
Breast Cancer: Pathology, Cytology, and Core Needle Biopsy Methods for Diagnosis

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Abstract

Successful cancer treatment relies on a combination of clinical examinations, imaging studies, and pathologic evaluations. Pathologic diagnosis should not be bypassed even when health care resources are very limited and clinical/radiologic findings are very suggestive of breast cancer. A timely and accurate pathologic diagnosis can be achieved by the use of appropriate tissue sampling techniques, optimal tissue processing, and competent interpretation of pathologic findings. Accurate preoperative pathologic diagnosis confirms the malignant nature of the lesion and documents baseline expression of prognostic and predictive biomarkers, thus enabling clinicians to make optimal therapeutic strategies such as planning the extent of the surgery. Methods of obtaining tissue samples and the cytohistological techniques and histopathological features of the spectrum of breast cancer pathology are discussed in detail.

Introduction

Breast cancer is the most common cancer in women in Western countries. Over the past decade, the incidence in many developing countries has been increasing at a more rapid rate than in developed countries, and breast cancer in these countries is often associated with poorer survival [1]. Of the breast cancer deaths around the world

in 2002, more than 50% occurred in countries with limited resources [2]. This is largely due to late presentation of the disease, limited resources for the diagnosis, and treatment in these countries.

To improve breast cancer outcomes in these countries, it is important to increase breast cancer awareness and accurately recognize breast cancer, especially at an early stage, because early diagnosis is lifesaving and cost-effective and requires less aggressive therapy.

In 2005, the Breast Health Global Initiative stratified levels of resources in countries with limited resources (from lowest to highest) into basic, limited, enhanced, and maximal [3]. In these countries, the economic realities appear to

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force physicians to support a paradigm shift away from sophisticated, expensive, and invasive modes of investigation in favor of cheaper, readily available, minimally invasive, yet reliable methods [4].

The commonly used sampling methods for preoperative pathologic diagnosis include fine-needle aspiration (FNA), core needle biopsy (CNB), and open surgical biopsy. Compared with open surgical biopsy, FNA and CNB are less invasive biopsy techniques in which the needle can be guided by palpation or imaging. For palpable lesions, the needle can be placed under the guidance of palpation; for nonpalpable mammographic abnormalities, the needle may be placed with image guidance.

FNA involves using a very small, thin needle to extract cells or fluid from the abnormal area. Since gaining its momentum in Europe in the 1950s, FNA has been adopted worldwide and has been proven to be safe, simple, fast, and cost-effective if properly performed [5–7] and if a quality cytopathology service is available. With FNA, it is possible to make “one-stop” diagnosis at outpatient clinics. A number of studies have demonstrated the reliability of FNA in the evaluation of breast lesions, with relatively high sensitivity and specificity, especially when combined with image guidance.

However, the success of FNA requires not only an excellent aspirator to obtain satisfactory aspirates but also breast cytopathologic expertise in interpreting the breast aspirates [8–10]. Recently, the popularity of FNA has been decreasing, which can be attributed in part to a shortage of training opportunities and paucity of well-trained or experienced cytopathologists at individual centers, leading to more diagnostic errors and higher rates of inadequate samples. Consequently, clinicians have become reluctant to rely on this technique for preoperative diagnosis. In addition, FNA has intrinsic limitations, largely because of the lack of reliable histologic architecture. Diagnostic difficulty may be encountered in certain lesions, even by experienced cytopathologists.

Nevertheless, FNA is considered suitable for low-resource settings particularly at the basic

level. Currently, this technique continues to be used as first-line of pathologic investigation for breast lesions in some developed countries and in many developing countries [11–20].

Because of the limitations of FNA, many institutes in the developed countries have replaced FNA by other tissue-based diagnostic procedures such as CNB [8, 21–24]. Image-guided CNB encompasses ultrasound-guided CNB (USG-CNB) and mammographically guided vacuum-assisted core biopsy (VACB). Core tissue allows for histologic examination with which pathologists are familiar and thus is suitable in centers where experienced cytopathologists are not available. CNB provides a more definitive pathologic diagnosis than FNA for some lesions and also facilitates biomarker studies, similar to other surgical specimens. Although CNB is more invasive, time-consuming, and more expensive than FNA, it is less invasive and relatively more cost-effective than open surgical biopsy and is easily performed with minimal scarring [25].

Indications

The decision as to which method needs to be used depends on availability of technique and expertise, the lesion’s nature (size, location, and consistency), the diagnostic performance, the physician’s preference, and the patient’s economic situation.

FNA

- For a newly identified, clinically and/or radiologically obvious malignancy of the breast, CNB is the sampling method usually preferred for histologic evaluation and biomarker studies. However, in countries with limited resources, FNA may be used as the sole sampling technique or as the first-line diagnostic tool for pathologic evaluation.
- When a major lesion of primary breast carcinoma is identified, it is important to know whether there is cancer elsewhere in the same quadrant (multifocal disease) or in different

quadrants (multicentric disease) before surgery is planned, especially when breast-conserving surgery is being considered. FNA is the preferred sampling method for assessing these satellite lesions.

- FNA can be reliably used for pathologic confirmation of an inoperable advanced-stage breast cancer before systemic therapy.
- During post-surgery follow-up of breast cancer patients, FNA is a preferred sampling method for newly developed chest wall lesions and for documentation of recurrence vs. reactive changes/fat necrosis.
- For breast lesions that appear radiologically benign or probably benign, FNA is preferred as the first-line sampling technique, and a benign diagnosis could save cost, time, and patient anxiety. Notably, benign breast lesions are far more common than breast cancer.
- For cystic lesions of the breast that are benign in the majority of cases, FNA is sufficient to obtain diagnostic tissue and sometimes even has served as a therapeutic procedure.
- Metastatic tumors of extramammary origin, although rare, can occur in the breast. FNA is usually sufficient for the diagnosis on the basis of cytologic features and/or immunoperoxidase findings [26, 27].
- Infectious diseases of the breast, including abscess, mastitis, and tuberculosis, can be reliably diagnosed by pathologic examination of FNA samples in conjunction with microorganism cultures of the aspirates [28, 29].
- Lymphoma of the breast can be sampled via FNA, and the aspirates are superior to core tissue for flow cytometric immunophenotyping [30, 31].
- Preoperative identification of metastatic disease of the local–regional lymph nodes (e.g., axillary, supraclavicular, infraclavicular, and internal mammary lymph nodes) has become an integral part of breast examination for patients with newly diagnosed breast cancer and is preferably conducted with FNA because it is the most accurate and cost-effective, yet safe, method. Axillary staging via FNA can spare sentinel node biopsy if axillary lymph

node cytology is positive and can be followed by immediate axillary dissection or triage of advanced cases for adjuvant/neoadjuvant treatment [32].

CNB

- All the previously mentioned lesions, except for cyst contents, can be sampled by CNB. Nonpalpable and image-detected abnormalities, such as microcalcifications and architectural distortion, should be sampled by CNB [24, 25, 33]. VACB is more accurate for these lesions than USG-CNB [34–36].
- CNB may be used as a second-line diagnostic tool for lesions in which FNA fails to yield sufficient cells or in which FNA is unable to reach an unequivocal diagnosis [25, 37].
- CNB is the preferred diagnostic technique when determination of in situ carcinoma vs. invasive carcinoma/tumor subtyping is required, usually for newly identified, clinically, and/or radiologically apparent primary breast carcinoma, especially for patients who are candidates for neoadjuvant chemotherapy.

Limitations and Disadvantages

FNA

- FNA may yield low cellular aspirates and result in an unsatisfactory sample for lesions with abundant fibrotic or desmoplastic stroma, such as invasive lobular carcinoma.
- The intrinsic limitation of FNA is sampling error due to small sample size and the lack of reliable histologic architecture. Therefore, even when the aspirate is adequate in cellularity, a diagnosis may not be accurate. This does not necessarily reflect the inability of a pathologist to recognize a specific entity but is rather due to the inherent nature of the breast lesions. For example, in benign lesions, it may be difficult at times to distinguish between fibroadenoma and benign/low-grade phyllodes

tumor [38–41], fibroadenoma and fibrocystic change, and fibroadenoma and papillary lesions [42]. Occasionally, it may be challenging to distinguish myxoid fibroadenoma from colloid carcinoma [43–45] and to distinguish benign sclerosing lesions from low-grade carcinoma [46, 47]. In borderline or low-grade lesions, such as atypical ductal or lobular hyperplasia, papillary lesions, in situ low-grade ductal or lobular carcinoma, some tubular carcinomas, and invasive lobular carcinoma [48–51], it may be difficult to render a definitive diagnosis or distinguish one from the other. It has been reported that false-negative or equivocal cytologic diagnoses are associated with tubular carcinoma [52–58] and invasive lobular carcinoma [59–65] because their cytologic features may overlap with those of benign proliferative diseases. In malignant lesions, it may not be possible to distinguish invasive from in situ carcinoma [66–69] or to accurately specify tumor subtype in some cases. Previous studies reported cytologic features that can be used to predict invasion [66, 68–71]. These include malignant cell clusters forming tubular structures, single tumor cells with intracytoplasmic lumina, proliferation of fibroblasts and elastoid stromal fragments, and the infiltration of malignant cells into fibrofatty fragments. However, overlapping features can be seen in both invasive and in situ lesions. Over-relying on these criteria can lead to misdiagnosis.

CNB

- CNB has similar limitations to those of FNA such as sampling error due to the small amount of tissue obtained with CNB. Although less frequently than with FNA, misinterpretation can occasionally occur with CNB. For example, some phyllodes tumors on excision were initially diagnosed as fibroadenoma by CNB, in part due to intratumoral heterogeneity. Likewise, it may be problematic to distinguish benign papillary lesions from atypical or malignant ones, between atypical ductal hyperplasia and low-grade

ductal carcinoma in situ, and between complex sclerosing lesion/radial scar and tubular carcinoma [72–74]. Approximately one-fifth of lesions diagnosed via CNB as ductal carcinoma in situ are associated with invasive component on excision [8, 75, 76].

- CNB requires local anesthesia, is unable to provide the on-site immediate assessment that FNA can provide, and takes longer to report results than FNA. Although touch imprints of CNB tissue may be evaluated during immediate assessment, the accuracy in predicting the histology of corresponding CNB is suboptimal [77].
- CNB is unable to aspirate fluid collections [78, 79].
- It may be difficult for CNB to sample some lesions that are close to the skin, near the chest wall, or in the axilla, as well as some types of calcifications [9, 74, 80].
- CNB, especially using a large bore needle, may be associated with histologic changes of the biopsy sites including hemosiderin deposition, fibrosis, foreign-body reaction, and infarction of some lesions such as papilloma [81].
- CNB may occasionally lead to reduction of tumor size, which can affect the decision for subsequent chemotherapy in tumors of borderline size [82]. Post-VACB ultrasonographic appearance mimicking malignancy has been reported [83].

Diagnostic Accuracy

FNA

The success of FNA depends on the nature of the lesion, the skill of the aspirator, and the experience of the interpreter. Availability of immediate assessment and feedback also affects the success rate.

A number of publications have demonstrated the high overall accuracy of FNA in the diagnosis of breast lesions. A large-scale study of 2,375 lesions from Thailand showed sensitivity, specificity, positive predictive value, and negative

predictive values of 84.4%, 99.5%, 99.8%, and 84.3%, respectively; overall diagnostic accuracy of 91.3%; and false-positive and false-negative rates of 0.5% and 16.7%, respectively [11]. In a study from the United States that evaluated the utility of FNA in 1,158 clinically suspicious, palpable breast masses in women under and over the age of 40 years, the sensitivity, specificity, and positive predictive value in both groups were 97–99%, although the negative predictive value in women over age 40 years was 86%. The overall false-positive rate was <1% and false-negative rate was 9% [84].

Even for imaging-detected nonpalpable lesions, FNA cytologic evaluation is highly accurate when practiced in a multidisciplinary setting [85, 86]. In a study at The University of Texas MD Anderson Cancer Center evaluating nonpalpable, noncystic breast lesions sampled with ultrasound-guided FNA, the sensitivity and specificity in the diagnosis of cancer were 91% and 77%, respectively, and the false-positive and false-negative rates were 1% and 2%, respectively [79]. Combined with more recent studies, the overall sensitivity was 76–99%, specificity 60–100%, positive predictive value 94–100%, negative predictive value 67–96%, diagnostic accuracy 72–95%, false-positive rate 0–3%, and false-negative rate 3–18% [11–13, 17–19, 80, 84, 87].

False-positive results are uncommon and are usually due to interpretative error, and occasionally due to improper specimen preparation (e.g., distortion of the cells from vigorous smear spreading, air-drying artifact). False-negative diagnoses are more likely the effects of sampling error rather than interpretative problems.

CNB

As is the case for FNA, the success of CNB depends on the nature of the lesions, competence of the aspirator, and experience of the pathologist. A number of studies have reported very high sensitivity (91–99%), specificity (96–100%), positive predictive value (100%), and negative predictive value (100%), which are better than

results for FNA for both palpable and nonpalpable lesions [88–91]. Also, the sensitivity of CNB increases with the number of cores taken (1 core, 76.2%; 2 cores, 80.9%; 3 cores, 89.2%; 4 cores, 95.2%) [92].

Sample Collection, Preparation, and Staining

FNA

It is controversial whether clinician or pathologist should perform the aspiration; however, practice makes perfect. Knowledge of the consistency of the lesion during physical examination or aspiration is helpful for predicting the nature of the lesion. For example, a hard lesion gritty to the needle is likely to be malignant, and a freely movable, well-defined, and rubbery mass is suggestive of a fibroadenoma.

Generally, for palpable breast masses, two to four needle passes are made. The needle gauge can be 22–25, depending on the quality of the lesion. The needle may be attached to a disposable syringe that is mounted to a pistol grip-like syringe holder to apply suction. A local anesthetic is usually not used, because the swelling that results can obscure the nodule. If a breast lesion is densely fibrous, a larger needle with suction is preferred, and, in this circumstance, local anesthesia may be advisable. One should release suction when blood or material is first seen in the hub of the needle. The needle is withdrawn from the lesion without any vacuum suction because the cells otherwise may end up in the syringe (rather than staying in the needle) and are difficult to expel onto slides. Fluid-filled cysts are the exception: if fluid is obtained, negative pressure should be maintained until the cyst is completely evacuated. Any residual mass requires re-aspiration of the solid component to avoid missing malignant cysts.

To prepare smears, the needle is removed from the syringe, which is filled with air. Pushing this air with the plunger of the syringe, a small drop of aspirated material is expressed onto each slide and smears prepared. After making direct smears,

air-dried Diff-Quik-stained and alcohol- or Carnoy-fixed Papanicolaou-stained smears are routinely made. Diff-Quik staining preferably highlights cytoplasmic features and background material, whereas Papanicolaou staining is better for the evaluation of nuclear characteristics (i.e., nuclear membrane, chromatin, and nucleolus). In some laboratories, hematoxylin-eosin stain is used to stain the smears; in others laboratories, liquid-based preparations (e.g., ThinPrep, SurePath) may be made, either together with or to replace direct smears [93–96]. The advantages claimed for the liquid-based technique include better preservation of cellular morphology, and also the cytologic diagnosis can be made from one slide while the remaining material can be used for biomarkers. This technique, however, is not widely accepted since it results in shrinkage artifacts and a diminution in the background material that is often useful for diagnosis.

On-site immediate assessment for specimen adequacy is important to ensure high diagnostic accuracy and to reduce the incidence of unsatisfactory aspirates. Ideally, the immediate assessment should be performed by an experienced cytopathologist who should correlate the cytologic findings of direct smears available at the time of aspiration with the clinical and radiologic findings (triple test) to determine whether the FNA contains material representative of the target lesion. Mismatched triple test results require re-aspiration or concurrent CNB. There is no specific requirement for a minimum number of ductal cells to be present to fulfill adequacy of the aspirates of solid nodules [97]. An FNA is considered “inadequate” if the sample is nonrepresentative (such as scant cellularity incompatible with clinical and/or radiologic findings) or if the smears show significant distortion or artifacts and cannot be interpreted. Immediate assessment can also warrant a proper triage of material for cell block and/or ancillary studies. Cell block material should be collected for immediate assessment for cases in which architectural features might be crucial for making a definitive diagnosis because cell block material retains, at least partially, histologic architecture of the lesion. In addition, cell

block is the preferred sample type for immunoperoxidase studies. A separate dedicated pass (if additional needle pass is deemed feasible by the aspirator) and needle rinse or tissue fragments scraped from a thick smear are all suitable for cell block. After centrifugation, the pellet is fixed in formalin, embedded in paraffin, and then sectioned and stained with hematoxylin-eosin, a process virtually identical to that used for surgical tissue specimens. Unstained cell block sections are used for immunoperoxidase studies. In cases where non-Hodgkin lymphoma is suspected based on immediate assessment, fresh cells should be collected as cell suspension for flow cytometric immunophenotyping. Likewise, in cases where an infectious process needs to be excluded, fresh samples should be collected for microorganism cultures.

CNB

CNB is usually conducted with image guidance. Both USG-CNB and VACB require local anesthesia. The former involves using a large-bore cutting needle (usually 14-gauge) and automated gun to remove one cylindrical core of breast tissue per insertion; three to four cores are routinely required to maximize the chance of definitive diagnosis. VACB is a semi-invasive, mini-resection biopsy procedure and involves using a vacuum-powered instrument (usually 9-gauge) to remove multiple pieces of breast tissue during one needle insertion. The tissue cores are fixed in formalin, embedded in paraffin, and then sectioned and stained with hematoxylin-eosin. Unstained sections are used for immunoperoxidase studies.

Complications

Complications in FNA are rare, and the most common ones are pain and bleeding [98–100]. The latter can be decreased by using a smaller bore needle and applying firm pressure after the needle exits the lesion. The other potential complications are vasovagal reactions, infection, and

pneumothorax; infarction, epithelial displacement, and needle track malignant seeding are extremely rare and are attributed to the use of large-bore needles [20, 80, 101–104]. All these complications can also occur with CNB, with discomfort and hematomas being the most common complications [79, 105].

Pathologic Report

Regardless of the type of sampling methods (FNA or CNB), the pathologic interpretation should be made on the basis of triple test whenever possible. Application of triple test can significantly reduce false-negative and false-positive interpretation in cytology diagnosis, with resulting false-negative rates of 0.4–1.7% [106]. When a discrepancy is encountered or a definitive diagnosis cannot be reached, a more invasive procedure (subsequent CNB or open surgical biopsy) should be considered for further evaluation. Cytologic examination should start with low magnification followed by high magnification. Features that need to be examined are listed next.

Low-Power Examination

- Background: necrotic debris, mucin, myxoid material, lipoproteinaceous material, inflammatory cells, blood elements, bipolar naked nuclei.
- Cellularity: high cellularity is often seen in neoplastic and proliferative disease.
- Cell arrangement: sizes and shapes of epithelial groups, monolayered cohesive sheet, three-dimensional cluster, papillary group, loosely cohesive or discohesive clusters, isolated cells.

High-Power Examination

- Cell type and proportion: epithelial cells, apocrine metaplastic cells, myoepithelial cells, mesenchymal cells, histiocytes.

- Size and shape of individual lesional cells.
- Cytoplasmic features: amount, granularity, vacuolization, intracytoplasmic lumina.
- Nuclear features: size and shape, nuclear/cytoplasmic ratio, location, pleomorphism, nuclear membrane irregularity, chromatin pattern, size of nucleolus, mitosis.

The National Cancer Institute has recommended five categories of diagnosis in breast aspiration cytology in order to bring a degree of uniformity to the diagnostic reporting [107]. These categories are unsatisfactory (C1); benign lesion (C2); atypical, probably benign (C3); suspicious, probably malignant (C4); and malignant (C5). For benign and malignant lesions, a general category of benign or malignant is better followed by a specific diagnosis whenever possible. For lesions with equivocal diagnosis, it is informative to include possible differential diagnosis and the likelihood of malignancy to allow clinicians to determine whether a close follow-up or histologic confirmation is appropriate [20, 108].

Unsatisfactory aspirate (C1) is often due to scant cellularity, poor preservation or distortion of the lesional cells, significant obscuring blood, or inflammatory components. The reported unsatisfactory rates are as high as 34% [11, 109]. This problem can be minimized by practicing aspiration skills, doing multiple needle passes for each lesion, and having a cytopathologist to perform on-site assessment. Studies have shown that the nondiagnostic rate was 20% without on-site assessment but was less than 1% with on-site assessment [11, 110]. In addition, FNA with ultrasound guidance should have a higher sensitivity than unguided FNA [85, 111].

Common Cytologic Features of Benign Lesions (Fig. 2.1)

- Variable cellularity
- Cohesive flat sheets of epithelial cells with honeycomb appearance; slight crowding when hyperplastic
- Small or slightly enlarged nuclei, evenly spaced from each other

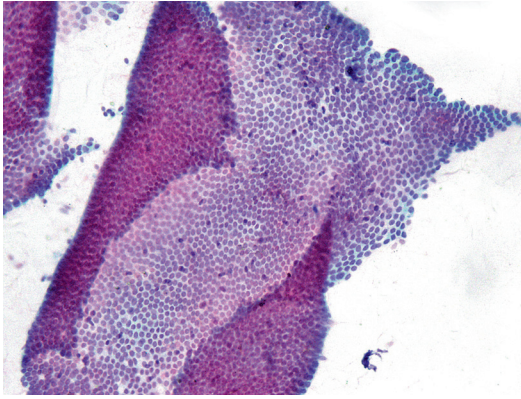


Fig. 2.1 Fine needle aspiration (FNA) smear of benign non-proliferative ductal epithelial cells, characterized by a cohesive flat sheet with honeycomb appearance. Interspersed within the monolayered ductal cells are myoepithelial cells with darker small individual nuclei (Papanicolaou stain)

- Smooth nuclear outline, fine chromatin, small to inconspicuous nucleoli
- Variable amounts of myoepithelial cells that appear as darker small individual nuclei within the epithelial fragments and in the background

Cytologic features of carcinomas vary significantly according to the histologic type, degree of differentiation, and the extent of stromal reaction. While some invasive ductal carcinoma (such as tubular carcinoma), lobular carcinoma, and mucinous carcinoma often show mild cytologic atypia and subtle malignant features (Fig. 2.2), the most commonly encountered ductal carcinomas show the following features (Fig. 2.3):

- Hypercellular aspirates
- Tumor cells forming three-dimensional, syncytial, or loosely cohesive clusters with numerous dispersed epithelial cells with intact cytoplasm
- Nuclear atypia encompassing enlarged, hyperchromatic, and pleomorphic nuclei, increased nuclear/cytoplasmic ratio, irregular nuclear membrane, coarse chromatin, presence of mitotic figures, and prominent nucleoli
- Absence of or rare myoepithelial cells
- Tumor necrosis

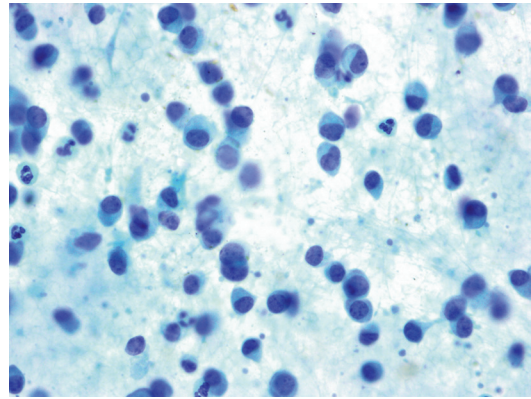


Fig. 2.2 FNA smear of a lobular carcinoma of the breast, characterized by eccentrically located nuclei with mild to moderate nuclear atypia and arranged in loosely cohesive clusters or single files (Papanicolaou stain)

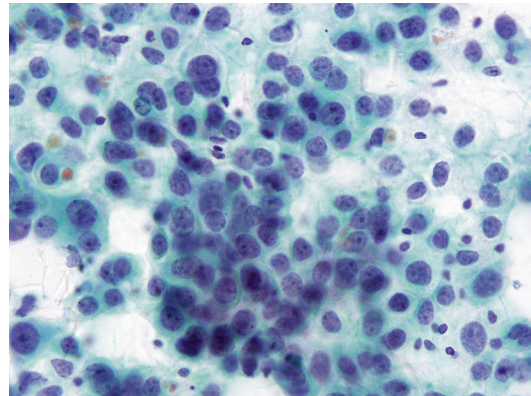


Fig. 2.3 FNA smear of a ductal carcinoma of the breast, characterized by hypercellular aspirate, pronounced nuclear atypia, and cellular pleomorphism (Papanicolaou stain)

With a cytologic diagnosis of breast carcinoma, tumor typing and nuclear grading should be incorporated into the diagnosis whenever possible. Tumor typing is based on cellularity, arrangement of cell groups, cell morphology, nuclear size and pleomorphism, and background content [112]. Several scoring systems have been developed including Robinson's grading system, Mouriquand's grading system, and Fisher's modification of Black's nuclear grading method [113–116]. The Robinson's grading system has been reportedly the easier and better one due to its superior objectivity and reproducibility [117, 118].

Tumor type and grade determined on FNA samples correlate quite well with the corresponding histologic grade and may assist in clinical decision-making [112, 114, 116, 117, 119].

The equivocal categories of C3 and C4 account for 5–10% of breast aspiration cases. Of note, 20–50% of C3 cases (atypical, probably benign) and 80–95% of C4 cases (suspicious, probably malignant) turn out to be malignant in final diagnosis [14, 120–125]. Lesions with equivocal cytologic diagnosis require additional histologic confirmation for definitive diagnosis.

Owing to the lower diagnostic yield and accuracy rate of FNA in certain lesions, cost-effectiveness of FNA becomes a debatable issue. Although the cost for FNA as a single sampling procedure is cheaper than the cost for CNB, especially in cases where there is a palpable lesion and FNA is conducted without imaging guidance, the total cost for obtaining a reliable final diagnosis will be higher than for CNB alone for lesions that are initially sampled with FNA subsequently require histologic (CNB or open surgical biopsy) confirmation [126]. Therefore, it is important to select diagnostic modality based on clinical/radiologic indications.

Ancillary Studies

Ancillary studies used in breast lesions are mostly immunoperoxidase stains and occasionally special stains such as mucin or fungal staining. Both CNB and FNA samples can be used for the ancillary tests, with CNB being the preferred type. For FNA samples, cell block, direct smear, and liquid-based preparation are all suitable for ancillary studies, but cell block is optimal since it is analogous to surgical pathology material.

In cases in which a cell block is not available or contains insufficient cells, direct smear and liquid-based preparation can be tried for immunostains as long as the sample is reasonably cellular [127–129]. If the cells of interest are present on only a single or a few smears when a panel of immunostains is needed, a cell-transfer technique—in which the original smear material is divided into several pieces and then transferred

onto multiple slides—may facilitate multiple immunomarker studies [130–133]. This technique can avoid a repeat biopsy solely for immunophenotyping of lesions.

There are several disadvantages of immunostaining on direct smear and liquid-based preparations:

1. Such sample types lack proper control tissue, which should be processed and fixed in the same way as the test specimen at each run of immunostaining
2. High background staining, which is usually associated with crowding of cells in a thick smear, or poor cytoplasmic preservation may lead to misinterpretation
3. Because of the lack of a reliable histologic architecture in the aspirated material, the mistaking of entrapped benign ductal cells, cells of ADH or DCIS for invasive tumor cells, can occur, leading to misinterpretation of biomarker results
4. Due to sampling error, tumor necrosis, or tumor fibrosis, it is a common limitation that only a small amount of cells are available for immunostaining, which may lead to a false-negative interpretation in tumors that express some markers only focally and heterogeneously

Therefore, caution should be exercised in the interpretation of immunostaining results on any samples that have limited lesional cells.

Prognostic and predictive factors most commonly tested in breast cancer samples are estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Knowing the status of these biomarkers is crucial for the assessment of a patient's eligibility for endocrine therapy and anti-HER2-targeted therapies, respectively. Although the biomarkers are usually tested in surgically resected or CNB specimens of newly diagnosed primary breast carcinoma and require standardized fixation conditions (i.e., fixation in 10% neutral buffered formalin for 6–48 h for ER, PR, and HER2) [134–136], the markers are also frequently tested in cytologic specimens to determine their status in metastatic carcinoma, since metastatic tumors are often sampled via FNA.

Clinicians frequently request retesting of these markers in metastatic tumors even though the receptor status of the patient's primary tumor is known. It is believed that, due to tumor heterogeneity and possible clonal evolution during biologic progression of the tumor, metastatic deposits may show loss or gain of the expression of these receptors and demonstrate a receptor status different from the status in the corresponding primary tumors. Also, the receptor activity in metastatic breast cancer may be altered after intervening systemic therapy (chemotherapy or targeted therapy). Therefore, assessment of these markers in a metastatic setting has a direct effect on the management of metastatic disease. In some cases in which the primary origin of a metastatic tumor is uncertain, receptor status (especially ER) is performed on FNA material of a metastatic carcinoma to verify or rule out a breast origin. Rarely, an FNA sample of a primary breast carcinoma is used for testing these markers when cytologic samples are the only sample type available for biomarker study.

With decent cell block material, ER, PR, and HER2 can be tested using immunostaining; HER2 can also be tested via fluorescence in situ hybridization (FISH). Without cell block, direct smear or liquid-based preparation may be used. For ER and PR immunostaining, previous studies have shown that preprepared direct smears can be used [137–139]. However, preprepared smears should be made prospectively at the time of aspiration. In routine practice, a retrospective requisition for biomarker studies may be received after a cytologic diagnosis has been completed and cell block tissue or preprepared smears are not available. Under such circumstances, the existing Papanicolaou-stained smears that have been used for routine cytologic diagnosis may be used for ER and PR staining. A study at MD Anderson Cancer Center compared ER staining results between smears and corresponding tissue sections and reported that ER staining can be performed directly on previously Papanicolaou-stained smears (without destaining) and that antigen retrieval greatly improved ER detectability and staining intensity without causing false positivity [140]. This technique allows the

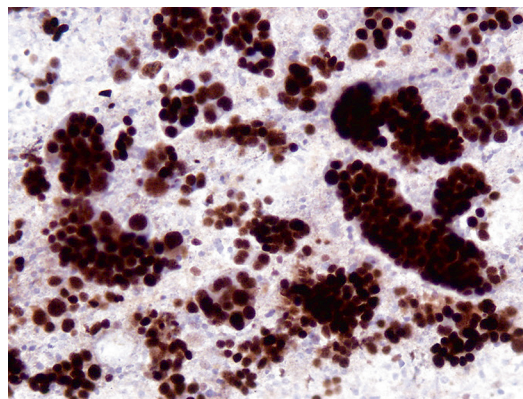


Fig. 2.4 Estrogen receptor was determined with immunocytochemical staining on a direct smear of a breast ductal carcinoma and was positive in approximately 95% tumor cells

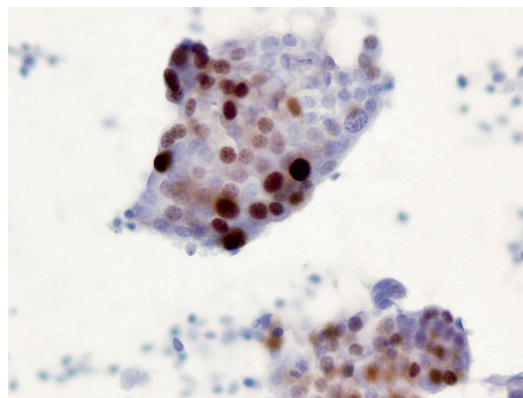


Fig. 2.5 Progesterone receptor was determined with immunocytochemical staining on a direct smear of a breast ductal carcinoma and was positive in approximately 40% tumor cells

use of archived slides for retrospective analysis of hormone receptor status, to visualize cytologic features and amounts of tumor cells on the slides prior to the tests, and thereby to enable selection of the “most representative” slide for staining (Figs. 2.4 and 2.5). In some centers, liquid-based monolayer preparation is used for ER and PR staining [141].

For HER2, immunostaining of HER2 on direct smear or liquid-based preparation is not standardized and is insufficiently reliable for clinical use because it is associated with high variability in sample preparation, fixation, staining, and

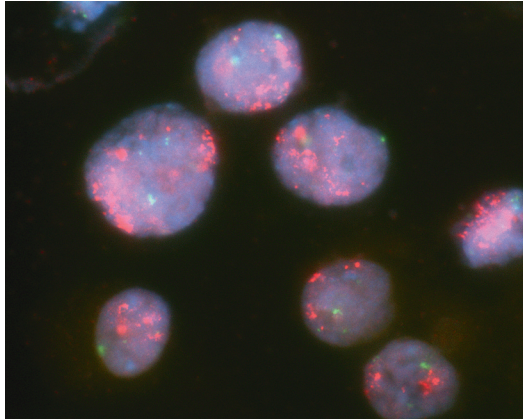


Fig. 2.6 HER2 testing using fluorescence in situ hybridization was performed on a direct smear of a breast ductal carcinoma and showed HER2 gene amplification

interpretation [142–144]. Therefore, the FISH method to detect HER2 gene amplification should be used instead. A number of studies have shown that HER2 status determined using FISH can be reliably evaluated in cytologic slides, with a concordance between cytologic samples and paired tissue sections of 91–100% [142, 145–148]. Compared with paraffin sections for FISH testing, the use of cytologic smears or touch imprint has the advantage of assessing monolayered tumor cells and enumerating all the HER2 signals within an entire nucleus without a truncating artifact (Fig. 2.6).

Chromogenic in situ hybridization is a bright field in situ hybridization method and is reportedly equally reliable to FISH in the determination of HER2 gene amplification [149–151]. This method detects HER2 copy number with a conventional peroxidase reaction and allows enumeration of gene copy number using a regular microscope along with histologic evaluation. Few studies have indicated that chromogenic in situ hybridization can potentially be performed on FNA material, including cell block sections, direct smears, or cytopsins, and the reported sensitivity, specificity, and accuracy were 84.0%, 87.9%, and 86.2%, respectively [152, 153]. Utility of this technique for testing HER2 status on cytologic samples requires validation in large studies.

Overall, the decision to perform which test on which sample type should be made on the basis of sample type and expertise available at each institution. If a laboratory chooses to perform prognostic and predictive marker studies on cytology specimens, the reliability of preanalytic and analytic methods should be validated according to the current guidelines [135, 136]. For laboratories that do not have specific experience with smears and liquid-based preparations, an effort should be made to obtain cellular cell block tissue for these tests. Of note, ER, PR, and HER2 status should be assessed on the invasive component of the breast carcinoma. Because cytologic specimens cannot reliably discriminate invasive from in situ components, interpretation of ER, PR, and HER2 status in a primary setting should proceed cautiously, especially if the tumor is small.

Stability of Prognostic and Predictive Biomarkers

Evaluation of the stability of hormone receptor and HER2 status during disease progression or after intervening systemic endocrine therapy or chemotherapy is of clinical significance. Using validated methods for ER testing and FISH for HER2 testing in metastatic breast carcinoma (mostly on direct smears), researchers at MD Anderson Cancer Center compared the results with those of primary tumors and observed a high level of stability for both markers. The concordance rates between primary and paired metastatic breast carcinoma were 92.5% for ER and 97% for HER2 [154, 155]. When evaluating patients with HER2-positive primary breast carcinoma, these researchers found that a positive-to-negative conversion of HER2 status occurred in 15% of metastatic breast carcinoma; the loss of HER2 positivity in metastatic carcinoma occurred in similar rates in both trastuzumab-treated and trastuzumab-naïve control groups, indicating that the loss of HER2-positive status was probably unrelated to intervening trastuzumab-based therapy [156, 157]. Nevertheless, given the importance of these markers for clinical

management, an effort should be made to retest their status in metastatic breast carcinoma.

Future Directions

To date, the common approach in identifying prognostic and predictive variables is to test one or a few markers in a cohort of patients, usually retrospectively. The resulting information may not fully capture the biologic heterogeneity in tumor growth, invasion, and metastasis and cannot accurately determine the risk of relapse for individual patients. Over the last decade, molecular testing, especially gene expression profiling microarray, has been used to identify more sophisticated prognostic and predictive factors for breast cancer patients. Gene combinations (i.e., gene signatures) seem more accurate than any single gene measurement alone.

It is reasonable to assume that CNB contains higher quality and amounts of RNA than does FNA for molecular testing. Several studies have demonstrated that both FNA and CNB samples yield adequate amounts of total RNA for microarray in experienced hands [158–160]. A learning curve has been observed during sample procurement via FNA. According to a study from MD Anderson Cancer Center, the success rate of gene expression profiling began at 70–75%, and then increased with practice to 97% [159]. It is not surprising that FNA and CNB show different cellular compositions, with a high proportion of carcinoma cells in FNA samples and more stromal cells and lymphocytes in CNB samples [158]. Selection of the preferred sample type for genomic studies should depend on whether the focus is on tumor cells only or on the tumor as well as its microenvironment (stroma).

Suitability of FNA samples for gene expression microarray has been shown in a number of studies that tried to identify prognostic and predictive variables, chemotherapy response predictor, and drug resistance mechanism [161–165]. During the course of systemic therapy, serial FNA may be an acceptable tool for tissue procurement in monitoring the response of tumor to the therapy and treatment-induced biomarker

changes [166]. Investigators from multiple institutions assessed the ER and HER2 expression data derived from comprehensive expression microarray data and observed a significant correlation between mRNA expression of ER and HER2 and the routinely determined status via immunostaining and/or FISH, with overall accuracies around 90% [159]. In that study, mRNA cutoff values of ER and HER2 were defined using tumors sampled via FNA, and the performance of each cutoff was validated in two independent datasets (one FNA specimens and the other surgical specimens) obtained from seven institutions across five countries. These findings indicate that it is promising to generate ER and HER2 information from comprehensive microarray data; integration of ER and HER2 mRNA expression data with multigene signatures from the same microarray data may refine and improve their predictive power for tumor response to target therapies and therefore allow for optimizing clinical decision-making and tailoring of therapeutic regimens on an individual basis.

FNA of breast lesions preserved in PreservCyt medium seems an acceptable sample type for protein profiling evaluation by the surface-enhanced laser desorption–ionization time of flight (SELDI-TOF) methodology [167].

The application of promoter hypermethylation has been investigated in liquid-based aspiration specimens, and this technique has been shown to improve diagnostic accuracy of breast lesions in which cytological assessment is indeterminate or suspicious for malignancy. It therefore might be a valuable ancillary tool for cytology diagnosis of breast carcinoma [168, 169].

Breast Cancer Risk Assessment

Identification of women at high risk for developing breast cancer is an important step in cancer prevention because these women may benefit from preventive intervention such as anti-estrogen agents or surgery [170–172]. The risk stratification is assessed on the basis of the Gail risk score and pathologic findings. Nipple fluid aspiration, ductal lavage, random periareolar FNA (RPFNA),

and CNB have been used for tissue acquisition [173]. Some researchers performed nipple aspiration followed by ductal lavage [174]; however, the latter two methods were both associated with low diagnostic yield and some discomfort. In addition, to date there are no data available regarding the efficacy or mortality reduction for ductal lavage used as a screening or diagnostic tool. RPFNA seems a better option for obtaining ductal and lobular cells and is the most accepted method by study participants [175–177]. The aspirated cells can be evaluated morphologically as well as for several biomarkers (epidermal growth factor receptor, ER, p53 protein, HER2, insulin-like growth factor 1, etc.) [176, 178]. A diagnosis of hyperplasia with atypia is associated with a high risk of developing breast cancer [173, 177]. Using FISH to screen for aneusomy in RPFNA samples, researchers at MD Anderson Cancer Center found that aberrations of chromosomal number were common in women at high risk for breast cancer; high-risk patients had significantly more monosomy of chromosomes 1, 11, and 17 and significantly more polysomy of chromosome 8 compared with low-risk patients [179].

Conclusions

Breast FNA and CNB are both useful for diagnosis, risk stratification, and biomarker testing.

Both procedures have their own specific advantages and limitations and can complement each other. While there is widespread preference for CNB in most developed countries, FNA is still a valuable initial procedure for evaluating palpable breast lesions in many developing countries in view of its ease, simplicity, affordability, safety, rapidity, low cost, and high degree of accuracy. There is no consensus as to which modality is preferable in breast lesion diagnosis. The option should be determined by several factors: availability of the necessary equipment and expertise, clinical/radiologic indications, the likelihood of achieving a definitive diagnosis, the need for biomarker studies, patient economic status, and the preferences of the managing clinician

and the patient. On the public side, education on the availability of the affordable and less invasive diagnostic techniques may encourage women to seek care at earlier stages of the disease. In the future, genomic and proteomic techniques hold great promise to complement the existing diagnostic modalities for evaluating breast lesions.

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