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Fine Needle Aspiration of the Breast for Diagnosis of Preinvasive Neoplasia

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Abstract Fine needle aspiration (FNA) of the breast is a well-tolerated procedure used to evaluate palpable breast masses, has a reported mean specificity of 99%, and a reported sensitivity of 70–99%. The false positive rate varies from 0–0.4% in most larger series, with a reported false negative rate ranging from 0.7–22%; however, higher false negative rates have been reported in tumors under 2 cm in diameter. The FNA technique uses a fine, 20 gauge or less, needle and is not associated with a significant risk of tumor growing out the needle tract.

FNA cytology is not effectively used if a breast mass cannot be palpated or distinguished from fibrous tissue within the breast. The procedure can be applied to nonpalpable masses detected by mammography by employing stereotactic techniques. The cytologic samples obtained from FNA can be used to distinguish atypical ductal hyperplasia from *in situ* or invasive ductal carcinoma; however, cytologic criteria to effectively distinguish ductal carcinoma *in situ* (DCIS) from invasive adenocarcinoma are not definitive in many cases, and are dependent on variables related to the type of intraductal tumor, the size and character of the cell groups, and the presence of single or disaggregated tumor cells. Employing current cytologic criteria, lobular carcinoma *in situ* (LCIS) may be distinguished from invasive lobular carcinoma in some cases; however, the individual LCIS cells are not morphologically distinct from lobular carcinoma cells. Atypical lobular hyperplasia has cellular features essentially the same as those seen in LCIS.

Needle biopsy (NB) employs larger needles of 14–16 gauge. Stereotactic guidance for NB can be augmented with cytopathology by preceding the biopsy with FNA, and/or by collecting the cellular sample available when washing the needle after the tissue sample is removed. These needle biopsy washings are often highly cellular and are complementary to the tissue diagnosis.

FNA samples or NBs, if adequately cellular, are applicable for DNA analysis by static image analysis (flow cytometry). Flow cytometry is of limited practical value where cellularity or tumor representation is poor because morphologic confirmation cannot be established. These samples can also be used to calculate tumor proliferative fraction, employing Ki-67 antigen. Quantitation of nuclear organizer (AgNOR) regions and expression of HER-2/*neu* and p53 proteins can be accomplished in these samples; estrogen and progesterone receptors can also be detected and quantitated.

Key words: Breast fine needle aspiration, cytopathology, ductal carcinoma *in situ*, HER-2, lobular carcinoma *in situ*, ploidy analysis, proliferation markers

Fine needle aspiration (FNA) of the breast for the tumor diagnosis was first reported by Martin and Ellis in 1934 [1]; however, its use was not widely accepted due to concerns regarding tumor spread by the procedure. Franzèn and Zajicek reintroduced FNA in 1960 [2], and their

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subsequent works renewed interest and clinical use of the procedure [3,4]. Breast FNA is a simple, accurate and well-tolerated procedure to evaluate palpable breast masses, and to effectively distinguish benign from malignant masses with a reported mean specificity of 99%, and a sensitivity of 70–99% [3,5]. The speed of preparation and reporting is an additional advantage of this procedure [6]. The false positive rate ranges from 0–0.4% in most larger series, with a reported false negative rate ranging from 0.7–22%; however, higher false negative rates have been reported in tumors under 2 cm in diameter [3,5]. The majority of false negative needle aspirates are due to lack of diagnostic cytologic material (or sample error) [5].

The FNA technique usually uses a needle of 20 gauge or less, from which cytologic smears are prepared. If there is sufficient cellular material, a cell block may also be prepared. The FNA procedure is not associated with a risk of tumor growing out the needle tract (Table I).

Current mammographically directed, stereotactically guided biopsy techniques often rely on needle biopsy (NB) to establish histopathologic findings [7]. This technique employs local anesthesia and usually a large needle, 16 gauge or larger, although 20 gauge needles are used by some groups. These needles, unlike those used for FNA, are stiff and lend themselves to accurate placement. In addition, they can be employed with a triggered mechanism which simplifies the technique from the physician's standpoint. The major disadvantages of needles of this size are that local anesthesia is needed and a small incision in the skin is necessary to permit access to the soft tissues of the breast. In addition, larger biopsy needles are recognized to produce some risk of tumor outgrowth in the needle tract following the NB. In such cases it is presumed that the needle tract is excised with the tumor, if a tumor is present. NBs of this type, as compared to FNA, are more limited in patient tolerance, and carry a risk of hematoma and reactive changes in the breast which could have untoward effects on prognoses if a tumor is indeed present. Fibrous changes, which may obscure subsequent mammographic findings, may occur in the breast following such needle biopsies.

FNA of breast masses provides cytologic material sufficient to distinguish benign from neoplastic breast lesions, and can determine tumor nuclear grade and identify certain tumor types, including lobular neoplasia, medullary carcinoma, colloid carcinoma, and apocrine carcinoma. Certain benign lesions, including fibroadenoma, granular cell tumor, intraductal papilloma, and breast abscess, can also be recognized. Some benign changes, including fat necrosis, radiation changes within the breast, and hematomas, can be distinctive, but may be associated with significant cellular atypia and account for false positive cases in some series [4,5,8]. FNA cytology is not effective if a breast mass cannot be palpated or distinguished from fibrous tissue contiguous within the breast. It has been applied to nonpalpable masses detected by mammography, em-

TABLE I. Fine Needle Aspiration (FNA) Cytology *versus* Needle Biopsy (NB)

	FNA	NB
Needle size	20 gauge or less	14–16 gauge
Cellular sample	Yes	No (needle washings)
Tissue sample	No (cell block)	Yes
Local anesthesia	No	Yes
Risk of tumor in needle tract	No	Recognized risk
Readily applicable to stereotactic guidance	Yes (but seldom used)	Yes

ploying stereotactic techniques in conjunction with needle biopsy [9–11].

Atypical ductal hyperplasia can be distinguished from ductal carcinoma *in situ* (DCIS) and ductal-type adenocarcinoma by the presence of myoepithelial cells about and within the cellular groups. In addition, there is relative uniformity and orderliness of the cell groups. Atypical hyperplasia may be difficult to distinguish from benign changes, or atypia that cannot be otherwise specified [12]. Static image analysis of the cellular sample for DNA ploidy may be of value in such cases [13]. Atypical ductal hyperplasia is a specific histopathologic diagnosis. The cytologic findings reveal atypical cells with myoepithelial cells, but cannot assess the number of ducts involved or the degree of proliferation [14].

The cytologic samples obtained from FNA are adequate to identify breast neoplasia; however, they do not definitively distinguish DCIS from invasive adenocarcinoma [15–19]. FNA can complement needle biopsy in evaluating nonpalpable breast masses, and may increase the detection rate in calcified lesions [9–11].

The cytologic features of FNA material from DCIS have been described as cellular specimens with numerous isolated cells, few neoplastic cell groups, and apparent loss of cell adhesion. The cells have a high nuclear/cytoplasmic ratio, and the nuclei have prominent macronucleoli. The cell groups in DCIS may be mixed with benign epithelial cells and macrophages, and have more regular nuclear spacing and less pronounced nuclear overlapping than those found in invasive carcinoma [19]. Individual cells of DCIS are not morphologically distinct from the cells of invasive adenocarcinoma. However, cell cluster uniformity and orderliness may be more evident than in ductal-type carcinoma. Generally, there are fewer single cells and fewer discohesive tumor cell groups in carcinoma *in situ* (Table II).

In a study of 85 cases, the sensitivity of detecting DCIS by FNA has been reported as 0.70, with clinical examination having a sensitivity of 0.61 and mammography a sensitivity of 0.74. Clinical exam and mammography had a combined sensitivity of 0.93 [20]. In evaluating the FNA results of these 85 DCIS cases, only 34% (29 cases) had definitive cytologic evidence of neoplasia and 28% (23) were suspicious for neoplasia. Twenty-seven percent (24) had negative cytologic findings and 11% (9) were unsatisfactory [20].

In a study of 843 breast FNA cases, 9 were from patients with *in situ* carcinoma; of these, 2 were interpreted as benign, 4 as suspicious, and 3 as probable or definitive carcinoma [21]. In a report of 12 DCIS cases, 6 of comedo type and 6 of cribriform and solid type, from a series including 355 invasive ductal carcinoma cases, the authors were unable to identify cytologic features that would reliably distinguish intraductal from invasive-type carcinoma [18].

Some cytologic differences have been observed

TABLE II. Cytopathological Findings

Feature	Atypical Ductal Hyperplasia	Ductal Carcinoma *In Situ*	Ductal Carcinoma
Cell clusters	Yes	Yes	Yes
Size	Variable	Variable	Variable
Uniformity	Yes	Yes	No
Orderly	Yes	Yes	No
Cell pleomorphism	Yes	Yes	Yes
Single cells with groups	Some	Some	Prominent
Myoepithelial cells	Prominent	Rare	Rare
Necrotic background	No	Rare	Yes

between lobular carcinoma and lobular carcinoma *in situ* (LCIS) (Table III). Although both have neoplastic-appearing cells with cytoplasmic inclusions, lobular carcinoma samples generally are more cellular and have a higher fraction of solitary tumor cells. The nuclei are generally more pleomorphic with more prominent nuclear hyperchromasia, chromatin clumping, and radial dispersion of nuclear chromatin [22]. If numerous cell groups are found, the cellular features may be difficult to differentiate from the usual adenocarcinoma. These differences do not appear sufficiently distinctive to make a definitive separation of LCIS from lobular carcinoma on cytology alone. These criteria have not been sufficiently tested in any well-controlled trial to date. Metastatic or primary small cell carcinomas may resemble lobular carcinoma [5].

Atypical lobular hyperplasia has cellular features essentially the same as those seen in LCIS [22,23]. Histologically, this distinction is usually made by the relative percent of terminal duct lobular units involved in the lobule as determined from histologic sections [14]. This type of quantitative assessment is not possible with FNA cytology. In such cases, the cellular sample may contain individual cells or some cell groups that support an interpretation of carcinoma; however, the cellular sample may be limited, resulting in an interpretation of suspicious for adenocarcinoma, or positive, but inclusive for adenocarcinoma. In such cases, biopsy of the mass is necessary for diagnosis (Table III).

A recognizable false negative rate primarily related to insufficient cellular sampling is associated with FNA of the breast. Approximately 25% of DCIS may be interpreted as negative by FNA, and 9% as unsatisfactory [20]. It is not unusual in breast biopsy samples to identify invasive carcinoma in one or more small foci associated with DCIS or LCIS. Sampling problems also occur with NB techniques, although this has not been studied in detail. The risk of false negative findings in NB requires further investigation, as does the question of whether a lower false negative rate is found when FNA is used alone or with NB. Single NBs of breast masses may give false negative findings in up to 20% of reported cases, and has generally been found less accurate than FNA in evaluating palpable masses [5,24].

The NB technique can be augmented with cytopathology by collecting the cellular sample available when the needle washing sample is collected or by performing FNA in conjunction with the biopsy [9,10]. These NB washings are often cellular and can support the NB tissue diagnosis, or even diagnose tumors if the needle biopsy is marginal or not diagnostic. Multiple

TABLE III. Cytopathological Findings*

Feature	Atypical Lobular Hyperplasia	Lobular Carcinoma *In Situ*	Lobular Carcinoma
Cellularity	Minimal	Minimal	Moderate
Cell clusters	Rare	Rare	Rare
Size	Small	Small	Small
Uniformity	Yes	Yes	No
Orderly	No	No	No
Cell pleomorphism	Slight	Slight	Moderate
Single cells with groups	Some	Some	Prominent
Cytoplasmic inclusions	Yes	Yes	Yes
Necrotic background	No	No	No

*Modified from Salhany KE and Page DL [22]

stereotactically guided NBs are usually performed with 3–5 separate NB samples, so that the risk of missing the diagnostic lesion or losing diagnostic cellular material is reduced.

If cytopathologic features alone are not sufficient to distinguish the DCIS cells from invasive carcinoma, can other features make this distinction? Nuclear grading, DNA ploidy, thymidine labelling, Ki-67, nucleolar organizer regions (AgNOR), and HER-2/*neu* protein immunostaining have been evaluated in a number of studies comparing DCIS and adenocarcinoma [25].

The nuclear grade of a recognized tumor can be determined by the cytologic findings of FNA, because nuclear size, nuclear chromatin pattern, and evidence of nucleoli are applied in grading (Table IV) [22,26,27]. Cytologic nuclear grading from FNA material has been reported to correlate with nuclear grade from histologic sections in 95% of cases [41]. Although DCIS with high nuclear grade in a histologic section identifies a subset associated with a higher rate of local recurrence [27], the nuclear grade of the DCIS can be highly variable and does not distinguish DCIS from invasive carcinoma.

Cellular samples from FNAs, which include tumor cells, are generally satisfactory for measuring DNA ploidy by static image analysis. In general, FNA samples from breast tissues have limited cellularity, precluding the use of flow cytometry for DNA ploidy analysis or calculating tumor S-phase fraction. With flow cytometry, analyzed cells are lost for histologic or cytologic evaluation. Usually a minimum of 25,000 cells are needed for analysis. In spite of these limitations, some success with flow cytometry has been reported [28,29]. Ploidy studies by flow cytometry on DCIS have demonstrated that 41–46% of cases are aneuploid (Table V) [26,30]. This is similar to the frequency of aneuploidy in invasive ductal-type breast adenocarcinomas [16]. DNA ploidy analysis of DCIS associated with invasive carcinoma by static image analysis may be informative in comparing cell populations, but requires careful control to avoid falsely identifying the sample as aneuploid [31]. There is an association between high nuclear grade and aneuploidy in DCIS. However, the presence or absence of a DNA aneuploid cell population does not distinguish DCIS from invasive ductal carcinoma. DNA ploidy calculations, and estrogen and progesterone receptors, are not of value in making the distinction between invasive and noninvasive neoplasia of the breast.

TABLE IV. Nuclear Grading in Breast Carcinoma [40,41]

Nuclear Grade	Nuclear Size	Nuclear Chromatin Pattern	Nucleoli
Grade 1	Uniform Minimal enlargement	Fine, evenly dispersed	Indistinct
Grade 2	Some variation 2× the size of Grade 1	Slightly coarse with some clumping	Present but not prominent
Grade 3	Highly variable 3× the size of Grade 1	Coarse and clumped with clearing	Prominent

TABLE V. Aneuploidy by Flow Cytometry in Ductal Carcinoma *In Situ* (DCIS)

Total Cases of DCIS	Number Aneuploid	% Aneuploid	Reference
56	23	41%	[26]
74	34	46%	[30]

The tumor S-phase fraction can be estimated by detecting cell cycle proteins such as Ki-67 as measured by immunoperoxidase. Such estimates are more readily made from NB specimens than FNAs. There is inconclusive data regarding the value of Ki-67 in distinguishing DCIS from invasive carcinoma [16]. Although Ki-67 expression is generally lower in DCIS than in invasive carcinoma [32–34], the Ki-67 score is reportedly higher in DCIS than in tubular or colloid carcinoma [32]. Thymidine labelling studies have demonstrated a higher labelling in comedo DCIS than *in* other types of DCIS or invasive carcinoma [35].

AgNORs have been studied in DCIS and invasive ductal-type adenocarcinoma (DTCA) (Table VI). In general, AgNOR counts are higher in DTCA than in DCIS [16,33,36,37]. In one study, 89% of the DCIS cases had lower AgNOR counts than the adenocarcinoma group. However, there was significant overlap in the AgNOR counts, eroding the reliability of distinguishing the cells of DCIS from DTCA in cellular samples (Table VI).

Study of the expression of HER-2/*neu* protein expression in DCIS, including comedo or solid as well as papillary or cribriform types compared to DCIS with minimal invasion or invasive adenocarcinoma, has demonstrated that DCIS of comedo type expresses HER-2/*neu* protein in 2/3 of the cases, whereas 1/3 or less of the invasive carcinomas express the protein. Of interest is that the papillary or cribriform DCIS did not express HER-2/*neu* protein in this limited study [38,39].

Although these findings regarding DCIS and adenocarcinoma do not distinguish these lesions, they do document the similarities between these two neoplastic cell populations. They do not discriminate the features that permit the invasive cellular component to invade or metastasize. These studies do, however, strongly suggest that there are two subsets of DCIS, one with a high risk of invasion and one with a low risk. Focused work has distinguished comedo, cribriform, and micropapillary types of DCIS. Tumors with a high nuclear grade have a higher thymidine labelling index, greater expression of the cell cycle protein Ki-67, higher frequency of overexpression of HER-2/*neu* protein (Table VII), and a recognized risk of recurrence and subsequent development of DTCA. DCIS with comedo necrosis appears to make up a large subset of this high risk group [27].

High nuclear grade intraductal carcinomas can

TABLE VI. Ductal Carcinoma *In Situ* (DCIS) and Invasive Ductal-Type Adenocarcinoma (DTCA) and AgNor Counts

Tumor Type	Cases Reported	AgNor Count	Reference
DCIS	1	6.2	[36]
DCIS	5	17.8 (SD 2.29)	[37]
DCIS	28	7.7 ± 2.7	[16]
DTCA	28	10.7 ± 4.1	[16]
DTCA	70	13.77 (SD 5.19) (4.6–26.97 range)	[33]
DTCA	79	5.5	[36]
DTCA	5	16.9 (SD 10.9)	[37]
LCIS	2	11.4 (SD 6.22)	[37]
Lobular carcinoma	3	4.3	[36]
Lobular carcinoma	5	9.7 (SD 1.66)	[37]

TABLE VII. Ductal Carcinoma *In Situ* (DCIS) Evidence for Two Cell Types

Type	Recurrence Rate [27,42]	HER-2/*neu* Protein Over-expression [39]	Thymidine Labelling Index [35]	Ki-67 Protein [32]
High nuclear Grade 2–3 comedo necrosis[a]	↑	Yes	Higher	Higher
Intermediate group	?↑	Variable	Variable	Variable
Low nuclear Grade 1[b], no necrosis	—	No	Lower	Lower

[a] Includes comedo carcinoma with necrosis as well as other subtypes with necrosis.
[b] Includes nuclear Grade 1 DCIS of all types, predominately cribriform and micropapillary without necrosis

be identified by FNA cytology, but are not usually distinguishable from invasive adenocarcinomas of similar cell type [16,18,19,39]. Current information supports that NB is preferable to FNA alone in evaluating nonpalpable, mammographically detected breast lesions. The role of FNA as an adjunct to NB to reduce the risk of false negative findings remains to be explored.

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