Fine-Needle Aspiration of Gray Zone Lesions of the Breast:
Fibroadenoma Versus Ductal Carcinoma

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While breast lesions have characteristic cytological features, some lesions, particularly adenocarcinoma and fibroadenoma, may present with overlapping features causing erroneous diagnoses. The current study aimed to define significant cytomorphologic features predictive of fibroadenoma and adenocarcinoma, respectively. Further, we intended to evaluate the predictive characteristics for differentiation between gray zone lesions and to identify root causes contributing to misdiagnoses. First, direct smears prepared from 14 histology-confirmed fibroadenomas and 14 adenocarcinomas were reviewed and characteristics of commonly encountered morphologic features were assessed. We then retrospectively and blindly reviewed nine cytohistologic discrepant cases using the significant characteristic as a guideline, in order to assess whether these discrepant cases could be correctly categorized. Morphologic characteristics predictive of fibroadenoma included moderate cellularity, large, folded cellular sheets/aggregates, staghorn projections, smooth and round borders, monolayers, honeycomb arrangement, smaller nuclear size, and background bipolar cells. Predictive characteristics of adenocarcinoma included high cellularity, loose cohesive sheets/aggregates, pointed projections, irregular borders, larger nuclear size, irregular nuclear membrane, prominent nucleoli, and single atypical epithelial cells. Retrospective, blind review correctly re-classified seven out of nine cytohistologic discrepant cases, including five false negative cases and two false positive cases. Root causes contributing to the misdiagnoses were large branching sheets of carcinoma mimicking folded sheets of fibroadenoma; fibroblasts mimicking myoepithelial cells; apocrine cells mimicking carcinoma cells; and not recognizing the loose myxoid matrix presenting as soap bubbles in fibroadenoma. In conclusion, this study identified significant characteristics that can assist in achieving accurate diagnosis in a subpopulation of breast aspirates that present with overlapping features. Diagn. Cytopathol. 2013;41:806–811.

Key Words: fine needle aspiration; breast; fibroadenoma; ductal carcinoma; gray zone lesions

Fine-needle aspiration, one of the three components of the triple test, is an important and the least invasive tool for preoperative evaluation of breast lesions and planning of patient care.[1,2] Reported diagnostic sensitivity and specificity ranges from 89.9% to 97.2% and from 71.9% to 99.9%, respectively.[2–7] Diagnostic challenges in gray zone lesions of the breast are frequently attributed to overlapping cytologic features of benign and malignant entities inherent to their intrinsic natures. An incidence of 2% of gray zone lesions has been documented and fibroadenoma comprises nearly 50% of such lesions.[8]

Although fibroadenoma has well-defined cytomorphologic features, it is not uncommon that it may deviate from the classic presentation by exhibiting marked cytologic atypia. In contrast, well-differentiated adenocarcinoma with bland cytomorphological features and minimal dyscohesion may mimic fibroadenoma. Thus, false positive and false negative diagnoses may be encountered.[5,9–13] Further, there are a few, isolated case reports describing concurrent fibroadenoma and adenocarcinoma.[14–16]

This study was conducted with the following goals: first, to identify the significant diagnostic features of fibroadenoma and adenocarcinoma, respectively, second, to evaluate the predictive role of those significant features in differentiating the gray zone lesions with overlapping features and, third, to identify the root causes that contributed to the false diagnoses.

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Materials and Methods

All fine-needle aspirations were performed using a 23- or 25-gauge needle attached to a 10-ml syringe in a plastic or metal holder. For each aspiration procedure, averages of 4 passes were obtained and two direct smears were made from each pass. The majority of the direct smears were fixed in 95% ethanol and stained with Papanicolaou stain. Three of the false negative cases had direct smears which were air dried and stained with Diff-Quik stain. Cytologic evaluation as described below was performed on all available smears.

The study was conducted in two phases. First, direct smears prepared from 14 fibroadenomas and 14 adenocarcinomas (two ductal carcinoma in situ and 12 well to moderately differentiated invasive ductal adenocarcinomas) were reviewed to identify significant diagnostic features. All cases were histologically confirmed. For each of the individual cases, detailed characteristics of the following commonly encountered morphologic features were assessed and recorded (Table I): (1) cellularity; (2) morphology of large cellular sheets or aggregates; (3) cellular arrangement; (4) cytologic features of epithelial cells; (5) single cells in the background; (6) presence of fibromyxoid tissue fragments. In the second phase of the study, we sought to evaluate the diagnostic value of the significant features identified in the first phase. Using the significant characteristic as a guideline, we conducted a retrospective, blind review of four histology-confirmed false positive and five histology-confirmed false negative cases that were retrieved from our cytology consult files, and assessed whether these cytohistologically discrepant cases could be correctly categorized. A diagnosis was rendered and root causes contributing to the false diagnosis were documented upon completion of the review for each of the cases.

The associations between morphologic features and histology-confirmed diagnosis (adenocarcinoma versus fibroadenoma) were evaluated by Fisher's Exact test. The odds ratios of the features, with 95% confidence intervals were calculated in the software package R.

Table I. Cytomorphologic Features Assessed in Fibroadenoma and Adenocarcinoma

Overall cellularity (percentage of slide coverage)
Low (<25%)
Moderate (25–50%)
High (>50%)
Pattern of cellular sheets/aggregates
With or without folding
Staghorn versus pointed projections
Cellular arrangement within sheets/aggregates
Smooth or round versus irregular or ragged borders
Monolayer, honeycombing versus overlapping
Tight versus loose cohesion
Nuclear features
Nuclear size > 3× red blood cell versus < 3× red blood cell
Nuclear membrane regularity
Chromatin texture
Nucleolear prominence
Background single cells
Atypical epithelial cells
Atypical apocrine cells
Bipolar cells
Fibroblasts
Lymphocytes.
Presence of fibromyxoid tissue fragments

Results

Review of direct smears prepared from 14 fibroadenomas and 14 adenocarcinomas identified several morphologic characteristics predictive of fibroadenoma and adenocarcinoma, respectively. The odds ratios of predictive features, with 95% confidence intervals, are presented in Table II. Accordingly, moderate cellularity, large, folded cellular sheets or aggregates, staghorn projections, smooth and round borders of the sheets or aggregates, monolayer, honeycomb arrangement of the cells within the sheets or aggregates, smaller nuclear size (< 3× red blood cell), as well as the presence of background bipolar cells, were associated with fibroadenoma (Fig. 1). On the other hand, high cellularity, loose cohesive sheets or aggregates, pointed projections, irregular borders of the sheets or aggregates, larger nuclear size (> 3× red blood cell),

Table II. Cytomorphologic Characteristics Predictive of Fibroadenoma vs. Adenocarcinoma

Feature	*Adenocarcinoma (n = 14)*	*Fibroadenoma (n = 14)*	*Odds ratio (95% CI)*	*P-value*
High cellularity	11 (79%)	3 (21%)	11.9 (1.73, 119.5)	0.007
Folded large sheets/aggregates	2 (14%)	13 (93%)	0.017 (0.0003,0.20)	<0.0001
Staghorn projections	2 (14%)	9 (64%)	0.10 (0.008, 0.74)	0.018
Pointed projections	9 (64%)	0 (0%)	∞ (3.55, ∞)	0.0006
Smooth, round borders	0 (0%)	14 (100%)	0 (0, 0.043)	<0.0001
Irregular borders	7 (50%)	0 (0%)	∞ (2.04, ∞)	0.006
Honeycombing arrangement	2 (14%)	10 (71%)	0.076 (0.006,0.56)	0.006
Loose cohesive sheets or aggregates	14 (100%)	4 (28%)	∞ (4.68, ∞)	0.0001
Nuclear size > 3× red blood cell	14 (100%)	4 (28%)	∞ (4.68, ∞)	0.0001
Nuclear size < 3× red blood cell	0 (0%)	10 (71%)	0 (0, 0.21)	0.0001
Irregular nuclear membrane	14 (100%)	0 (0%)	∞ (23.1, ∞)	<0.0001
Prominent nucleoli	8 (57%)	2 (14%)	7.4 (1.03, 92.8)	0.05
Bipolar cells	0 (0%)	14 (100%)	0 (0, 0.43)	<0.0001
Atypical epithelial cells	14 (100%)	0 (0%)	∞ (23.1, ∞)	<0.0001

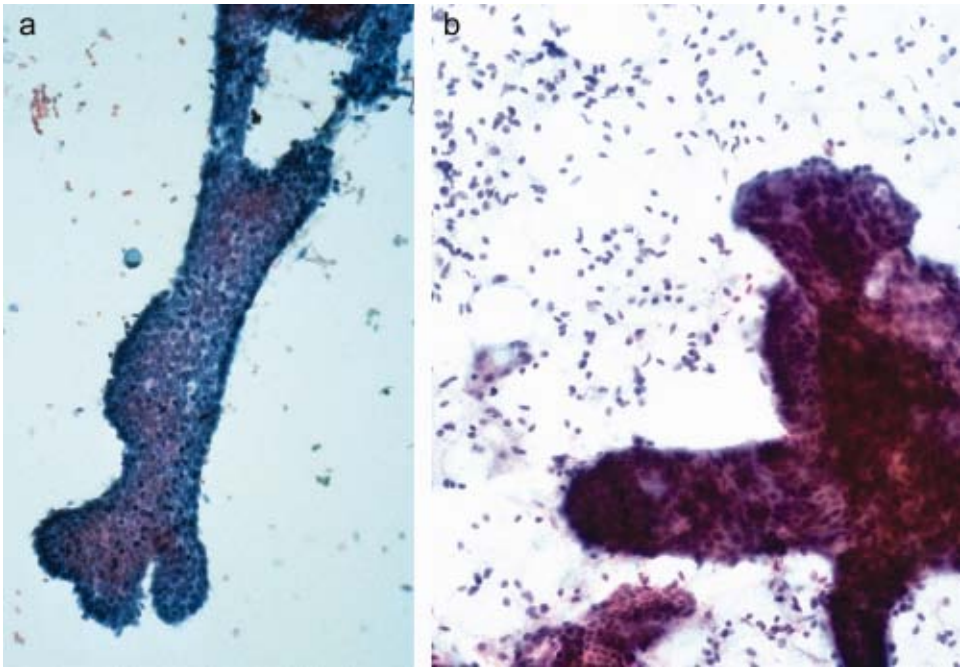

Fig. 1. Cytologic features predictive of fibroadenoma. Cellular smears contain large, folded sheets or aggregates with staghorn projections and smooth and round border. Nucleoli are arranged in a monolayer, honeycomb pattern. Numerous bipolar cells are present in the background (Papanicolaou stain, 20×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

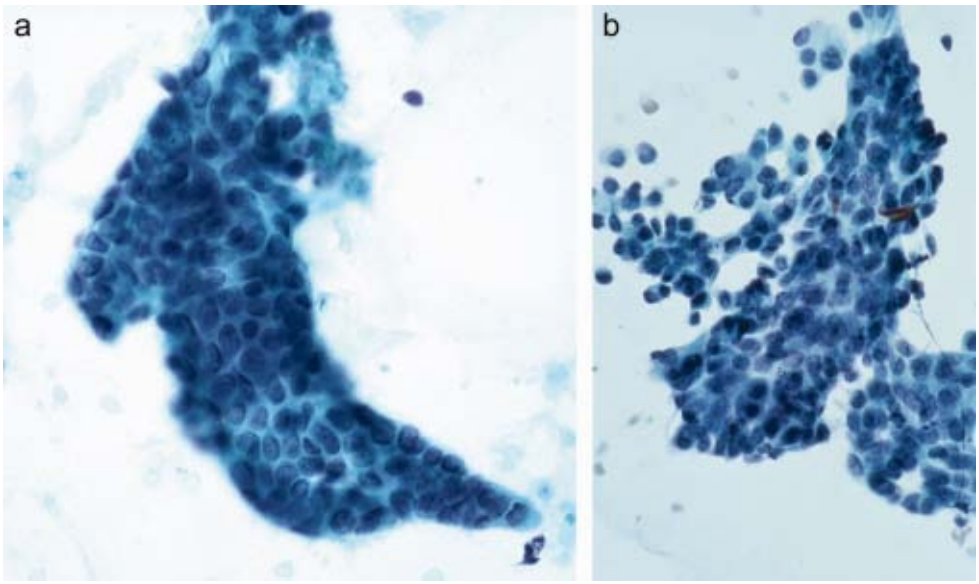

Fig. 2. Cytologic features predictive of adenocarcinoma. Cellular smears contain loose cohesive sheets or aggregates with loss of honeycomb arrangement, pointed projections, and irregular borders. Enlarged nuclei with irregular nuclear membranes and prominent nucleoli, as well as single atypical epithelial cells are appreciated (Papanicolaou stain, 40×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

irregular nuclear membrane, prominent nucleoli, and presence of single atypical epithelial cells were predictive of adenocarcinoma (Fig. 2).

A retrospective, blind review of the nine cytohistologically discrepant cases that applied the aforementioned significant characteristics correctly re-classified all five false negative cases as adenocarcinoma. These cases presented with numerous large sheets that sometimes branched or folded in a background of variable number of stripped nuclei assumed to be of fibroblastic origin that were misinterpreted as bipolar cells (Fig. 3). Upon further review, it was noticed that the fragments frequently had at least

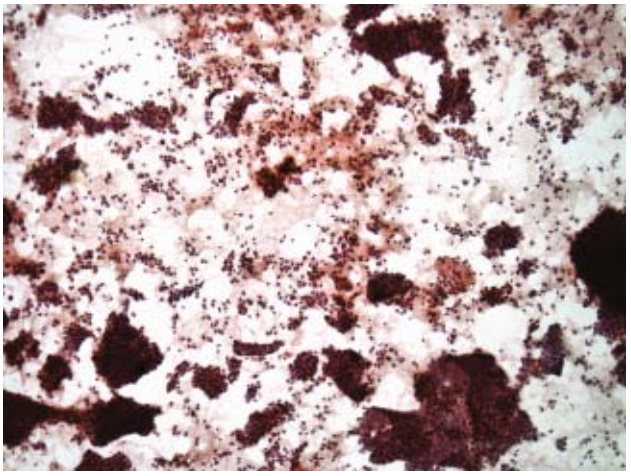

Fig. 3. False negative case. Cellular smear showed numerous large, branched sheets. Some had pointed projections and irregular borders. Background stripped nuclei probably of fibroblastic origin was misinterpreted as bipolar cells (Papanicolaou stain, 10×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

one sharply pointed border, had short cell cords extending directly from the edge, or had ragged and irregular borders, with cells falling off the border and blending with the background. Initial false negative diagnosis occurred in one case due to overlooking a carcinoma component that was superimposed on the fibroadenoma. Two of the four false positive cases were correctly diagnosed as fibroadenoma. We noticed that these two cases contained numerous singly scattered cells in the background admixed with a soap bubble-like material initially thought to be technical artifact. In retrospect, it was recognized that this material corresponded to the loose myxoid matrix, and that the atypical single cells were unusually discohesive apocrine cells. We failed to correct the remaining two false positive cases, in which tight and loose clusters of atypical epithelial cells were interspersed with extensive inflammatory infiltrates. The atypical cells appeared to have increased nuclear/cytoplasmic ratio, coarse chromatin, and prominent nucleoli. Upon review of the surgical specimens, the same features were identified and clearly represented reactive changes in the background of the traditional architecture of fibroadenoma (Fig. 4).

Discussion

Although both fibroadenoma and adenocarcinoma have classic cytomorphologic features and a correct definitive diagnosis can be established in most instances; both entities may deviate from their classic presentations, resulting in histology-confirmed false negative and false positive cytologic results that have been reported.[5,9–13] The current study investigated challenging gray zone lesions of breast, particularly fibroadenoma and adenocarcinoma, which mimicked each other due to the presence of overlapping cytomorphological features associated with the intrinsic nature of the lesions. This study did not include lesions in which the diagnostic gray zone occurred in suboptimal specimens with limited cellularity, obscuring blood, or poor preparation, etc. We evaluated characteristic morphologic criteria that are commonly encountered and well established in the literature as useful in the differentiation of benign from malignant breast lesions.[17–20] We identified the significant architectural and cytologic features that should alert the reviewers to microscopic mimics and improve the cytohistology concordance of such gray zone lesions.

According to the synopsis developed and approved at the National Cancer Institute-sponsored multidisciplinary conference,[21] either air-dried direct smears stained with Romanowsky-type stains or alcohol-fixed, Papanicolaou-stained direct smears is regarded as an optimal method for preparing cytologic material obtained from breast fine-needle aspiration. It is not unusual that different laboratories set up their preparatory procedures bases on their pathologists' preferences and consequently, it is not uncommon that diagnoses were established on the basis of Papanicolaou staining alone in several published studies.[20,22,23] The current study was based on discrepant cases that were retrieved from our consultation files and that were predominantly submitted as alcohol-fixed smears. Consequently, we chose to perform the statistical evaluation solely of alcohol-fixed, Papanicolaou-stained direct smears in the first phase. One should be aware that some cellular and stromal elements may be less conspicuous with Papanicolaou staining compared with Romanowsky-type stains. For two of four false positive cases included in the current study, loose myxoid matrix of fibroadenoma had a soap bubble-like appearance that was initially misinterpreted as artifact. Only three of the false negative cases had air-dried smears and none contained fibromyxoid matrix. Unfortunately, none of the false positive cases had air-dried smears to assess and therefore we are unable to predict whether Diff-Quik stain could have better identified the myxoid matrix. We believe however, that it is the unusual soap bubble-like appearance that caused its misinterpretation on the Papanicolaou stain rather than being actually highlighted or not by any particular stain.

We observed that nearly 80% of histology-confirmed adenocarcinomas presented with hypercellularity. However, 20% of histology-confirmed fibroadenomas also revealed hypercellularity. The latter was previously described by others.[10,12]

Similar to fibroadenoma, well differentiated adenocarcinoma may reveal various sizes of tissue fragments. Tissue fragments in fibroadenoma showed staghorn projections that were characteristically smooth in at least some of its

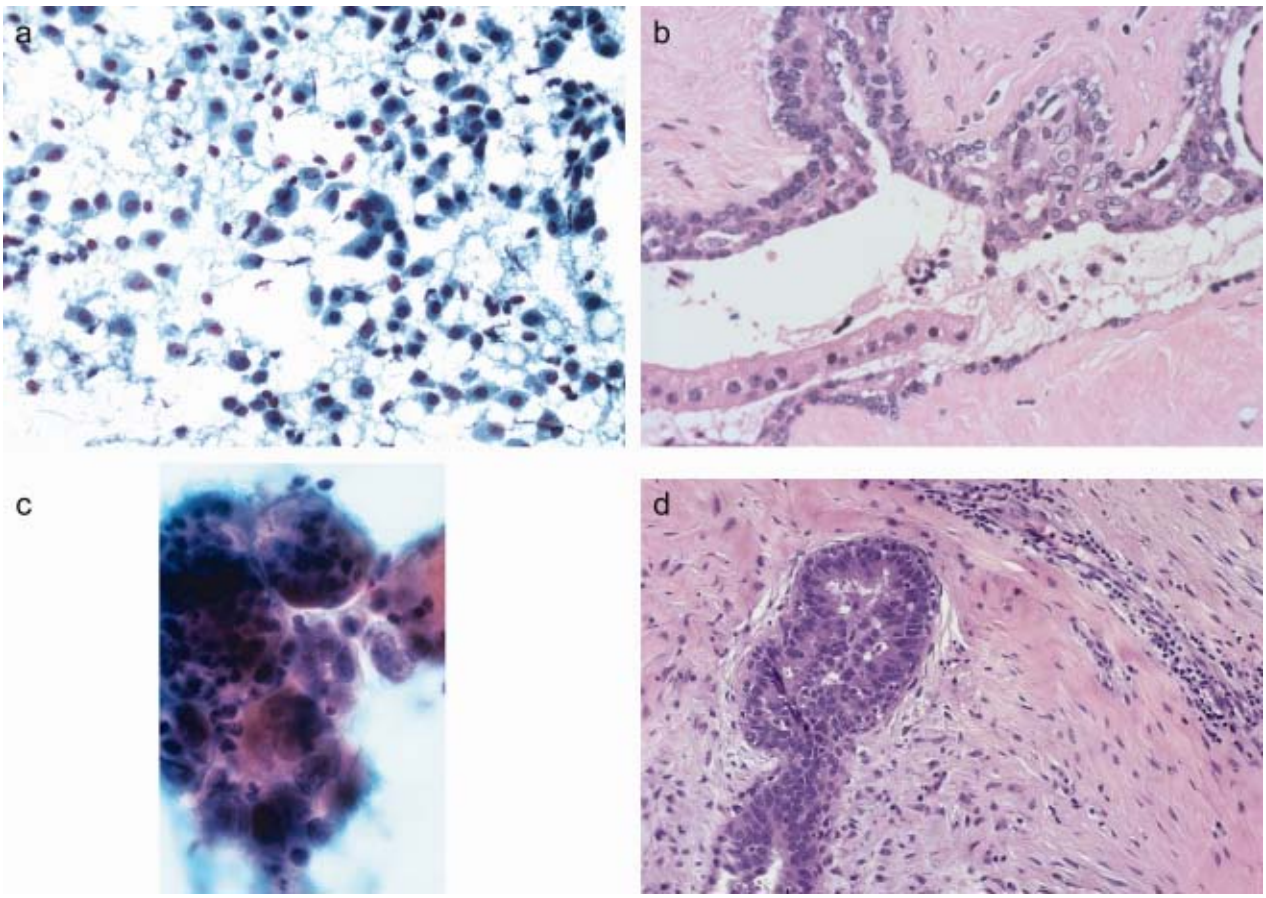

Fig. 4. False positive cases. **A**: Cellular smear contained single, markedly atypical apocrine cells with admixed with a soap bubble-like loose myxoid matrix (Papanicolaou stain, 40×); (**B**) the corresponding histologic specimen revealed fibroadenoma with apocrine metaplasia (H & E stain, 20×). **C**: Tight and loose clusters of atypical epithelial cells were interspersed with inflammatory infiltrates (Papanicolaou stain, 60×); **D**: The same features were identified and clearly represented reactive changes in the background of the traditional architecture of fibroadenoma (H&E stain, 20×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

borders. In contrast, finger-like projections and fragments in adenocarcinoma tended to have irregular borders, frequently with pointed edges or short cords of cells extending at the edge of the small fragments corresponding to the invasive trabeculae and cords seen on the histologic sections. The cellular groups also frequently appeared to have cells falling off their borders, suggesting discohesion. It is noteworthy to mention that well differentiated adenocarcinoma may present as tissue fragments showing subtle architectural atypia and mimicking a monolayer, honeycombing arrangement. This deceptive pitfall may be avoided by further review at higher magnification to evaluate the borders and the individual cellular features.

It is not surprising to find that both cytologic and nuclear atypia were predictive of adenocarcinoma. More importantly, atypical cells may not always represent carcinoma cells, and correct identification of cell types/origin is vital to avoid false positive diagnosis. A few reported cases documented that fibroblasts with prominent nucleoli and atypical apocrine cells were causes of false positive diagnosis.[24,25] In the current study, two false positive diagnoses were correctly reclassified as fibroadenoma upon recognition of atypical and discohesive apocrine cells that were initially misinterpreted as carcinoma cells. In addition, the current study demonstrates that the presence of severely atypical epithelial cells associated with reactive conditions, such as inflammation, is another cause of false positive diagnoses. Correct identification of cell types and origins is also crucial to avoiding false negative diagnoses; all five false negative diagnoses presented herein could have been prevented had cell types been correctly identified. Initially, background stripped nuclei assumed to be of fibroblastic origin was misinterpreted as bipolar cells in these cases. In this regard, immunostaining with the myoepithelial cell marker p63, when used in conjunction with morphologic evaluation, may be useful to correctly categorize problematic cases.[22,26]

It was noticed in Table II that some of the morphological features only presented in fibroadenoma or ductal carcinoma. For example, 10 out of 14 fibroadenomas showed

nuclear size $< 3\times$ red blood cell and bipolar cells presented in all 14 fibroadenomas; on the other hand, irregular nuclear membrane and atypical epithelial cells in 7 and 14 ductal carcinomas. These findings should be carefully interpreted since both entities are known to present with overlapping features. It is by no means certain that a correct diagnosis should be established based upon a single, isolated cytologic feature. Furthermore, features such as mitosis and necrosis are generally associated with malignancy and would usually trigger at least a suspicious diagnosis. Since all our discrepant cases lacked these features, we did not consider them useful in the evaluation of overlapping lesions since by definition they lacked the definitive diagnostic features.

It is understandable that various histologic changes seen in fibroadenoma or ductal carcinoma could affect cytologic diagnosis. The correlation between histologic subtypes of fibroadenoma or ductal carcinoma and cytologic diagnosis has been described previously.[18,20] Accordingly, mastopathic type fibroadenoma compared to common and organoid types had a significantly higher chance of falling into the "indeterminate" of "suspicious for malignancy" diagnostic category; mixed ductal/mucinous carcinoma may pose a diagnostic challenge to distinguish from benign entities with abundant extracellular mucinous material in cytology specimens. The current study focused on identification of significant characteristics that can assist in achieving accurate diagnoses in a subpopulation of breast aspirates that present with overlapping features. Root causes contributing to the erroneous diagnoses included: cases of carcinoma presenting with large branching sheets, mimicking folded sheets and staghorns traditionally associated with fibroadenoma; stripped fibroblasts mimicking myoepithelial cells; unusual discohesion of apocrine cells, mimicking single atypical cells traditionally seen in carcinoma; not recognizing the loose myxoid matrix presenting as soap bubbles in fibroadenoma.

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