Breast Cytology: Current Issues and Future Directions

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Abstract: Breast cytology, in particularly fine needle aspiration biopsy (FNAB), has been used for many years as a diagnostic tool for managing patients with breast lesions. In experienced hands, FNAB is highly sensitive and specific. Other benefits include its low cost, minimal invasiveness, and ability to provide same-day diagnosis. Despite all these benefits, FNAB has gradually been replaced by core needle biopsy (CNB) because of its high error rates when there is a lack of experienced cytopathologists, its inability to distinguish between invasive and in situ carcinoma, and most importantly, its inability to provide adequate and suitable materials for quantitative evaluation of HER2 and other prognostic markers. Other uses of breast cytology include touch preparation cytology for intraoperative evaluation of sentinel lymph nodes and surgical margins of lumpectomy specimens and for providing same-day diagnosis of CNB. In addition, breast cytology, such as ductal lavage and nipple fluid cytology, has also found applications in risk assessment for women at high risk for developing breast cancer. With the increased utilization of molecular technologies, genomic and proteomic studies have been successfully applied to breast cytologic preparations. It would not be far fetched to predict that in the very near future, the clinical application of molecular analyses will be routine ancillary testing in breast cytology, thus allowing early cancer detection, and improved tumor characterization as well as prediction of patients’ outcomes and therapeutic responses.

Keywords: Breast cytology, FNAB, CNB, breast cancer.

INTRODUCTION

Breast cytology, particularly fine needle aspiration biopsy (FNAB), has been an integral part in the management of women with breast lesions. Although its use has gradually reduced in the United States, Canada, and the United Kingdom, FNAB continues to be used worldwide, especially in developing countries, for the initial management of breast lesions [1-10]. In addition, breast cytology has been established as a viable alternative for intraoperative examination of breast specimens. More recently, breast cytology, such as ductal lavage, has been utilized as an individual risk assessment tool for women at high risk for developing breast cancer. In this review article, the benefits and limitations of FNAB in the diagnosis of breast lesions are examined. We shall also discuss the use of breast cytology in intraoperative examination of breast specimens and as a risk assessment tool. Last but not least, the applications of molecular testing to breast cytology will also be explored.

FINE NEEDLE ASPIRATION BIOPSY

FNAB was first introduced in 1930 [11]. Over the years, it has been widely accepted as a first line diagnostic procedure for patients with breast lesions. However, since the beginning of the new millennium, there is a gradual decline in the popularity of FNAB and an increased usage of core needle biopsy (CNB) in the preoperative assessment of breast cancer in the United States, Canada, and the United Kingdom [1-3] The reasons for the loss of popularity for FNAB include high error rates due to a lack of experienced cytopathologists at individual laboratories. In addition, the belief that the inability of FNAB to provide adequate and suitable samples for assessment of prognostic markers also contributes to the decline. Nevertheless, FNAB continues to be used worldwide, especially in developing countries, for the management of breast lesions [4-10].

Current Indications of FNAB in the Management of Breast Lesions

Currently, the indications for FNA of breast lesions include
1. Evaluation of cystic lesions.
2. Diagnosis of recurrent or metastatic breast cancer.
3. Confirmation of locally advanced cancer.
4. Axillary staging of patients with invasive breast cancer.

Diagnostic Accuracy

One of the arguments for the replacement of FNAB by CNB is the high error rate due to a lack of experienced cytopathologists. However, in experienced hands, FNAB is a highly accurate diagnostic procedure. Recent review demonstrates that breast FNAB has a sensitivity ranging from 76% to 99%, a specificity from 60% to 100%, and a diagnostic accuracy from 72% to 95% [12]. For palpable breast lesions, both the sensitivity and specificity are over 95% [13, 14]. While the specificity for both FNAB and CNB approaches 100%, FNAB may be more sensitive (97%) vs 90% for CNB in the diagnosis of palpable breast cancers [15].

For nonpalpable breast lesions, FNAB under image guidance is also comparable to image-guided CNB. In one study, 1,885 FNAB with ultrasound guidance of nonpalpable
lesions over a 68-year period was analyzed [16]. Based on combined histologic and clinical follow-up, the authors reported a sensitivity of 97% and specificity of 99% for ultrasound guided FNAB when definitive benign and malignant diagnoses were considered. Based on a multicenter study evaluating over 2,400 women who underwent image-guided FNAB followed by image-guided CNB of nonpalpable breast abnormalities, the sensitivity (89%) of CNB for diagnosing all lesions was significantly lower than that of FNAB (97%) [17].

The positive and negative predictive values of breast FNAB range from 94% to 100% and from 67% to 96%, respectively [12]. False positive diagnoses are rare (0-3%) and are usually the result of interpretative error [12]. Pregnancy related changes, fibroadenoma, therapeutic changes, fat necrosis, and papillary lesions are some examples that can lead to false positive interpretations [10]. On the other hand, the false negative diagnoses have been reported to be in the range of 3% to 18% and can be attributed to either sampling or interpretative errors [12]. Low-grade ductal carcinoma, lobular carcinoma, and mucinous carcinoma, are some examples of lesions that may be associated with underinterpretation [10, 18]. However, the majority of the false negatives are due to sampling errors. To reduce false negative diagnoses, it is important to apply the triple test for accurate diagnosis in each patient [9]. The triple test is the combination of clinical, radiologic, and cytologic findings in arriving at a diagnosis. If all three components are negative, the negative predictive value approaches 100% [19]. On the other hand, if any discordance exists among any of the 3 components, further investigations, such as excisional biopsy, should be contemplated.

**Distinction Between In Situ and Invasive Carcinoma**

Because of the difference in the management in regard to sentinel node biopsy and the use of pre-operative neoadjuvant chemotherapy, it is imperative to determine if a breast cancer is in situ or invasive. Because of the lack of reliable cytologic features to distinguish between in situ and invasive breast carcinoma on FNAB [20-22], histologic examination is preferred. However, CNB suffers from sampling errors too; up to 20% of cases diagnosed as in situ carcinoma only on CNB were found to be associated with invasive carcinoma on subsequent surgery [23, 24]. As a matter of fact, over 95% of FNAB with a malignant diagnosis in the presence of either a palpable mass or a speculated appearance on imaging showed invasion on histologic follow up [25, 26].

**On Site Assessment**

The advantage of FNAB is that it is fast, inexpensive, and minimally invasive. The results are rapidly available. A preliminary diagnosis can be given by a cytopathologist within 5-10 minutes after the procedure. In most cases, a definitive diagnosis can be given within 24 hours. Because a diagnosis can be made available on the same day as the procedure, this would enable “one-stop shopping” in a multidisciplinary setting, i.e. allowing the clinicians to discuss the diagnosis with the patient, proceed with further investigation, and help with subsequent management and treatment without delay.

Another benefit of on-site assessment is the reduction in the number of unsatisfactory or inadequate samples. Additional passes can be performed immediately when a specimen is designated as inadequate. Nasuti et al. has shown that with on-site adequacy assessment, the non-diagnostic rate was less than 1% whereas the non-diagnostic rate was 20% when on site evaluation was not performed [27]. Unfortunately, reimbursement for pathologists performing on site assessment was inadequate [28]. Furthermore, not all centers have the expertise to provide such service.

**Ancillary Studies and Molecular Testing**

Perhaps the strongest argument against FNAB is its inability to provide adequate and suitable samples for the evaluation of predictive factors, such as estrogen and progesterone receptors (ER and PR) and HER2. As a result, more expensive and invasive procedures, such as CNB, are required for obtaining additional tissue for ancillary testing once a malignant cytologic diagnosis is made. Breast FNAB is, therefore, judged as incurring additional costs and delays in patient management.

One of the challenges in utilizing cytologic samples for evaluating predictive and prognostic biomarkers is meeting the fixation requirement recommended by the recently published American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP) guidelines for quantitative analysis of HER2 expression [29] According to the recommendations, breast specimens should be fixed for a minimum of 6 hours and a maximum of 48 hours duration in 10% neutral-buffered formalin. Most cytologic samples, including direct smears, liquid-based preparations, and cell block preparations, are often fixed in 95% ethanol. A recent study shows that there was only a moderate positive agreement of 73% (weighted Kappa of 0.57) between ethanol-fixed and formalin-fixed tissue samples with HER2 immunohistochemistry [30]. The lack of agreement is mainly attributed to the fact that ethanol-fixed cell block preparations more frequently demonstrate overexpression of HER2 immunostaining when compared to formalin fixed tissue samples, resulting in false positive results. On the other hand, excellent correlation for ER and PR results were noted between ethanol-fixed cell block preparations and formalin-fixed tissue samples [30].

Cytology material obtained by FNAB has been shown to provide good quality of DNA and RNA with a yield that is comparable to that of CNB. In a study comparing FNAB to CNB for transcriptional profiling, the authors showed that FNAB samples were more representative of tumor cells and had 80% tumor cells versus 50% for CNB and 5% stromal cells in FNAB versus 30% in CNB [31]. Recent developments in molecular technologies, such as gene expression profiling, could complement traditional cytology by allowing early cancer detection, and improved tumor characterization as well as prediction of patient outcomes and therapeutic responses.

**Staging of Regional Lymph Nodes**

Preoperative determination of involvement of regional lymph nodes can influence the selection of treatment options. For patients with T1 and T2 breast cancer, a positive cytologic diagnosis avoids unnecessary sentinel lymph node
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Inadequate samples are reported in less than 10% of cases with on-site assessment of adequacy [36-38]. The overall sensitivity and specificity of ultrasound-guided FNAB for the evaluation of axillary lymph nodes for preoperative staging of breast cancer range from 25% to 95% and from 97% to 100%, respectively [35, 37, 39-44]. False positive diagnoses are rare, less than 2% [37, 44, 45]. The common causes of false-negative results include sampling error, small metastases (<5mm), involvement of few (<3) lymph nodes, and interpretive errors [46].

Other Indications for FNAB

In addition to being a diagnostic tool, FNAB has also been utilized to collect breast tissue for monitoring of therapeutic response of women receiving neoadjuvant chemotherapy and for risk assessment in asymptomatic women without suspicious lesions on physical examination or mammography.

Serial FNAB: Preoperative neoadjuvant chemotherapy is the standard of care in locally advanced breast cancer with the intention to expand surgical options and to improve survival. The extent of tumor response to neoadjuvant chemotherapy correlates with disease free and overall survival [47-50]. Modification of their treatment regimes may be warranted if patients who are expected to have no or partial response could be identified early. Taking a series of FNABs of a tumor during the course of therapy can provide useful information about treatment-induced changes in biomarkers, which in turn may be helpful in monitoring patient response to neoadjuvant chemotherapy [51-53]. For example, a decrease in proliferation indices as measured by Ki-67 and an increase in Bel-2 expression in serial FNAB taken after 21 days of neoadjuvant chemotherapy consisting of mitoxantrone and methotrexate correlated with complete response in patients with primary breast cancer [52]. More recently, based on FNAB-derived cDNA microarray expression profiling, the number of genes that changes after one cycle of neoadjuvant chemotherapy (adriamycin and cyclophosphamide) was 10 times greater in tumors with complete response than those with partial or no response [53].

Random Periareolar Fine Needle Aspiration (RPFNA): RPFNA consists of aspirating 2 random sites approximately 1 cm from the nipple areolar complex in both the upper-outer and upper-inner quadrants of each breast [54]. The premise of RPFNA is based on the belief that random tissue sampling may be able to detect proliferative changes associated with increased breast cancer risk if these changes are widespread within the breast [55]. When 4 to 5 passes were taken per site, adequate cytology for morphologic assessment was achieved in 94% of women [54]. Asymptomatic women who were found to have cytologic atypia on RPFNA were more likely to develop carcinoma than those without atypia within 3 years [54]. Limitations of RPFNA include intra- and inter-observer variation and inability to precisely locate an area of severe atypia.

TOUCH PREPARATION

Touch preparation (TP) cytology, also known as imprint cytology, consists of smearing fresh tissue onto a glass slide which is then stained. There has been great interest recently in the use of TP cytology in the management of breast cancer patients. Some examples include intraoperative evaluation of sentinel lymph nodes and surgical margins of lumpectomy specimens, as well as the provision of immediate assessment of CNB.

Breast Core Needle Biopsies

As mentioned earlier, CNB has gradually replaced FNAB as the diagnostic procedure of choice for patients with breast lesions. However, patients and physicians must traditionally
Fig. (2). Touch preparation of a sentinel lymph node, low-power showing loosely cohesive groups and clusters of tumor cells in a background of numerous small lymphoctes (H&E 20X), (A). Higher magnification showing an irregular cluster of tumor cells with large epithelial cells that display increased nuclear to cytoplasmic ratio and prominent nucleoli (40X) (B). Frozen section of lymph node showing sheets of epithelial tumor cells surrounded by lymphocytes consistent with metastatic breast carcinoma (C).
margins accounts for up to 25% local recurrence as compared to less than 4% local recurrence in patients with negative margins; [66-70] therefore, obtaining clear margins is critical to minimize local recurrence. TP cytology of the margins of lumpectomy specimens has been shown to rapidly and reliably evaluate margins with a diagnostic accuracy ranging from 73% to 100% [71-73]. The advantages of TP cytology over frozen sections include avoidance of problems associated with freezing and cutting adipose tissue as well as the ability to evaluate the entire marginal surface area of the resected specimens.

**NIPPLE FLUID CYTOLOGY**

Nipple discharge is relatively uncommon and can be due to either physiologic or pathologic conditions; the latter is most commonly associated with intraductal papillomas (Fig. 3). About 10% of patients with nipple discharge are found to have an underlying malignancy [74]. In general, nipple discharge fluid cytology is considered to be an ineffective diagnostic tool. Recent studies reported that the sensitivity and specificity of nipple discharge fluid cytology in diagnosing malignancy range from 16% to 46% and from 60% to 62%, respectively [75, 76].

![Image](image.png)

**Fig. (3).** Nipple discharge specimen showing numerous foamy macrophages and few ductal epithelial cells suggestive of benign cyst (Papanicolaou stain 20 X).

Attempts have been made to obtain nipple secretions from massaging/pumping the breast or by aspirating the nipple from asymptomatic women for the purpose of breast cancer screening. In a mass screening study using nipple fluids obtained from breast massage, only 0.1% of 150,000 Japanese women were found to have malignancy [77]. Similarly, nipple fluid aspiration also has a low sensitivity of detection of breast cancer; cancer cells were demonstrated in less than half of the nipple aspirate fluids obtained from patients who underwent breast cancer surgery [78-80]. Therefore, nipple aspirate fluid (NAF) cytology is of limited use in the current clinical setting.

**DUCTAL LAVAGE**

Ductal lavage (DL) was introduced in the last decade as a minimally invasive procedure to identify cellular atypia in mammary ducts. The underlying premise is that the presence of atypical ductal cells is predictive of increased risk of developing breast cancer [81, 82]. DL, as an individualized assessment tool, is Food and Drug Administration (FDA)-approved only for women who are at an increased risk for developing breast carcinoma but without suspicious lesions on physical examination or mammography.

DL yields far more cells, with a median number of 13,500 versus 120 cells, when compared to NAF samples; about 80% of samples were classified as adequate [83]. Based on the degree of cytologic atypia, DL specimens can be classified as benign, mild atypia, marked atypia, and malignant [84]. When DL demonstrates unequivocal malignant cells, further investigation, such as magnetic resonance imaging, is warranted to localize any occult lesions. However, several studies have reported low sensitivity of DL in detecting breast carcinoma in patients undergoing mastectomy for carcinoma [85, 86]. A plausible explanation is that DL only samples cells from intraductal processes; about 15 to 30% of invasive cancers lack an intraductal components [87, 88]. For DL specimens with atypia below the level of malignancy, the clinical significance of such findings is not known. A wide variety of benign conditions such as papilloma, fibroadenoma, duct ectasia, and endogenous and iatrogenic hormonal states, can give rise to varying degrees of atypia, resulting in poor correlation of the degree of cytologic abnormality with various proliferative breast disease [84]. Furthermore, there is poor interobserver reproducibility in interpretation of DL specimens among pathologists [84].

Currently, DL is not considered a screening tool for the general population. One reason is that it is not FDA-approved for women who have no known risk factors, the group that would most benefit from such a risk assessment tool. Another disadvantage of DL is that both clinicians and pathologists have to undergo training to be able to perform the procedure and interpret the findings, respectively. Even for women who are at high risk for developing breast cancer, DL is only one of the few diagnostic tools for individualized risk assessment and should not be construed as a substitute for more established means of diagnosis such as clinical examination and mammography.

**CIRCULATING TUMOR CELLS**

The detection of circulating tumor cells (CTCs) in the peripheral blood is one of the newest tools in the management of many human cancers including breast cancer. A variety of methods have been developed to detect CTCs in peripheral blood. However, to date, the only FDA-approved method for detection of CTCs in patients with metastatic breast, colon, and prostate cancers is the CellSearch System (Veridex, Raritan, NJ) which combines cytomorphology and immunology for the detection of CTCs. Briefly, using antibody coated magnetic beads, the CellSearch System captures circulating cells expressing epithelial cell adhesion molecule (EpCAM). The captured cells are then labeled with fluorescent monoclonal antibodies specific for leukocytes (CD45) and epithelial cells (CK 8, 18, and 19). Fluorescent marker-labeled circulating cells are then displayed on the computer screen with the aid of an automated microscope. CTCs are defined as CK+/CD45- nucleated cells. High interobserver agreement and high instrument reproducibility are noted [89, 90].

Based on a large cohort of breast cancer patients, a high CTC count measured using the CellSearch assay at the time
of diagnosis was found to be an independent adverse prognostic factor [91]. In addition, the same study also demonstrated that after the completion of chemotherapy, disease progression was inevitable in patients with metastatic breast cancer if the initial positive CTC assay failed to fall below 5 cells per 7.5 ml of blood [91]. The latter observation suggests that the CTC assay by CellSearch may be helpful in monitoring therapeutic response. Tumor cells derived from the so-called “normal genotype” of invasive breast carcinoma are typically negative for EpCAM expression and may constitute a false negative interpretation [92].

**MOLECULAR ANALYSIS AND BREAST CYTOLOGY**

With the advent of molecular technologies, abundant evidence has emerged to support the notion that each patient’s breast cancer possesses a unique molecular signature that may impart prognostic significance and influence therapeutic decisions [93-95]. For example, it has been shown that gene expression profiles are more powerful than traditional clinico-pathologic parameters in predicting disease outcomes in young patients with breast cancers [93]. Gene expression profiling can also be helpful in identifying patients who would not benefit from chemotherapy, avoiding exposure to the toxicities and risks of such treatment [96]. Therefore, comprehensive molecular profiling would influence our understanding of the classification, prognosis, and therapeutic response of breast cancers.

Several studies have shown that 70% (based on a single pass) to 100% (based on 3 to 4 passes) of FNAB aspirates can yield a sufficient amount (>1 ug) of mRNA for molecular testing such as cDNA microarray analyses [31, 97, 98]. Symmans et al. demonstrated that transcriptional profiles from FNAB and CNB of the same tumors were generally similar [31]. A few years later, the same group of investigators were able to separate ER-negative and ER-positive tumors based on gene expression profiles obtained from FNAB of breast cancers [99].

FNAB samples can also been used for proteomic analyses. Using surface enhanced laser desorption-ionization time of flight (SELDI-TOF) methodology, similar reproducible protein profiles were noted for the majority of FNAB from various benign breast lesions whereas protein profiles obtained from the aspirates of malignant lesions were visually different from those of benign breast lesions as well as between different subtypes of carcinomas [100]. In a more recent study, cellular samples derived from archival cytology aspirate smears and frozen FNAB samples were subjected to reverse phase protein microarray (RPPM) technology [101]. Adequate amounts of protein were extracted from both preparations to allow the quantification of individual phosphorylated and nonphosphorylated proteins. Potential applications of RPPM include in vivo monitoring of cell-signaling proteins before and after treatment, thus enhancing the ability to prescribe individualized therapy regimens through the mapping of aberrant cell-signaling patterns [101].

Molecular testing can also be used to aid in the assessment of the short-term risk of developing breast cancer. Current risk models, such as the Gail Model, which are based on personal and family history, have only limited individual discriminatory value [102]. Increasing attention has been given to the use of risk biomarkers to improve short-term predictive accuracy for the individual woman who is at risk of developing breast cancer and who may benefit from medical or surgical prevention options. Although cytomorphology may have limited value as a risk factor, breast fluids and aspirates obtained through NAF, DL, or RPFFNA in asymptomatic women can provide a rich source of biomarkers for risk assessment. A sufficient amount of RNA can be made available from RPFFNA samples for quantitative polymerase chain reaction (PCR) for 6 to 12 biomarkers [103]. Recently, it has been shown that quantitative evaluation of DNA hypermethylation using methylation-specific PCR can be readily preformed on NAF and DL samples [104]. These authors observed that methylation of CCND-2, p16, RAR-beta, and RASSF-1a was significantly more prevalent in tumor than in normal tissue specimens. In another study, using fluorescent in situ hybridization, chromosomal alterations identified in DL specimens obtained from women who underwent breast cancer surgery matched those identified in the corresponding resected breast cancers using comparative genomic hybridization [105]. Interestingly, only 10% of the DL samples were identified as malignant cytologically, whereas over half of the DL samples showed molecular changes characteristic of the tumor.

**CONCLUSIONS**

Breast cytology continues to play an integral part in the management of breast lesions. In experienced hands, FNAB is reliable and accurate for diagnosing breast cancers. One major obstacle is the challenge to provide suitable materials for meeting the current guidelines for the quantitative evaluation of HER2 expression. In addition to its role as a diagnostic tool, breast cytology has gradually established itself as an individual risk assessment tool for women at risk of developing breast cancer and a reliable alternative to frozen sectioning during intraoperative evaluation of sentinel lymph nodes and margins of lumpectomy specimens. With the rapid increase in the diversity and utilization of molecular technologies, many investigators have demonstrated the feasibility of applying genomic and proteomic studies to breast cytology. It would not be far fetched to predict that the clinical application of molecular analyses to cytologic samples of the breast will be routine in the near future.

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