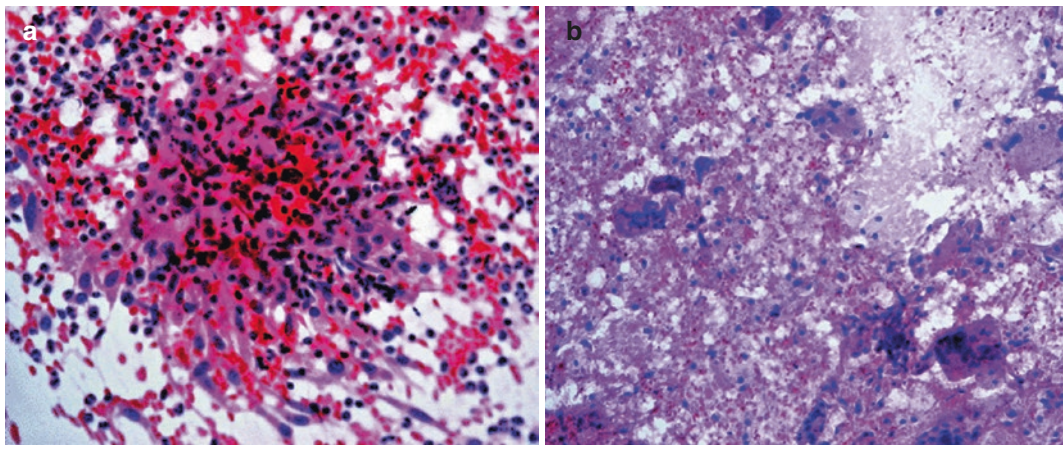

Fig. 6.3 (**a**, **b**) Mammary tuberculosis. (**a**) Cellular smear shows epithelioid granulomas, with lymphocytes and neutrophils in the background. (**b**) Cellular smear highlights Langhans giant cells and necrosis

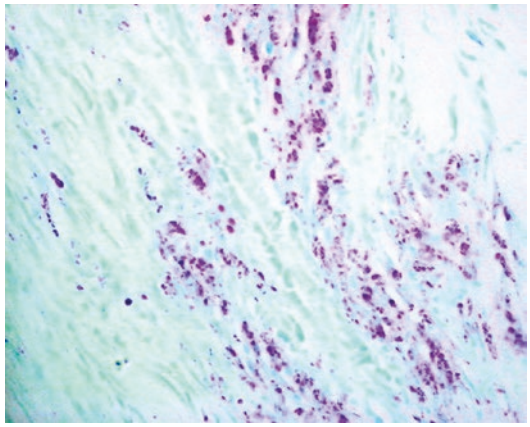

Fig. 6.4 Mammary tuberculosis. Alcohol-acid-resistant bacilli are demonstrated in a tissue section by Ziehl-Neelsen staining

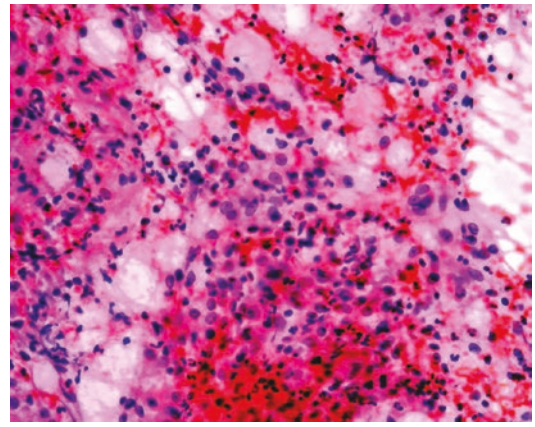

Fig. 6.6 Idiopathic granulomatous mastitis. FNAC smears show epithelioid macrophages, giant cells, and neutrophils, but necrosis is absent

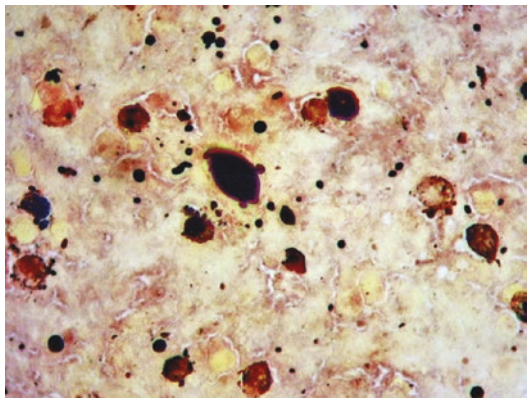

Fig. 6.5 Breast paracoccidioidomycosis. Note the yeast cells with multiple budding "steering wheels"

When all other causes of granulomatous infections of the breast are excluded, the diagnosis of idiopathic granulomatous mastitis (IGM) can be made. This condition affects young women aged between 20 and 40 years and is frequently associated with 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. 6.6) (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.

A morphologic variant of IGM is cystic neutrophilic granulomatous mastitis (CNGM). This rare granulomatous inflammatory process mainly affects young females who are parous or currently pregnant (Wu and Turashvili 2020). It is strongly associated with Gram-positive *Corynebacterium* species (Taylor et al. 2003). Usually, it is unilateral, although 8.5% of patients present with bilateral disease. The common manifestations include breast mass, nipple inversion, and sinus formation mimicking breast cancer clinically. Although FNAC could aid in the diagnosis of this clinically worrisome entity, there are no well-defined cytology criteria for diagnosing CNGM. Cytologic diagnosis might be limited by the significant cytomorphologic overlap with other granulomatous mastitis. A study suggests the possibility of CNGM should be raised in the presence of well-formed clear space representing dissolved lipid in conjunction with epithelioid histiocytes, granulomas, multinucleated giant cells, and neutrophils on ThinPrep or cell block preparation, and proper specimen triage for microbiology work up and an additional sample for microbiology culture can potentially help facilitate the diagnosis.

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 diseases 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.

6.6 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 fibrosis, the material obtained is scarce and non-diagnostic.

6.7 Fat Necrosis

Fat necrosis of the breast commonly affects large breasts and presents as a mass. It is an inflammatory reaction commonly secondary to injury of adipose tissue, mostly reported in perimenopausal women after trauma, surgery, biopsy, or radiotherapy. As fat necrosis is often mass forming and is firm to hard on 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. 6.7). 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, 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.

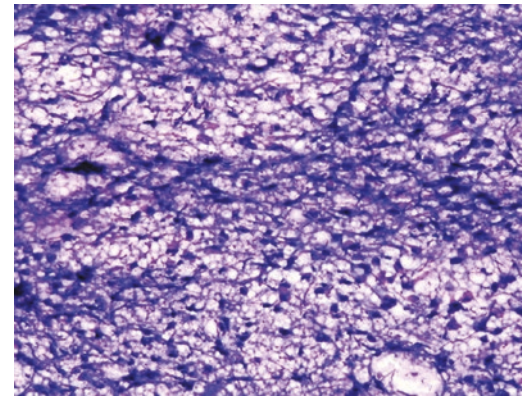

Fig. 6.7 Fat necrosis. Moderately cellular smears with foamy mononuclear macrophages, disintegrated or collapsed fat cells, neutrophils, and fibrofatty tissue fragments (MGG stain)

6.8 Summary

- Most inflammatory lesions of the breast can simulate malignancy clinically and radiologically, especially 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 pres-

ence 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 IGM. This condition appears to follow pregnancy, and the classic histological picture is of a granulomatous inflammatory reaction around lobules. CNGM is best considered a morphologic variant with lipid spaces within the lesion.
- 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 a 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 to 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

7

Catarina Callé, Fernando Schmitt, Gary Tse, and Puay-Hoon Tan

It is frequent to classify FCC as cystic or solid lesions, and as non-proliferative or proliferative lesions. Non-proliferative lesions include cysts, apocrine metaplasia, and fibrosis. Proliferative lesions comprise a vast group of diagnoses, characterized by epithelial proliferation, ductal or lobular, with or without atypia. Those without atypia include sclerosing adenosis, apocrine adenosis, radial scar and complex sclerosing lesion, columnar cell changes (CCC), and usual ductal hyperplasia (UDH) (Guray and Sahin 2006). 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 characteristic of FCC and are summarized in Table 7.1.

Table 7.1 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

7.1 Non-proliferative Lesions

Evidence reveals that breast biopsies have non-proliferative lesions reported in up to 70% of cases. The entities that compose the group of non-proliferative FCC are cysts, apocrine metaplasia, and fibrosis, and all can be associated with calcifications (Guray and Sahin 2006). In this

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section, we describe the clinical, radiological, cytological, and histological features of these lesions.

7.1.1 Cysts

Cysts are the most common change in FCC, found in one-third of women aged between 30 and 50 years. The clinical presentation of cysts may be wide, ranging from asymptomatic to palpable masses associated with or without pain. Generally, in mammography or ultrasound, they appear as well-defined rounded masses. Cysts are better evaluated at ultrasound, showing circumscribed margins, sharp anterior and posterior walls, and no internal echoes and posterior enhancement (Fig. 7.1). Some may resemble fibroadenomas, papillomas, or when inflamed may resemble high-grade carcinoma of no specific type, carcinoma with medullary features or mucinous carcinoma.

Cysts are dilated terminal duct lobular units, with rounded or ovoid shape. Most are microscopic with no clinical relevance, but may unite forming multiple juxtaposed multilocular cysts, visible to imaging and palpation. The cyst size may vary over time and the majority disappears after FNAC.

The aspirate is most frequently composed of a thin, watery, and lightly stained fluid, or it may be blood stained, brown or black, cloudy, or turbid, and varies in viscosity.

While performing an FNAC of a breast cyst, if the cyst does not fully aspirate, the fluid is thick or blood stained, or there are any clinical or imaging concerns, a cytology examination of the specimen is mandatory. In case the cyst is fully aspirated with no residual palpable or ultrasound lesion, without clinical and imaging concerns, even though the risk of malignancy is low, cytology is recommended.

Cytology smears of cysts have low cellularity, with a **proteinaceous background** ranging from thin to thick, with or without debris, and cholesterol crystals. **Foamy macrophages**, with vacuolated or finely granular cytoplasm, or with dense epithelioid cytoplasm are generally documented and may have small, round, or irregular nuclei. May be documented isolated or in aggregates, as multinucleated cells or siderophages. Cysts may be **lined by flat or atrophic epithelium** or by **apocrine metaplastic cells.** Tissue fragments of **ductal epithelial cells** with **myoepithelial cells** can be present in sparse numbers, and usually in flat sheets (Figs. 7.2 and 7.3)**.** A variable number of bare bipolar nuclei dispersed singly are also commonly present. **Apocrine metaplasia,** a

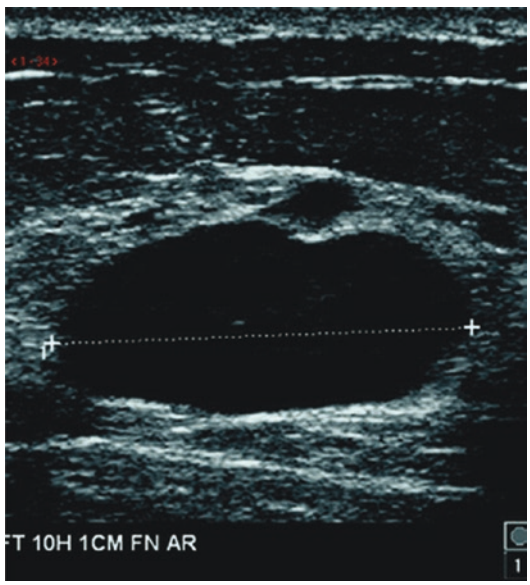

Fig. 7.1 Cystic lesion at ultrasound. Note the posterior enhancement of the ultrasound beam

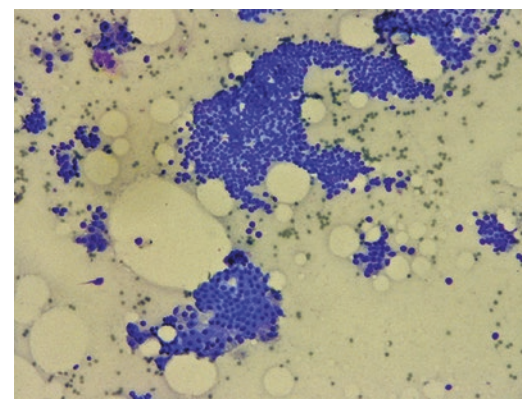

Fig. 7.2 Fibrocystic changes. Presence of apocrine cells and clusters of ductal epithelial cells are common findings in non-proliferative FCC (MGG stain)

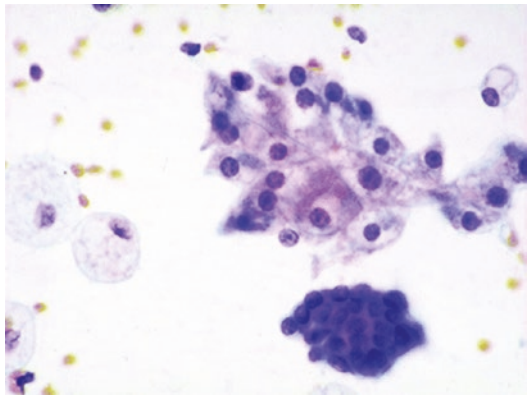

Fig. 7.3 Breast cyst. Note the presence of histiocytes, apocrine metaplasia, and a benign group of epithelial cells (MGG stain)

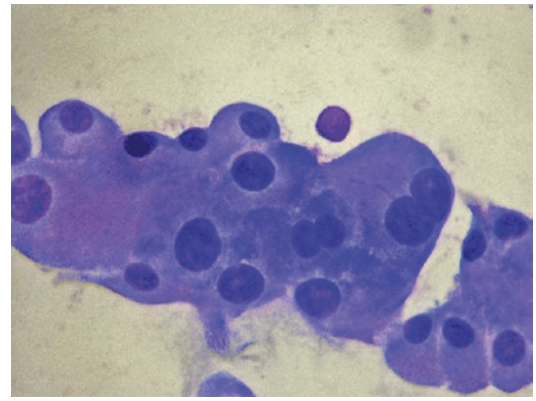

Fig. 7.4 Nuclear atypia in apocrine metaplastic cells (MGG stain)

common lining of cystic walls is commonly present in sheets of evenly spaced polygonal or single cells with the classic eosinophilic cytoplasm, and with round nuclei with distinct nucleoli. More details of apocrine metaplasia are described in the following paragraphs.

The consensus for cyst management is to follow up the patient, is without further therapy, due to its low risk of malignancy (ROM).

7.1.2 Apocrine Metaplasia

Apocrine metaplasia occurs commonly in the setting of FCC and is distinguished from apocrine adenoma, a very rare entity, based on the finding of a well-demarcated mass, commonly found in the adenoma. There are no size criteria to discriminate both (World Health Organization 2019).

Apocrine metaplasia is characterized by cells with abundant eosinophilic granular cytoplasm, and central regular round nuclei with prominent nucleoli. Binucleated cells may also be seen.

Apocrine cells may degenerate and appear atypical, with enlarged and hyperchromatic nuclei, and intracytoplasmic lumina (Fig. 7.4). Atypical apocrine metaplasia should be diagnosed only when the nuclei display a significant cytological atypia. One should bear in mind that these features are a frequent occurrence with infection.

7.1.3 Fibrosis

Fibrosis is part of the spectrum of FCC, and is generally a consequence of a ruptured cyst, that triggers a chronic inflammatory response with fibroblastic proliferation of the interlobular stroma.

7.1.4 Reporting Non-proliferative Lesions

According to the Yokohama System for reporting Breast Fine Needle Aspiration Biopsy Cytopathology, cysts and FCC should be rendered the benign category.

The advantages of using this system in FNAC reports are clear, allowing greater diagnostic clarity, and better communication in health care teams, with benefits for patient diagnosis and management. Clinical audits and interchange between different health institutes also profit from pathology reports that offer a detailed description of smears and render a diagnostic category (Montezuma et al. 2019).

Comments could be added depending on its features: "cyst contents," "cyst with apocrine cells," and "fibrocystic change."

"**Cyst contents**" should be rendered when smears are composed of a proteinaceous background with histiocytes and no apocrine epithelial cells. There should be an agreement with imaging and total collapse or no palpation after

fine needle aspiration. If apocrine sheets are added to the above description the diagnosis of "**cyst with apocrine cells"** should be rendered. If tissue fragments of ductal epithelial cells with myoepithelial cells are documented, the diagnosis **"fibrocystic change"** is made to expedite/ease correlation with ultrasound imaging (Field et al. 2020).

Example of a report according to the Yokohama System of a smear with a pattern of scattered small apocrine sheets in a proteinaceous background with occasional histiocytes:

Benign *Occasional apocrine sheets are seen in a proteinaceous background with histiocytes. Comment: the features are those of a cyst with apocrine cells.*

7.2 Proliferative Lesions

Proliferative FCC is a group of diverse diagnoses that has either a non-neoplastic proliferation of the number of acini in the terminal duct lobular units, defined as adenosis, or either a proliferation of the epithelial lining of these acini or ducts, with or without atypia. Intraductal or intralobular proliferative changes range from benign including usual epithelial hyperplasia and CCC, through atypical including flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH), and atypical lobular hyperplasia. The atypical spectrum merges with low-grade ductal carcinoma in situ and lobular carcinoma in situ (World Health Organization 2019; Yu et al. 2017). In this chapter, we will focus on benign, non-atypical proliferative breast lesions, that may present some overlapping cytological features with FCC.

7.2.1 Adenosis

7.2.1.1 Clinical and Radiology

Adenosis is characterized mainly by a lobulocentric proliferation of the terminal duct lobular unit (TDLU), composed both of myoepithelial and epithelial cells, and occurs most often as part of the spectrum of fibrocystic changes. Its clinical appearance will be most commonly a palpable mass with adenosis being responsible for a major part of the lesion. In case of adenosis limited to isolated lobules that do not constitute FCC, the lesion may be detected by mammogram when presenting with calcifications. Pregnancy and lactational physiological changes are common causes of adenosis, but the most frequent form is sclerosing adenosis. Apocrine adenosis, tubular adenosis, and blunt duct adenosis are other subtypes described in the literature. Microglandular adenosis, is a rare entity, and despite being called adenosis, differs substantially in its structural features from lesions conventionally termed adenosis (Hoda et al. 2020).

Sclerosing adenosis is composed of benign epithelial and myoepithelial proliferation, with a characteristic stromal fibrosis arranged in a lobulocentric pattern and may mimic invasive carcinoma. On imaging, it may resemble a mass with calcifications and architectural distortion.

7.2.1.2 Cytology

The smears of these lesions have a clean background, with very small cohesive ductular tissue fragments, moderately to highly cellular, with bland small nuclei, associated with small, dense stromal tissue fragments. Usually, myoepithelial cells with bare bipolar nuclei and dispersed single epithelial cells are documented in the background. A definitive diagnosis is generally not possible (Kundu et al. 2012).

Smears of microglandular adenosis 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. 7.5). This characteristic, which is also present in histology, makes the differential diagnosis of 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.

7.2.1.3 Histology

Adenosis is characterized by an increase in the number of acini or ductules per lobular unit, resulting in an increased size of the lobule.

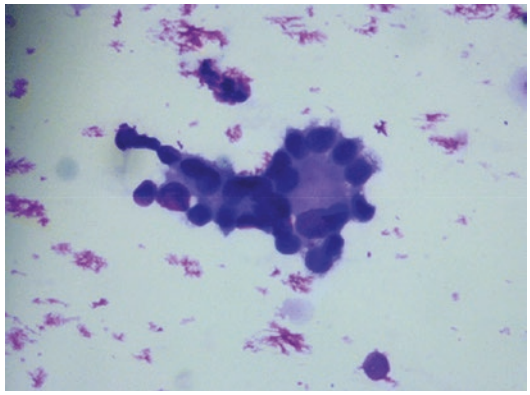

Fig. 7.5 Microglandular adenosis. Note tubular arrangement MGA (MGG stain)

Adenosis appears as a physiological phenomenon during pregnancy and lactation, leading to diffuse increase of the breast lobules. In nonpregnant 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 to 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 ductal carcinoma in situ (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 a single layer of columnar epithelium. Luminal epithelium can have mildly enlarged nuclei but without atypia, are currently included in CCC. Microglandular adenosis is a rare and very special form of adenosis. It is formed by small round ductules without myoepithelial lining, and they 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.

7.2.2 Radial Scar or Complex Sclerosing Lesion

7.2.2.1 Clinical and Radiology

Radial scars are commonly asymptomatic lesions detected casually, and on mammogram may be identical to carcinomas. Composed of a central sclerotic area of fibrosis and elastosis, distorting the parenchyma, accompanied by a benign glandular proliferation of small, distorted tubules. The epithelial proliferation may be of many types: UDH, ADH, DCIS, and lobular neoplasia (Hoda et al. 2020).

7.2.2.2 Cytology

Smears of these lesions feature FCC with epithelial hyperplasia, usually showing a proteinaceous background with foamy macrophages and dispersed bare bipolar nuclei. With moderate to high cellularity, and appreciable large ductal epithelial tissue fragments, monolayered sheets of apocrine cells and myoepithelial cells, are observed. Smaller tubular epithelial tissue fragments with scattered epithelial and myoepithelial cells may be documented. Mild nuclear atypia, with moderate nuclear enlargement and variation in size, is described in some cases. Myxoid stomal fragments, small sclerotic or elastotic tufts may also be encountered. The described features of radial scar in FNAC are not diagnostic (Orell 1999; Field et al. 2020).

The differential diagnosis of radial scars includes FCC; epithelial hyperplasia, but radial scar commonly has higher cellularity and occasional tubules; intraductal papilloma, but radial scar lacks the complex meshwork and stellate papillary fragments; when radial scars produce a high cellularity, a suspicion for malignancy may be raised.

7.2.2.3 Histology

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 complex sclerosing lesion is used if these lesions are larger than 1 cm, and sometimes they mimic invasive carcinomas clinically and mammographically.

7.2.3 Epithelial Hyperplasia/Usual Ductal Hyperplasia

7.2.3.1 Clinical and Radiology

Normal breast ducts or acini are lined by two layers of cells: luminal and myoepithelial. When there is a proliferation of the epithelial lining of these acini or ducts, a diagnosis of hyperplasia may be rendered. Intraductal proliferative lesions of the breast have traditionally been divided into three categories: UDH, ADH, and DCIS. In this section, we will only describe UDH.

UDH typically involves terminal duct lobular units or may occur in extralobular ducts. Clinically may be asymptomatic or present with a palpable lump. Ultrasound usually shows nonspecific features of fibrocystic changes, and on mammograms occasionally is associated with microcalcifications. There are several diagnoses where epithelial hyperplasia may be documented, with smears having hyperplastic ductal epithelial tissue fragments and bare bipolar nuclei: fibrocystic changes, intraductal papilloma, radial scars or complex sclerosing lesion with high cellularity, fibroadenomas, benign and borderline phyllodes tumors, gynaecomastia, nipple adenomas and CCC.

7.2.3.2 Cytology

Smears of this lesion may be moderate to high in cellularity, with mostly large flat ductal epithelial tissue fragments with myoepithelial cells. These epithelial cells are bland, showing no substantial nuclear atypia. Larger 3-D tissue fragments with irregularly arranged cells may be present, forming irregular slit-like secondary lumina, with nuclei that lack orientation, mild nuclear enlargement and pleomorphism, and numerous myoepithelial cells. Bare bipolar nuclei are documented in the background, wholly ovoid shape with fine, even chromatin and no nucleoli reassuring its benign features, important to distinguish these cells from stripped malignant nuclei (Field et al. 2020).

7.2.3.3 Histology

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 duct-lobular acini, which, as already mentioned, is defined as adenosis.

The distinction between different types of intraductal proliferation can only be made with histopathology criteria, but even in this material the distinction between ADH and DCIS is challenging.

Histologically the diagnosis of UDH is characterized by the expansion of ducts due to an irregularly arranged proliferation of epithelial cells with mildly pleomorphic nuclei, pseudoinclusions, small round nucleoli, and rare mitosis. Focal apocrine change can occur, and small slit-like irregular secondary lumina too. No necrosis is documented.

UDH is a very common lesion and traditionally has been presumed as a precursor of ADH and DCIS, however, only a few molecular changes have been identified. Only a small proportion of UDH harbor clonal populations of cells and present genomic alterations also described in ADH. The vast majority of UDH are thought not to progress, do not represent a precursor lesion (Boecker et al. 2002), and may be omitted in the pathology report.

7.2.4 Columnar Cell Changes

7.2.4.1 Clinical and Radiology

CCC are clonal alterations of the TDLU with enlarged, variably dilated acini lined by columnar epithelial cells. Are seen in FNAC of mammographic grouped, amorphous calcifications, or as an incidental finding, often in association with other benign changes, such as cysts and epithelial proliferative lesions (World Health Organization 2019).

7.2.4.2 Cytology

Smears of these lesions show ballooned terminal ductules, with a central lumen that can be demonstrated by focusing up and down on the fragment. Myoepithelial nuclei are seen on the outer surface, and there is columnar cell orientation at the margins of the fragments and columnar cells are present in the background. Calcifications are often present in a proteinaceous background (Field et al. 2020). CCC cannot be reliably diagnosed by FNAC because of significant overlapping FNAC features with papillary neoplasms and well-differentiated adenocarcinoma (Jensen and Kong 2007).

7.2.4.3 Histology

CCC are characterized by enlarged and dilated acini lined by columnar epithelial cells with apical cytoplasmatic snouts. Nuclei are commonly ovoid, regularly oriented perpendicular to the basement membrane, and have evenly dispersed chromatin and inconspicuous nucleoli. The epithelial cell lining should only be 1 or 2 cells thick. In case there is cellular stratification or tufting of more than 2 cells the diagnosis of columnar cell hyperplasia should be rendered. Microcalcifications and luminal secretions are frequent. CCC do not show cytological atypia, and in case cytological atypia is present the diagnosis of FEA should be rendered.

CCC have strong and diffuse nuclear staining for ER and lack staining for CK5/6. This lesion is frequently documented in association with other benign changes such as cysts and epithelial proliferative lesions. CCC and FEA have been described as the earliest stage in the low-grade breast neoplasia pathway and represent non-obligate precursors to ADH, DCIS, and low-grade invasive breast carcinomas. Both CCC and columnar cell hyperplasia may have genomic abnormalities, with loss of heterozygosity (losses

of 16q), the genetics hallmark of low-grade lesions. Although UDH has been contemplated as the precursor to ADH, current evidence suggests that CCC and FEA are more reasonable precursors. This is suggested due to the similar immunohistochemical and molecular genetic profiles. However, the risk for the subsequent development of breast cancer after a diagnosis of CCC is slightly increased (relative risk: ~1.5), this value is not clearly independent of the risk associated with concomitant proliferative lesions. In conclusion, CCC have a very low risk of progression to invasive breast carcinoma (World Health Organization 2019).

7.2.5 Reporting Proliferative Lesions

According to the Yokohama System for reporting Breast Fine Needle Aspiration Biopsy Cytopathology adenosis, radial scars, UDH and CCC should be rendered the benign category.

The specific diagnosis of proliferative changes in FNAC requires a detailed and careful evaluation, using the key cytological diagnostic criteria. An effort must be made to maximize the correlation between imaging and clinical history to avoid false-positive diagnoses of carcinoma. Smears should be clearly described, and a list of differential diagnoses should be given, accentuating the most expected diagnosis.

Example: Highly cellular smears showing a pattern of frequent cohesive large and some smaller epithelial tissue fragments and folded sheets, with regularly arranged rounded nuclei showing minimal nuclear enlargement or pleomorphism and with associated myoepithelial nuclei and bare bipolar nuclei in a clean background. There are few small epithelial tissue fragments or dispersed single cells.

Benign: These highly cellular smears show a pattern of predominantly large epithelial tissue fragments with plentiful myoepithelial nuclei, and bare bipolar nuclei in the clean background.

Comment: The features are those of epithelial hyperplasia (Field et al. 2020).

7.3 Summary

- Proliferative and non-proliferative FCC are common lesions, may present as palpable or non-palpable masses and are frequently sampled by FNAC.
- Non-proliferative FCC and FCC with UDH or CCC are not a risk for subsequent cancer development.
- Sclerosing adenosis (found in association with FCC) may have overlapping cytological findings with UDH.
- Radial scar/complex sclerosing lesion is one of the most frequent causes of discrepancy between the radiological image (suspicious/malignant) and cytological diagnosis (benign) in the preoperative workup of breast lesions; in many cases it is possible to characterize the benign nature of these lesions in cytology.
- Reliable identification and subclassification of FCC with proliferative lesions on cytology can be very difficult due to the overlap of cytological features. It is more important to define the cytological pattern as benign, suspicious, or malignant instead of giving a specific "histological" diagnosis, so the right follow-up is rendered to each patient.

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Fibroadenoma

8

Mihir Gudi, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

8.1 Clinical, Radiological, and Epidemiological Findings

Fibroadenoma is the most common benign breast neoplasm that usually occurs in young women of childbearing 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 lesions have been detected by mammography as well-defined masses. On ultrasound examination, the fibroadenoma appears as a solid, round, or oval mass with distinct margins (Fig. 8.1). Calcifications may be observed in fibroadenomas from older women (Mendoza et al. 2011).

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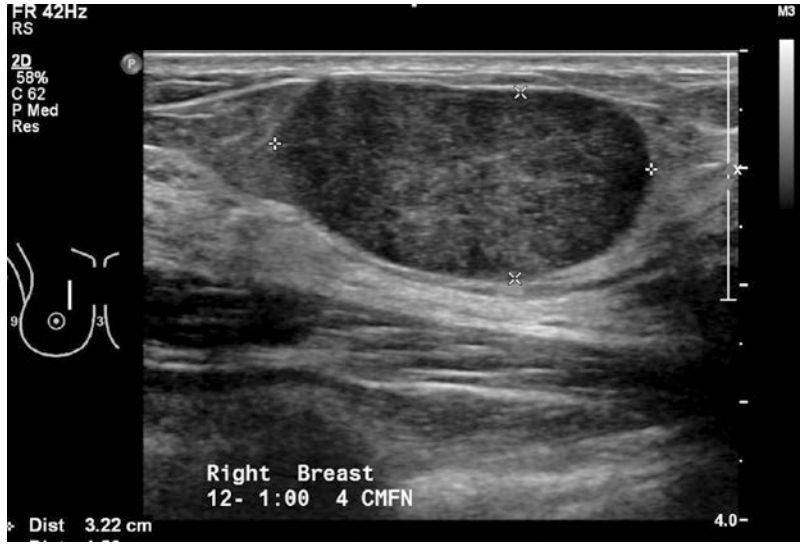

Fig. 8.1 Ultrasound image demonstrates an oval, circumscribed, hypoechoic mass

8.2 Cytologic Findings

Diagnosis of fibroadenoma on FNAC is highly accurate with a 79.3% predictive value in one series of 362 cases (López-Ferrer et al. 1999). 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. 8.2a–g). Branching epithelial sheets are commoner in aspirates from fibroadenomas with an intracanalicular growth pattern (Mendoza et al. 2011). 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. 8.3). Presence of usual ductal hyperplasia within the fibroadenoma can lead to the presence of larger branched proliferative epithelial aggregates in the aspirates (Fig. 8.4a, 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. 8.5).

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. 8.6). The fibroadenoma is the most common cause of a false-positive diagnosis in FNAC of breast lesions. 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 fibroad-

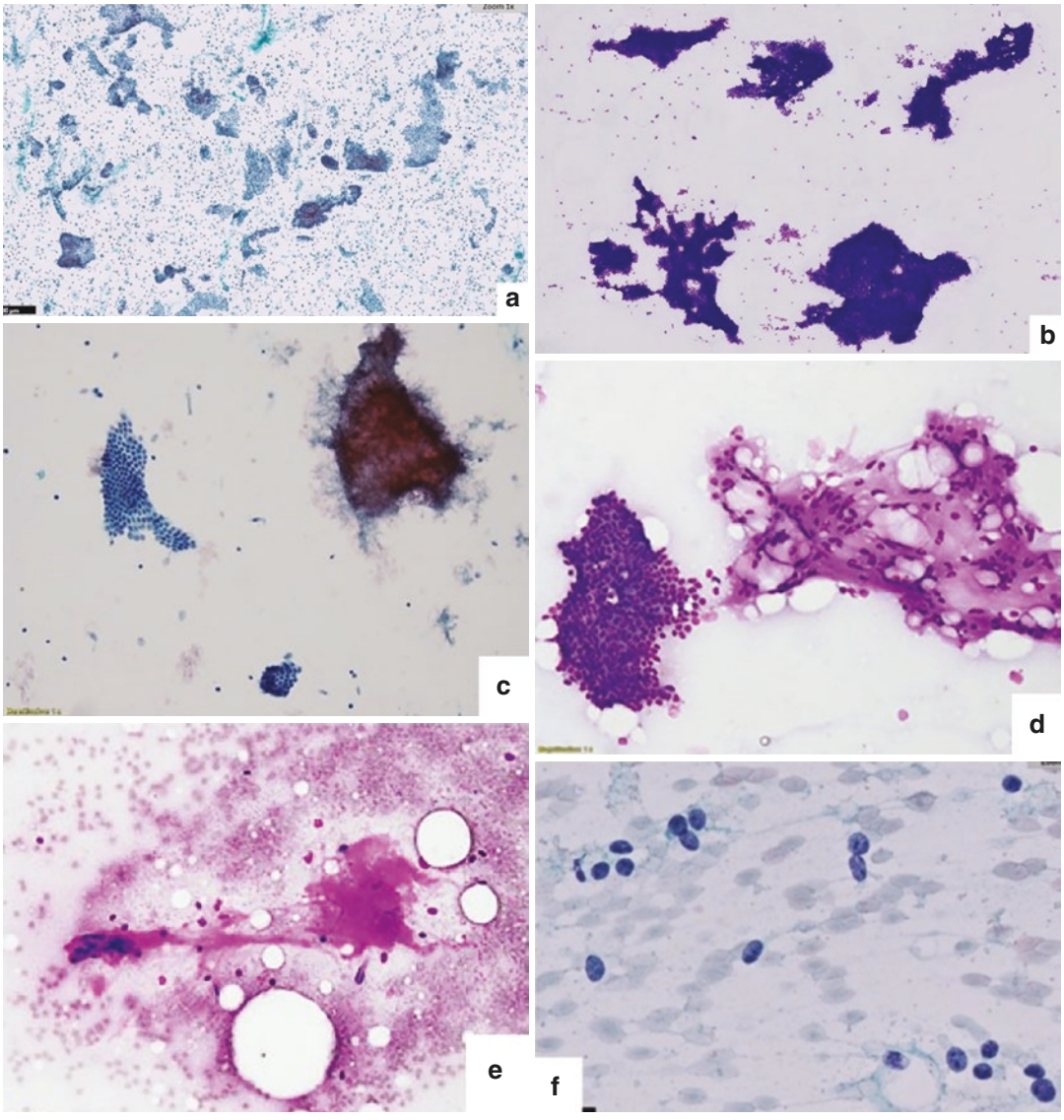

Fig. 8.2 (**A**) Flat cohesive branching sheets of ductal epithelial cells interspersed with bare nuclei, PAP (**A**) and MGG (**B**). (**C**, **D**) Fibrillary stromal material admixed with cohesive sheets of ductal cells and bare nuclei PAP (**C**) and MGG (**D**). (**E**) Metachromatic stromal material (MGG). (**F**) Kissing pairs of small bare nuclei (PAP). (**G**) H & E stain of a core biopsy from a typical fibroadenoma

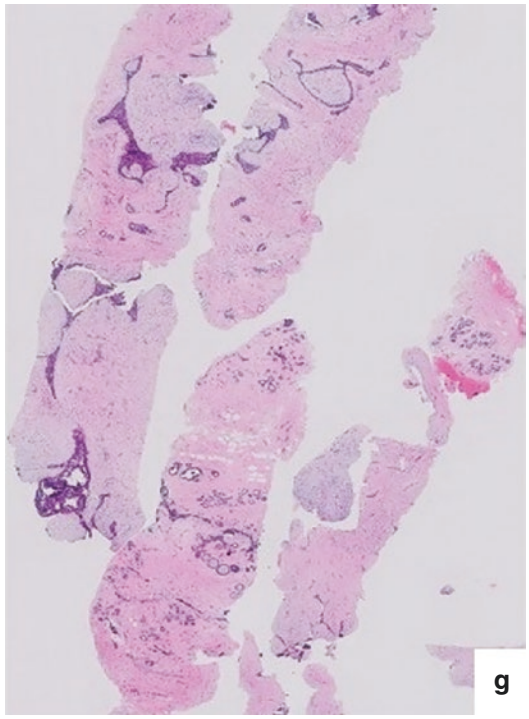

Fig. 8.2 (continued)

enoma 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 (Fig. 8.7) (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 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. If significant nuclear atypia is seen in a what otherwise appears to be a typical fibroadenoma aspirate, a diagnosis of fibroadenoma with atypia should be rendered, as low-grade DCIS/ LCIS or invasive tumor within or adjacent to a fibroadenoma can occur (Field et al. 2020). The role of the triple approach cannot be overemphasized.

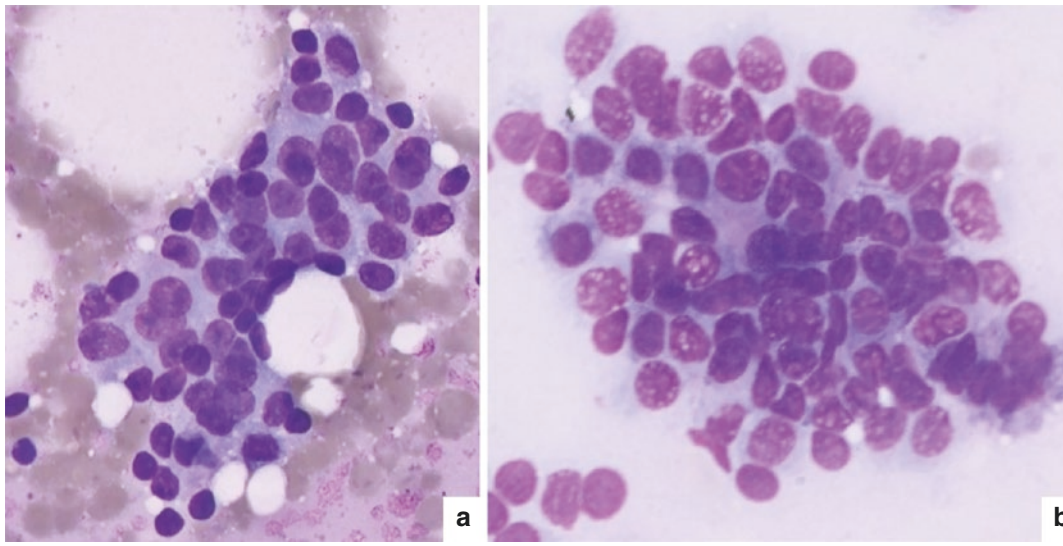

Fig. 8.3 Fibroadenoma. A closer view of the bimodal epithelial aggregate with small dark nuclei of myoepithelial cells at the periphery of the cluster (**A**), as well as intermixed among the ductal epithelial cells (**B**), (MGG)

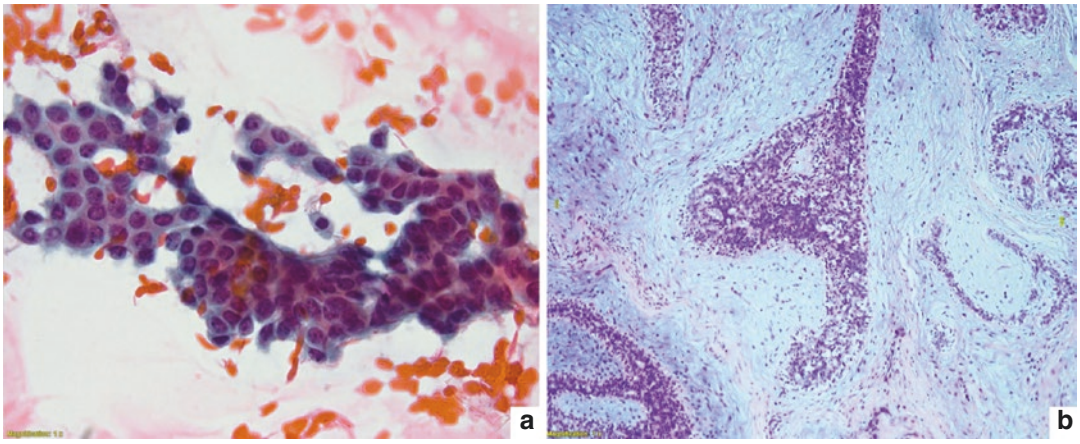

Fig. 8.4 Fibroadenoma with usual ductal hyperplasia. (**a**) Large and branched cohesive epithelial sheet with admixed myoepithelial cells. There is a degree of nuclear atypia and some nuclear overlapping (**b**) Corresponding histology shows a fibroadenoma with the epithelial component demonstrating usual ductal hyperplasia

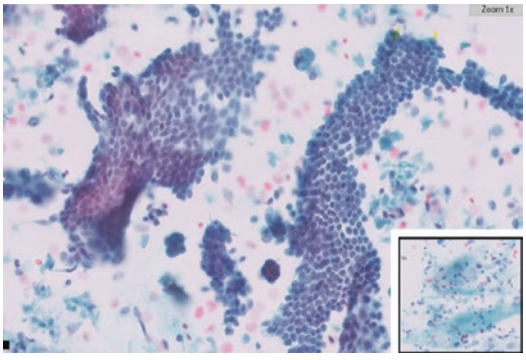

Fig. 8.5 Fibroadenoma with usual ductal hyperplasia. Large bimodal epithelial aggregate shows ductal epithelial cells admixed with darker nuclei of myoepithelial cells. A loose myxoid stromal clump with elongated nuclei is noted (inset)

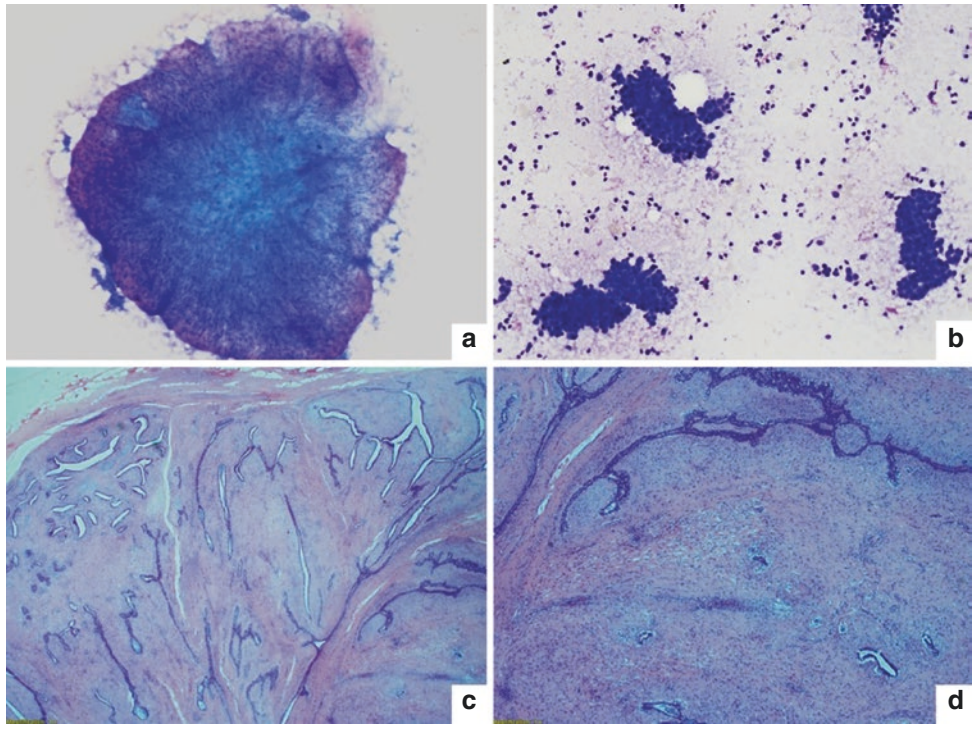

Fig. 8.6 Cellular fibroadenoma. Diff-Quik smear shows cohesive epithelial clusters (**b**) accompanied by more cellular myxoid stromal clumps (**a**). H&E sections show a cellular fibroadenoma with a pronounced intracanalicular growth pattern and a mildly cellular intervening stroma (**c**, **d**)

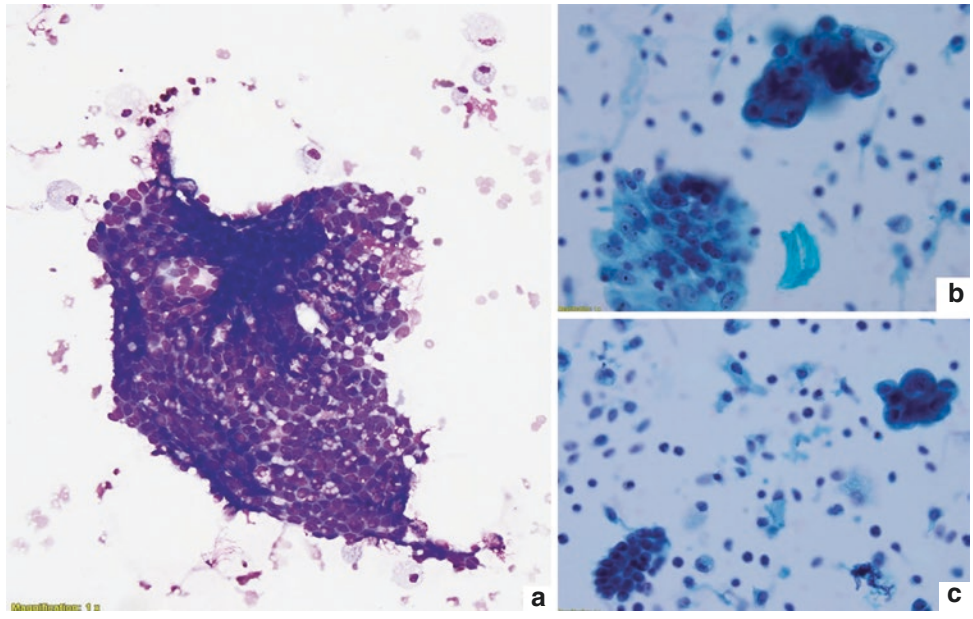

Fig. 8.7 Fibroadenoma with lactational change. (**a**) Flat cohesive sheet of ductal epithelial cells with vacuolated cytoplasm and foamy histiocytes in the background; MGG. (**b**, **c**) A hyperchromatic cluster of ductal cells with vacuolated and foamy cytoplasm, containing nuclei with prominent nucleoli, admixed with many bare nuclei in the background (PAP); features seen in pregnancy and lactation. (**d**) H&E from the core biopsy showed a fibroadenoma and background lactational change

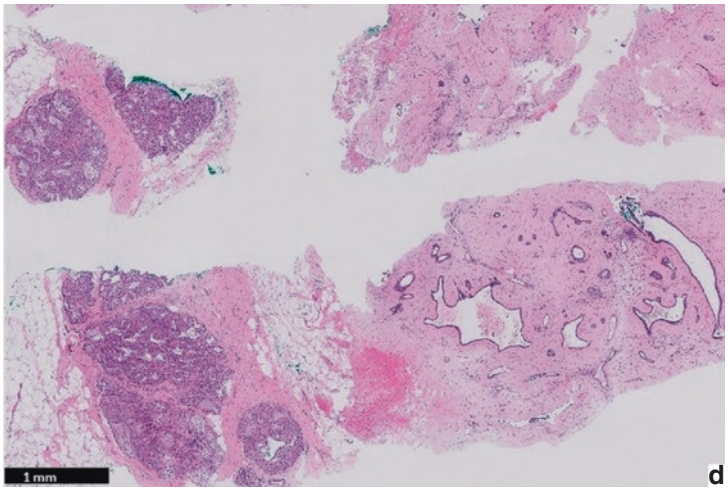

Fig. 8.7 (continued)

8.3 Differential Diagnosis

8.3.1 Fibrocystic Changes

While fibrocystic change can usually be readily distinguished from fibroadenoma by the lower cellularity and presence of foam and apocrine cells, occasional aspirate yields from fibroadenoma can show similar appearances (Fig. 8.8). 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).

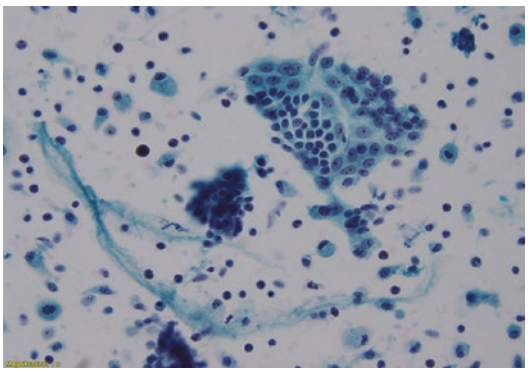

Fig. 8.8 Fibroadenoma with cystic change shows cohesive clusters of ductal cells, some with apocrine features, many bare nuclei, cyst macrophages, and a wispy stromal fragment (PAP)

8.3.2 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).

8.3.3 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 (Fig. 8.9). 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).

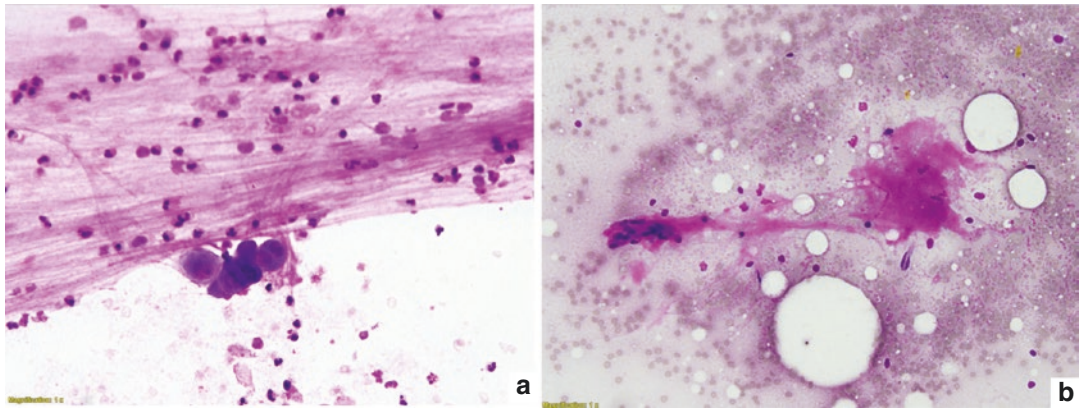

Fig. 8.9 Background fluid mucin with magenta hue from an aspirate of a mucinous carcinoma (**a**) in contrast to brighter pink fibrillary stromal material in a myxoid fibroadenoma (**b**); MGG

8.3.4 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 (Fig. 8.10), 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).

8.3.5 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.

8.3.6 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 a relative lack of stag-horn 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).

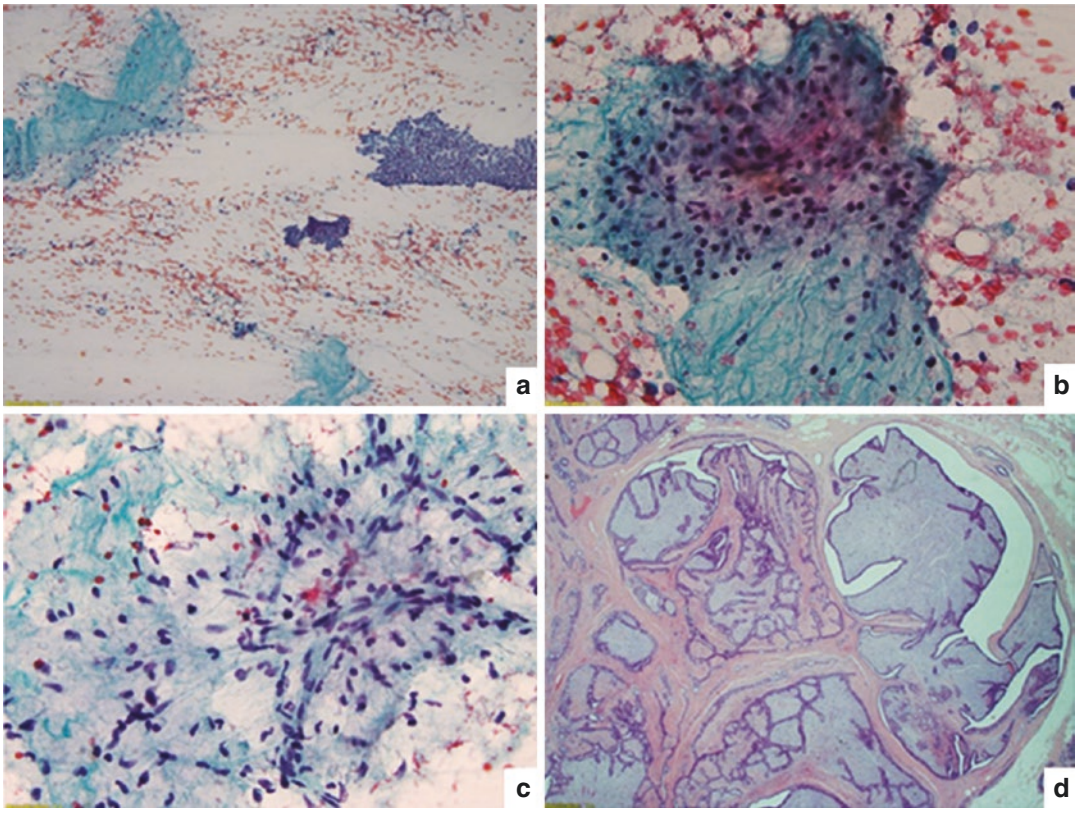

Fig. 8.10 Benign phyllodes tumor with increased stromal fragments (**a**) PAP. Increased stromal cells within the stromal fragments (**b** and **c**) PAP. H&E sections from a benign phyllodes tumor with fronded architecture and well-circumscribed borders (**d**)

8.3.7 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).

8.3.8 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.

8.3.9 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 tongue-like projections, and columnar cells (Fig. 8.11). Fibrovascular cores when identified are helpful in corroborating a papillary lesion along with associated foam cells. Myoepithelial cells tend to be fewer than in the fibroadenoma (Michael and Buschmann 2002). Central papillomas are usually solitary and seen subareolarly.

8.3.10 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 (Fig. 8.12). It is prudent to recommend histological confirmation when cytologic atypia is discovered, in order not to overlook 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. 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

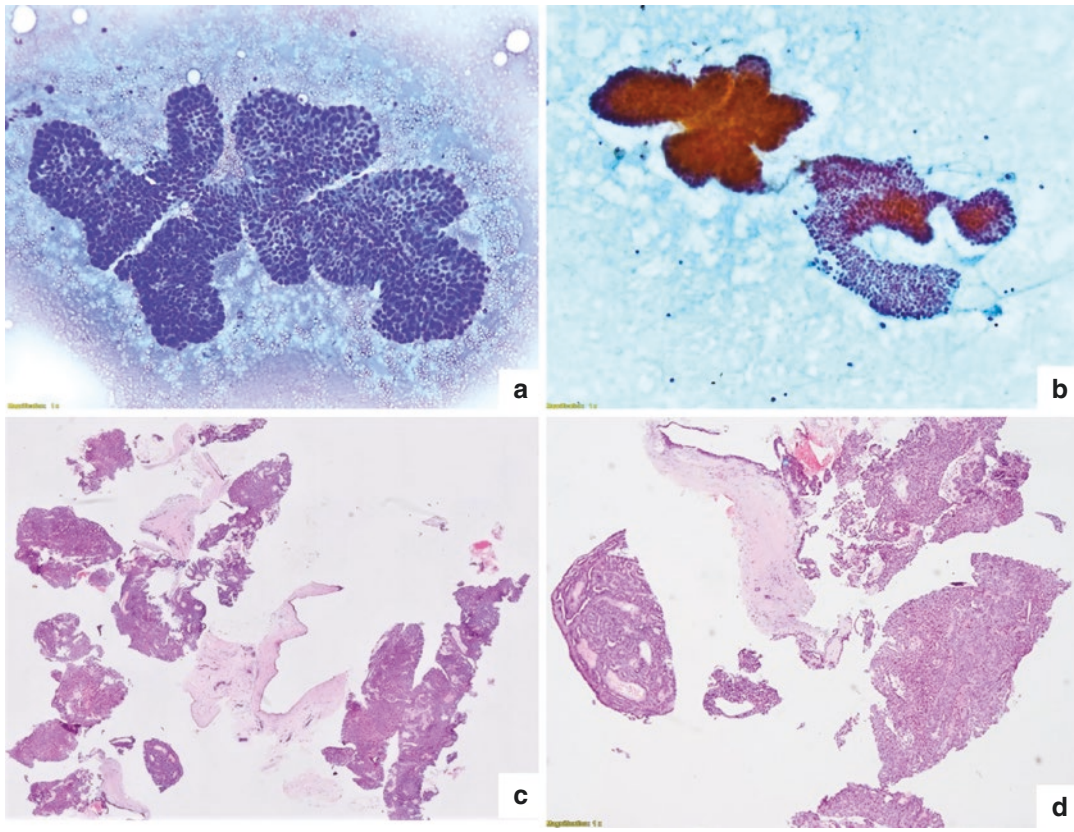

Fig. 8.11 Papilloma with three-dimensional papillary clusters of ductal cells in a background of blood and macrophages (**a**. MGG; **b**. PAP). H&E stained section showing fragments of a distended duct with freelying fragments of a papilloma (**c, d**)

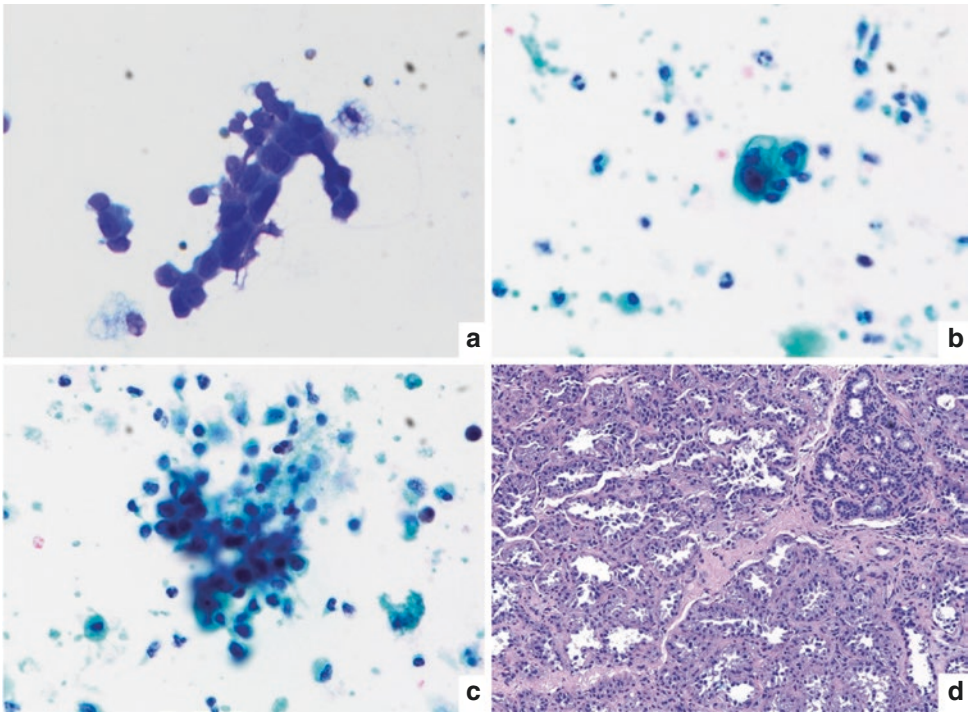

Fig. 8.12 Lactational adenoma (**a**). A loosely cohesive cluster of enlarged ductal cells with moderate amounts of pale cytoplasm; MGG. (**b**). A three-dimensional cluster of ductal cells with hyperchromatic nuclei, vacuolated cytoplasm, and background cystic proteinaceous debris PAP. (**c**). A loosely cohesive cluster of enlarged ductal cells with moderate amounts of pale cytoplasm and background proteinaceous cystic debris; PAP. (**d**). H&E stain showing a lactational adenoma/change

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 (Fig. 8.13) 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).

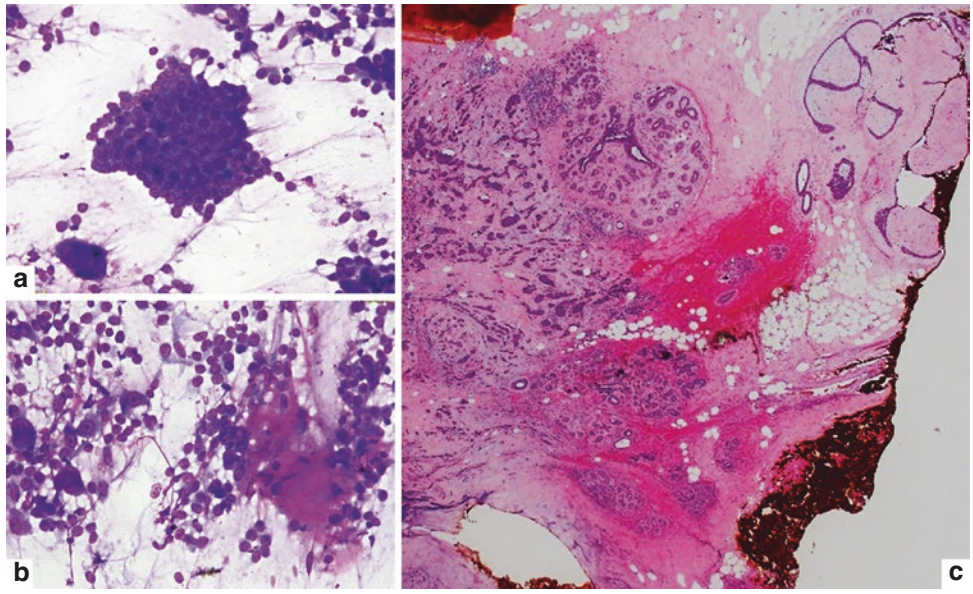

Fig. 8.13 (**a**, **b**) MGG. Fibroadenoma infiltrated by invasive ductal carcinoma. (**a**). Flat cohesive sheet of ductal cells surrounded by bare nuclei. (**b**). Stromal fragment from the fibroadenoma surrounded by dispersed atypical cells including pleomorphic forms from a carcinoma. (**c**). H&E stain showing an invasive ductal carcinoma infiltrating into a fibroadenoma

8.4 Histologic Correlations

Macroscopically circumscribed, encapsulated, and lobulated, the fibroadenoma shows two main histological patterns of intracanalicular and pericanalicular growth which are without clinical significance, apart from the more frequent association of intracanalicular fibroadenomas with MED12 gene mutations which have been discovered to be an early step in the pathogenesis of fibroepithelial neoplasms. The admixture of epithelial and stromal elements gives rise to the cytological appearances of epithelial aggregates within the 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. 8.14); the juvenile fibroadenoma which is usually diagnosed in adolescents and are characterized by increased stromal cellularity with usual epithelial hyperplasia and a pericanalicular growth pattern; and the complex fibroadenoma that contains cysts larger than 3 mm, sclerosing adenosis, epithelial calcifications, or papillary apocrine hyperplasia.

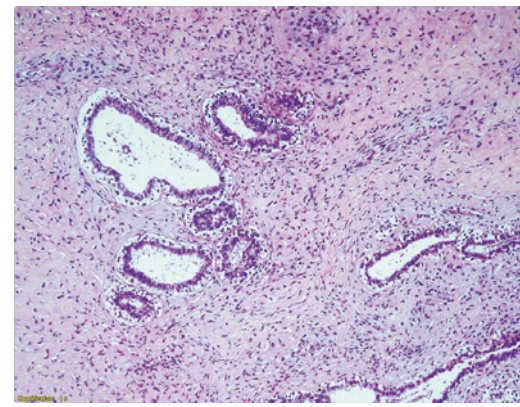

Fig. 8.14 Cellular fibroadenoma. Cellular stromal component intermixed with epithelial elements

8.5 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

9

Joshua Li, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

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.

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9.1 Phyllodes Tumors

9.1.1 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. 8. 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 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

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tumors tend to show stromal fragments of low to moderate cellularity (Fig. 9.1a, b), whereas borderline to malignant phyllodes tumors show moderate to high stromal cellularity (Bhattarai et al. 2000) (Figs. 9.2a, b and 9.3a, 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. 9.4). 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). The other frequently used defining criteria were the presence of atypical single cells in the background. In benign phyllodes tumors, such single

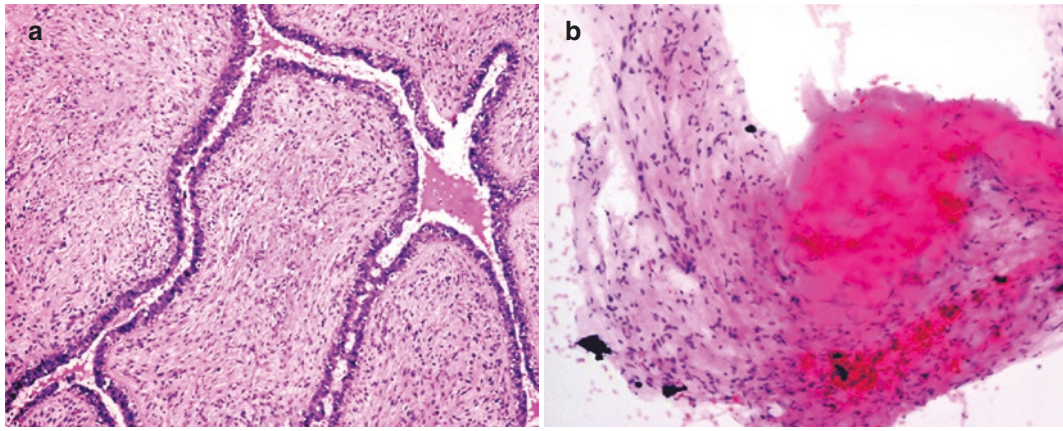

Fig. 9.1 (**a**, **b**) Benign phyllodes tumor. (**a**) Histology shows mild stromal hypercellularity. (**b**) Cellular stromal fragments are of low to moderate cellularity on cytology

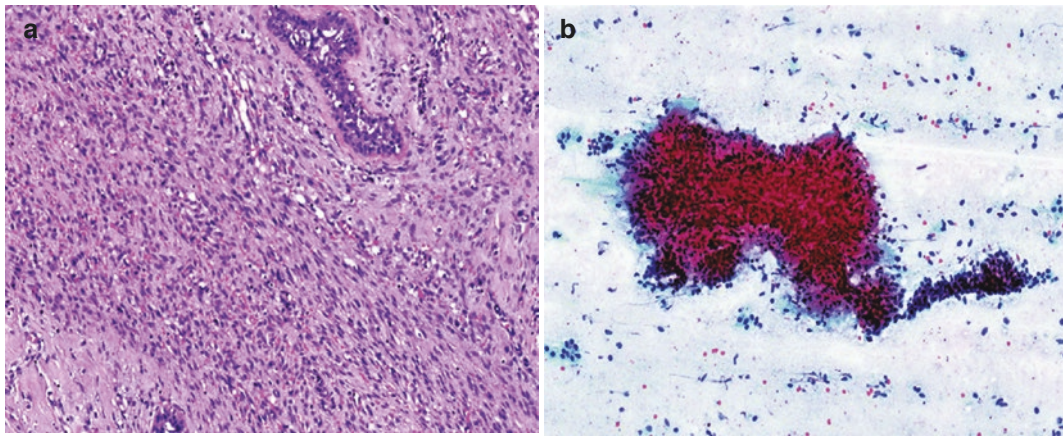

Fig. 9.2 (**a**) Phyllodes tumor of borderline malignancy. Cellular stroma with mild to moderate stromal atypia and scattered mitoses. (**b**) Phyllodes tumor. Cellular stromal clump is closely associated with an epithelial cell aggregate

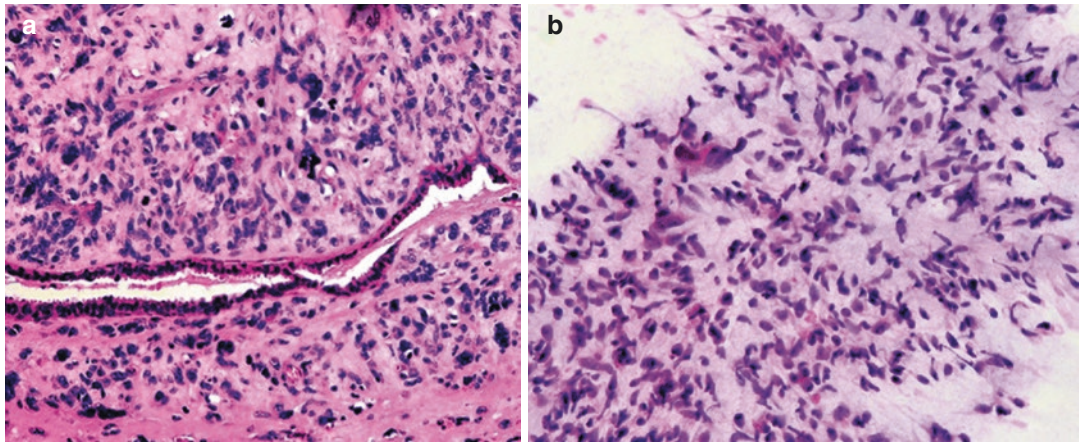

Fig. 9.3 (**a**, **b**) Malignant phyllodes tumor. (**a**) Stroma is of high cellularity and prominent pleomorphism. (**b**) Stromal fragments are of high cellularity and prominent pleomorphism on cytology

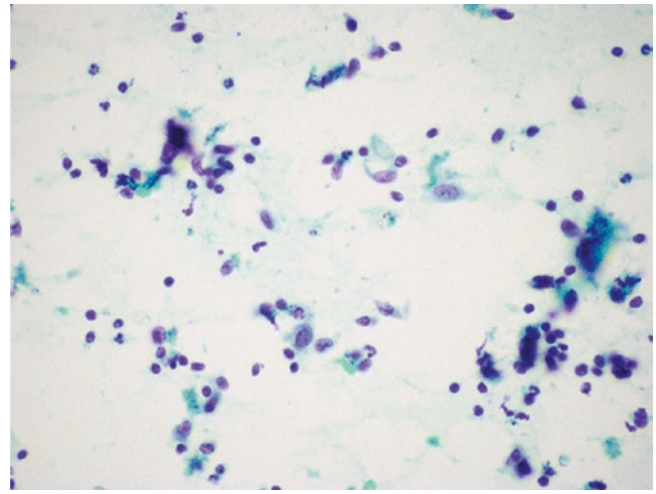

Fig. 9.4 High magnification of the plump and spindled cells, some of which show elongated cytoplasmic extensions in a smear of a malignant phyllodes tumor

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. 9.5). Malignant heterologous elements can also be present in rare cases (Kuppusamy et al. 2021). Some authors reported the use of p53 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).

9.1.2 Differential Diagnosis

For benign phyllodes tumor, the immediate differential diagnosis is fibroadenoma. Whereas

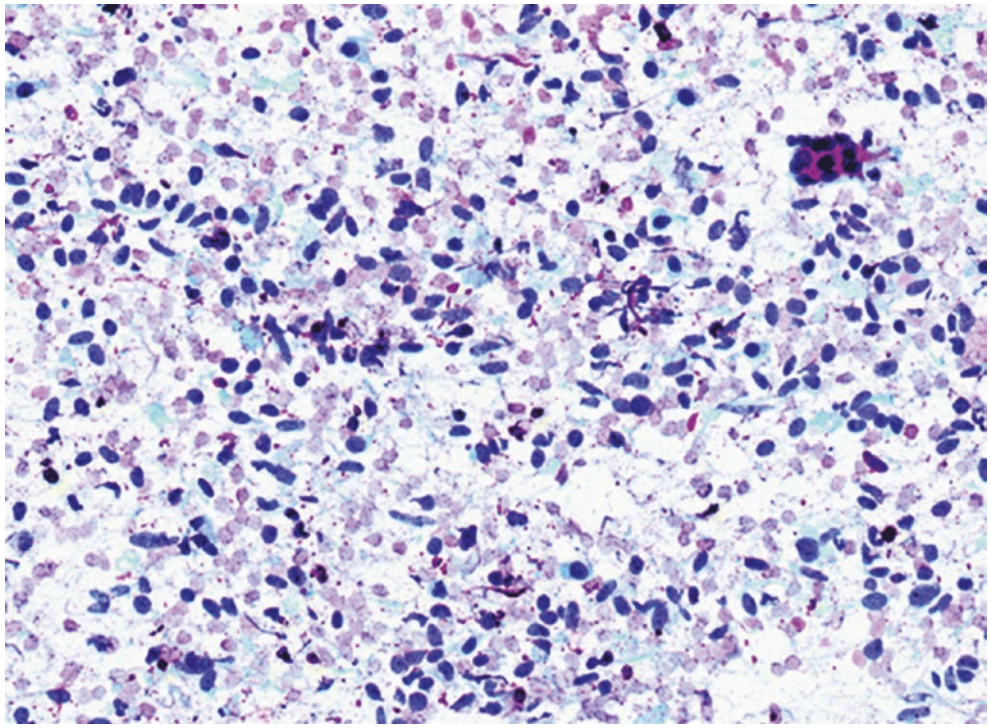

Fig. 9.5 Phyllodes tumor. Dispersed cells show both spindle and ovoid nuclei with variability in nuclear sizes

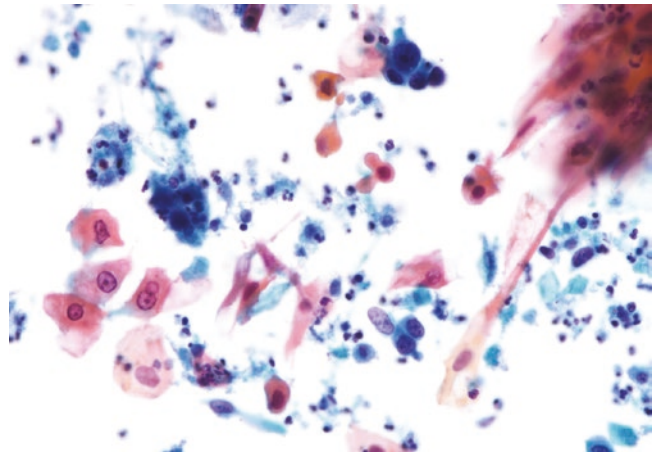

Fig. 9.6 Malignant keratinizing squamous cells as seen in squamous cell metaplastic carcinoma

borderline and malignant phyllodes tumors overlap with different types of metaplastic carcinomas, especially the high-grade ones. The stromal fragments from metaplastic carcinomas vary in degree of atypia, and may not be distinguishable from those from phyllodes tumor. In contrast, the presence of malignant ductal and/or squamous epithelial cells are characteristic features that indicate a diagnosis of metaplastic carcinoma (Lui et al. 2007) (Fig. 9.6).

9.2 Hamartoma

9.2.1 Clinical and Epidemiological Findings

Hamartomas are thought to comprise about 5% of all benign breast tumors, and they occur mostly in women in their 40 s 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.

9.2.2 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. 9.7a, b). These can be present in up to 55% of 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.

9.2.3 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).

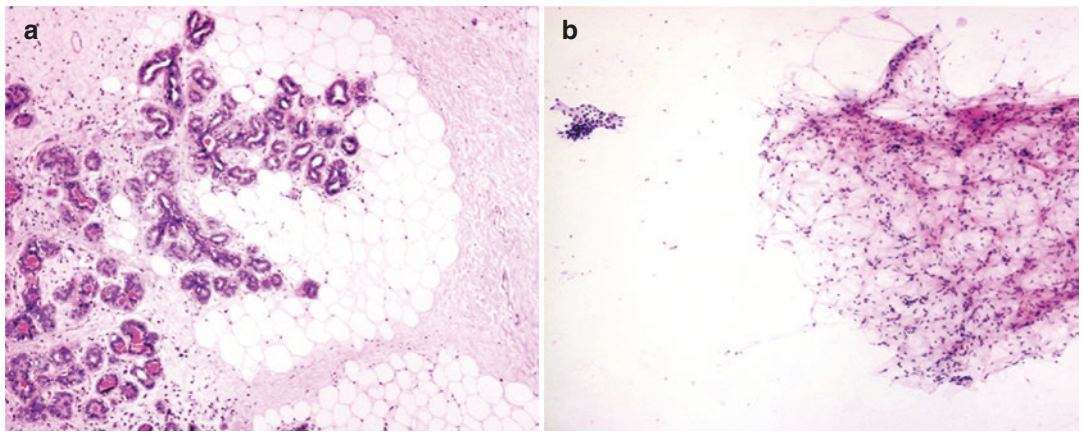

Fig. 9.7 (**a**, **b**) Hamartoma. (**a**) Histology shows mature adipose tissue with admixed breast lobules. (**b**) Cytology shows epithelial cells arranged in branching sheets, in proximity to fibrofatty tissue

9.2.4 Management

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

9.3 Pseudoangiomatous Stromal Hyperplasia (PASH)

9.3.1 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).

9.3.2 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 (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. 9.8a–c).

9.3.3 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 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 few cellular stromal fragments in PASH, but this is probably a moot point as both lesions are benign, and the clinical management is the same.

9.3.4 Management

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

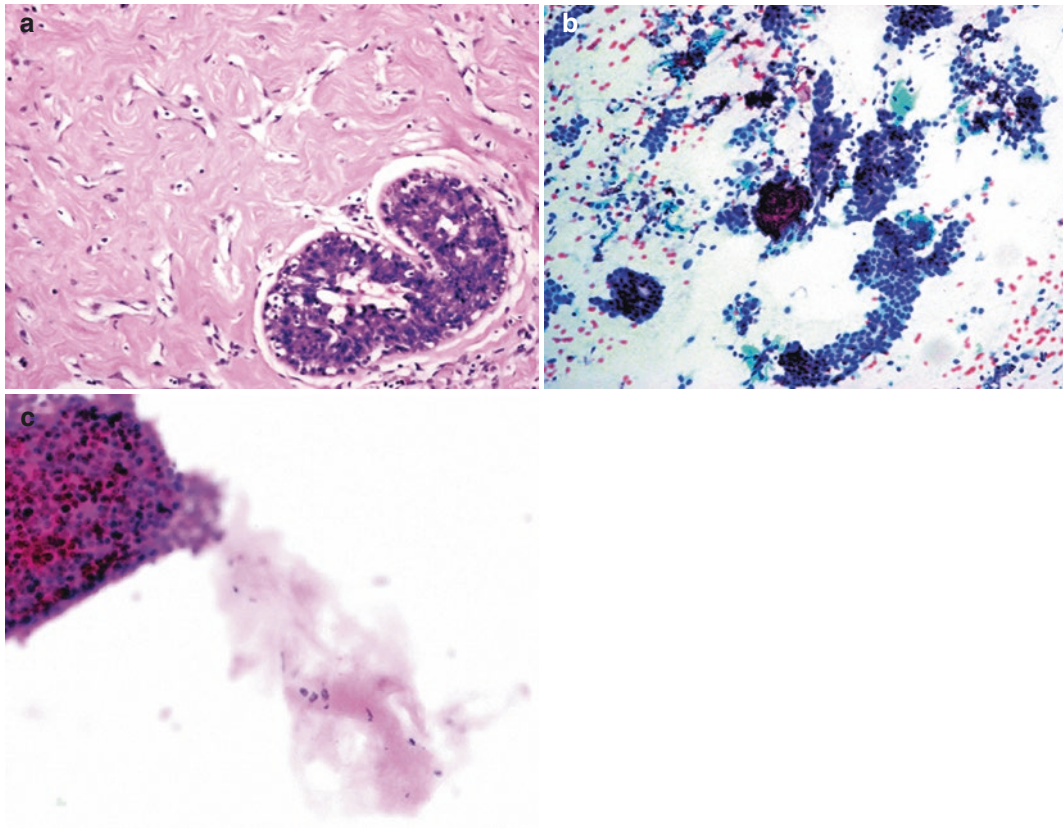

Fig. 9.8 (**a**) PASH. Histology shows anastomosing empty slits lined by myofibroblasts resembling vascular spaces. (**b**) Cytologic preparation of PASH shows moderate cellularity. The variable epithelial clusters are solid or with very rare branching. (**c**) Cytology of PASH shows hypocellular stromal fragments

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

Joshua Li, Gary Tse, Puay-Hoon Tan, Fernando Schmitt, and Nour Sneige

10.1 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 fine needle aspiration cytology (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

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with atypia (FEA), ADH, low-grade DCIS and LN (atypical lobular hyperplasia and lobular carcinoma in situ).

10.2 Non-Proliferative Breast Disease

10.2.1 Clinical and Epidemiological Findings

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

10.2.2 Cytologic Findings

Cytologically mild epithelial hyperplasia shows simple, small to medium-sized epithelial cell groups with occasional intercellular spaces and conspicuous myoepithelial cells (Figs. 10.1, 10.2, and 10.3). Occasionally apocrine metaplastic cells may also be seen.

10.2.3 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. 7.

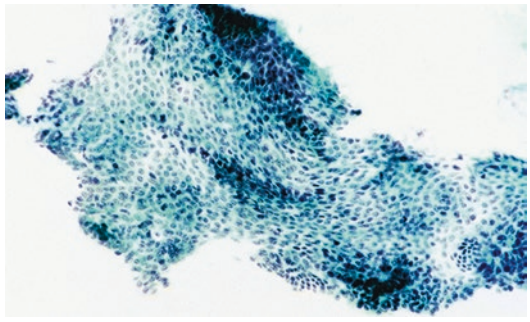


Fig. 10.1 Large sheets of benign ductal epithelial cells are seen, with the individual epithelial cells showing bland and normochromatic nuclei with a regular architecture

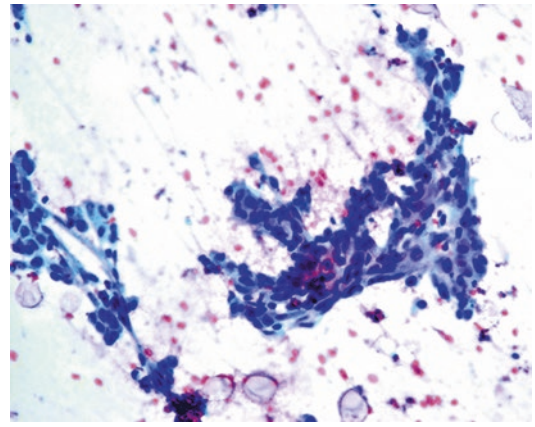


Fig. 10.2 Small fragments of epithelial cells showing minimal nuclear enlargement and crowding, admixed with myoepithelial cells

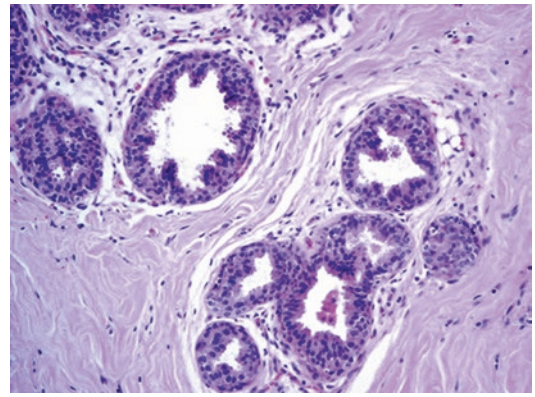


Fig. 10.3 Histologic picture of mild epithelial hyperplasia with a mild increase in the number of epithelial cell layers within the lobules. The epithelial cells show bland morphology

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 (Ducatman et al. 1992; Sneige 2000).

10.3 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% (Arisio et al. 1998; Feichter et al. 1997; Ishikawa et al. 2007; Mendoza et al. 2011; Rosa and Masood 2011). 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) in this category. The cytological changes of sclerosing adenosis and papillomas are discussed in Chaps. 7 and 11 and are not included here.

10.3.1 Moderate and Florid Epithelial Hyperplasia

10.3.1.1 Clinical and Epidemiological Findings

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

10.3.1.2 Cytologic Findings

Cytologically, the proliferating cell masses may show large complex structures with visible intercellular spaces or cell bridges (Dawson et al. 1995; Sneige and Staerkel 1994; Thomas et al. 1995). In florid epithelial hyperplasia, the architectural characteristics can be well demonstrated in the FNAC, with the irregularly stretched out intercellular spaces or tapered bridges composed of epithelial cells that are aligned parallel to the long axis of the bridges (Figs. 10.4, 10.5, and 10.6). 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

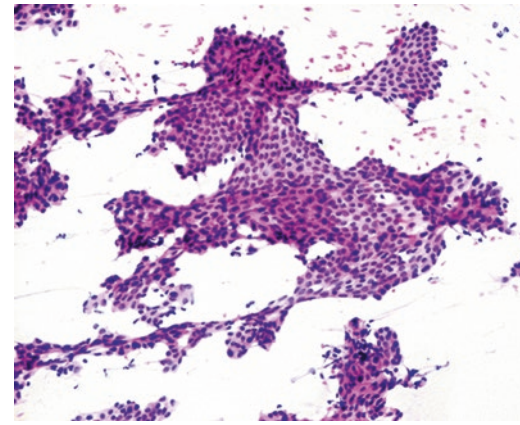


Fig. 10.4 Florid epithelial hyperplasia showing a sheet of benign ductal epithelial cells. Myoepithelial cells can be identified at the periphery. The epithelial cells possess spindled nuclei, with some suggestion of nuclear streaming

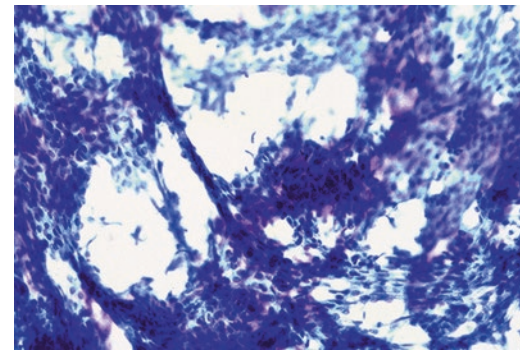


Fig. 10.5 Florid epithelial hyperplasia showing large sheets of benign epithelial cells with somewhat spindled nuclei, with cells that are aligned along the periphery of the fragment in a parallel direction

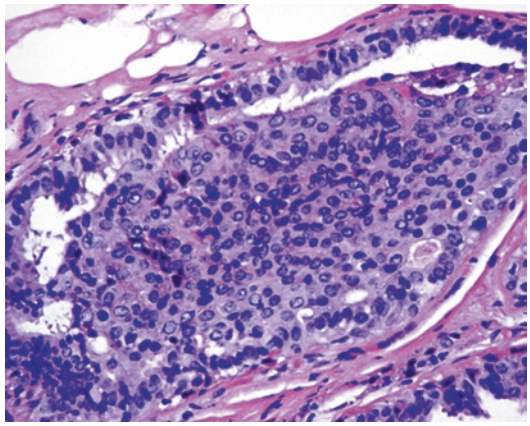


Fig. 10.6 Histology of florid epithelial hyperplasia showing cohesive proliferation of benign epithelial cells with nuclear streaming. The secondary lumina are peripherally located and slit-like

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).

10.3.1.3 Histologic Correlations

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

10.3.2 Sclerosing Adenosis

10.3.2.1 Clinical and Epidemiological Findings

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

10.3.2.2 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 over-interpretation of FNAC smears (Fig. 10.7).

10.3.2.3 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. 10.8).

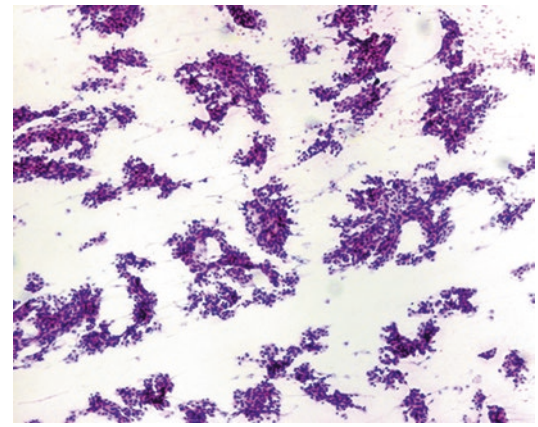


Fig. 10.7 Sclerosing adenosis shows a smear with high cellularity and the epithelial cell clusters show cohesive tubules surrounded by myoepithelial cells. Some single cells and bare nuclei are noted in the background

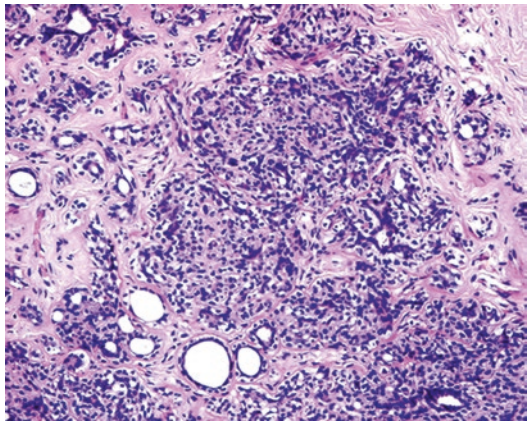


Fig. 10.8 Histology of sclerosing adenosis, with pseudoinfiltrative tubules of epithelial cells surrounded by myoepithelial cells in a densely fibrotic stroma

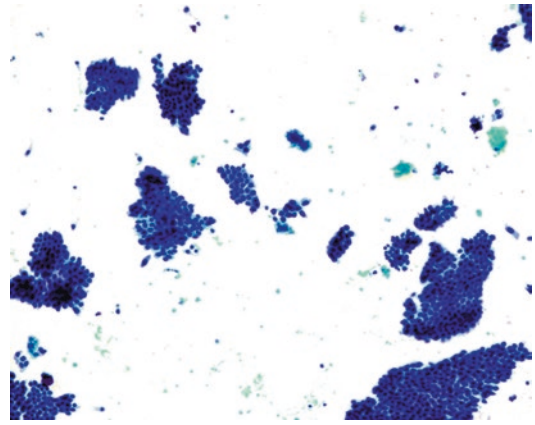


Fig. 10.9 Aspirate of columnar cell changes showing a relatively uniform cell population with mildly enlarged and variable nuclei. These cells form three-dimensional cell groups and show crowding and some loss of polarity. A vague suggestion of peripheral palisading is also seen

10.3.3 Columnar Cell Changes

10.3.3.1 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.

10.3.3.2 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. 10.9). Background single cells occurred in about 70% of the cases, and these cells ranged from cuboidal to columnar (Saqi et al. 2004).

10.3.3.3 Histologic Correlations

Columnar cell changes typically show dilated ductal lobular units, with the lining cells being

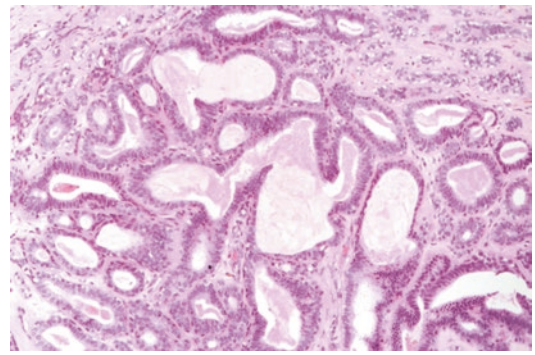


Fig. 10.10 Histologic features of columnar cell changes showing dilated ductal lobular spaces that are lined by epithelial cells with dense nuclei and columnar cytologic appearances

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. 10.10). 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.

10.4 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% (Al-Kaisi 1994; Lim et al. 2004; Mulford and Dawson 1994; Tran et al. 2010; Wang and Ducatman 1997; Yu et al. 2017). 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% (Arisio et al. 1998; Feichter et al. 1997; Ishikawa et al. 2007; Park and Ham 1997; Rosa and Masood 2011). 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 (Arisio et al. 1998; Mendoza et al. 2011). 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 (Bulgaresi et al. 2005; Park and Ham 1997). 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 detail. 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 a significant intraluminal mass. In ADH and 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 lies 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 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 to 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.

10.4.1 Atypical Ductal Hyperplasia

10.4.1.1 Clinical and Epidemiological Findings

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

10.4.1.2 Cytologic Findings

The FNAC of ADH shows variable cytology. The cells show atypical proliferative changes characterized by monotonous, evenly spaced epithelial cells harboring 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 without atypia may be present. Scattered foamy histiocytes and calcifications may be seen (Figs. 10.11, 10.12, and 10.13).

10.4.1.3 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).

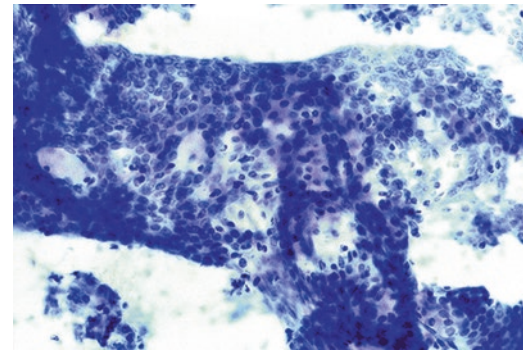


Fig. 10.11 Cytologic preparation of atypical ductal hyperplasia showing sheets of monotonous, evenly spaced epithelial cells exhibiting round, slightly enlarged nuclei with fine chromatin and inconspicuous nucleoli. Occasional myoepithelial cells may be seen. The epithelial cells are arranged in a vague cribriform pattern

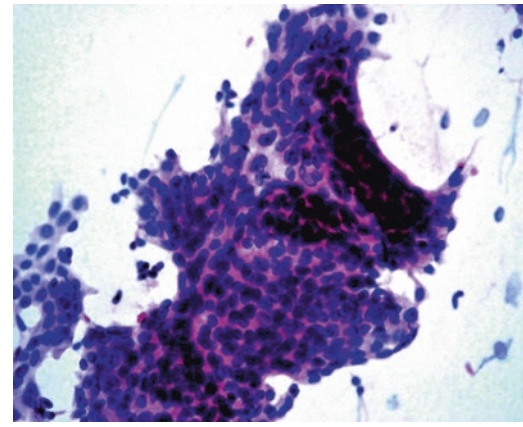


Fig. 10.12 Aspirate of atypical ductal hyperplasia showing monotonous epithelial cells with slight nuclear pleomorphism and nuclear crowding. Myoepithelial cells can be discerned in the periphery

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

10.4.1.4 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

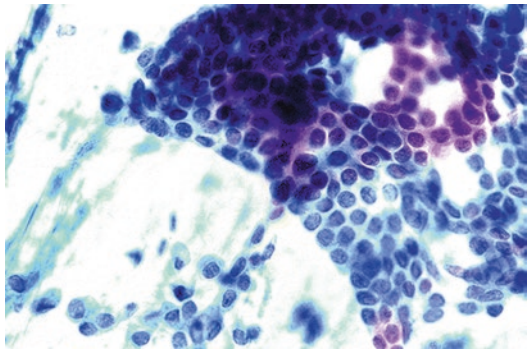


Fig. 10.13 Aspirate of atypical ductal hyperplasia showing epithelial cells with fine chromatin and inconspicuous nucleoli

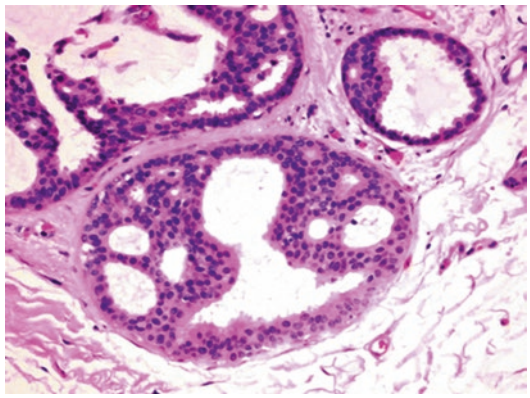


Fig. 10.14 Histology of atypical ductal hyperplasia showing intraductal luminal epithelial proliferation with mass formation. The cells show round and dark staining nuclei and form rounded lumens with a geometric pattern

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 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. 10.14 and 10.15). Hence, the differentiation between ADH and DCIS, is quantitative, and not qualitative.

10.4.1.5 Management

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

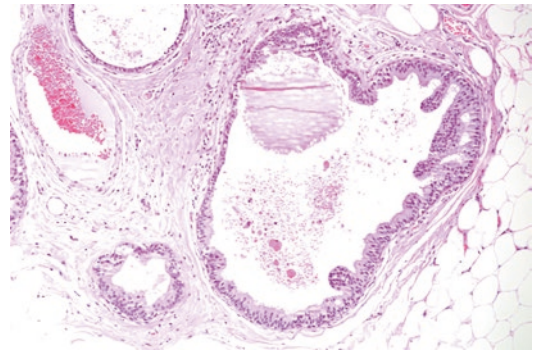


Fig. 10.15 Atypical ductal hyperplasia showing relatively uniform atypical epithelial cells partially involving a duct space and forming small lumens

10.4.2 Ductal Carcinoma In Situ, Low Grade

10.4.2.1 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.

10.4.2.2 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. 10.16, 10.17, 10.18, 10.19, 10.20, and 10.21). As the degree of loss of 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).

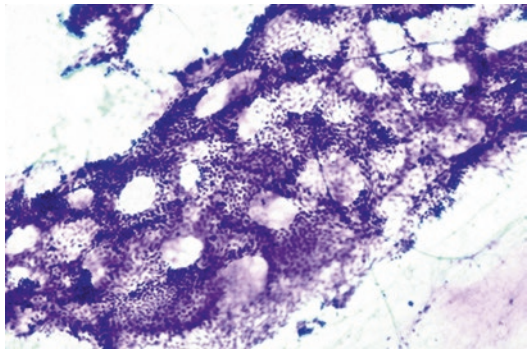


Fig. 10.16 Ductal carcinoma in situ in an aspirate showing a sheet of malignant cells with monotonous appearances forming a large cribriform tissue fragment, which is apparent in the FNAC preparation

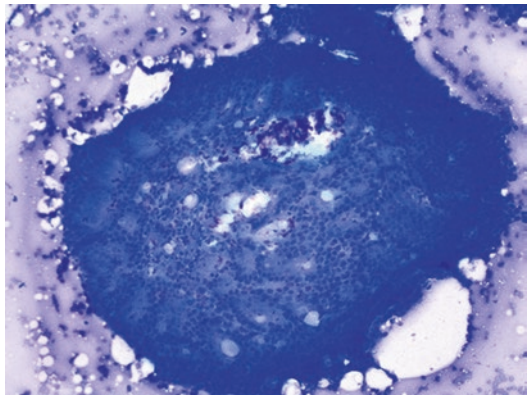


Fig. 10.17 Ductal carcinoma in situ in FNAC showing an essentially solid pattern with rare central lumen formation. Cribriforming can be identified in part of the epithelial structure

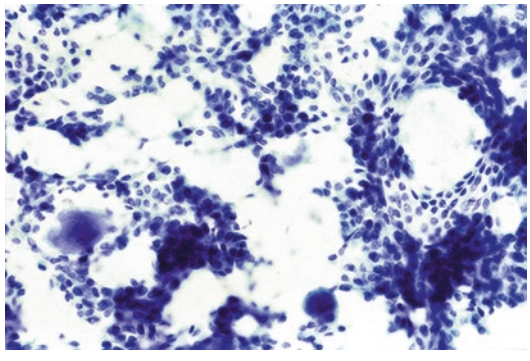


Fig. 10.18 Ductal carcinoma in situ in an aspirate showing details of cribriforming with small luminal calcifications identified

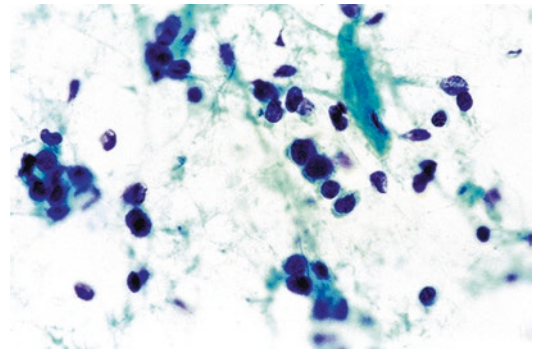


Fig. 10.19 Ductal carcinoma in situ showing single low-grade malignant cells with mild nuclear variation and a clean background

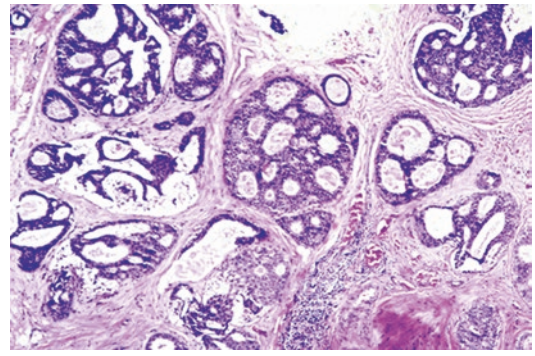


Fig. 10.20 Histologic appearance of low-grade ductal carcinoma in situ showing distension of the ducts and lobules by a monotonous cell population forming sievelike, geometric patterns

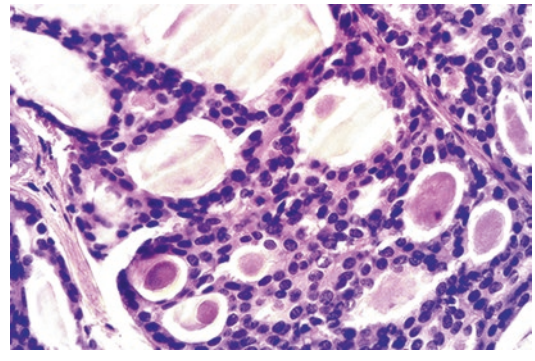


Fig. 10.21 Histologic appearance of ductal carcinoma in situ showing monotonous tumor cells with only mild nuclear atypism

10.4.2.3 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 (Field et al. 2020; Lee et al. 2021).

10.4.2.4 Histologic Correlations

Low-grade DCIS histologically tends to show monomorphic malignant cells that are oval to cuboidal, and these cells are arranged in a 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. Necrosis is not a characteristic feature of low-grade DCIS.

10.4.2.5 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.

10.4.3 Lobular Neoplasia

10.4.3.1 Clinical and Epidemiological Findings

LN encompasses both atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS). Both lesions differ by 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 with the adjacent benign changes rather than with the lesion itself.

10.4.3.2 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. 10.22 and 10.23). The pleomorphic variant features nuclei of larger size and greater pleomorphism (Auger and Hüttner 1997) (Fig. 10.24a, b).

10.4.3.3 Differential Diagnosis

The cellular features are similar in ALH and LCIS and in invasive lobular carcinoma, but as ALH and LCIS are usually clinically asymptom-

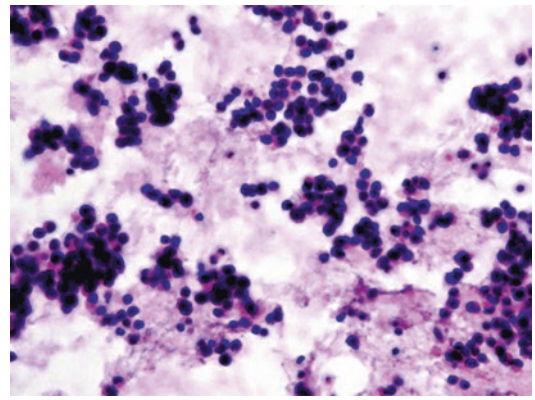


Fig. 10.22 Lobular neoplasia showing poorly cohesive abnormal cells with mild nuclear atypism. Vague single-cell strands can be identified

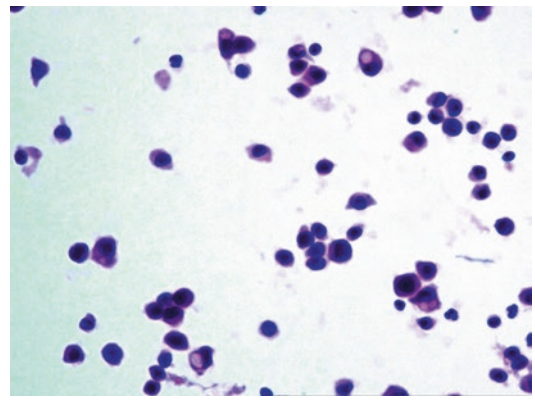
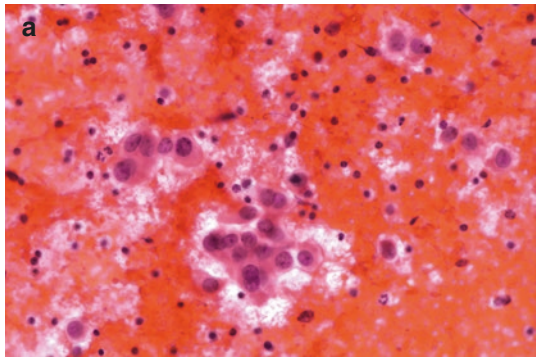


Fig. 10.23 Lobular neoplasia showing malignant cells with mild nuclear atypism. They tend to be small, uniform, with eccentric nuclei and also show intracytoplasmic vacuoles. Vague single cell file can be discerned



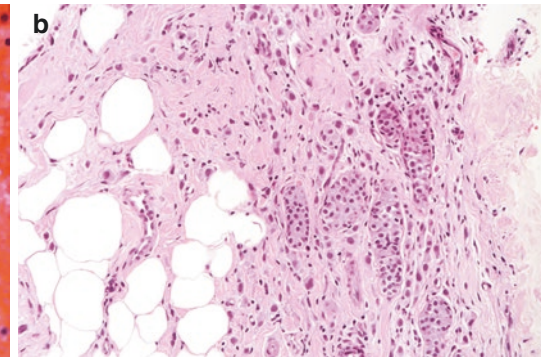


Fig. 10.24 (**a**) Compared to classical lobular neoplasia, the pleomorphic variant features larger nuclear size with greater pleomorphism (**b**) follow-up biopsy showing pleomorphic lobular carcinoma with in situ components

atic, 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.

10.4.3.4 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. 10.25). The cells show rounded nuclei with fine chromatin and inconspicuous nucleoli. The cytoplasm is eosinophilic to somewhat clear and may contain intracytoplasmic vacuoles.

10.4.3.5 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.

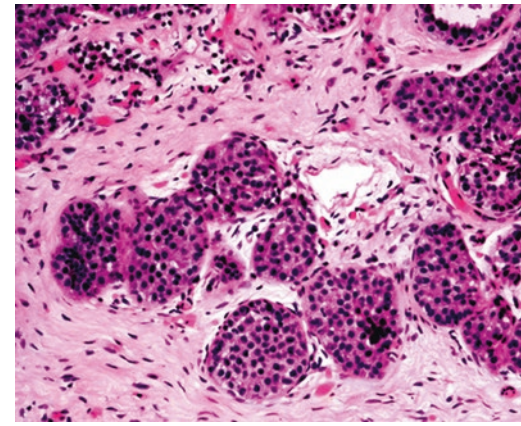


Fig. 10.25 Histology of LCIS showing distension of the ducts and lobules by uniform malignant cells. These cells form solid epithelial proliferation with the absence of lumen formation

10.5 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 are 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 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.

10.5.1 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 most 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%).

10.5.2 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. 10.26). 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 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 (Bonzanini et al. 2001; McKee et al. 2001; Shin and Sneige 1998). In addition, it was also reported that most of the invasive cancers showing

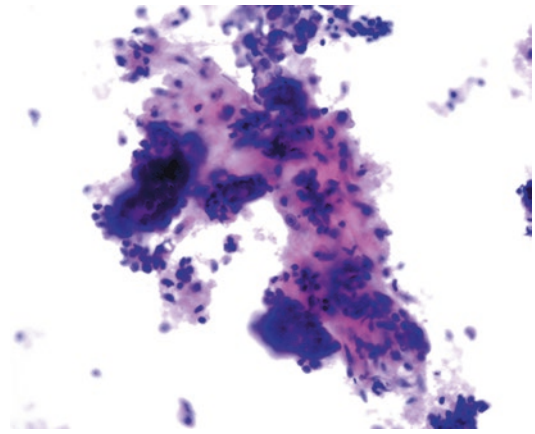


Fig. 10.26 Cytologic preparation of a high-grade invasive ductal carcinoma showing tumor cells invading into stromal fragments

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 a 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. 10.27), with such being seen in 42–72% of invasive aspirates but only in up to 20% of the aspirates of in situ carcinoma (Bofin et al. 2004; McKee et al. 2001). 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 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 disease 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.

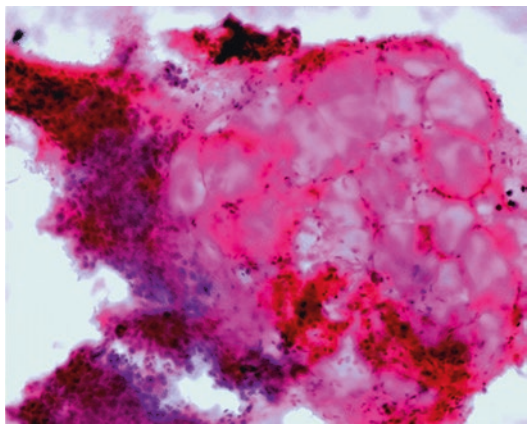


Fig. 10.27 Cytologic preparation of a high-grade invasive ductal carcinoma showing the presence of tumor cells within adipose tissue fragments

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

11

Andrew S. Field, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

11.1 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, which 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 (Masood et al. 2003) but 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.

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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 crushed artifact will cause dispersal in the tail of the smear away from the label. Crucially in breast FNAC, 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.

11.2 Intraductal Papilloma

11.2.1 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 contralateral breast of up to 2X, 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.

11.2.2 Cytologic Findings

Papillomas usually show diagnostic meshwork and stellate papillary tissue fragments, and less commonly, true papillary tissue fragments in FNAC (Field and Mak 2007a, 2007b). The meshwork tissue fragments have a crisscrossing mesh of usually sclerotic and elastotic thin stromal strands containing tubules of bland ductal cells and myoepithelial cells resembling adenosis (Figs. 11.1 and 11.2). The stellate papillary tissue fragments may have a similar sclerotic or a more fibroelastotic branching stellate cores, including thin non-staining elastotic fibrils, covered in hyperplastic sheets of ductal cells with myoepithelial cells (Figs. 11.3 and 11.4). The stellate papillary 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. A third type

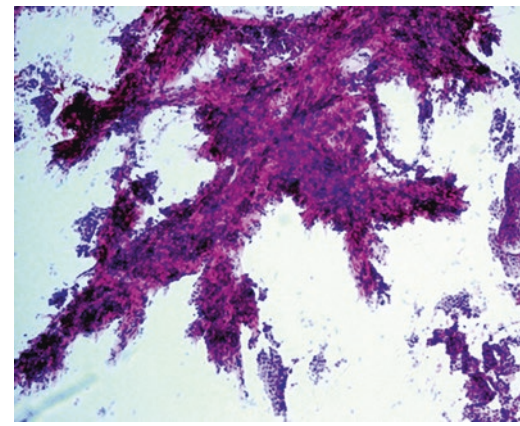

Fig. 11.1 Cytologic preparation showing true papillary fragments with discernible fibrovascular cores. Sheets of benign hyperplastic epithelial cells are seen in the background

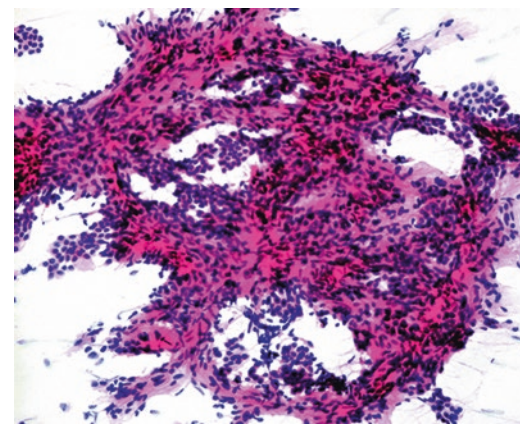

Fig. 11.2 Cytologic smear of a meshwork fragment displaying stromal strands containing epithelial tubules and partially covered by epithelial cells

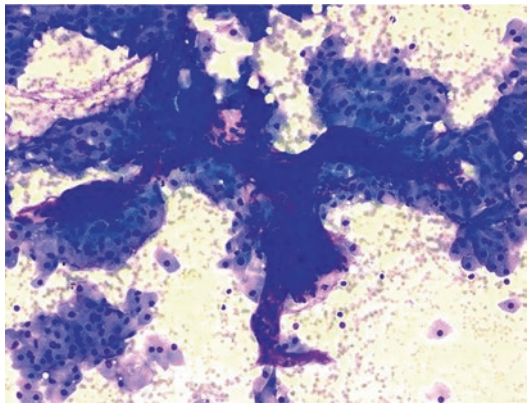

Fig. 11.3 Papillary fragment with sclerotic fibrovascular cores containing thin blood vessels, and partially covered in epithelium showing apocrine change

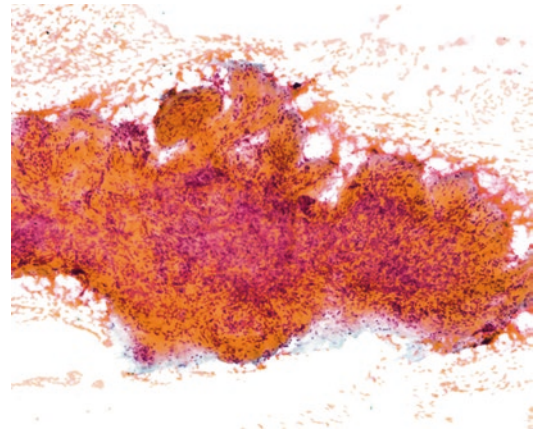

Fig. 11.5 Naked papillary fronds consisting of broad fibrotic stromal fragments with minimal ductal epithelium

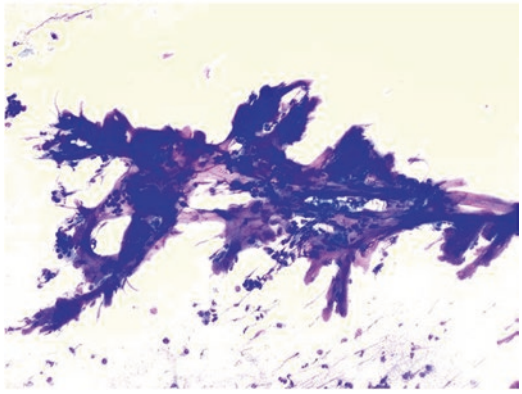

Fig. 11.4 Papillary stellate fragment showing the fibrotic core with branching

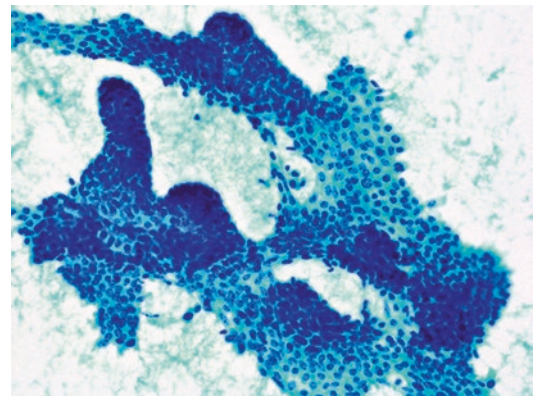

Fig. 11.6 Epithelial fragments showing micropapillae formation. Micropapillae are devoid of genuine fibrovascular cores. The epithelial cells show slight nuclear pleomorphism and crowding

of tissue fragment consisting of fibrotic broad stromal fragments with minimal ductal epithelium, termed "naked papillary fronds," albeit not common, is also typical of papillomas (Fig. 11.5) (Jamidi et al. 2021).

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 a hemoserous nipple discharge.

Once the meshwork and stellate papillary tissue fragments are recognized, the attached epithelium 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 nuclear crowding and overlapping, but without fibrovascular cores (Figs. 11.6 and 11.7). True papillae are relatively uncommon, although tufting of epithelium presenting in cytology as irregular ductal tissue fragments may occur.

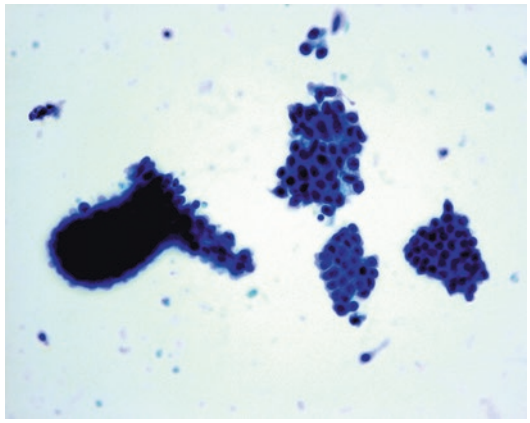

Fig. 11.7 Small epithelial fragments of micropapillae. These epithelial cells show rounded and slightly hyperchromatic nuclei, and fibrovascular cores are absent

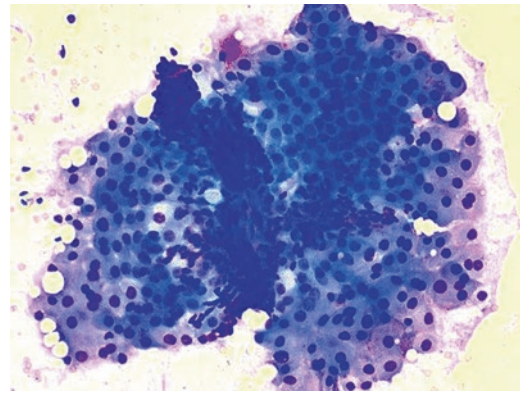

Fig. 11.8 Juvenile papillomatosis showing a large fragment with identifiable fibrovascular cores in the center, surrounded by hyperplastic epithelial cells, many of which show apocrine metaplasia

Infarction of papillomas can produce atypical, small rounded epithelial fragments, but recognition of the other features of intraductal 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 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 papillary tissue fragments but having very thin, magenta fibrovascular strands as cores, often with prominent myoepithelial cells (Figs. 11.8 and 11.9). These cores lack the elastotic fibrils of papilloma stellate papillary tissue fragments. There is a proteinaceous background with variable but usually scant histiocytes.

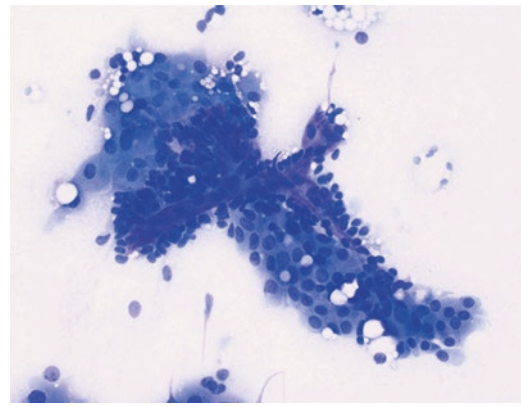

Fig. 11.9 Another papillary fragment in juvenile papillomatosis showing myoepithelial cells and hyperplastic epithelial cells showing apocrine metaplasia

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 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 "intraductal papillomas with epithelial hyperplasia with atypia" and excision rather than a core biopsy recommended. The recognition of the intraductal papilloma criteria assists in avoiding a false-pos-

itive diagnosis of malignancy, and ductectomy or other appropriate surgery can be recommended.

11.2.3 Differential Diagnosis

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

11.2.3.1 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. 11.10).

11.2.3.2 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 with possible cribriform or micropapillary areas, may also be seen (Fig. 11.11).

11.2.3.3 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 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. 11.12).

11.2.3.4 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 frag-

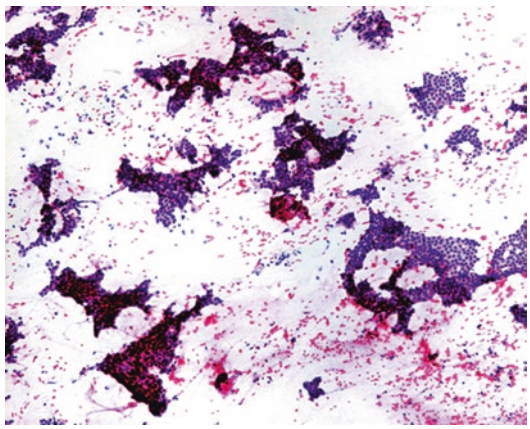

Fig. 11.10 Aspirate of epithelial hyperplasia showing sheets of ductal epithelial cells admixed with myoepithelial cells. Some of the epithelial sheets show three-dimensional architecture

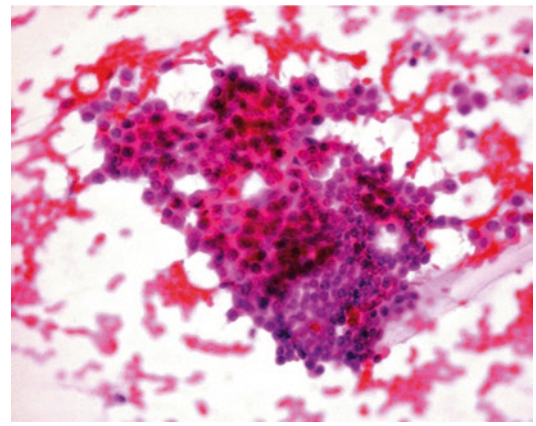

Fig. 11.11 Aspirate of ADH showing hyperplastic epithelial cells forming vague structures of lumen formation and rudimentary cribriforming

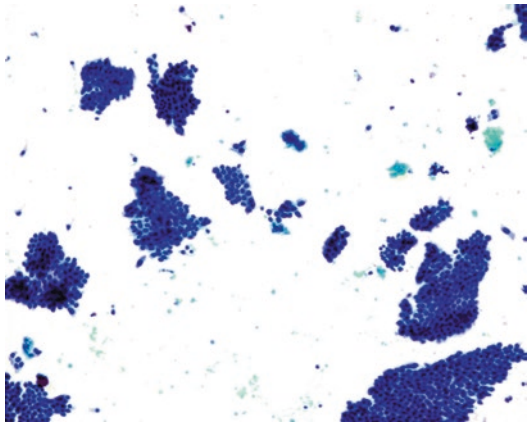

Fig. 11.12 Columnar cell changes showing ballooned tissue fragments with uniform monotonous epithelial cells. Occasional single columnar cells are noted in the background

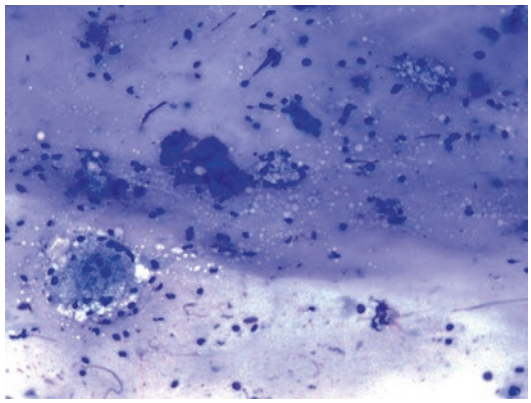

Fig. 11.13 Radial scar shows dispersed ductal cells and hyperplastic tissue fragments, but lack of meshwork or stellate tissue fragments

ments. 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 in the absence of meshwork or stellate tissue fragments (Field and Mak 2007a, 2007b) (Fig. 11.13).

11.2.3.5 Fibroadenoma

The typical cytologic appearance of fibroadenoma consists of branching "staghorn" epithelial fragments, stromal fragments, and abundant bare bipolar nuclei. Myoepithelial cells can be identified within the epithelial fragments that are

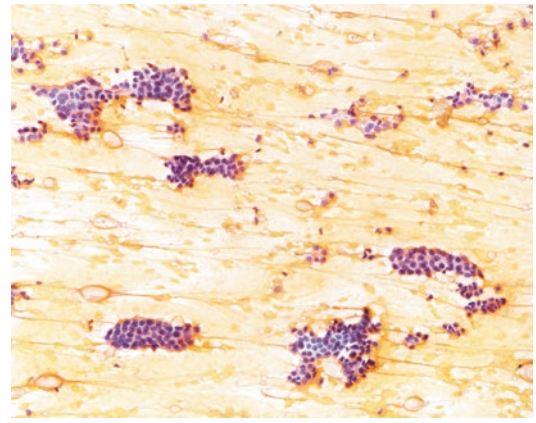

Fig. 11.14 Tubular adenoma with an abundance of tubular formation

formed by benign ductal cells. The stromal fragments are frequently of low cellularity and a fibromyxoid appearance, in contrast to the branching fibroelastotic or sclerotic stroma in the fibrovascular cores of papilloma.

11.2.3.6 Nipple Adenoma and Tubular Adenoma

The ductal epithelial cells in nipple adenomas and tubular adenomas are cohesive and aggregate into three-dimensional ball-like or tubular structures. The background cell population is mostly bare bipolar cells, whereas dispersed ductal cells are uncommon. In contrast to papillomas, stromal fragments are absent (Fig. 11.14).

11.2.4 Histologic Correlations

The histological features of intraductal papillomas vary from a simple single lesion with fibrovascular cores covered in a ductal cell layer including myoepithelial cells, within a duct lined by similar epithelium, to papillomas that ramify through a network of adjacent ducts (Figs. 11.15 and 11.16). The fibrovascular cores may be sclerotic or very broad, and the intervening epithelial component may show changes ranging from marked epithelial hyperplasia, ADH to low- to intermediate grade DCIS, which may be papillary. Recognition of these lesions in surgical

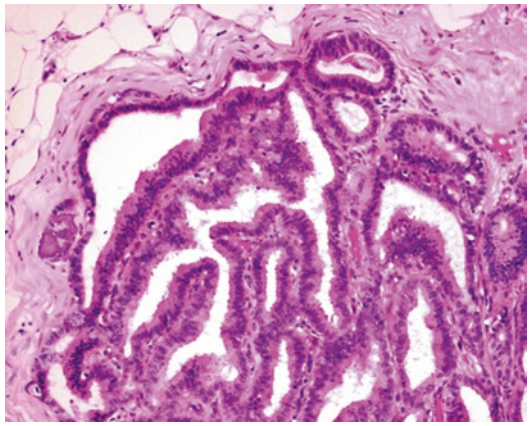

Fig. 11.15 Histologic picture of a papilloma showing broad fibrovascular cores covered by a ductal cell layer with myoepithelial cells, within a dilated duct lined by benign ductal epithelium

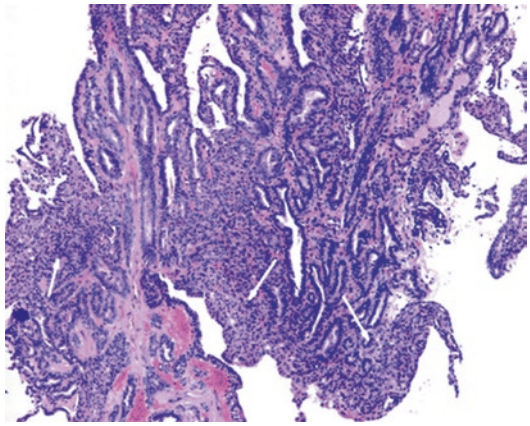

Fig. 11.16 Histologic picture of a papilloma showing broad fibrovascular cores composed of sclerotic fibrotic tissue. The branching of the fibrovascular cores is irregular. Foci of solid florid epithelial hyperplasia are seen within the papilloma (*arrow*)

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 intraductal papillomas and their variable epithelial hyperplasia, which is not usually prominently papillary but rather sheet-like with epithelial streaming and slit-like spaces and prominent myoepithelial cells. However, true papillary tissue fragments with thin fibrovascular cores covered in bland ductal and myoepithelial cell layers do occur in papillomas (Field and Mak 2007a, 2007b).

11.3 Papillary DCIS

11.3.1 Clinical and Epidemiological Findings

The clinical presentation of papillary DCIS is similar to intraductal papilloma. The radiological appearance is also similar to papilloma. Because of the significant clinical and radiological overlap, accurate differentiation will be based on pathological assessment.

11.3.2 Cytological Findings

In papillary DCIS 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 are no meshwork or stellate papillary tissue fragments as seen in intraductal 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. 11.17, 11.18, and 11.19).

Microcalcifications associated with papillary DCIS 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 hyperpla-

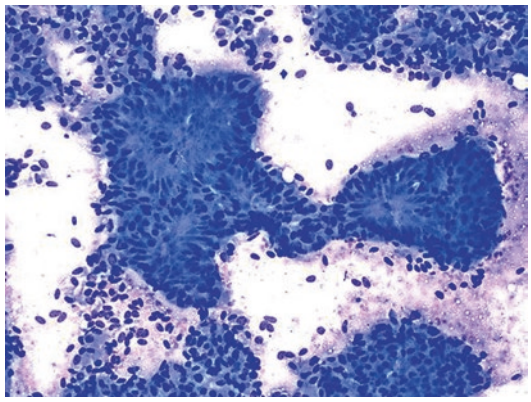

Fig. 11.17 Epithelial papillary fragments in papillary DCIS showing mildly atypical epithelial cells often showing a columnar orientation along the thin fibrovascular strands, and the paucity of myoepithelial cells

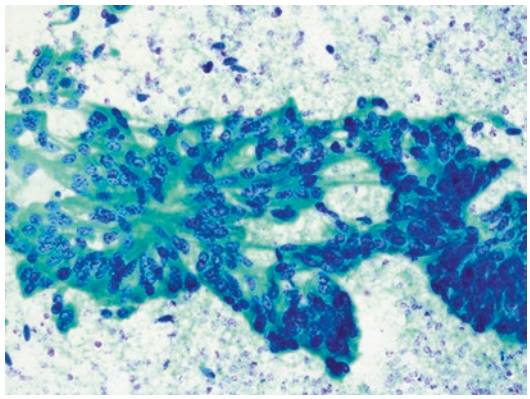

Fig. 11.18 Cytologic details of the epithelial cells in papillary DCIS, showing a single-cell population of epithelial cells with a columnar orientation and mild nuclear atypia. No myoepithelial cells identified

sia associated with a papilloma, but there will be no 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" (Field et al. 2020). In addition, some of these cases of atypia will be found to be low-grade invasive carcinomas, no special type, and evidence of invasive carcinomas such as the presence of crowded, rigid atypical tubules, tufted sclerotic stromal fragments infil-

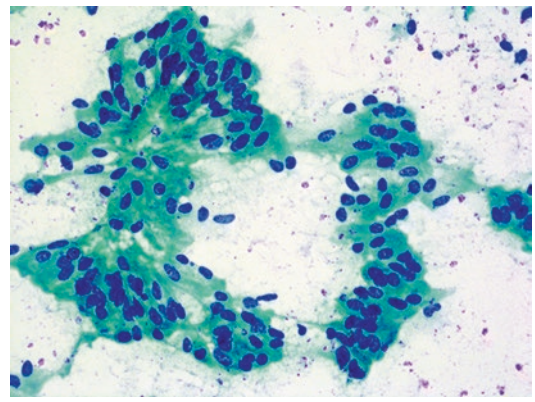

Fig. 11.19 Cytologic details of the epithelial cells in papillary DCIS, showing a single-cell population of epithelial cells with columnar orientation and mild nuclear atypia. No myoepithelial cells identified

trated by atypical epithelial strands, and prominent dispersal of atypical epithelial cells should be searched for.

11.3.3 Differential Diagnosis

11.3.3.1 Benign Papillary Lesions

Within the telltale tissue fragments present within the FNAC, the smaller size of tissue fragments, increased nuclear size and mild atypia and the presence of complex and slender fibrovascular cores are subtle but good clues to suggest papillary DCIS, which can then be confirmed by the lack of myoepithelial cells on the tissue fragments, and the lack of bare bipolar nuclei and the abundance of dispersed ductal cells 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.

11.3.3.2 Other Types of Low-Grade DCIS

The surgical management of most low-grade DCIS, including papillary DCIS, is similar; in addition, very often these different patterns of ductal carcinomas in situ are present in a mixed pattern in the subsequent surgical pathology. 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 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 "crater-like" or "pockmark" depressions which represent a cribriform architecture.

11.3.3.3 Invasive Low-Grade Ductal Carcinoma

Distinguishing papillary DCIS (and other intraductal carcinomas of low and intermediate grade with cribriform or solid sheet patterns) from invasive low-grade carcinoma, no special type 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.

11.3.4 Histologic Correlations

Papillary DCIS usually shows low-grade carcinoma cells present within and distending ducts. These cells are arranged in a fine and arborizing 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. 11.20 and 11.21). 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 intraductal papilloma, papillary DCIS tends to show higher epithelial cellularity and finer and thinner fibrovascular cores with more elaborate patterns.

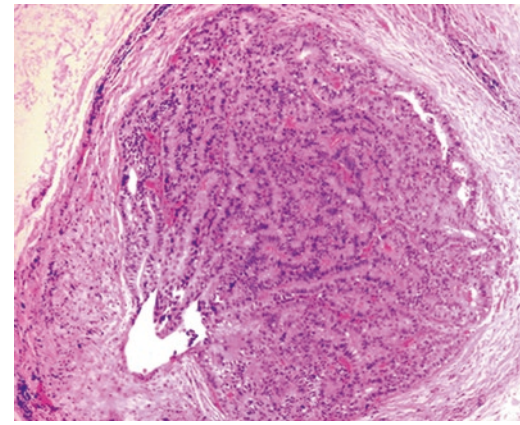

Fig. 11.20 Papillary DCIS showing proliferation of monotonous epithelial cells lining fine and elaborate thin fibrovascular cores

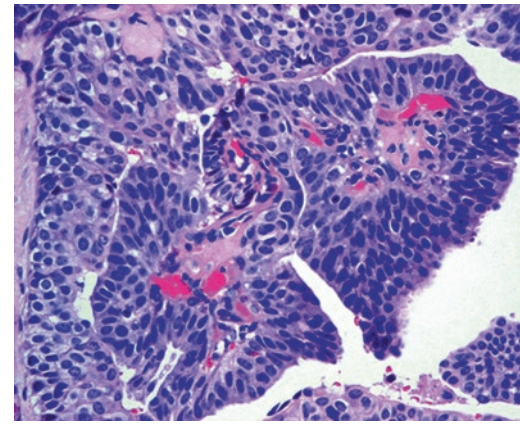

Fig. 11.21 Histologic details of papillary carcinoma in situ showing layers of mildly atypical epithelial cells over thin and delicate fibrovascular cores, devoid of a myoepithelial cell layer

11.4 Solid Papillary Carcinoma

11.4.1 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 is considered invasive.

11.4.2 Cytologic Findings

In FNAC material, the features include 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 micropapillae are not seen, although cribriform and solid tissue fragments can be present. The ductal cells can exhibit neuroendocrine differentiation characterized by granular or finely vacuolated cytoplasm and a plasmacytoid appearance with speckled nuclear chromatin and eccentric cytoplasm (Figs. 11.22, 11.23, 11.24, and 11.25).

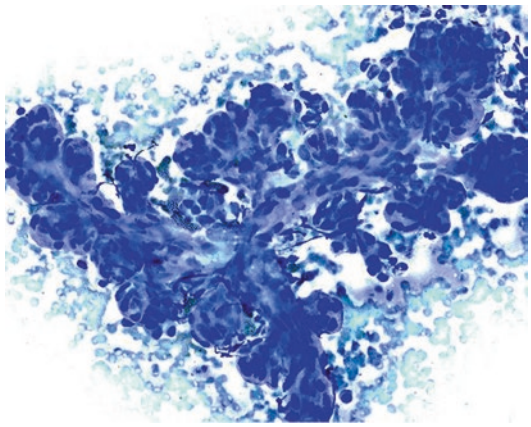

Fig. 11.22 Solid papillary carcinoma showing epithelial fragments with distinctive fibrovascular tissue fragments with bulbous tips

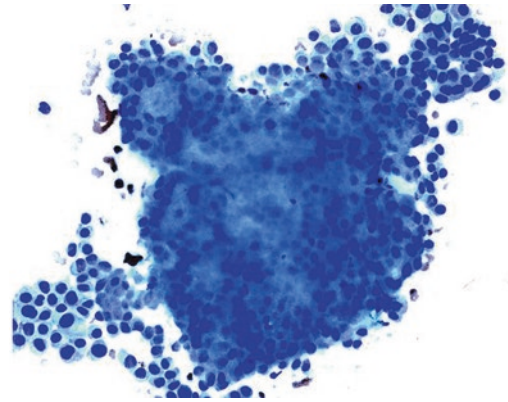

Fig. 11.23 Large cellular fragment of solid papillary carcinoma consisting of epithelium showing nuclei devoid of prominent nucleoli and granular eccentric cytoplasm, suggestive of neuroendocrine differentiation

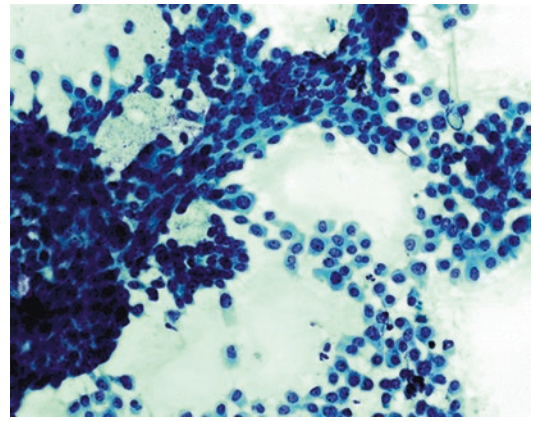

Fig. 11.24 Cellular details of solid papillary carcinoma showing epithelial cells with eccentric cytoplasm and moderate to abundant granular eccentric cytoplasm, suggestive of neuroendocrine differentiation. Abundant single cells with similar morphology are usually seen in the background

11.4.3 Differential Diagnosis

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

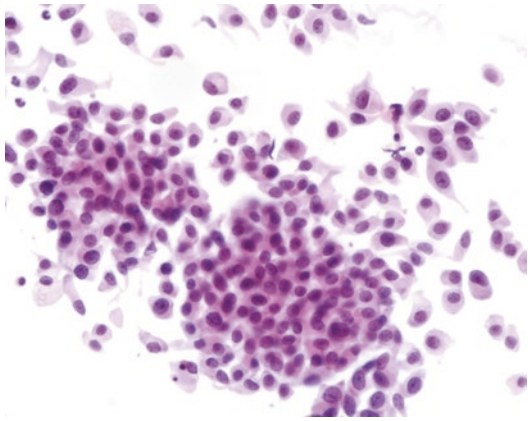

Fig. 11.25 Neuroendocrine differentiation evidenced by plasmacytoid cells with speckled nuclear chromatin and eccentric cytoplasm

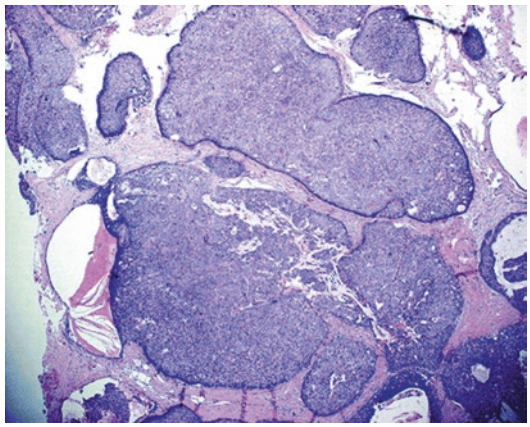

Fig. 11.26 Histology of a solid papillary carcinoma showing ducts filled and expanded by a solid proliferation of atypical cells, with single or multiple fibrovascular capillary coils in the midst of the epithelial cells

11.4.4 Histologic Correlations

Solid papillary carcinoma shows multiple ducts expanded by a solid and focally cribriform proliferation of atypical cells, with single or multiple capillary coils scattered in 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 suggestive of neuroendocrine differentiation (Figs. 11.26 and 11.27). Many may express neuroendocrine markers including synaptophysin and chromogranin. The ducts may

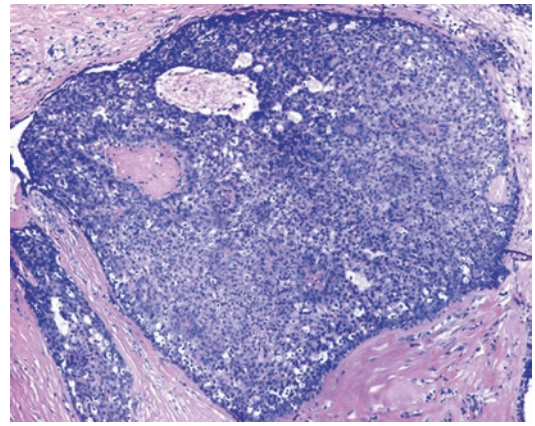

Fig. 11.27 Solid papillary carcinoma with solid epithelial cells expanding the entire ductal space; only several small fibrovascular cores can be identified. The cells show spindled nuclei, and the architectural pattern may give an impression of nuclear streaming

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, accompanied by a desmoplastic stroma and are completely devoid of rimming myoepithelial cells, these alterations are regarded as an invasive disease (WHO 2019).

11.5 Encapsulated Papillary Carcinoma

11.5.1 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 or complex rounded 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 in the papillary fronds of the encapsulated papillary carcinoma, this tumor has been regarded as representing an indolent form of invasive cancer

with a rounded pushing front, or carcinoma in transition from in situ to invasive phase. The Working Group of the WHO Classification of Breast Tumours (2019) regards encapsulated papillary carcinoma as in situ (Tis) disease.

11.5.2 Cytologic Findings

Low-grade or "clinging" intraductal carcinoma involving cysts produces cytological smears with 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. 11.28 and 11.29). A diagnosis of "suspicious of low-grade intraduct carcinoma, with or without an invasive component," can be made.

Encapsulated papillary carcinomas produce similar cytology to papillary DCIS. They may be associated with invasive carcinoma, no special type.

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.

11.5.3 Differential Diagnosis

11.5.3.1 Benign Papillary Lesions

Encapsulated papillary carcinoma is usually associated with cystic change. The old blood, granular proteinaceous material, and siderophages may give a false impression of benign cystic or papillary lesions. However, the nuclear atypia and lack of myoepithelial cells on high-power assessment are distinguishing features. Other helpful clues include a relative increase in ductal cells and decreased stromal fragments, and a lack of the distinctive stellate papillary tissue fragments and associated ductal epithelial tissue fragments and apocrine sheets commonly seen in intraductal papilloma.

11.5.3.2 High-Grade DCIS

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 calcifica-

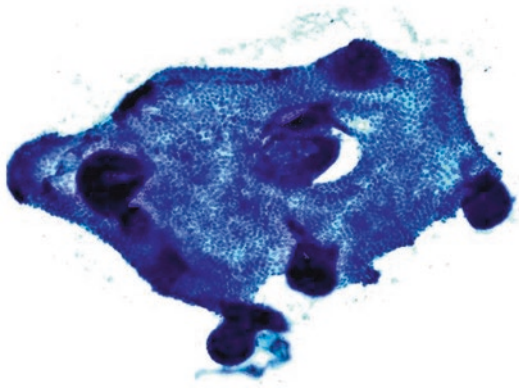

Fig. 11.28 Intracystic papillary carcinoma showing micropapillary tissue fragments with a mild degree of nuclear crowding and distinctive rigid micropapillae

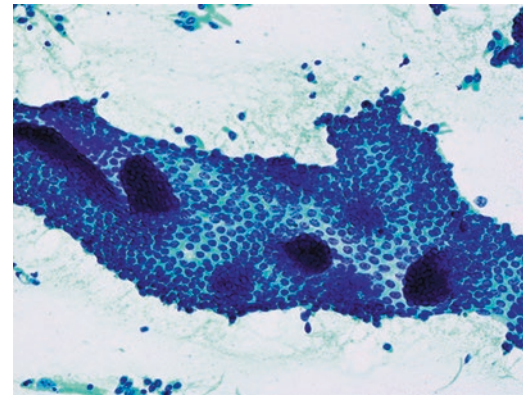

Fig. 11.29 Cytologic cellular details of intracystic papillary carcinoma showing mild nuclear atypia, together with rigid micropapillae

tions. True papillary tissue fragments are not seen, and these cases will be signed out as "suspicious of high-grade DCIS with or without invasive component."

11.5.3.3 Carcinoma of Other Types

Carcinoma of varying types, including tubular, invasive cribriform, invasive 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.

11.5.4 Histologic Correlations

Surgical pathology of these uncommon lesions usually shows papillary epithelium lacking myoepithelial cells arranged along delicate fibrovascular fronds taking up a variable amount of the cystic space. Crowding can occur and the papillary fronds and diagnostic arborescent papillary architecture may be only focal (Fig. 11.30). The epithelial layer lining the cystically dilated space 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 cytological preparations. Most authors will consider this lesion to behave in a manner similar to carcinoma in situ and should be managed as such. If high nuclear grade and mitotic activity are present or the tumor shows HER2 positivity or is triple negative, the lesion should be regarded as an invasive carcinoma, no special type, and graded accordingly (WHO 2019).

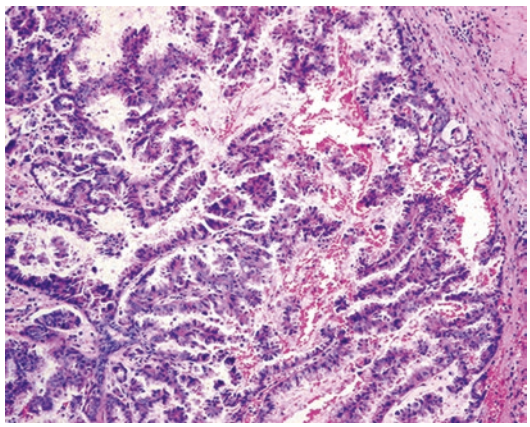

Fig. 11.30 Histology of encapsulated papillary carcinoma showing papillary epithelium lacking myoepithelial cells lining delicate fibrovascular fronds taking up a variable amount of the cystic space, which is devoid of myoepithelial cell lining

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Mucinous Lesions

12

Catarina Callé, Fernando Schmitt, Gary Tse, and Puay-Hoon Tan

12.1 Introduction

Mucinous breast lesions refer to a broad spectrum of entities, ranging from benign fibrocystic changes with luminal mucin to mucocele-like lesions, invasive mucinous carcinoma, and cyst-adenocarcinoma mucinous (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 (Tse et al. 2013).

Mucins are classified into two categories: membrane-bound mucins, involved in signal transduction (MUC1, MUC3, and MUC4) and gel-forming secreted mucins released into the extracellular space (MUC2, MUC5AC, and MUC6). MUC1 is located at the apical surface of most normal breast epithelium, in neoplastic epithelium there may be loss of polarity and an increase in expression. Mucinous carcinoma characteristically produces MUC2 and MUC6 (a rare finding in other mammary carcinomas). MUC1, MUC3, MUC4, and MUC5AC expression is not associated with specific tumor types (Ginter et al. 2020; Harrison and Dillon 2018).

Apart from lesions associated with extracellular mucin, lobular neoplasia, 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 (Tse et al. 2013). Since the last edition of the WHO Breast Tumors Classification, it is now perceived that some invasive lobular carcinomas may be associated with extracellular mucin production, an extremely rare feature.

While FNAC of mucinous lesions show overlapping features and can potentially be mistaken for one another, there are some distinctive cyto-

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logical features apart from the presence of mucinous material in the background (Tse et al. 2013).

12.2 Mucocele-Like Lesions

12.2.1 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 (Tse et al. 2013).

12.2.2 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. 12.1). 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 (Tse et al. 2013).

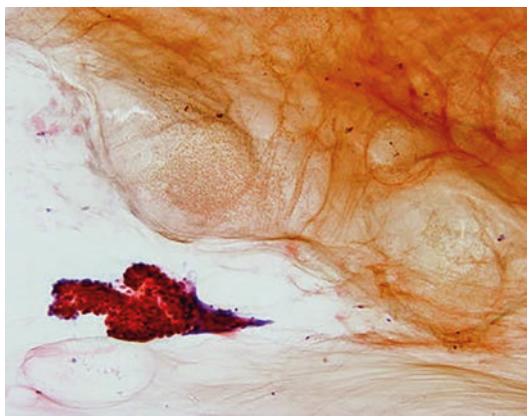

Fig. 12.1 Mucocele-like lesion. Benign bimodal epithelial cluster floats within abundant mucinous material

12.2.3 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 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 the 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 (Tse et al. 2013).

12.2.4 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 (Tse et al. 2013).

12.2.5 Reporting

According to the Yokohoma System for reporting Breast Fine Needle Aspiration Biopsy Cytopathology mucocele-like lesions should be rendered the atypical category. "Atypical in breast FNAB cytology is defined as the presence of cytological features seen predominantly in benign processes or lesions but with the addition of some features that are uncommon in benign lesions and which may be seen in malignant lesions" (Field et al. 2020).

Example: "A mucinous background with low cellularity consisting of occasional single cells or small tissue fragments showing minimal nuclear enlargement or pleomorphism. *Atypical*: There is abundant mucin in the background with only scanty dispersed small tissue fragments and single epithelial cells showing minimal pleomorphism. *Comment*: the features favor a mucocele-like lesion but mucinous carcinoma cannot be excluded. Core biopsy is recommended" (Field et al. 2020).

12.3 Mucinous Carcinoma

12.3.1 Clinical and Epidemiological Findings

Mucinous carcinoma (MC) of the breast, also known as mucoid, colloid, or gelatinous carcinoma, is a special subtype of invasive breast carcinoma, that represents about 2% of all breast carcinomas, with an excellent prognosis, with a 10-year survivals rate of 89%, occurring in women of older age group (WHO 2019). Mammographically, it may mimic 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.

12.3.2 Cytologic Findings

Aspirates from MC include tridimensional epithelial clusters and single cells of small to medium size, with mild to moderate nuclear atypia and occasional intracytoplasmatic vacuoles. Extracellular mucin is observed in the background (Fig. 12.2) and may contain few capillary vessels (Jayaram et al. 2000; Ventura et al. 2003). The epithelial cells can be bland and mimic benign clusters, but the presence of intact cells with retained cytoplasm is often seen (Fig. 12.3). Nuclear size 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 (Tse et al. 2013). FNAC is highly accu-

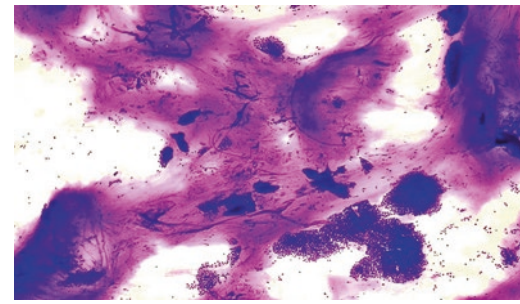

Fig. 12.2 Mucinous carcinoma. Diff-Quick stain shows cohesive groups of epithelial cells within a mucoid background

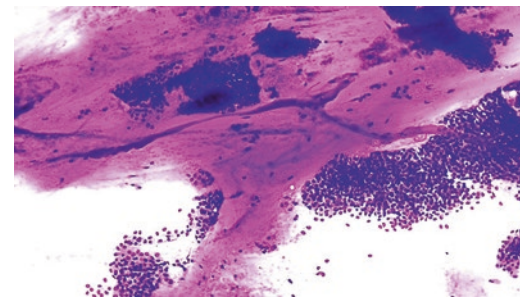

Fig. 12.3 Mucinous carcinoma. High magnification of an epithelial cluster showing relatively uniform nuclei. Metachromatic mucoid material is noted. A few dissociated cells with retention of cytoplasm are seen

rate at predicting carcinoma with mucinous differentiation although it is not possible to reliably predict if the lesion represents pure MC or a mixed carcinoma (Shield et al. 2016).

12.3.3 Histologic Correlations

MC is a rare histologic type of estrogen receptor (ER)-positive/HER2-negative breast cancer (BC), 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 pure mucinous carcinoma with excellent outcome (Lee et al. 2021) (Fig. 12.4a, 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 MC. 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 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 (Tse et al. 2013).

Two main forms of MC have been described: types A and B, with type AB having transitional features (Capella et al. 1980). Type A, 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 is the more cellular form with endocrine differentiation and sometimes signet ring cells, and these lesions are less likely to be underdiagnosed as benign. 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 (Tse et al. 2013; Weigelt et al. 2009). In a recent study, the authors performed an exploratory analysis of the mutational repertoire in types A and B pure MC, that revealed no statistically significant differences between both. This study also shows that MC of the breast is genetically heterogeneous and lacks a pathognomonic fusion gene or somatic mutation (Pareja et al. 2019).

When invasive carcinoma forms mucin in between 10 and 90% of the tumor, with the rest comprising invasive breast carcinoma of no special type (IBC-NST), the term mixed mucinous carcinoma is used, with an attendant less favorable prognosis. FNAC of these mixed tumors are unlikely to be overlooked, as the IBC-NST component will demonstrate cytological features of IBC-NST carcinoma.

12.3.4 Management

The diagnosis of mucinous cancer on FNAC, in conjunction with corroborating clinicoradiologi-

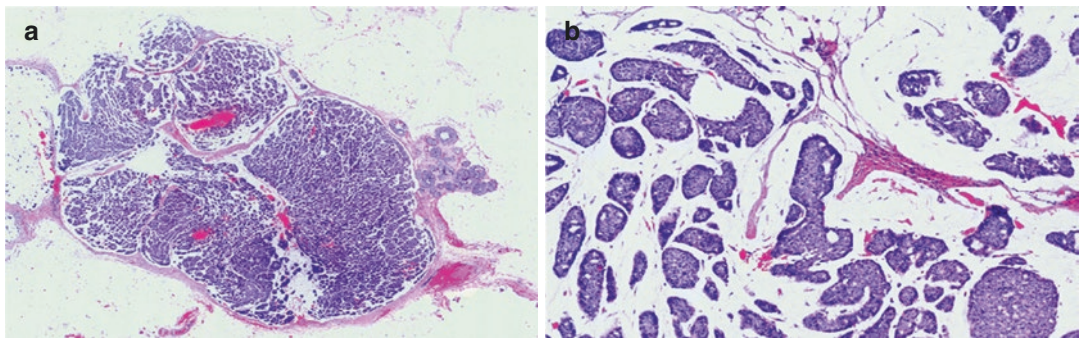

Fig. 12.4 (**a**) Scanning view of the circumscribed mucinous carcinoma, with islands of DCIS at the periphery. (**b**) Higher magnification shows solid nests of tumor cells within mucin pools

cal findings, can be therapeutically managed accordingly.

12.3.5 Reporting

According to the Yokohoma System for reporting Breast Fine Needle Aspiration Biopsy Cytopathology mucinous carcinoma should be rendered the suspicious of malignancy or the malignant category.

Example: "Mucinous background with low cellularity consisting of occasional single cells or small tissue fragments showing mild to moderate nuclear enlargement or pleomorphism. *Suspicious of malignancy*. There are scattered moderately atypical epithelial cells in a background of abundant fibrillary mucin. *Comment*: suspicious of mucinous carcinoma. Core needle biopsy or simple excision biopsy is recommended" (Field et al. 2020).

Example: "There is a fibrillary mucinous background with moderate epithelial cellularity consisting of single epithelial cells and small tissue fragments showing mild to moderate nuclear enlargement or pleomorphism. *Malignant*. These moderately cellular smears show moderately atypical epithelial cells in a background of abundant fibrillary mucin. *Comment*: The features are those of mucinous carcinoma" (Field et al. 2020).

12.4 Mucinous Cystadenocarcinoma

12.4.1 Clinical and Epidemiological Findings

Mucinous cystadenocarcinoma is an extremely rare primary invasive carcinoma of the breast, included for the first time in the last edition of the WHO Breast Classification of Tumours 2019. Most reported in post-menopausal Asian women, as a palpable mass with a median tumor size of 3 cm (WHO 2019).

Follow-up has been short, and most reported cases had a relatively good prognosis, with only a few patients reporting lymph node metastasis at presentation and none with distant metastasis (Nayak et al. 2018).

12.4.2 Cytologic Findings

Smears of this lesion have a background with abundant extracellular mucin and necrotic material. The neoplastic cells are arranged in aggregates composed of columnar shape cells, which contain intracellular mucin and an atypical nuclei in the cell's periphery, with coarse chromatin and prominent nucleoli (Kim et al. 2012; Sentani et al. 2012).

12.4.3 Histologic Correlations

Grossly, the mucinous cystadenocarcinoma is a well-circumscribed solid and cystic mass, containing gelatinous material. Histologically is characterized by large cysts lined by atypical tall columnar cells rich in intracytoplasmatic mucin, with similar features of pancreatobiliary and ovarian mucinous cystadenocarcinoma. The literature describes stratification, tufting, and papillary structures, with basally positioned nuclei, abundant intracytoplasmatic mucin and extracellular mucin, filling the cystic spaces, that most commonly have rounded contour but lack myoepithelial cells at the periphery. Most are ER, PR, and HER2 negative (Koenig and Tavassoli 1998; Nayak et al. 2018).

The differential diagnosis with primary breast tumors includes mucinous carcinoma and encapsulated papillary carcinoma, the latter lacks intracytoplasmatic mucin, and both are typically diffusely positive for ER and PR.

12.4.4 Management

The diagnosis of mucinous cystadenocarcinoma, a rare tumor subtype with only a few descriptions of the cytological findings, has to be made cautiously. Most reported cases had a relatively good prognosis, but follow-up time has been limited (Nayak et al. 2018).

12.5 Mucinous Papillary Neoplasms

12.5.1 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/intraductal papillary carcinoma, encapsulated papillary carcinoma, solid papillary carcinoma (in situ and invasive) and invasive papillary carcinoma (WHO 2019). 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 bloodstained 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 (Tse et al. 2013).

12.5.2 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. 12.5).

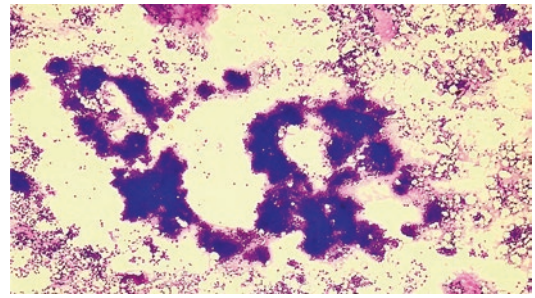

Fig. 12.5 Diff-Quick stain of the fine needle aspirate shows papillary epithelial structures within a background containing mucin as well as dispersed intact cells

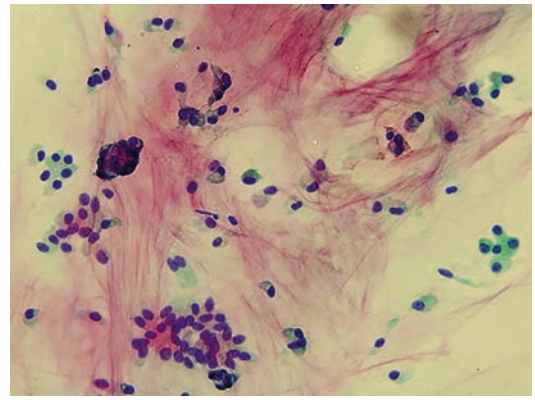

Fig. 12.6 Polygonal and columnar cells within a mucinous background

Columnar cells, are sometimes seen, in small groups or individually disposed (Fig. 12.6). 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 (Tse et al. 2013).

12.5.3 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. 12.7). The histological appearances of

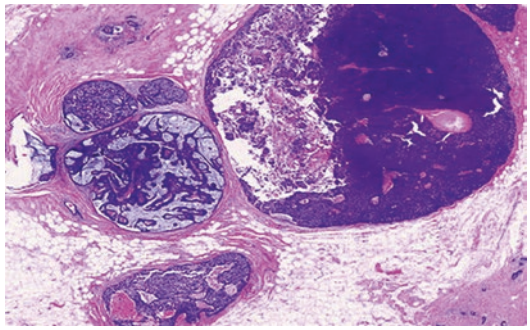

Fig. 12.7 Low magnification of solid papillary carcinoma with luminal mucin in a few distended duct spaces

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 (Tse et al. 2013).

12.5.4 Management

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

12.5.5 Reporting

Cytological findings of a papillary lesion often fall into the suspicious category, as appearances of benign papillomas and those that harbor malignancy shows substantial overlap (see Chap. 11) (Tse et al. 2013).

12.6 Invasive Lobular Carcinoma with Extracellular Mucin

Invasive lobular carcinoma accounts for 5–15% of all invasive breast carcinomas. While intracellular mucin production is a common characteristic of ILC, especially when signet ring cells are present, extracellular mucin production is an extremely rare feature. Since the last edition of the WHO is now perceived that invasive lobular carcinomas may be associated with extracellular mucin production, an extremely rare feature. Until 2019 only 23 cases were described in the literature (WHO 2019; Singh et al. 2019).

12.7 Differential Diagnosis

12.7.1 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 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 (Begum et al. 2009; Tse et al. 2013).

12.7.2 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 (Tse et al. 2013).

12.7.3 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; Tse et al. 2013).

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Carcinoma and Variants

13

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13.1 Clinical Findings

Invasive breast carcinoma is an infiltrative 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.

13.2 Radiologic Findings

Most commonly, breast cancer is identified as a mass lesion, often ill-defined or as a spiculated mass on 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.

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13.3 Pathologic Findings

13.3.1 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, or may be soft and mucoid as in mucinous carcinoma. Some special variants have distinct macroscopic appearances. Invasive 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 glistening 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.

13.3.2 Histologic Findings

The 2019 WHO classification requires a specialized pattern in over 90% of the tumor area to classify a breast carcinoma as "special type." If the special subtype is present in 10–90% of the tumor, with the remaining component of no special type (NST), it is classified as mixed NST and special type (WHO 2019). 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 (tumor-infiltrating lymphocytes, TILs) are frequent, as well as a circumscribed macroscopic appearance. The special types show some distinct characteristics that are reflected 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 moderate 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 *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 WHO proposed classification of these tumors is descriptive and divides them into 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 carcinoma of the

breast is 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, forming long fascicles with fingerlike extensions infiltrating the adjacent breast parenchyma. Squamous cell carcinoma usually presents 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 *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 disease* is not a specific variant of breast carcinoma, it is mentioned here because it can be sampled by cytology. The defining histologic feature of Paget disease is the presence of malignant glandular epithelial cells within the squamous epithelium (epidermis) of the nipple. Cytologically, these cells are high grade, with large pleomorphic nuclei and abundant eosinophilic cytoplasm. The nipple may vary from being macroscopically normal to being erythematous and ulcerated. Underlying the Paget's disease can be an in situ or invasive carcinoma.

Some special types of cancers are now subsumed under the rubric of invasive carcinoma NST according to the WHO 2019 breast tumor classification. These include the previously designated medullary carcinoma which was beset with interobserver reproducibility issues, and which is now regarded as an invasive cancer NST with medullary pattern, typically featuring prominent TILs. Other tumors included as special morphological patterns within the group of invasive cancer NST are oncocytic, lipid-rich, glycogen-rich, clear cell, pleomorphic, choriocarcinomatous, melanotic, and sebaceous cancer. Tumors with neuroendocrine differentiation are also classified as invasive carcinoma NST, although it is acknowledged that neuroendocrine tumors of the breast are a challenging entity that continues to be debated upon (Rakha and Tan 2022).

13.4 Cytologic Findings

13.4.1 General Findings

Breast carcinoma is a heterogeneous entity from clinical, radiological, morphological, and molecular perspectives. Despite that, there are general criteria 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 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. 13.1). Some carcinoma aspirates can contain a population of naked epithelial/tumor cell nuclei and these should not be mistaken as the naked bare nuclei of benign aspirates and are usually easily distinguished due to the apparent anaplasia they exhibit (Kocjan et al. 2013) 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 there are 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 Chap. 18. There are some criteria suggestive of invasions 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. Nevertheless FNA is still useful in diagnosing breast cancers. As in all breast lesions, FNA of 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 certain in cytology, imaging is very useful to solve this problem (Kocjan et al. 2008).

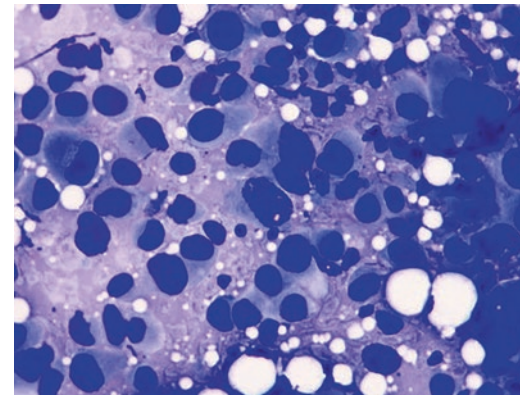

Fig. 13.1 Invasive breast carcinoma. Observe the main criteria of malignancy in breast aspirates: lack of cohesiveness, nuclear pleomorphism, and presence of mitotic figures (MGG stain)

13.4.2 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. 13.1). 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 clean (Fig. 13.2). 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. While it is difficult to accurately classify breast carcinomas into three grades based on cytology like on histology; generally low-grade tumors show predominantly large irregular three-dimensional epithelial tissue fragments, with some smaller groups and dispersed single cells as opposed to high-grade carcinomas where the pattern is predominantly smaller tissue clusters with plentiful dispersed cells (Field et al. 2020). High-grade ductal carcinomas, with cells containing enlarged nuclei and prominent nucleoli, necrosis, and neutrophils, are more often associated with a hormone receptor-negative phenotype (Figs. 13.3, 13.4, 13.5 and 13.6) (Dufloth et al. 2009). As it is difficult to distinguish invasive carcinomas from DCIS, criteria of malignancy, together with 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.

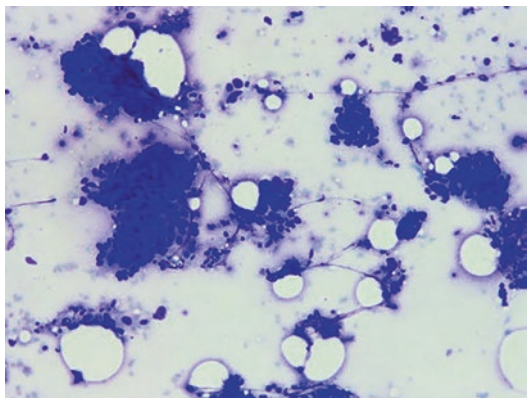

Fig. 13.2 Invasive ductal carcinoma. Groups of malignant epithelial cells with nuclear atypia. Note the background with necrotic debris and inflammatory cells (MGG stain)

13.4.3 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. 13.7). There is a proclivity for cells to disperse. 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. Cytoplasmic vacuolation may not always be present but when seen, can give the cells a signet ring appearance (Fig. 13.8a). 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

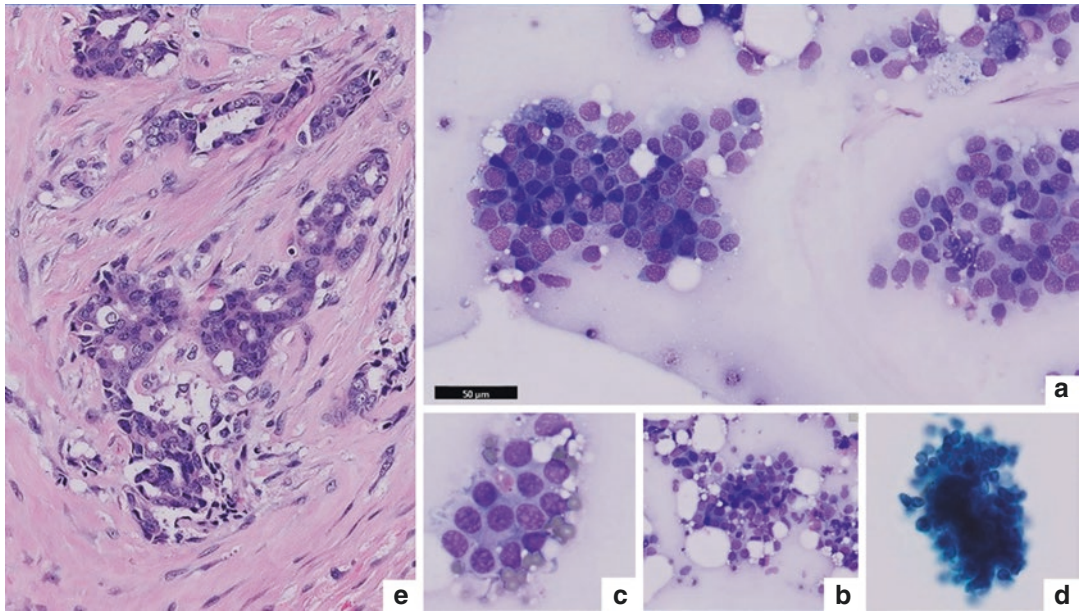

Fig. 13.3 Grade 1 Invasive ductal Carcinoma. (**a**–**c**) MGG stained smear showing clusters of mildly discohesive cells with mild nuclear pleomorphism and absent myoepithelial cells. The background appears clean. (**d**) Cluster of atypical ductal cells with minimal pleomorphism PAP. (**e**) H&E section showing a grade 1 invasive ductal carcinoma with tubule formation and surrounding desmoplastic stroma

classic lobular carcinoma. The pleomorphic variant features larger cell size with more nuclear atypia, prominent nucleoli, abundant cytoplasm with occasional multinucleated malignant cells and mitoses, and may be misclassified as ductal in up to 25% of FNAC smears (Green et al. 2005). ILC is one of the main reasons for false-negative diagnosis in breast aspirates. This is due to the low cell yield and small cell size. According to the literature, the overall sensitivity in the detection of malignancy in ILC cases is 76%. The discrepant cytologic findings with clinical and imaging findings are 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 of ductal carcinoma can be a problem. Smearing artifact with stripped nuclei may be seen. The small clusters and relatively bland linear rows of malignant cells in a classic ILC may be overlooked if there are concurrent larger epithelial sheets from a fibroadenoma or accompanying benign proliferative changes. In these instances, triple assessment is crucial. While cytoplasmic vacuoles are an important clue to the diagnoses of ILC, these may also be seen in other malignant lesions like mucinous and ductal carcinoma, as well as benign lesions like hyperplasia and apocrine change (Field et al. 2020).

13.4.4 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. 13.9). 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 the

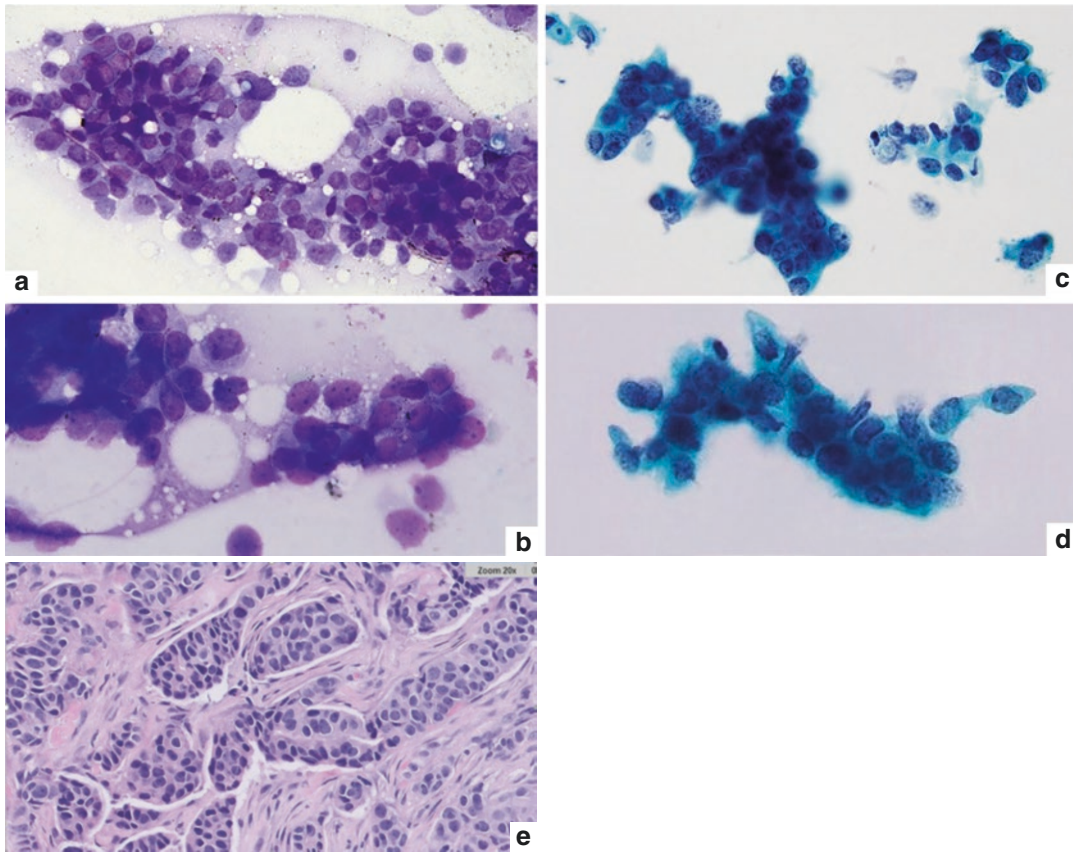

Fig. 13.4 (**a–d**) Grade 2 Invasive Ductal Carcinoma. (**a–b**) MGG shows moderate nuclear pleomorphism, discohesion, and multiple tiny nucleoli. (**c**, **d**) PAP stain showing moderate amounts of cytoplasm and tiny nucleoli. (**e**) H&E section from the same case shows clusters of moderately pleomorphic cells with no tubule formation and surrounding desmoplastic stroma

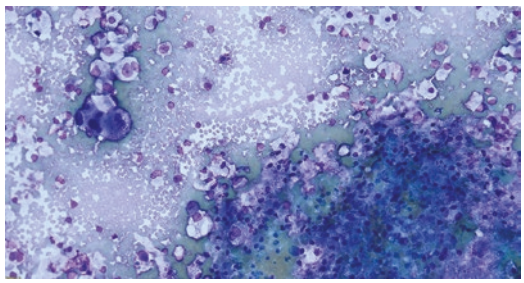

Fig. 13.5 High-grade invasive ductal carcinoma with necrosis and inflammatory cells in the background. The histology of this case revealed a triple-negative breast carcinoma (MGG stain)

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 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 clues to the correct diagnosis (Fig. 13.10). 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 fol-

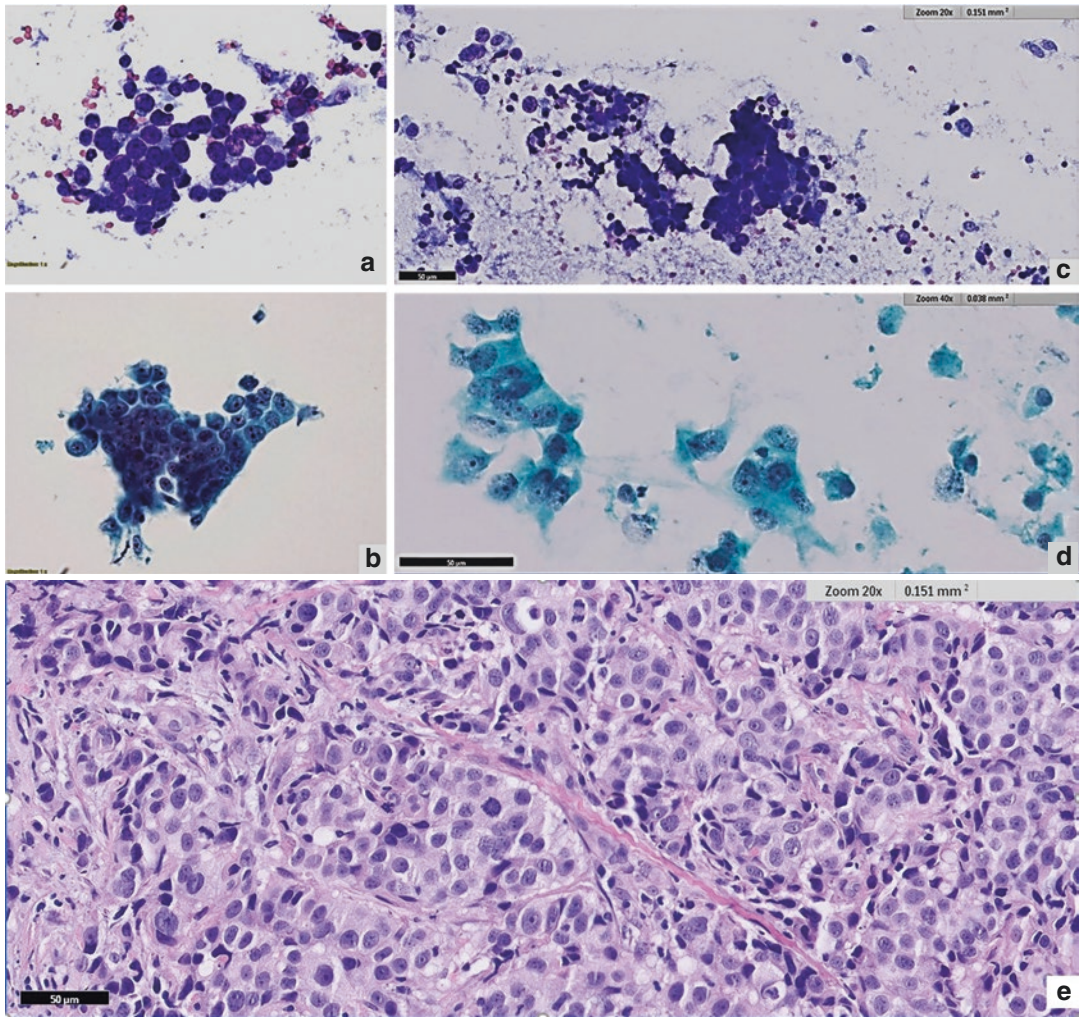

Fig. 13.6 Grade 3 Invasive ductal carcinoma. (**a**, **c**) MGG stain; (**b**, **d**) PAP stain. Clusters of pleomorphic cells showing discohesion, multiple tiny nucleoli better appreciated on the PAP, moderate amounts of cytoplasm, and background necrotic debris. (**e**) Histology from the same case shows a poorly differentiated ductal carcinoma with solid islands exhibiting no tubule formation and mitoses (*black arrows*)

lowed by excisional biopsy for a definitive diagnosis.

13.4.5 Invasive Cribriform Carcinoma

FNAC from invasive cribriform carcinoma shows cohesive sheets and three-dimensional cribriform clusters of bland-looking and mitotically inactive ductal cells in a bloodstained background (Figs. 13.11 and 13.12). 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 are usually 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. 13.11a). Sometimes, hemosiderin pigment is seen in the cytoplasm of giant

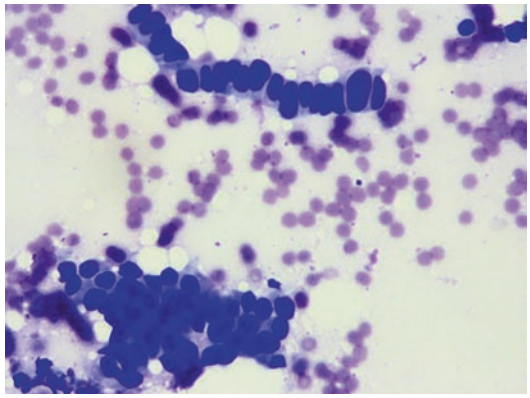

Fig. 13.7 Invasive lobular carcinoma. Note the malignant cells forming *small chains* in the aspirates (MGG stain)

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.

13.4.6 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 of

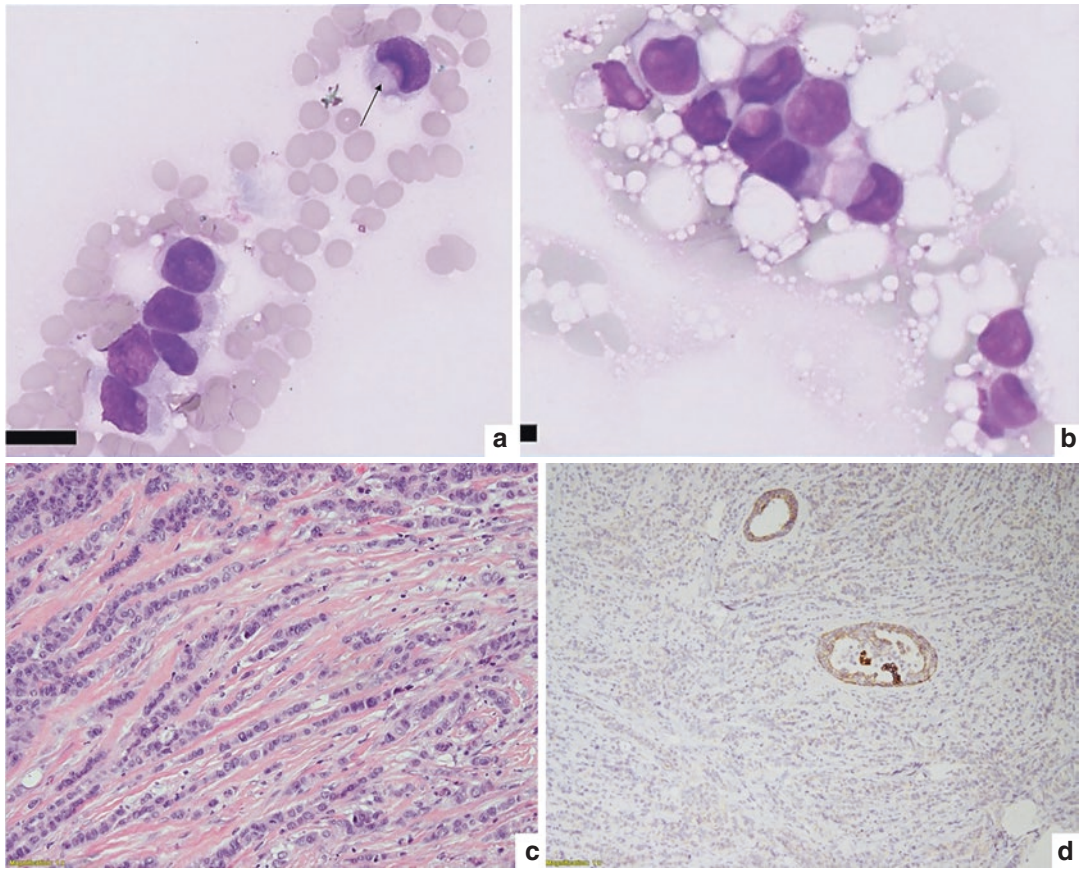

Fig. 13.8 (**a**, **b**) Invasive lobular carcinoma. MGG. Carcinoma cells are in chains with intracytoplasmic vacuoles (*black arrow*). (**c**) H&E section showing a classic invasive lobular carcinoma exhibiting a linear file pattern. (**d**) Negative staining for E-Cadherin within the tumor

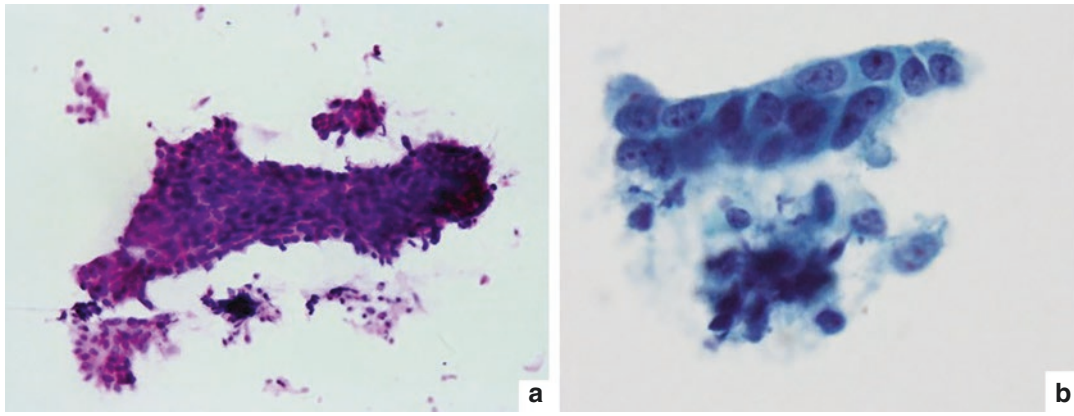

Fig. 13.9 Tubular carcinoma. Aspirate shows cohesive clusters of uniform, mildly pleomorphic tumor cells. The cells are arranged mostly in tubular structures with an angular appearance or comma-like pattern (**a**) HE and (**b**) PAP

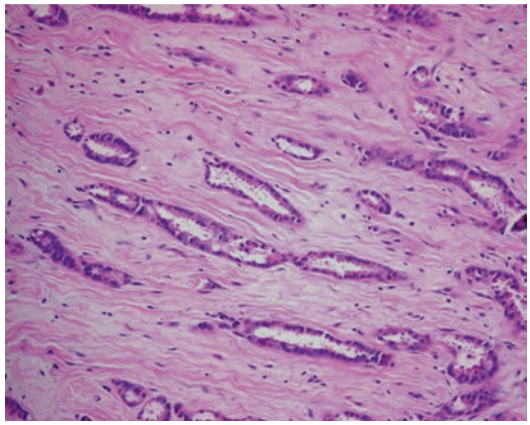

Fig. 13.10 Tubular carcinoma. Histology demonstrates angular tubules lacking significant cytonuclear atypia

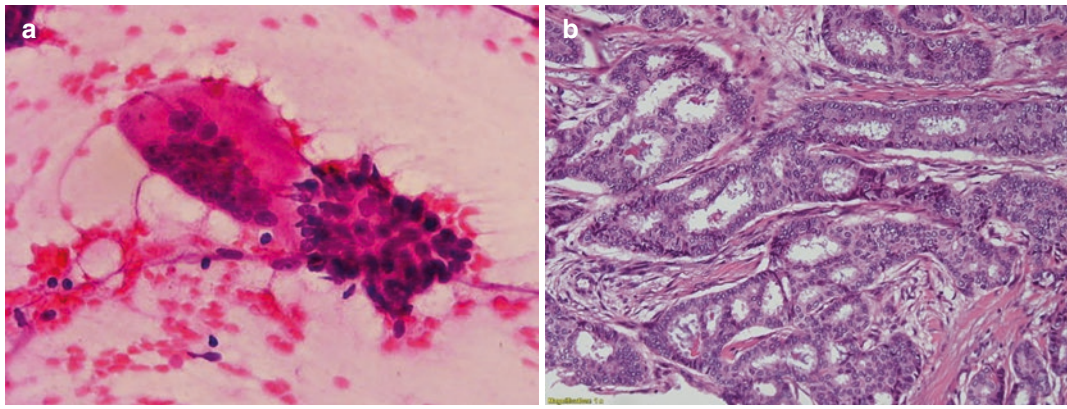

Fig. 13.11 Invasive cribriform carcinoma. (**a**) Note osteoclast-like multinucleated cells at the periphery of a group of mildly pleomorphic malignant cells with round to oval nuclei (H&E stain). (**b**) H&E section showing invasive cribriform islands from a Grade 1 invasive cribriform carcinoma

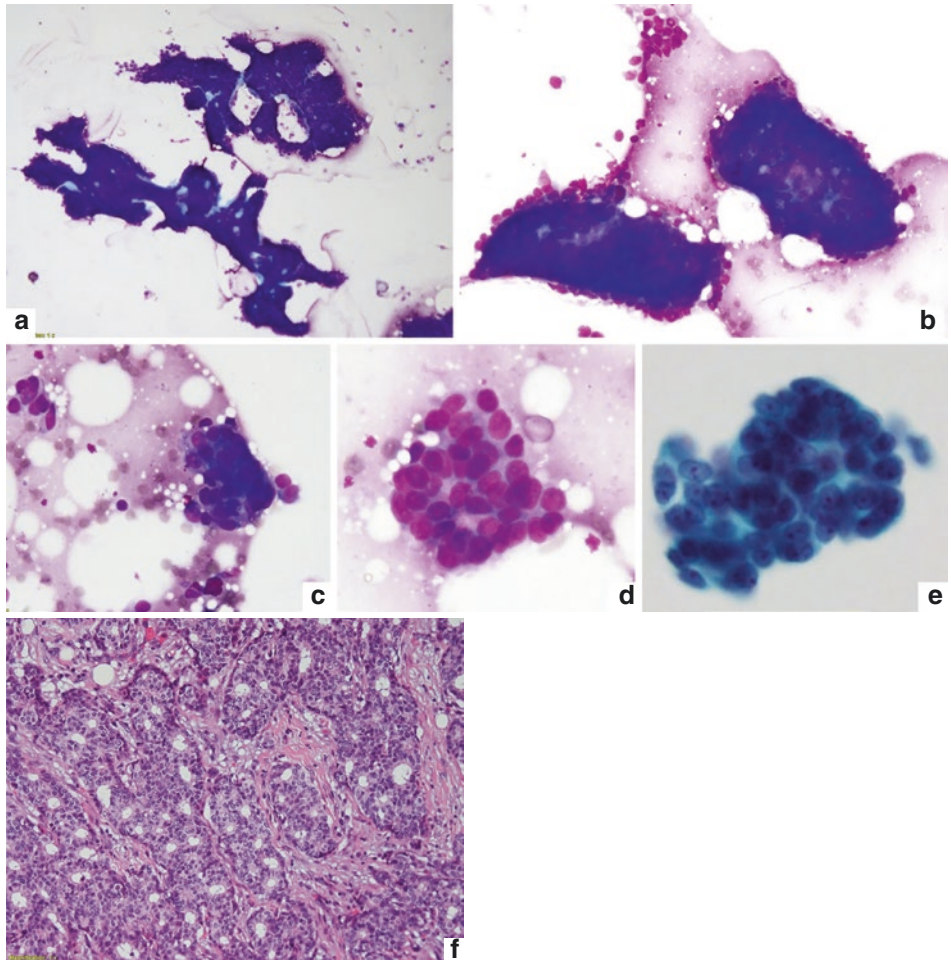

Fig. 13.12 Invasive cribriform carcinoma. (**a**) Branching sheets of ductal cells. MGG. Flat largely cohesive sheets of ductal cells with evenly placed nuclei; note rare bare nuclei in the background, lumens or cribriform spaces in the clusters with mild nuclear atypia and no myoepithelial cells (**b–d**) MGG and (**e**) PAP. (**f**) H&E section of a classic invasive cribriform carcinoma with invasive islands composed of epithelial clusters with minimal nuclear atypia accompanied by punched out spaces

filmy, wispy blue-green material on Papanicolaou's stain (Figs. 13.12, 13.13, 13.14 and 13.15). 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

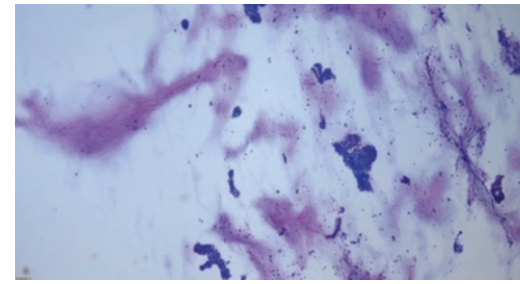

Fig. 13.13 Mucinous carcinoma. Note three-dimensional groups of neoplastic cells surrounded by abundant extracellular mucinous material which is metachromatic on MGG staining (MGG stain)

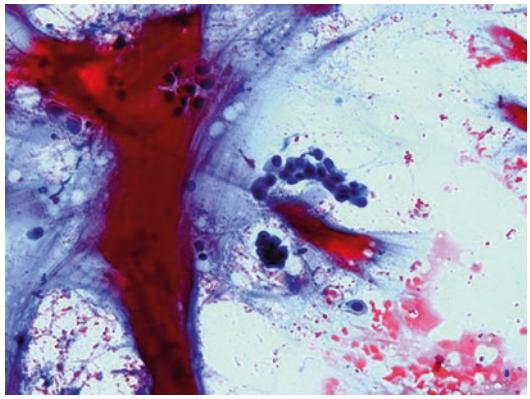

Fig. 13.14 Mucinous carcinoma. Papanicolaou staining highlights the background mucin as a wispy blue-green material (Pap stain)

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 a 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.

13.4.7 Invasive Carcinoma NST with Medullary Pattern

The definitive diagnosis of breast carcinoma NST with medullary pattern is difficult to achieve based only on cytology (Fig. 13.16). The term medullary carcinoma is no longer included in the fifth Edition of the WHO classification of breast tumors and is now regarded as one end of the spectrum of TILs-rich invasive breast carcinoma NST rather than a distinct morphological subtype. This entity can be suspected on FNAC when there are prominent lymphoplasmacytic infiltrates accompanying high-grade malignant cells, in conjunction with clinical and imaging findings of a usually well-circumscribed, mobile mass. Such "medullary features" can be recognized in aspirates, and can be clinically useful because these tumors are associated with *BRCA1* mutation and the basal-like phenotype (Da Silva and Lakhani 2010). Aspirates from carcinoma with medullary pattern are usually cellular with large pleomorphic tumor cells in a background of lymphocytes and plasma cells (Fig. 13.17). 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 material and a few small groups of atypical epithelial cells, or the tumor nuclei may appear bare without cytoplasm or with only a thin rim of cytoplasm. Based on cytological features, the differential diagnosis includes high-grade ductal carcinoma with inflammatory infiltrates. In this case, the aspirates consist predominantly of pleomorphic cells arranged in three-dimensional clusters rather than syncytial groups. Carcinoma with medullary pattern presenting at the axillary 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 primarily or secondarily involving the breast is a differential diagnosis. Lymphomas present as a discohesive population of malignant lymphoid cells, devoid of neoplastic epithelial cells.

13.4.8 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 (Fig. 13.18a, b). The nuclear atypia is usually high grade.

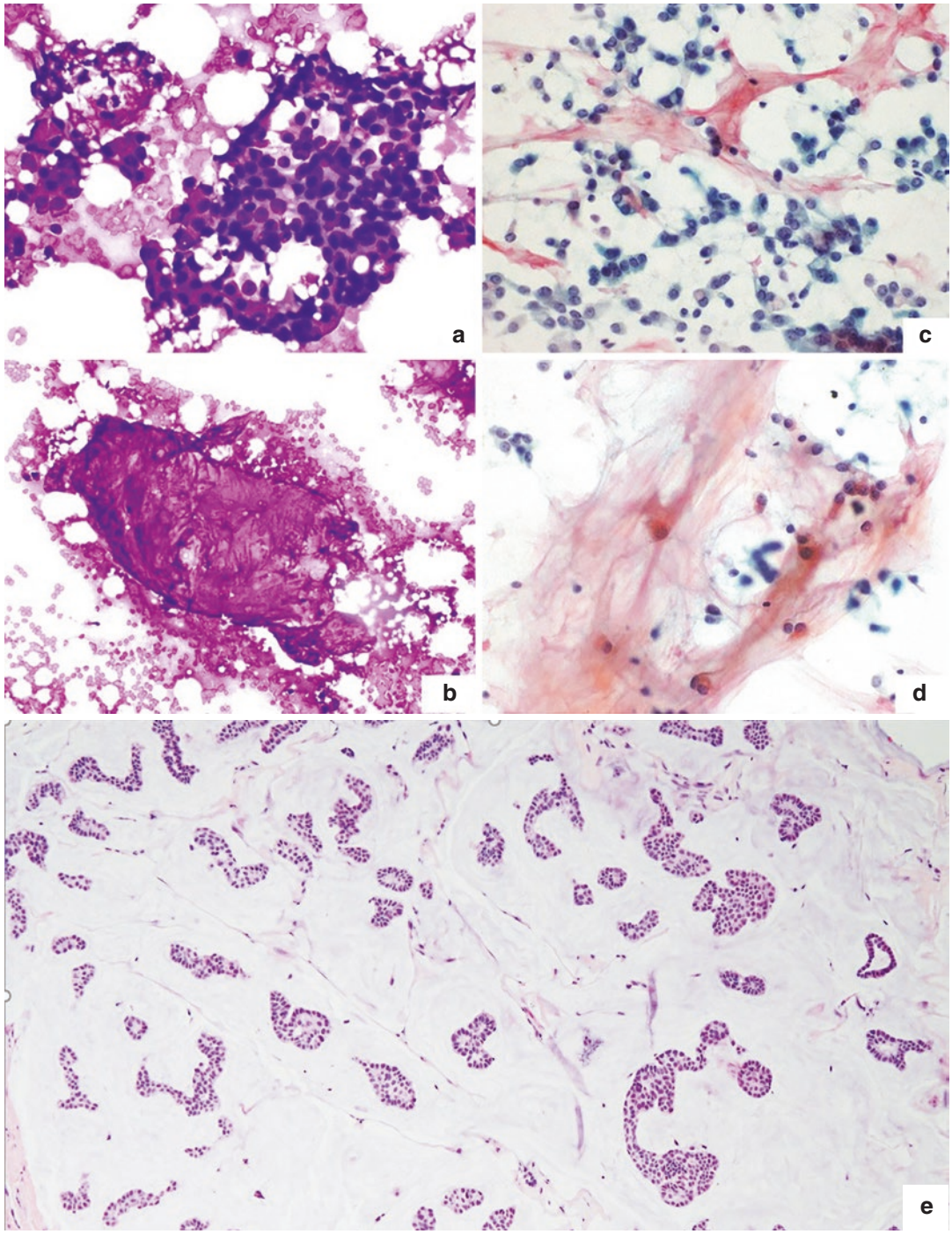

Fig. 13.15 (**a–b**) MGG stain showing clusters of mildly pleomorphic carcinoma cells and background magenta-colored staining mucin. **(c–d)** PAP stain showing mucin which is brownish gray in color, within which are floating carcinoma cells from a mucinous carcinoma. (**e**) H&E stain showing clusters of carcinoma cells floating in a pool of mucin

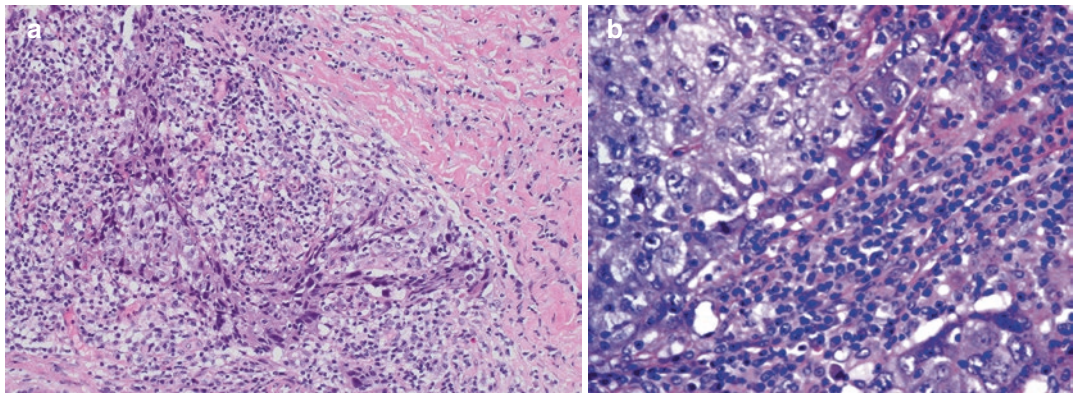

Fig. 13.16 (**a**, **b**) Carcinoma with medullary pattern/TILs-rich Invasive breast carcinoma. (**a**) Histology shows a well-defined margin, syncytial growth pattern, and prominent lymphocytic infiltrate (H&E stain). (**b**) High-power histology shows pleomorphic high-grade vesicular nuclei with prominent nucleoli, as well as numerous mitoses (H&E stain)

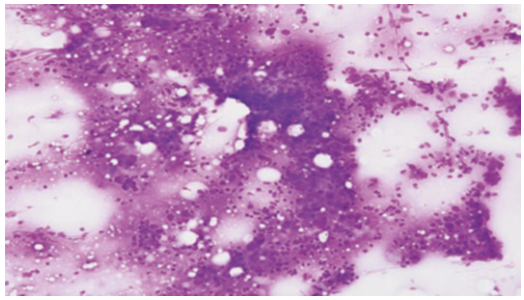

Fig. 13.17 Carcinoma with medullary pattern. Note large pleomorphic tumor cells in a background of lymphocytes (MGG stain)

Well-developed papillary fronds with fibrovascular cores are not seen. The smears tend to show micropapillary and rounded or irregularly shaped epithelial clusters which contain closely apposed cells, and may display molding in a "jigsaw" pattern. The external aspect of the epithelial fragments may show a mucinous blush (Field et al. 2020). 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.

13.4.9 Metaplastic Carcinoma

Metaplastic breast carcinomas (MBC) comprise a 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 (McCart Reed et al. 2019). 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 (Figs. 13.19 and 13.23). 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. 13.20). Occasionally, the sparse cellularity of liquid aspirates, a background of histiocytes and inflamma-

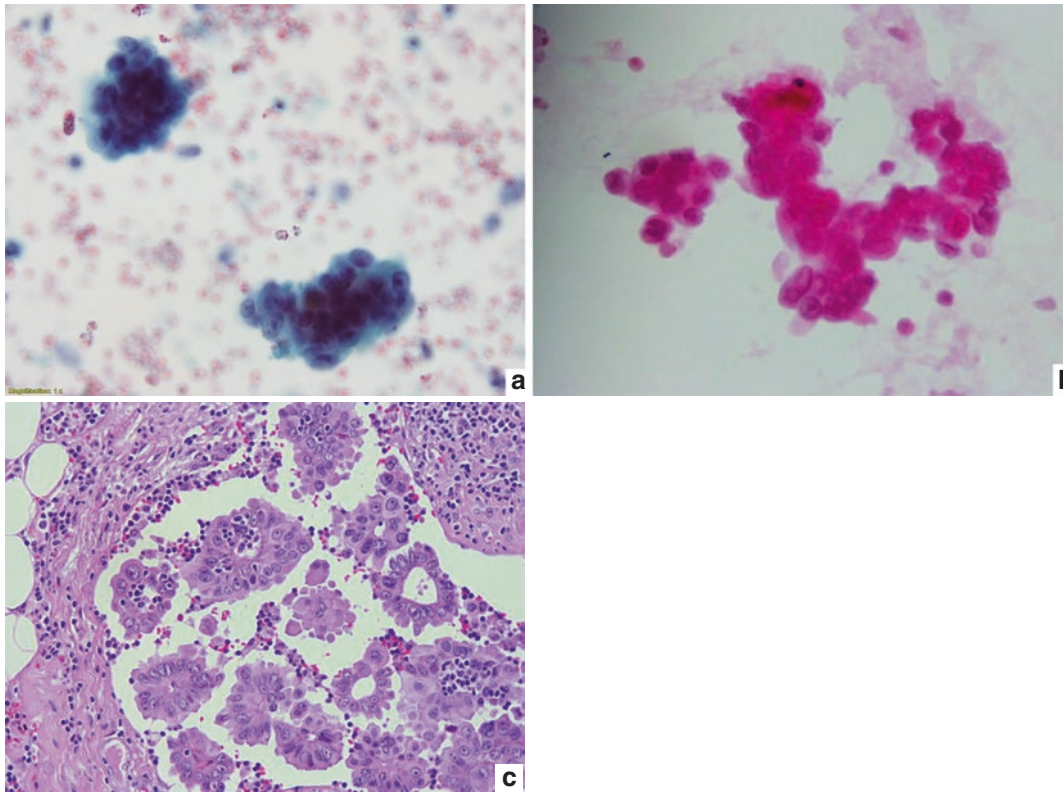

Fig. 13.18 Invasive micropapillary carcinoma. Tightly cohesive tumor cell clusters with a morular appearance (**a**) PAP and (**b**) H&E stain. (**c**) Invasive micropapillary carcinoma. Tumor cell clusters with central spaces residing within empty stromal spaces (HE stain)

tory cells, or the relatively monotonous appearance of ductal or spindle cells may cause problems in the diagnosis of malignancy (Fig. 13.21). In this situation, p63 is an extremely useful marker that can be used in cytological smears. p63 is positive in MBC and 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, 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. 13.22). Squamous metaplasia is the most common feature in metaplastic carcinomas, and a careful

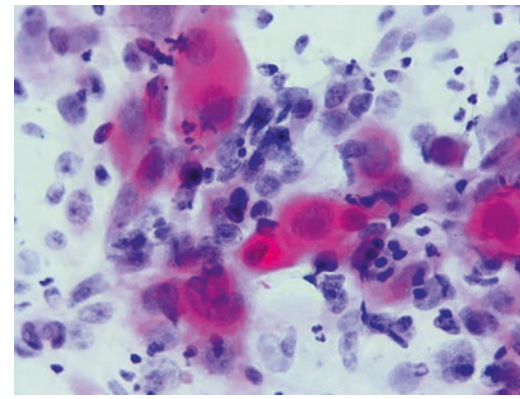

Fig. 13.19 Metaplastic breast carcinoma showing squamous cell differentiation (H&E stain)

scrutiny of all smears is necessary as squamous cells are often scarce. Sarcomas of the breast excluding malignant phyllodes tumors are

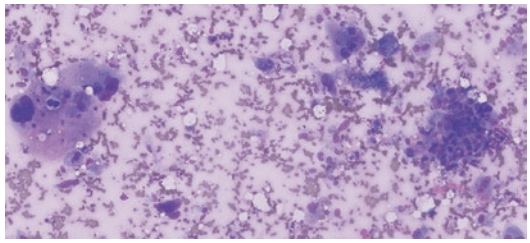

Fig. 13.20 Metaplastic breast carcinoma displaying malignant multinucleated giant cells (MGG stain)

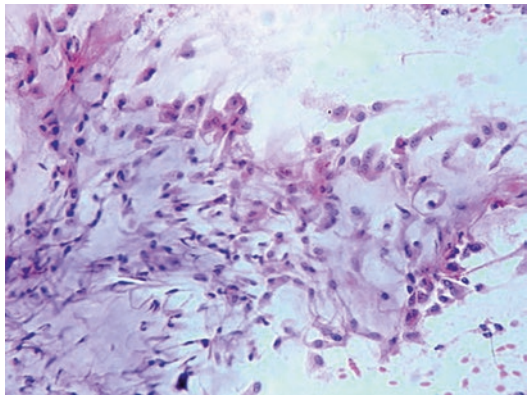

Fig. 13.21 Metaplastic breast carcinoma. Hypercellular smears with isolated spindle cells with bland appearance (H&E stain)

extremely uncommon. Therefore, in malignant smears with a predominance of neoplastic spindle cells, the possibility of metaplastic carcinoma should always 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 show bland spindle cells or groups of epithelial cells without significant

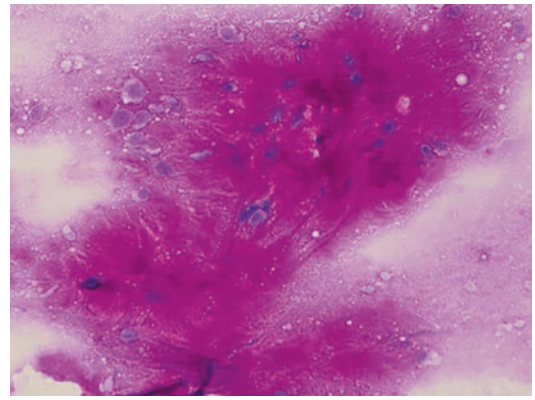

Fig. 13.22 Metaplastic breast carcinoma. Note dispersed malignant cells mixed with stroma resembling chondroid matrix (MGG stain)

atypia. Breast sarcomas are generally more pleomorphic and anaplastic, with absence of a neoplastic epithelial component. 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 (Fig 13.23c). 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.

13.4.10 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 and pleomorphic. The cytoplasm is abundant, finely granular, and amphophilic (Fig. 13.24). 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 malig-

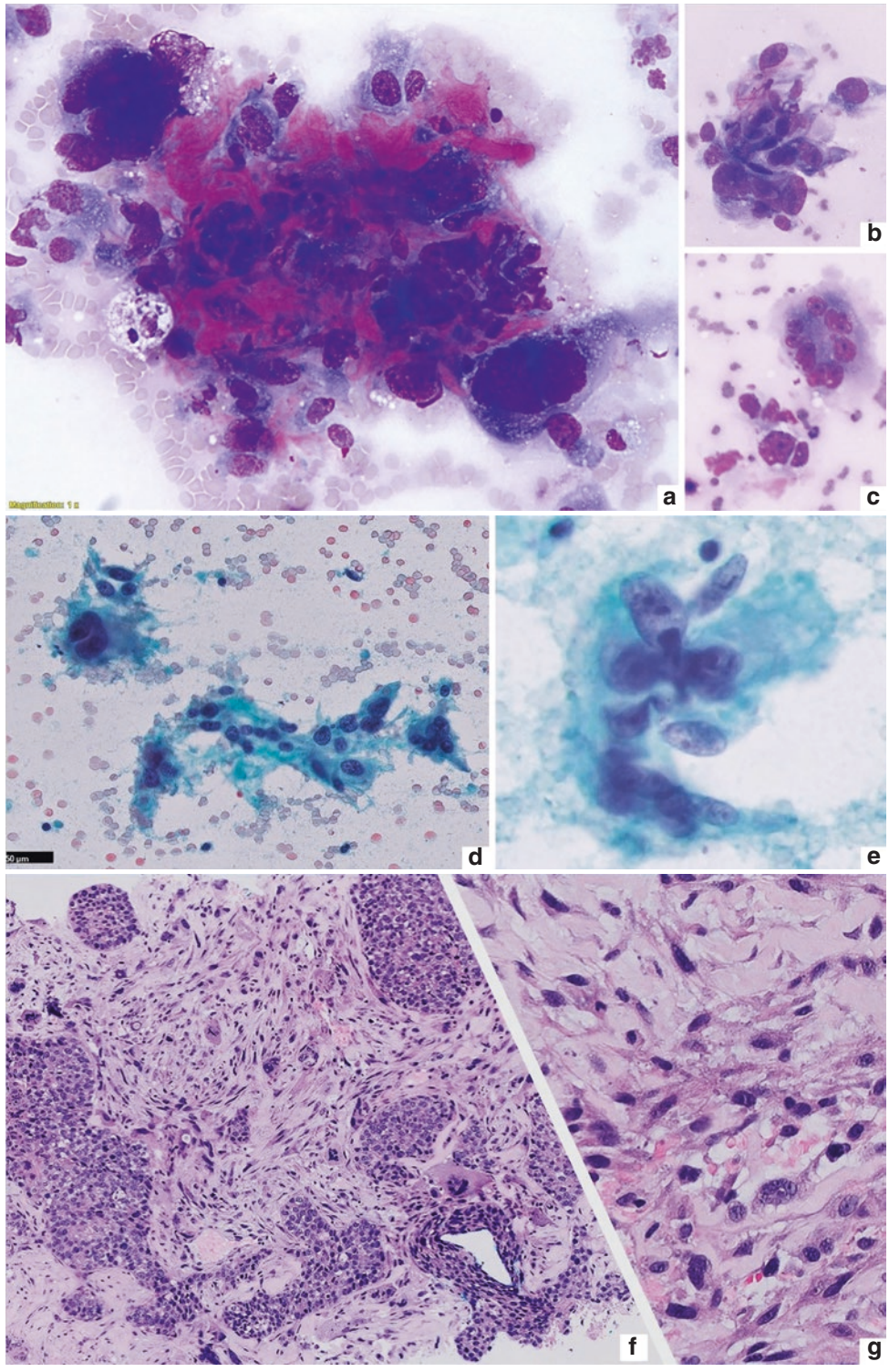

Fig. 13.23 (**a, b**) Metaplastic matrix producing carcinoma with large pleomorphic cells surrounded by magenta colored matrix (MGG); (**c**) Multinucleate tumor giant cell (MGG). (**d**, **e**) (PAP). Metaplastic carcinoma with large pleomorphic cells with spindle nuclei. (**f**) Metaplastic breast carcinoma H&E. Islands of pleomorphic cells surrounded by atypical spindle cells and multinucleate tumor giant cells with atypical mitoses (*black arrow*). (**g**) Atypical spindle cells with pleomorphic nuclei in the stroma surrounding the tumor islands

nancy when compared with benign apocrine lesions. Metaplastic apocrine epithelium can exhibit some nuclear atypia, especially in the context of fibrocystic change or inflammation but usually retains an ordered arrangement of nuclei with a low N:C ratio and without the coarse chromatin or large eosinophilic pleomorphic nucleoli of apocrine carcinoma. 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.

13.4.11 Papillary Carcinoma

Papillary carcinoma of the breast is a rare type 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 is non-papillary on histology follow-up. Fibrocystic change and fibroadenoma may closely simulate papilloma on cytology. The details have been discussed in Chap. 11. 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. 13.25, 13.26 and 13.27). The aspirates may be associated with blood. Presence of cell balls and absence of bipolar naked nuclei are two of the most distinctive findings favoring a malignant conclusion. As papillary-like fragments may be seen in hyperplastic epithelium from benign proliferative processes, with some degree of degenerative atypia in cystic lesions, discretion should be observed to avoid overcalling atypia within papillary-like fragments in the background of otherwise classic cyst contents and in the absence of radiological concern. Ancillary techniques can be useful in the distinction between 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

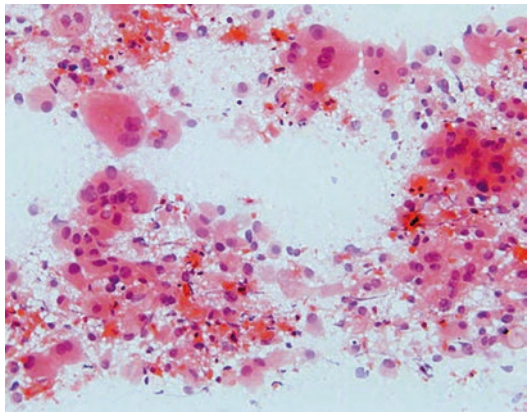

Fig. 13.24 Apocrine carcinoma. Pap-stained smear reveals abnormal cells with pleomorphic nuclei and copious cytoplasm, interspersed with degenerate necrotic cells with pyknotic nuclei

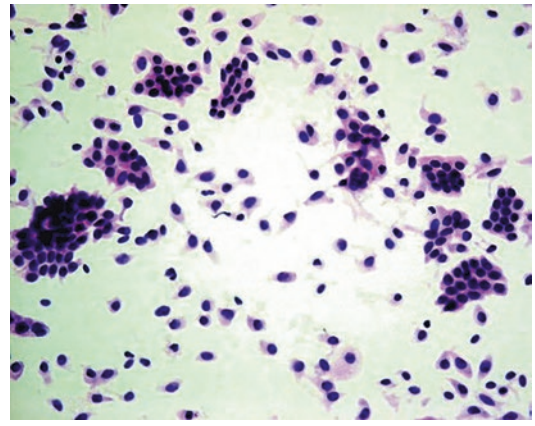

Fig. 13.25 Papillary carcinoma. Cytology shows papillae arranged in cell balls, isolated columnar cells, and absence of bipolar naked nuclei

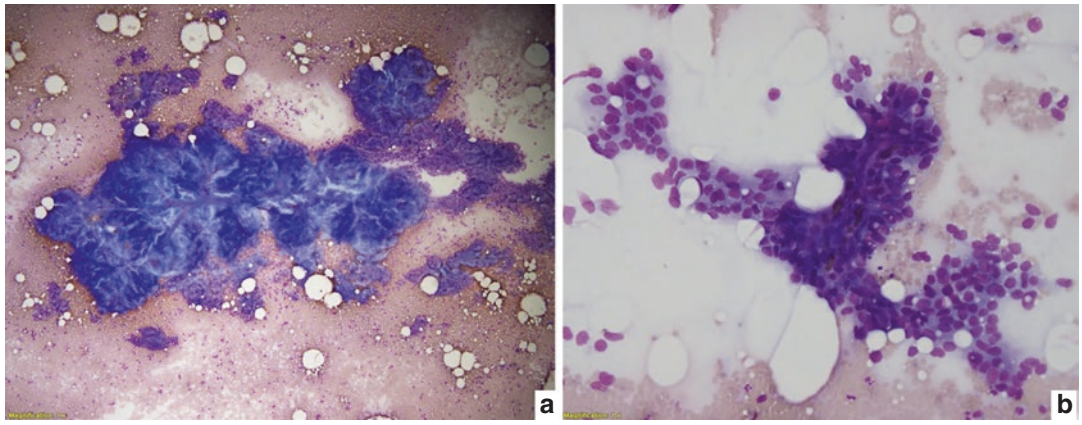

Fig. 13.26 Papillary Carcinoma. (**a**) MGG stain showing a papillary fragment with central fibrovascular core (**b**) MGG showing papillary-like aggregates composed of small monotonous epithelial cells from an encysted papillary carcinoma

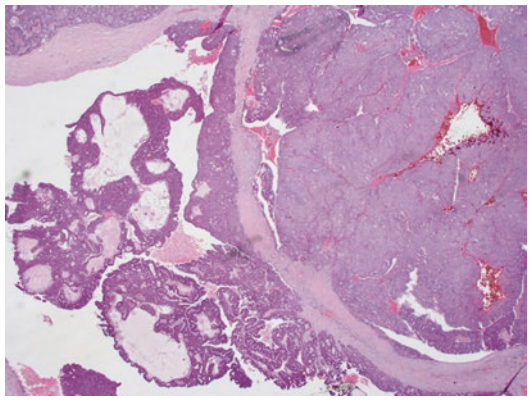

Fig. 13.27 H&E of an intracystic/encysted papillary carcinoma with a thickened fibrotic capsule containing a papillary tumor with fibrovascular cores

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

13.4.12 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 artifacts can be present.

13.4.13 Acinic Cell Carcinoma

This is a type 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 primarily from salivary glands. The smears of the well-differentiated cases show acinic cells with small and uniform nuclei, abundant and granular cytoplasm, and a 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. 13.28). In the breast, differentiation from tumors with similar histological appearances should be considered, 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 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.

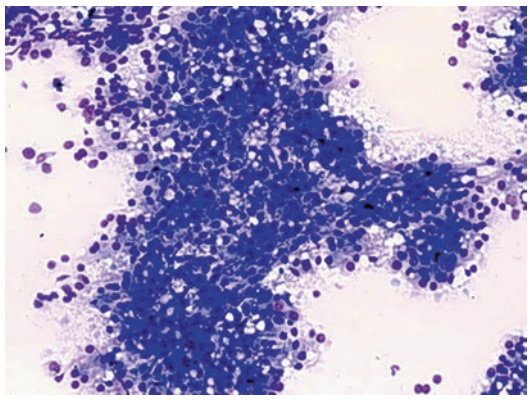

Fig. 13.28 Acinic cell carcinoma. Presence of vacuolated cells with moderate atypia can be seen (MGG stain)

13.4.14 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.

13.4.15 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 a single vacuole occupying most of the cytoplasm to numerous, that latter giving 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.

13.4.16 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 patterns. The most common appearance is of clusters of cohesive small uniform cells arranged around magenta-stained hyaline globules associated with tubular structures covered with uniform

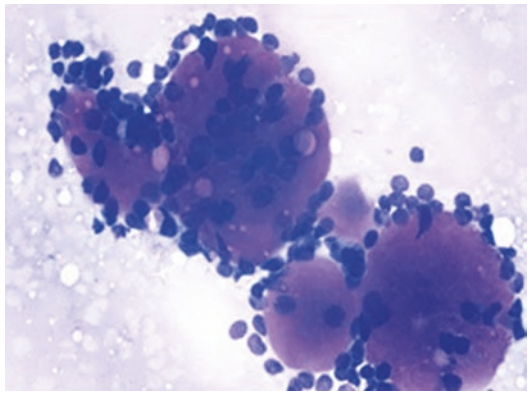

Fig. 13.29 Adenoid cystic carcinoma. Note the malignant cells surrounding the hyaline spheres

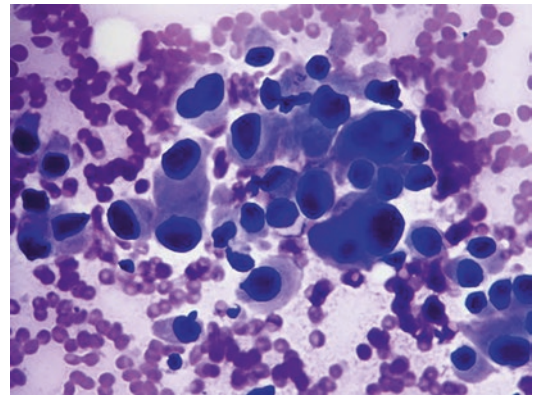

Fig. 13.30 Paget disease. Tumor cells are large, with abundant cytoplasm and large nuclei with prominent nucleoli

epithelial cells (Fig. 13.29). The individual cells are small and have round or ovoid nuclei which in smears are often naked, although a narrow rim of cytoplasm may be present. In less differentiated cases, there is a predominance of solid fragments of tumor cells.

13.4.17 Paget Disease

Paget 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. 13.30). The tumor cells are arranged individually and in clusters. The differential diagnoses include malignant melanoma (in general S100, HMB-45, and SOX10 positive) and squamous cell carcinoma. The malignant cells of Paget disease in general are positive for CK7, EMA, and HER2.

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Other Uncommon Lesions

14

Yunbi Ni, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

14.1 Primary Breast Lymphoma

14.1.1 Clinical Finding

Breast is an uncommon site for lymphoma. Primary breast lymphoma is a rare neoplasm accounting for 0.1–0.15% of breast malignancy (Domchek et al. 2002), and is defined as the presence of a primary breast lesion with no involvement in other extramammary sites. Indeed, it is less common than secondary lymphoma involving breast. The diagnosis of a primary breast lymphoma relies on the exclusion of lymphoma at other sites clinically and radiologically. Patients' age varies from 9 to 85 years old, with a median age of 58 years. Most patients present with a painless lump. Other symptoms and signs are local pain, local inflammation, palpable lymph nodes and incidental mammography finding (Jeanneret-Sozzi et al. 2008). Imaging findings are non-specific. Mammographically most lesions are oval-shaped, high-density masses, and the echo pattern is usually hypoechoic. Spiculated margin or calcification are uncommon. Diffuse large B cell lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), and follicular lymphoma accounts for the majority of primary breast lymphoma, although any subtype can occur. Breast is also a distinct site for Burkitt lymphoma and a specific site for breast implant-associated anaplastic large-cell lymphoma.

For high-grade lymphoma, FNAC is usually diagnostic of malignancy, and a specific diagnosis can be reached in conjunction with ancillary tests, including immunocytochemistry, flow cytometry and cytogenetic study. Although sometimes tissue biopsy is required for definite diagnosis and subtyping.

14.1.2 Cytologic Findings

14.1.2.1 Diffuse Large B Cell Lymphoma

Diffuse large B cell lymphoma is the most common subtype, accounting for 50% of primary breast

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lymphoma. Smears show discohesive large lymphoid cells in a background of small lymphocytes and histiocytes. Necrosis can be seen. The malignant cytologic features are prominent that usually a confident diagnosis of high-grade B cell lymphoma coupled with immunocytochemical staining is of no problem. One pitfall is the difficulty in the distinction of diffuse large B cell lymphoma from high-grade follicular lymphoma. A subsequent biopsy for confirmation and subtyping is usually required, although the treatment regimens for these two high-grade B cell lymphomas are similar.

14.1.2.2 Burkitt Lymphoma

Burkitt lymphoma is a highly aggressive non-Hodgkin B cell lymphoma, with three distinct clinical forms being recognized: endemic, sporadic, and immunodeficiency-associated. Primary Burkitt lymphoma of the breast usually affects female of puberty, pregnancy, or lactation. It is often bilateral and massive, with a rapid spread and a poor prognosis. The cytologic preparation is hypercellular. The neoplastic cells are typically of medium-size and they show a diffuse monotonous growth pattern. The nuclei are rounded with fine clumped and dispersed chromatin, with basophilic medium sized, paracentrally located nucleoli. The cytoplasm is deeply basophilic and usually contains lipid vacuoles. Numerous apoptotic bodies and scattered tangible body macrophages can be observed in the background. Ancillary study, including immunocytochemical staining and molecular study, is essential in the diagnosis. Immunocytochemically, tumor cells are of B cell lineage and are positive for germinal center markers, such as CD10 and BCL-6. Proliferative index assessed by Ki 67 is almost 100%. MYC protein is expressed in most cells. EBER is positive in EBV-positive cases. c-MYC translocation is characteristic but not specific.

14.1.2.3 Breast Implant-Associated Anaplastic Large Cell Lymphoma

Breast implant-associated anaplastic large cell lymphoma is a very rare implant-associated CD30-positive ALK-negative T-cell lymphoma. On average, it develops 8–10 years after the initial implants have been placed (Brody et al. 2015). The diagnosis on cytology is based on the identification of lymphoma cells from peri-implant seroma.

On cytology, the lymphoma cells are large and pleomorphic. Hallmark cells with horseshoe-shaped or kidney-shaped nuclei can be identified in most cases. Occasionally wreath-like kidney-shaped nuclei or Reed-Sternberg-like cells may be observed. However, large atypical cells can also be identified in reactive peri-implant seroma, which makes the evaluation more challenging. These confusing large atypical cells include histiocytes, immunoblasts, and pseudosynovial cells. Immunohistochemical staining is mandatory for diagnosis. Tumor cells are CD30 positive and T-cell markers are variably expressed. One also needs to note that immunoblasts in reactive peri-implant seroma are CD30 positive as well, but they can be confirmed by CD20 positivity. Breast implant-associated anaplastic large cell lymphoma is immunocytochemically negative for ALK1 and does not carry the ALK translocation. In doubtful cases, T-cell receptor gene rearrangement analysis may be performed.

For low-grade lymphoma, e.g., low-grade follicular lymphoma and MALT lymphoma, the utility of FNAC in establishing a solid diagnosis is limited. Cytomorphologically, they both show overlapping features with benign reactive process, being composed of mixed population of small atypical cells with irregular nuclei and rare large cells. In addition, the high content of reactive cells may impair detection of a monoclonal B cell population. Core needle biopsy or excisional biopsy is usually required even if the immunophenotype on cytology specimen is conclusive of a neoplastic process.

14.2 Metastases to the Breast

14.2.1 Clinical Finding

Metastases to the breast are malignancies in the breasts originating from an extramammary site. They are rare, representing 0.2–1.3% of breast

malignancies. They usually develop in the fifth or sixth decade, but pediatric patients have also been reported (DeLair et al. 2013). Females are more frequently involved than males. More often the patients have a prior history of malignant tumor, but metastases to the breast may also be the first sign of an extramammary breast primary malignancy. The most common origin of epithelial metastases is contralateral breast, and other commonly reported sites of origin include lung, skin, stomach, and ovary. Breast masses in childhood and adolescent need special attention. Majority of breast masses in this age group are benign, and a malignant breast neoplasm is more likely to be metastasis. About one-third of patients with breast metastases previously reported are younger than 20 years old (Shukla et al. 2005). Metastatic hematolymphoid malignancy and rhabdomyosarcoma are most common in this age group.

14.2.2 Cytologic Findings

The cytologic features vary among different types of metastatic tumors. Some show relatively characteristic features: typical nuclear features and melanin pigments in melanoma (Fig. 14.1), fine salt and pepper chromatin pattern in neuroendocrine tumor (especially well differentiated neuroendocrine tumor and small cell carcinoma) (Fig. 14.2), isolated islands of centrifugally orientated columnar cells in colorectal adenocarcinoma, and papillary architecture and psammoma bodies in serous carcinoma, etc. However, at least one-third of epithelial metastases to breast lack specific morphologic features and are high grade, making the diagnosis difficult (Fig. 14.3). A detailed history, clinical correlation, and immunocytochemistry helps in establishing an accurate diagnosis, thus avoiding unnecessary surgery and ensuring

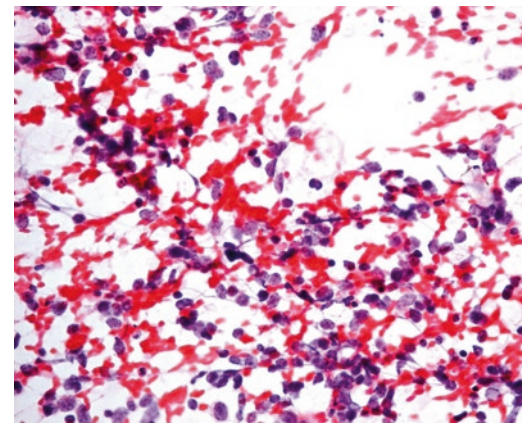

Fig. 14.2 Metastatic small cell carcinoma of lung primary. Smear shows small groups of ovoid medium-sized tumor cells with scanty cytoplasm and fine dispersed chromatin and indistinct nucleoli

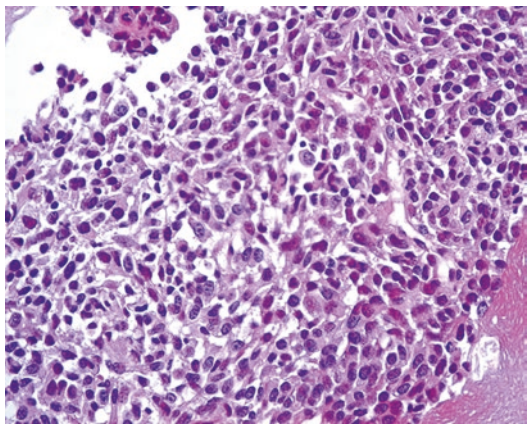

Fig. 14.1 Metastatic melanoma in breast. Cell block preparation shows tumor cells of high nuclear pleomorphism, hyperchromatic nuclei, and distinct nucleoli. However, melanin pigments in this case are inconspicuous

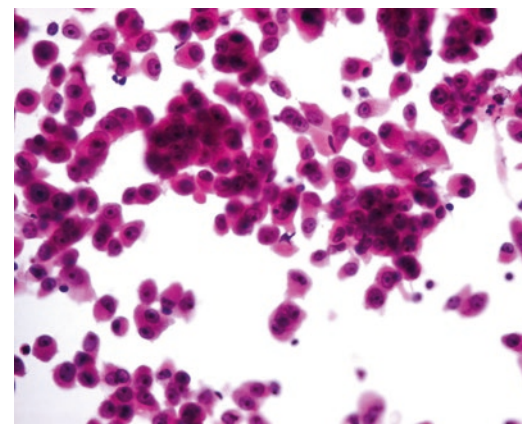

Fig. 14.3 Metastatic adenocarcinoma of lung primary. Cytospin preparation shows scattered and clusters of tumor cells with focal vague glandular formation. The tumor cells show pleomorphic nuclei, distinct nucleoli and moderate amount of cytoplasm

appropriate treatment. An immunocytochemical staining panel for breast carcinoma should be used, including ER, PR, GATA3, Mammaglobin, GADFP-15, and HER2, as well as specific makers for any suspected non breast primary, such as TTF1 for lung adenocarcinoma (Fig. 14.4), CDX2 and CK20 for colorectal carcinoma, WT1 and PAX8 for ovarian serous carcinoma, and HMB45 and melan A for melanoma (Fig. 14.5).

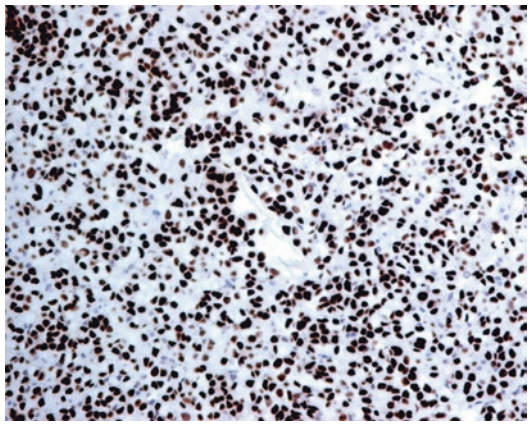

Fig. 14.4 Immunocytochemical staining shows positivity of TTF-1 in metastatic adenocarcinoma of lung primary

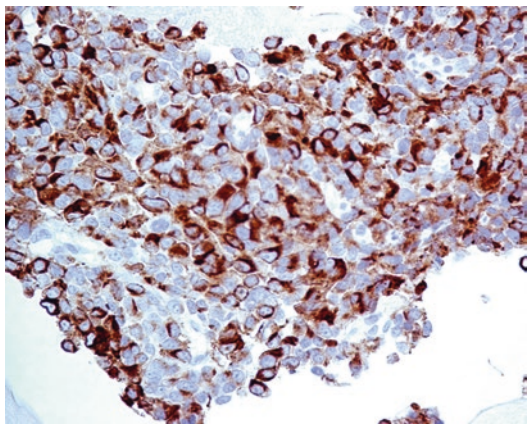

Fig. 14.5 Immunocytochemical staining shows positivity of HMB-45 in metastatic melanoma

14.3 Summary

- Primary breast lymphoma and metastases to the breast are uncommon. Patients usually present with non-specific breast masses. FNAC may be the first diagnostic procedure in some of these diseases, and it is useful in establishing an accurate diagnosis in most of these cases, thus avoiding unnecessary surgery and ensuring appropriate treatment.
- Diffuse large B cell lymphoma is the most common subtype of primary breast lymphoma. The malignant morphologic features are typically apparent that usually a confident morphological diagnosis of lymphoma coupled with immunohistochemical staining is of no problem. A subsequent biopsy for confirmation and subclassification is sometimes required.
- Primary Burkitt lymphoma shows a diffuse medium-size monotonous growth pattern. The nuclei are round with fine clumped and dispersed chromatin. Ancillary study, including immunohistochemical staining and molecular study are essential in the diagnosis.
- Breast implant-associated anaplastic large cell lymphoma is a very rare implant-associated CD30-positive ALK-negative T-cell lymphoma. The diagnosis on cytology is based on the identification of hallmark cells with horseshoe-shaped or kidney-shaped nuclei from peri-implant seroma.
- For low-grade follicular lymphoma and MALT lymphoma, the utility of FNAC in establishing a solid diagnosis is limited. Tissue biopsy is usually required even if the immunophenotype on cytology specimen is conclusive of a neoplastic process.
- Metastases to the breast are malignancies in the breasts originating from an extramammary site. The cytologic features vary on different types of metastatic tumors. A detailed history,

clinical correlation, and immunocytochemistry helps in establishing an accurate diagnosis, thus avoiding unnecessary surgery and ensuring appropriate treatment.

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Assessment of Axillary Nodes

15

Jill Su Lin Wong, Gay Hui Ho, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

15.1 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.

15.2 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. 15.1). 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.

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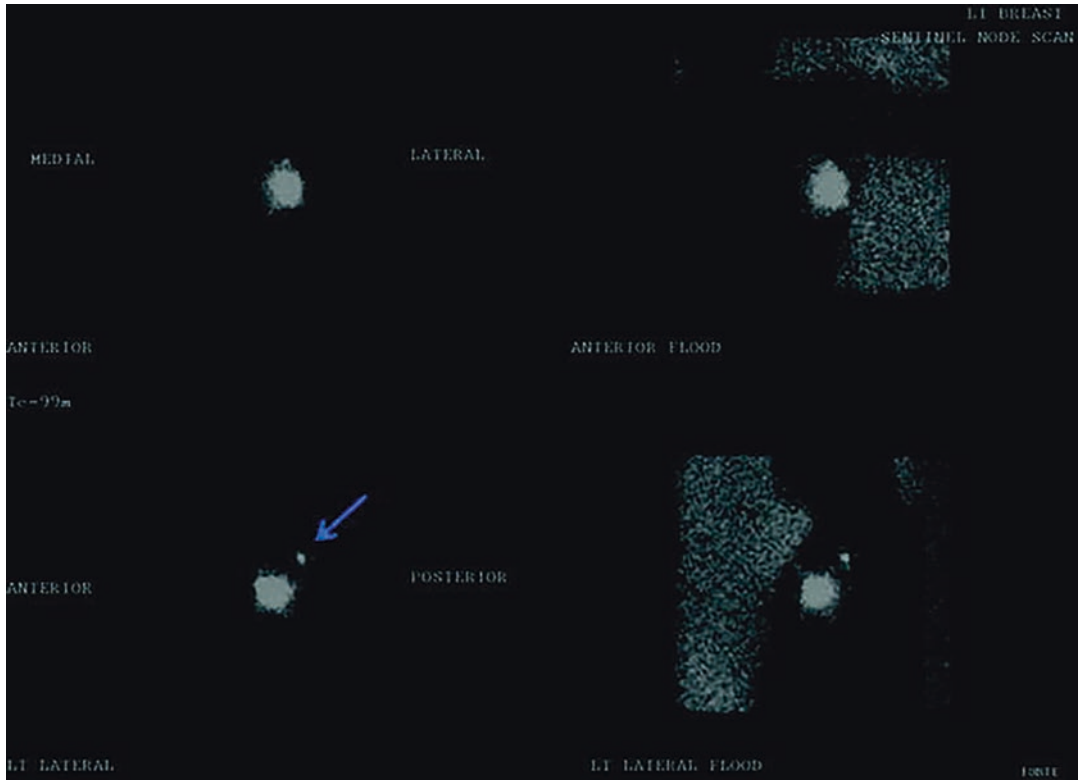

Fig. 15.1 A 68-year-old lady presented with a screen-detected invasive carcinoma. Mammography and US of the nodes were unremarkable. Prior to surgery, Tc99m sulfur colloid was injected into the dermis overlying the tumor. The larger area of tracer accumulation occurs over the tumor; the *arrow* points to the sentinel node superior and posterior to the tumor. Histologic examination of the sentinel nodes was negative for malignancy

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

15.3 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. 15.2). Ultrasound also demonstrates normal nodes which typically have a smooth thin hypoechoic cortex and an echogenic central

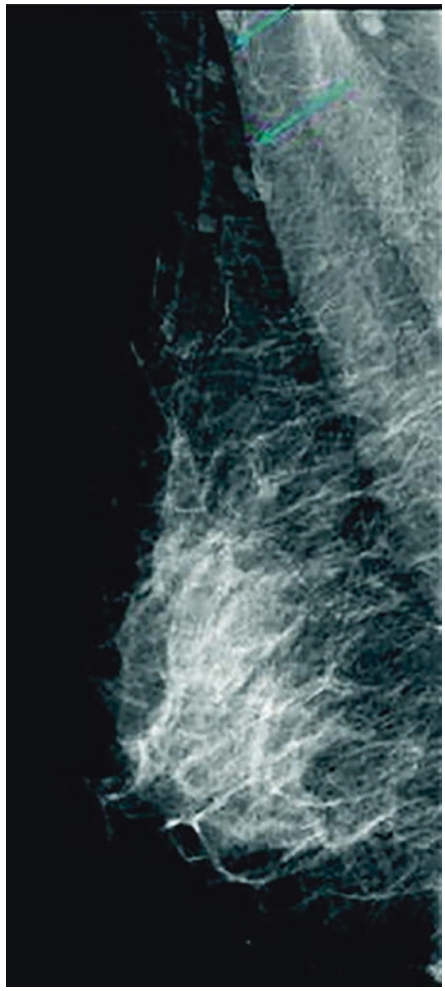

Fig. 15.2 A normal right mammogram shows normal small axillary nodes (*arrows*). They are small, bean-shaped opacities with low-density fat within a hilar concavity

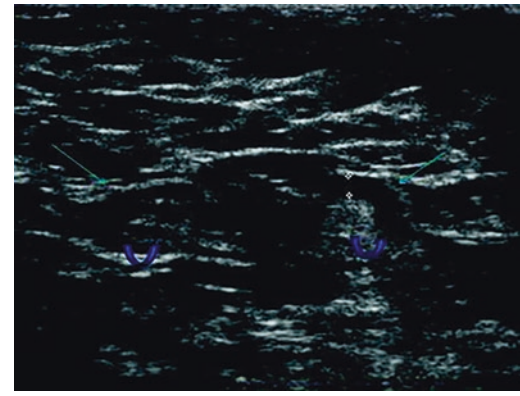

Fig. 15.3 Two axillary nodes (*blue arrows*) with normal appearances. The *calipers* outline the cortical thickness which is under 2 mm. The central echogenic fatty hilus is preserved in both nodes (*curved arrows*)

hilus (Fig. 15.3). 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. 15.4a). Their characteristic bean shape is lost, and they appear round with or without focal nodularity (Figs. 15.4b, c and 15.5a–c).

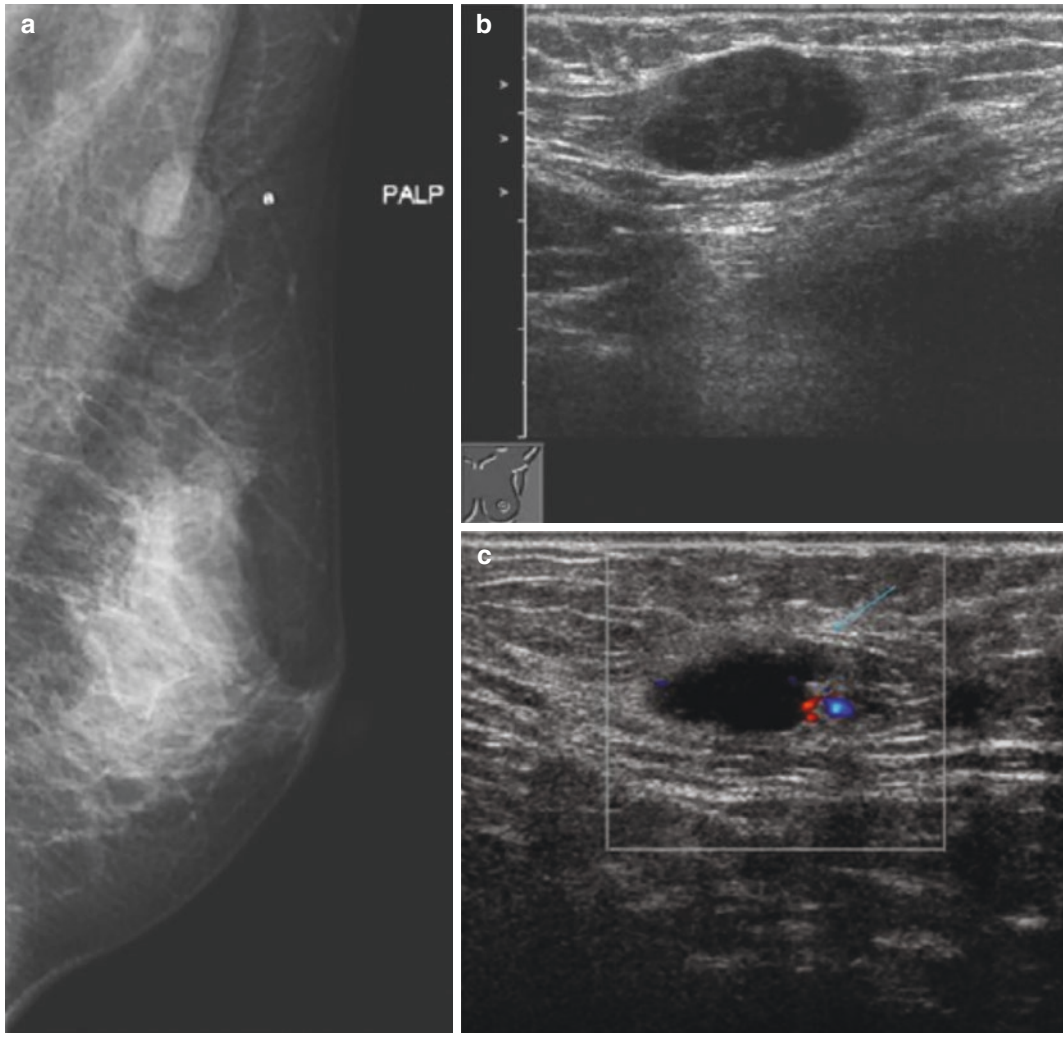

Fig. 15.4 (**a**) Left mammogram in a patient with newly diagnosed invasive carcinoma which cannot be seen on mammography. A skin marker has been placed over a palpable enlarged dense lymph node. The normal fatty hilus has been completely replaced in this node which has lost its normal bean shape and 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

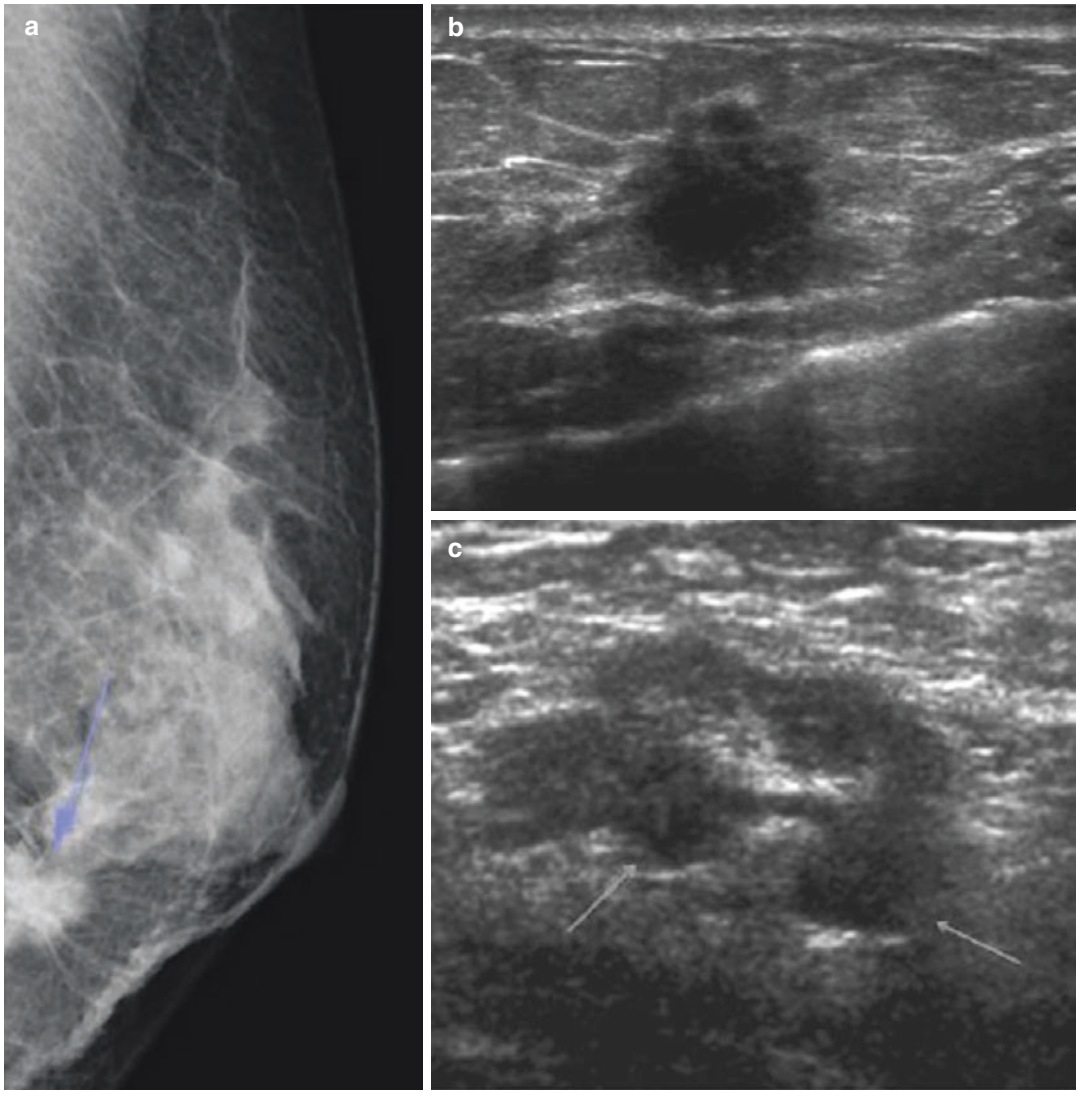

Fig. 15.5 (**a**) A 51-year-old patient presented with a screen-detected spiculated mass in the inferior aspect of the left breast (*arrow*). No enlarged nodes are seen on mammography. (**b**) Sonographic appearance of the breast mass, which revealed invasive carcinoma on core biopsy. (**c**) Ultrasound 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 extranodal extension

15.4 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.

15.5 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. 15.6). 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. 15.7 and 15.8).

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.

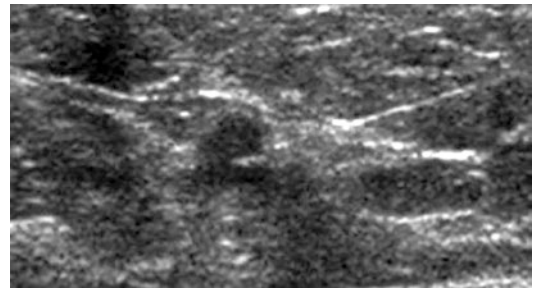

Fig. 15.6 A 58-year-old patient with a newly diagnosed invasive cancer. There is a non-palpable but sonographically suspicious node. This underwent FNA which yielded malignant cells. The needle is kept as horizontal as possible, that is, parallel to the transducer. This allows the length of the needle as well as its tip to be visualized in the same plane

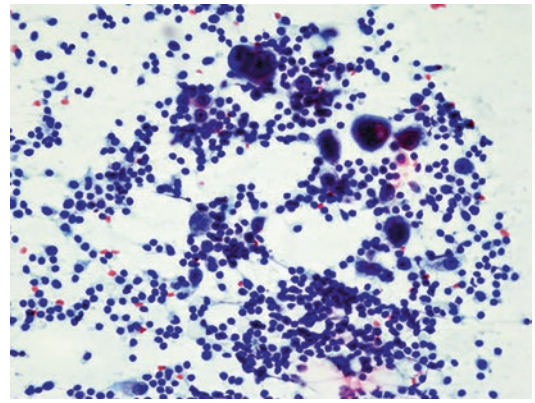

Fig. 15.7 Cytologic smear of a sentinel lymph node that is positive for metastatic carcinoma cells

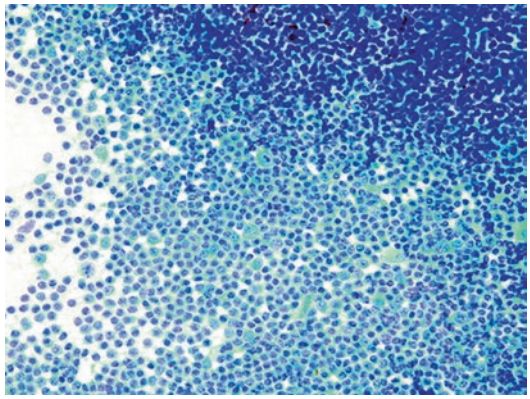

Fig. 15.8 Imprint smear (PAP) of a sentinel lymph node shows predominantly medium-sized lymphocytes with a few interspersed larger activated lymphoid cells

15.6 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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16 Special Ancillary Techniques: Immunohistochemistry

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16.1 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. 16.1). In most of the reported series, there was established correlation of Ki-67 with mitotic count, grade, and S phase fraction (Dalquen et al. 1997; Ostrowski et al. 2001; Pelosi et al. 1994). 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

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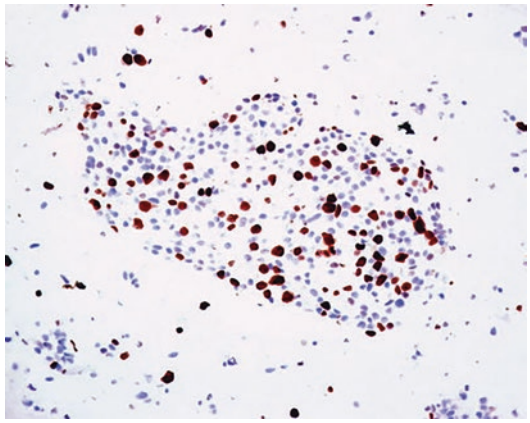

Fig. 16.1 Cell block of invasive breast carcinoma. Ki-67 expression was seen as nuclear staining in some of the tumor cells

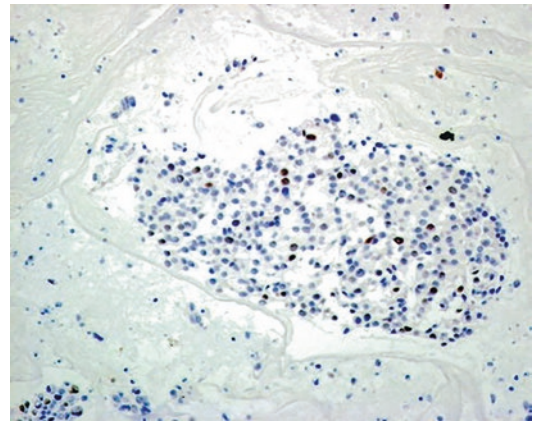

Fig. 16.2 Cell block of invasive breast carcinoma. Scattered p53 expression was seen as nuclear staining in some of the tumor cells

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 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 (Colecchia et al. 1995; Koutselini et al. 1991; Pelosi et al. 1994; Stephenson et al. 1994). Thus, p53 may potentially be useful in the characterization of cytologic preparations into either benign or malignant categories (Fig. 16.2). 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 (Colecchia et al. 1995; Koutselini et al. 1991).

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

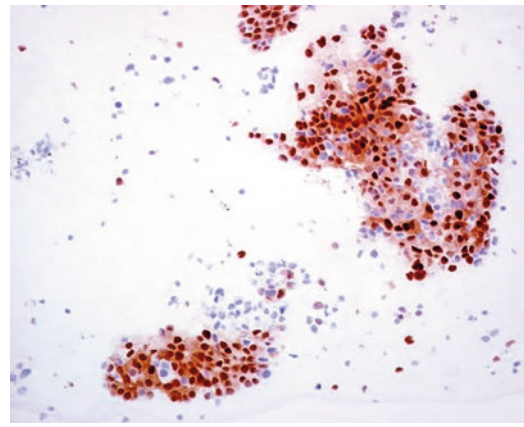

Fig. 16.3 Cell block of invasive breast carcinoma. Cyclin D1 was seen as nuclear staining in some of the tumor cells

and 40% for benign aspirates (Fig. 16.3). 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 single 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, and the staining results have to be corroborated with other diagnostic considerations (Fig. 16.4).

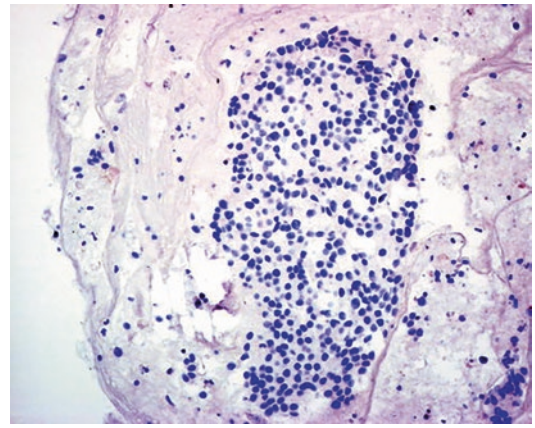

Fig. 16.4 Cell block of invasive breast carcinoma, showing absence of p63 nuclear staining in all of the tumor cells

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. 16.5).

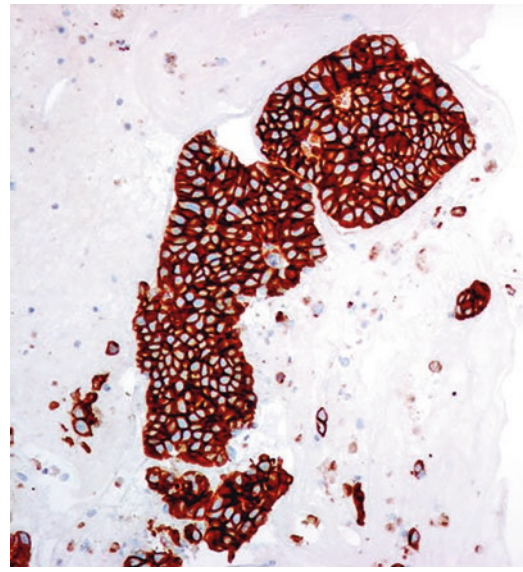

Fig. 16.5 Cell block of invasive breast carcinoma. E-cadherin expression as membrane staining was detected in most of the tumor cells

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).

It has also been demonstrated that assessment of DNA ploidy and S-phase fraction can be accurately done on FNAC materials, with good cor-

relation with the Ki67 index in the histologic excision tumor sample (Panwar et al. 2021). This is particularly so as FNAC samples are most amenable to flow cytometry analysis, and such approach will be most appropriate for patients undergoing neoadjuvant chemotherapy or in late metastatic cases or other situations where histologic biopsy / exision mataerials may not be available for tumor grade assessment (Gazic et al. 2008). More recently FNAC materials from breast cancers have been tested for a panel of 10 methylated markers assayed by Quantitative Multiplex-Methylation-Specific PCR (QM-MSP), and it was shown that the results were highly reliable. This may provide one more useful modality in diagnosing malignancy using FNAC, particularly in situations where histologic biopsy material may not be available (Downs et al. 2019).

16.2 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).

16.3 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

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 (Cano et al. 2003; Tafjord et al. 2002; Zoppi et al. 2002). 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. 16.6 and 16.7).

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 (Corkill and Katz 1994; Jorda et al. 1994; Moriki et al. 2004; Nizzoli et al. 2000, 2003) or cell blocks (Klorin and Keren 2003; Shabaik et al. 2011; 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 (Briffod et al. 2000) (Fig. 16.8). 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

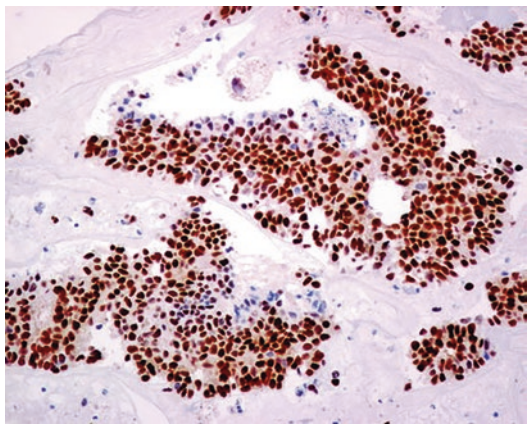

Fig. 16.6 Cell block of invasive breast carcinoma. ER was expressed as nuclear staining in most of the tumor cells

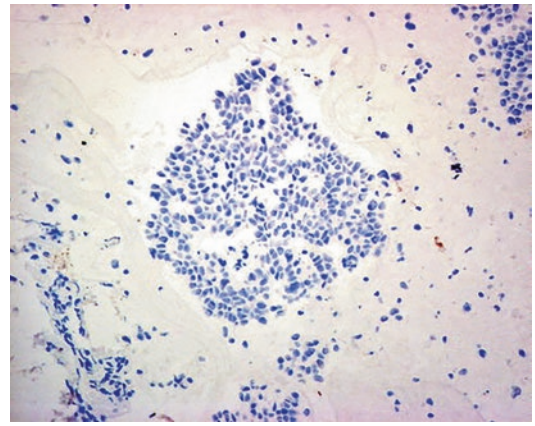

Fig. 16.7 Cell block of invasive breast carcinoma. PR was negative in the same case

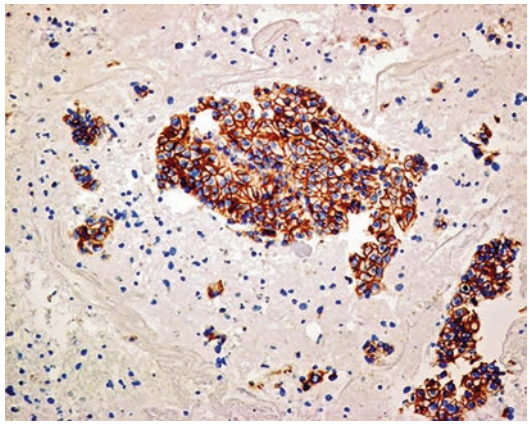

Fig. 16.8 Cell block of invasive breast carcinoma. Complete and strong membrane staining of HER2 was seen in most of the tumor cells

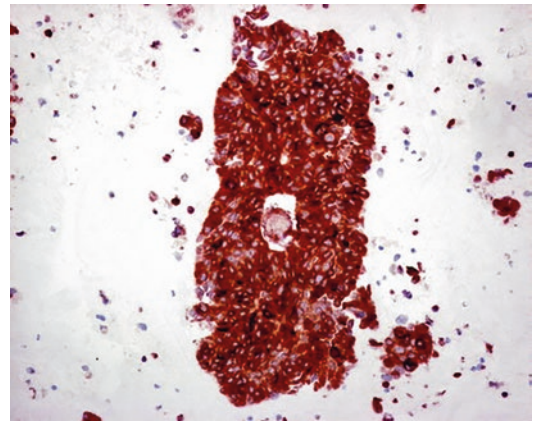

Fig. 16.9 Cell block of invasive breast carcinoma. Bcl2 was highly expressed in the cytoplasm of the tumor cells

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).

16.4 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. 16.9).

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17 Molecular Studies and Artificial Intelligence

Daniel Gomes Pinto, Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

17.1 Introduction

The application of molecular techniques has changed the practice of cytopathology. The use of molecular techniques on cytological samples is now widely accepted. In the context of breast cancer, the study of molecular markers has proven useful for the detection of emerging, resistant clones, and adequate selection of targeted therapy. Cytological samples have several advantages over tissue biopsies: they are easier to obtain, allow for the evaluation of sample adequacy at the time of the procedure, and provide well-preserved DNA and RNA for molecular testing, oftentimes better preserved than in tissue samples (Schmitt et al. 2008; Schmitt and Barroca 2011, 2012). Cytological samples are usually obtained through minimally invasive procedures, such as FNABs and effusion draining, enabling repeated testing required to monitor emerging resistances. Furthermore, sample preservation is often better than formalin-fixed, paraffin-embedded tissue, particulary when liquid fixatives are used.

The majority of molecular techniques can be applied to cytological samples with results comparable to tissue biopsies (Beca and Schmitt 2019; Pinto and Schmitt 2020). These include polymerase chain reaction (PCR), in situ PCR, in situ hybridization (ISH), microarrays and sequencing, including next-generation sequencing (NGS) (Di Lorito and Schmitt 2011; Pisapia et al. 2021). PCR methods work well in cytological samples, allowing for the detection of gross chromosomal alterations, such as deletions, translocations, and point mutations in individual genes. RT-PCR uses cDNA as a template for primer exon sequences, flanking the breakpoints of translocations. Current applications include diagnosis, most frequently in the context of hematolymphoid malignancies through the detection of clonal gene rearrangements, or for theranostic purposes in the context of solid tumors through the detection of gene mutations or rearrangements. NGS has likewise been shown to yield actionable results in cytological samples

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in high agreement with tissue biopsies. Preparing samples for NGS is a complex laboratory procedure involving the creation of libraries, PCR, sequencing and data analysis using modern software. NGS allows massive parallel sequencing of multiple genes (multiplexing), enabling the detection of several mutations, amplifications, and rearrangements in a single sample. Both of these techniques can be performed directly with freshly collected material either from FNAB, in liquid-based cytology samples, in cells scraped from FNAB slides or those preserved in cell blocks (using the same methods of extraction as in tissue). If cell blocks are used, however, it is worth noting that the same artifacts present in formaldehyde-fixed, paraffin-embedded tissue may be present, such as C > T single base substitutions.

When samples are collected fresh for the specific purpose of molecular testing, the needle should be washed in ethanol, methanol, or culture mediums like RPMI. The amount and quality of DNA obtained by FNAB is usually adequate, with between 50 and 100 cells in a given sample being sufficient to yield good results in PCR. This is possible because FNAB samples are less likely to have contamination by stroma, inflammatory cells or local structures, unlike tissue biopsies which has been shown in studies on molecular profiling using cDNA microarrays. Cellularity requirements may be higher for NGS, depending on the assay, but success has been reported with 1% or lower tumor cellularity. Of note, this technique has also been shown to extract actionable results not only from the aforementioned sample types, but also supernatants obtained from centrifuging cytological specimens. However, whether these are reliable enough to be used in the clinical practice remains an open area of research.

As previously mentioned, ISH can also be applied to cytological samples, using either fluorescent or chromogenic markers. These techniques enable the detection of numerical or structural aberrations in chromosomes, including deletions, insertions, translocations, or amplifications, most commonly for HER2 in carcinomas of the breast. These methods can reliably be performed directly on smears, air-dried or ethanol fixed, cell blocks and sediments prepared from liquids. Sediments usually are monolayered and are ideal for ISH techniques due to a lack of cellular overlapping.

Since cytological samples are usually limited in quantity, if an adequate cellularity is present and the technique is available, NGS is probably the most useful method.

Care must be taken, however, when performing any molecular tests in these samples due to differences in fixation methods, except for cell blocks. Protocols developed for paraffin-embeded tissue should not be applied directly to cytological samples. In fact, all methods should be validated via paired cytological-histological samples prior to clinical use (Pisapia et al. 2021; Schmitt and Barroca 2012; Schmitt 2011; Pang et al. 2011). In the setting of metastatic tumors, cytological samples may be the only material available for testing. Validation should be done in advance to ensure the samples are used to their maximum potential. Adequate controls should also be employed, using cytological material with known genomic alterations. These may be obtained from scrapings of surgical specimens and divided using cell transfer techniques (Pinto and Schmitt 2022; Ferguson et al. 2013). The importance of adequate controls cannot be understated, in fact, more than 50% of published papers on immunocytochemistry applied on cytological material did not mention which controls are used, and many laboratories use tissue sections as controls, and this may not be optimal for cytological stainings (Colasacco et al. 2011).

As discussed, cytological samples usually preserve the integrity of DNA and RNA which enable molecular testing. Liquid-based cytology samples, particularly if the fixative used is alcohol based, would suitably preserve cell samples with sufficient DNA and RNA quality for several molecular analyses such as NGS, PCR and RFLP (Longatto-Filho et al. 2009; Pisapia et al. 2021; Wholschlaeger et al. 2009). Air-dried and alcohol-fixated smears even after 10 years have also been shown to preserve DNA integrity of the materials, making the application of these methods viable in archival slides, and is particularly

useful for cases with only few slides and new materials cannot be obtained (Killian et al. 2010). However, whether this long term material preservation applies to RNA is still uncertain. As discussed, RNA analysis can be performed on liquid-based cytology samples, paraffin-embedded tissue or cell blocks. Given the fickle nature of these molecules, a better source are often fresh or frozen samples. Procedures for effectively freezing and storing human neoplastic and normal tissues have been developed and are currently in use in tumor banks across the world, encompassing specimen handling from collection to shipping, including storage, retrieval, and tracking. Research has shown that cells obtained from FNACs of breast cancer surgical specimens are an effective tissue-sparing method for cell collection and banking, also enabling the preservation of high-quality RNA (Eloy et al. 2009). This can be very useful in alternative to traditional tumor banking as it allows the entire tumor to be submitted for histological processing, which is particularly useful in cases of small neoplasms and increasingly common due to effective screening programs. For this purpose, cytopathologists play an essential role, both in collecting and in determining sample adequacy.

17.2 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. This diversity seems to arise from distinct genetic, epigenetic, and transcriptomic alterations in different neoplasms (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 proposed a molecular classification for breast cancer. These initial gene expression microarray-based studies led to the identification of at least five molecular subtypes of breast cancer: luminal A, luminal B, normal breast-like, HER2, and basal-like (Perou et al. 2000; Reis-Filho and Pusztai 2011). These seminal studies were based on the analysis of a limited number of samples and with somewhat different definitions for the various molecular groups, and have been modified and refined over time. The normal breast-like subgroup is now mostly regarded as an artifact of the pioneering microarray studies. Other subgroups have been proposed, particularly inside the triple-negative tumors. Clinically, tumors may be classified into those subtypes using a number of validated molecular assays. These have proven useful in predicting distant and late recurrences and in selecting patients for adjuvant and neoadjuvant chemotherapy. Most frequently, the molecular subtype is still estimated using immunohistochemistry for ER, PR, and HER2, with molecular tests being reserved for challenging cases, such as luminal B / HER2 negative tumors with few or no positive lymph nodes (Tsang and Tse 2020).

17.3 Molecular Studies on FNAB from Primary Breast Tumors

The current clinical management of breast cancer is still mostly based on traditional prognostic and predictive factors—histology, clinical parameters, and the well-studied ER, PR, HER2, and Ki67, all of which are associated with prognosis and treatment outcome. However, this classification system is not perfect as it fails to take into account tumor heterogeneity and subtle molecular alterations. The use of high-throughput molecular technologies enabled a better understanding of such complexity, by allowing the classification of breast tumors into biologically and clinically distinct groups based on their gene expression patterns. From a practical standpoint, breast cancer patients fall into three categories: the hormone receptor positive cases that are

treated with hormone receptor targeted therapies with or without additional chemotherapy, the HER2 positive cases that are treated with HER2-directed therapy, or those that are negative for hormone receptors and HER2, treated solely with chemotherapy or now with immunotherapy (Wilking et al. 2011), using anti-PD-L1 molecules. Thus, the expression of ER remains a prognostic and predictive factor of the utmost importance in breast cancer patients. Those with tumors expressing ER have longer disease-free interval and overall survival than patients with tumors lacking ER expression. In terms of the molecular classification, ER positive tumors encompass two subtypes: luminal A and luminal B. Luminal B tumors show co-expression of HER2 or a high-proliferative index. This translates into a possibly more aggressive behavior, and some of these patients will benefit from chemotherapy.

Samples obtained from breast cancer patients using FNAC are suitable for most molecular and theranostic studies in clinical practice, including immunocytochemistry. Cytological material can be used to reliably assess ER and PR status, as well as HER2, in high agreement with tissue. These studies can be performed in all kinds of cytological materials, however, attention must be paid to utilize proper antigen retrieval methods, and adequately and carefully validate protocols and techniques in-house. Overall, the best results are obtained from liquid-based cytology smears and cell blocks. In the latter, Ki67 may also be assessed and quantified (Pinto and Schmitt 2022).

When equivocal results are encountered in immunohistochemical studies for HER2, in situ hybridization (ISH) ought to be performed, either based on fluorescent dyes or chromogens (bright field). These methods are the gold standard methods for detecting HER2 amplifications and may be performed on FNAB samples in excellent correlation with tissue.

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 few therapeutical options (Badve et al. 2011). Evaluation of PD-L1 with immunohistochemistry on breast cytology speciments is, unfortunately, pending for validation. However, based on the experience from other organ systems, especially when the assay requires PD-L1 evaluation in inflammatory cells, the results are poorly reproducible on cytological samples.

Although studies on other organ systems have shown that most molecular studies may be performed on cytology samples, as long as protocols are adequately validated, the aforementioned molecular assays for subtyping still have not been approved for use in cytological samples.

From the practical standpoint, FNACs of the breast remain extremely useful in the context of breast cancer for theranostic testing, either in the context of a primary tumor or a local recurrence. In fact, local recurrences must now be re-tested for molecular markers, and in this context cytology should be the sampling method of choice, given the minimally invasive nature of the procedure.

17.4 Molecular Studies on FNAB from Metastatic Breast Tumors

The same holds true for metastatic breast cancer. When it is first diagnosed, clinical guidelines state that the neoplasm should be re-sampled to confirm the diagnosis, usually suspected by a combination of clinical and imaging findings, and for re-testing of theranostic markers. This is particularly relevant in this setting since metastatic clones may not have been the prevalent clones in the primary tumor, due to tumor heterogeneity and selection from therapy. Therefore, their underlying biology is frequently different and this is usually detected as a switch in expression of hormonal receptors between primary tumors and metastases, which may occur in a significant percentage of tumors (Grinda et al. 2021). Hence, ER, PR, HER2, and Ki67 should always be re-tested. Additionally, other therapeutically relevant markers should also be evaluated. These include BRCA1/2 mutations, which should also be assessed in HER2-negative tumors, and

PIK3CA mutations, which should be assessed in ER/PR positive, HER2 negative neoplasms. As discussed, FNAC samples are adequate for the outlined immunohistochemical studies and, based on current literature, should show excellent results when used for molecular testing of BRCA and PIK3CA (Pisapia et al. 2021). In the context of a metastatic recurrence, with the adequate clinical history, a diagnosis of breast cancer should be straightforward using cytology, even for pathologists less experienced with this technique. Given that it is a less invasive method when compared to tissue biopsies, and can be performed using imaging guidance with good yields, there is no reason not to resort to this technique in adequate clinical context.

17.5 Molecular Studies on FNAB Material Used for Frozen Tissue Banking of Breast Cancer

Methods for the classification of the breast cancer molecular subtype currently used in clinical practice have been validated in formaldehyde-fixed, paraffin-embedded tissue. Despite known artifacts, this is the most common sample type available. However, recent studies have shown that fresh-frozen samples provides better quality RNA for testing, with clinically relevant discordances in a minority of cases, particularly in luminal tumors (Lien et al. 2021). As such, some institutions still rely on high-quality, freshly-frozen human neoplastic and normal tissues stored in tumor banks for testing. However, these tumor banks are expensive to maintain and require specialized equipment and validated procedures for collection, storage, retrieval, shipping, and tracking of samples. Although molecular assays have yet to be validated and recommended by clinical guidelines for use in cytological specimens; it is logical to assume that since the cell samples may be obtained whole and fresh, the samples so obtained would not only be adequate, but also superior to tissue samples as they have not been subjected to fixation. In small tumors, obtaining tissue for tumor banks may be difficult, given the need to study the entirety of the tumor histologically. In this context, fine needle sampling can provide a tissue-sparing alternative. After collection, the needle should 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 extracted successfully from these samples (Eloy et al. 2009).

17.6 Artificial Intelligence in Cytology

Artificial intelligence (AI) encompasses several technologies which allow machines to perform tasks usually perceived to require human intelligence. When applied to pathology, it usually refers to methods of computer vision and algorithms of machine learning, which can be trained on large datasets and make accurate predictions based on the knowledge obtained.

Recently, one of these algorithms has been approved for clinical use by the FDA in the context of prostate cancer histology. Other medical fields, such as radiology, make more frequent use of the technology. Pathology in general, however, has been hindered by a lack of adequate training datasets—that is, the algorithms require digital images to be trained on, and pathology is still mostly an analog discipline.

Unlike radiology, there is no sensor to scan cells and tissues directly into as digital image, which makes digitization always an extra step in processing. Furthermore, the requirement of higher resolution and colored images results in larger storage requirements and a need for accurate color display. Although digital pathology has been available in one form or another since the 1990s, it has only been in the last decade that digital pathology became viable, through several technological advances in storage, displays, and the scanners (Farahani and Pantanowitz 2015). In fact, several societies dedicated to digital pathology have created guidelines for the purpose of the implementation of digital workflows. However, penetration of adoption is still unclear (Fraggetta et al. 2021).

Overall. cytology is behind Anatomic Pathology in general. This is because tissue sections are mostly two-dimensional, but FNAC smears are three dimensional. Two technical solutions have been put forward so far: Z-stacking and deep focusing. In Z-stacking, the slide is scanned at multiple depths around a point of focus, which enables fine focusing similar to in the microscope. Deep focus takes several scans at different depths and then combines these images into a single digitized slide in which everything is in focus. Both require higher scanning times compared to histology, since the slide has to be scanned more than once, and Z-stacking demands much higher storage, given that a single slide can represent several images. Deep focus could be the happy middle ground, but is very dissimilar to what pathologists are used to.

Further compounding this issue is a lack of standardization of scanning protocols, including what magnification to scan at and how to use Z-stacking and deep focus, a lack of clinical guidelines and little support from scanner manufacturers (Alrafiah 2022; Capitanio et al. 2018).

Nevertheless, several AI algortihms have been developed for use and tested in cytology samples.

Before tackling that subject, however, some background is warranted. These algorithms aim to, at some level, understand what a certain image represents and provide a relevant output. This is computer vision, the field of research concerning itself with the methodologies which enable computers to extract meaning from visual input. This area saw several developments arise during the mid-to-late twentieth century and the early 2000s. Its most significant advances came only in the late 2000s and 2010s, however, with the advent of the so-called "deep learning revolution," which enabled the extraordinary capabilities we now associate with artificial intelligence, from the accurate identification of objects and people in photographs to self-driving cars (Sejnowski 2018).

Most of AI research during the twentieth century was based on a logic and knowledge approach, which attempted to translate human reasoning to code. This led to good results in some fields, but not others. For example, these algortihms famously solved the game of chess, but Go, which has a number of possible board positions several orders of magnitude larger than chess, was forever out of reach. The "deep learning revolution" begun in the early 2000s, enabled by increasingly fast computers and a wealth of data, brought on by larger and more accessible storage, and the internet.

This new paradigm in AI research, which we are currently experiencing, uses several techniques, the details of which are outside the scope of this text. However, so-called artificial neural networks, frequently used in computer vision, are illustrative and worth special mention. These networks are layered algorithms, loosely inspired on the strutucture and functioning of the human brain. They are composed by several nodes, called artificial neurons, connected to each other through synapses. Artificial neurons are organized in input, output, and hidden layers. Input neurons receive data as a real number and pass it along to the next layer of neurons, the hidden layers, where calculations are actually performed. Results are then passed along to the output layer and read by the user. These calculations consist on the application of a non-linear function to the sum of a specific inputs of the neuron, using specific weights and biases, which can be adjusted as part of the training process. Whereas input and output layers are always one neuron "deep," there can be many hidden layers. When there is a large number of these, a network is considered deep. It is this "deepness" which enables these algorithms to learn complex and abstract representations of reality.

For an image to be analyzed by an AI algorithm, several steps have to take place. First is pre-processing, through which images are standardized, using blurring, rotation, color correction, and other techniques. Then features, information about the shape of the object are extracted. These features can then be fed to an artificial neural network classification algorithm, as described above. Of course, these algorithms are useless until the aforementioned weights and biases are adjusted, and this is done through training on a specific dataset. This dataset may be labeled or unlabelled, resulting in supervised or unsupervised learning, respectively.

In gynecological cytology, automatic screening has been available for more than two decades. However, these are not based on deep learning algorithms, traditionally, and recent studies have shown several shortcomings in the classical methods employed. Some vendors have recently started rolling out commercial products taking advantage of deep learning. This could prove a fruitful endeavor, given that several publications have reported high accuracies in distinguishing between negative and positive cytology slides, as well as in the accurate classification according to the Bethesda system.

Successful implementations have also been reported on several organ systems, from thyroid and the pancreas to urine and effusions. Most publications focus on the detection of malignancy, and show that algorithms used so far have very high specificities for this purpose, with high, but slightly lower, sensitivities. Diagnostic values are tendentially lower when considering the accurate classification into all categories of a given system.

On the particular case of breast cytology, algorithms have been able to distinguish between benign and malignant smears with a high sensitivity (above 80%) and a specificity of 100%. They have been shown to be able to differentiate ductal and lobular carcinomas, and between fibroadenomas and carcinomas (Alrafiah 2022; Landau and Pantanowitz 2019).

Other than in the context of gynecological cytology, none of these solutions have so far entered into clinical practice. In this setting and in histology, however, several algorithms are already approved for diagnostic use, both in Europe and the USA. It is reasonable to expect that as experience with these technologies increases, as digitization becomes more common and as advantages are better characterized and understood, that the number of approved algorithms and users will increase. Cytology is hindered by the fact that the slides are harder to digitize, but one could argue that it is perhaps the field most likely to benefit from these techniques – classification systems have well-defined criteria and a very finite number of categories, the very problem that these deep learning algorithms are primed for. Furthermore, one of the most important jobs of a cytopathologist is telling apart a benign from a malignant specimen. Even if a given algorithm fails at a correct classification, if it can identify malignancy with sensitivities close to cytopathologists, human observation could be reserved for specimens signaled by the machine, preserving important time and effort.

As a last note, machine learning algorithms also enable objective and reproducible quantification measurements, namely of immunohistochemical markers. Some of these are already approved for diagnostic use in histology and in clinical practice in some centers. If applied to cytology, they could serve to alleviate concerns some users might have with the technique and bring breast FNACs into a wider audience. Looking forward this may even trigger mass adoption, particularly if these are incorporated into clinical guidelines for selecting therapy.

In conclusion, AI is a recent and very active field of research in many areas of medicine. These algorithms perform tasks previously reserved to humans, oftentimes, with a high success rate. In a clinical setting, they can already be used in histology and gynecological cytology. In the breast, new developments are expectable in the coming years as digitization sees wider adoption and vendors come to support the technology.

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18 Comparison of Aspiration and Core Needle Biopsy

Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

18.1 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 they do not requrie 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, though the use of FNAC has diminished significantly over the years in many institutions in preference for core biopsy.

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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. During the FNAC process, 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 make 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 imaging guidance using a gauge 11 core needle, with larger bore needles also being used. It allows for the removal of multiple tissue samples by vacuum aspiration and

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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.

18.2 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 for the procedure is also longer. 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.

For 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 relatively 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 is less amenable for 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 for cytologic assessment. 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.

18.3 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 such lesions require pathologic diagnosis. The efficacy of 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 (Boerner et al. 1999; Ibrahim et al. 2001). 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 hypocellular, 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 non-palpable breast lesions over needle core biopsies (Lacambra et al. 2011).

18.4 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 lesions 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 benign lesions were reported as atypia on FNAC (Tran et al. 2010). So far no specific 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 cytologic assessor. 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 understanding of the molecular mechanisms. It is now established 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 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 cases 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 of 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.

18.5 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 differs 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 node 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 (Klijanienko et al. 2004; Mckee et al. 2001; Sauer et al. 2006). Among these features, infiltration into fibrous tissue fragments 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 (Mckee et al. 2001; Sauer et al. 2006). 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 (number of sampled cores). 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.

18.6 Papillary Lesions

Papillary lesions of the breast represent a highly problematic area for accurate cytologic diagnosis. The cytologic details 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 (which encompasses a spectrum of tumor types including in situ and invasive), papillomas can also be complicated by superimposed florid epi-

thelial 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). Notwithstanding the generally low accuracy of FNAC in diagnosing papillary lesions, the question is whether it is 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, with core biopsy diagnosis of a papillary breast lesion being categorized as a lesion of uncertain malignant potential warranting clinical-radiological-pathological discussion for subsequent management (Deb and Tan 2022). 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 distinction of benign from 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.

18.7 Fibroepithelial Lesions

Fibroepithelial lesions refer commonly to fibroadenomas and phyllodes tumors. Fibroadenomas are benign, whereas phyllodes tumors may behave in a variable fashion, from a totally benign clinical course, to a propensity for local recurrences, and even giving rise to rare distant metastases in the malignant form. Complete excision with adequate margins is considered the optimal treatment for phyllodes tumors, but there is no similar margin requirement for fibroadenomas. The WHO Classification of Breast Tumors (2019) recommends favoring a diagnosis of fibroadenoma or applying the term "benign fibroepithelial lesion" when not all characteristic features of benign phyllodes tumors are seen, 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 epithe-

lial 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; Lerwill et al. 2022). 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.

18.8 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 efficacies 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 and deferred to excisional histology. 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

19

Gary Tse, Puay-Hoon Tan, and Fernando Schmitt

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 are 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.

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Our increased understanding of the underlying molecular mechanisms of breast carcinogenesis has led to the widespread use of molecular classification in breast cancers, and this is now standard of care in the classification of breast cancers, in addition and supplementary to the traditional histologic classification. The routine clinical use of molecular classification entails and immunohistochemical assessment of hormone receptors, HER2, other basal cytokeratins and proliferation marker (Ki67). Knowledge of the "molecular status" of the tumor confers advantage in the management, particularly in the choice of therapy (including personalized targeted therapy). Breast cytology also possesses the ability for allowing these immunohistochemical tests to be done on the cytology materials, thus obviating a more aggressive, traumatic, needle core biopsy that requires more time and even larger instrument (mammotome). This is particularly relevant in the metastatic setting, where a cytologic aspirate provides materials for optimal testings of the tumor. 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 PR 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. 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.

The International Academy of Cytology had pioneered a unified approach in the cytologic diagnosis of cytologic specimens in different organs systems. In the breast the Yokohama system has been proposed (Field et al. 2020). This classification has proven to be easy to use, and showed high concordance among different users (Marabi et al. 2021). With a unified diagnostic approach and criteria, the Yokohama system will gain wide acceptance and facilitate a common language among all cytopathologists.

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 FNAC 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 progression 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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