Diagnostic Value of Imprint Cytology During Image-Guided Core Biopsy in Improving Breast Health Care

Shahla Masood, Dian Feng, Osman Tutuncuoglu, Gabor Fischer, Maryam Bakhshandeh, Roger L. Bertholf, and David Wolfson
Department of Pathology, University of Florida College of Medicine-Jacksonville, Jacksonville, Florida

Abstract. The aim of this study was to investigate the accuracy of imprint cytology (IC) of breast core biopsy under ultrasound guidance and to assess the value of a rapid on-site preliminary diagnosis of breast lesions. A total of 437 breast core needle biopsies under ultrasound guidance with touch imprint cytology, histology, and final diagnosis were reviewed. These cases were collected from archived files at our institution. Of 437 core biopsies, IC classified 241 (55%) as benign; 22 (5%) as probably benign; 28 (6%) as probably malignant; 107 (25%) as malignant; and 39 (9%) as inadequate for IC diagnosis. Histological classifications for the 437 cases were: 285 (65%) benign; 132 (30%) malignant; 16 (4%) atypical hyperplasia; and 4 (1%) inadequate specimen. The overall sensitivity and specificity indices of IC were 95% and 96%, respectively, for benign and probably benign lesions vs malignant and probably malignant breast lesions. The overall positive and negative predictive values were 91% and 97%, respectively. The overall accuracy was 95% (379 of 398 cases, excluding specimens inadequate for IC diagnosis). IC of ultrasound-guided core needle biopsy provides a rapid and reliable preliminary diagnosis for breast lesions; it also serves as a means to verify the adequacy of biopsy specimens and to optimize the biopsy procedure. Use of IC may reduce anxiety in patients with benign lesions and expedite the diagnosis and assessment of treatment options in patients with breast cancer.

Introduction

Rapid diagnostic centers that offer both radiology and cytology services have enhanced the care of patients with breast lesions [1,2]. While fine needle aspiration cytology has high diagnostic sensitivity and specificity for diagnosing breast cancer, the choice of treatment options (e.g., neo-adjuvant chemotherapy, hormonal therapy, and post-mastectomy radiation) depends on formal pathological assessment, making the use of pre-operative core biopsy popular [3-7]. However, core biopsies are not amenable to immediate diagnosis, which rapid diagnostic centers are intended to provide [8-13]. In order to achieve immediate cytological diagnosis and high quality histological assessment from a single diagnostic procedure, we compared imprint cytology (IC) vs histology of ultrasound-guided core biopsies of breast lesions. The aim of this study was to assess the feasibility and value of IC in diagnosing breast lesions.

Materials and Methods

This is a retrospective analysis of 437 symptomatic or asymptomatic patients who had ultrasound-guided core biopsy of breast lesions that, on radiological examination with BIRAD ≥4, were reported by the radiologist as suspicious for breast cancer. Core biopsies were obtained under local anesthesia using a 14-18 gauge automated needle and making 2-7 passes per lesion. The cores were gently rolled against glass microscope slides to obtain imprint smears, and then were immersed in formalin. At least two smears were prepared from each core. One slide was air dried and stained with Diff-Quik for immediate on-site assessment by cytopathology fellows; the other slide was spray-fixed with 95% ethyl alcohol and was later stained with Papanicolaou for further cytomorphological assessment. The core biopsies were embedded in paraffin, cut in 4 μm sections, and stained with H&E.

These cases were collected from archival cases at the Department of Pathology, University of Florida Health Science Center-Jacksonville, from 2005 to 2009. The IC diagnosis was made independently from the IC diagnosis; the diagnoses were grouped as follows: inadequate (less than 4 clusters of mammary epithelial cells); benign; probably benign;
probably malignant; and malignant according to diagnostic criteria [14]. When the IC results were obviously benign or malignant breast lesions, no further core biopsy passes were required after the minimum of two core samples. When the IC results were inadequate or undetermined, additional passes of core biopsy were requested, up to a maximum of six. The IC diagnoses were compared with the corresponding histopathological findings and final diagnoses.

Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of IC for benign vs malignant lesions, as well as overall benign and probably benign vs malignant and probably malignant, were calculated as follows:

- **Sensitivity** = TP/(TP+FN);
- **Specificity** = TN/(TN+FP);
- **Positive predictive value** = TP/(TP+FP);
- **Negative predictive value** = TN/(TN+FN);
- **Accuracy** = (TP+TN)/(TP+FP+TN+FN);

where TP = true positive; TN = true negative; FP = false positive; and FN = false negative.

### Results

All patients were female. The concordance of IC and histology is summarized in Table 1. Of 437 IC cases, 241 (55%) were benign; 22 (5%) were probably benign; 28 (6%) were probably malignant; 107 (25%) were malignant; and 39 (9%) were inadequate specimen. The corresponding distribution of histological diagnoses was 285 (65%) benign; 132 (30%) malignant; 16 (4%) atypical hyperplasia; and 4 (1%) inadequate specimen.

When the benign and probably benign cases were grouped together (as in Table 2), and the malignant and probably malignant cases were similarly grouped, and 39 subjects with inadequate specimens for imprint cytology were excluded, the overall sensitivity and specificity indices were 95% (123/130) and 96% (256/268), respectively; these calculations also grouped biopsy specimens with atypical hyperplasia as benign.

When the 39 cases with inadequate IC specimens were included in the total, the sensitivity and specificity indices were 93% (123/132) and 85% (256/301), respectively. The false negative rate of IC was 2.7% for histologically malignant lesions and 3.4% for atypical hyperplasia.

The predictive value and the accuracy of diagnostic procedures are influenced by the pre-test probability of disease. When applied to patients who had undergone core biopsies, which primarily includes patients with a high suspicion of disease, the positive and negative predictive values of IC were 91% (123/135) and 97% (256/263), respectively, and the overall accuracy was 95% (379/398). For strictly benign versus strictly malignant breast lesions, the sensitivity and specificity were 95% (102/107) and 98% (236/241), respectively. Since the numbers of false positive and false negative results in the benign and malignant groups were the same (5), the positive and negative predictive values were also 95% and 98%, respectively. The accuracy of IC for identifying benign vs malignant cases was 97% (338/348).

The clinical performance of IC can also be viewed in the context of how the results influence practice. For example, if IC is being used as a screening test, and all patients with IC findings other than malignant were referred for core biopsy, then IC would have correctly identified 102 of the 132 cases in which the core biopsy detected malignancy, and the sensitivity of the screening test would be 77% (102/132). Similarly, if the assessment of benign by IC were used to rule out malignancy, then 236 (benign + atypical hyperplasia) of the total of 301 core biopsy-benign (and atypical hyperplasia) cases were correctly identified by IC, corresponding to a specificity of 78% (236/301).

Of the 39 cases in which IC revealed an inadequate core biopsy, the majority were histologically benign (33 cases, 85%), consisting of stromal sclerosis (25 cases, 75%) with or without mammary ductal epithelium or microcalcification, scar tissue associated with post-radiational changes (3 cases, 9%), fat necrosis (3 cases, 9%), and periductal chronic inflammation (2 cases, 6%). Four cases (10%) were inadequate for diagnosis, showing only fat and blood on the smears, and only vascular and fibroadipose tissue on the core biopsy. However, 2 cases (5%) were malignant, exhibiting only blood on IC but showing scant infiltrating ductal carcinoma in a sclerotic background in the histological sections. Of 241 benign lesions by IC, 95% were benign and 3% were classified as atypical hyperplasia by histology.

The accuracy of IC for identifying benign lesions including atypical hyperplasia was 98%. Most benign breast lesions were comprised of fibrocystic changes (Fig. 1, panels A and B), proliferative breast disease without atypia, intramammary lymph node, and granulomatous reaction. These benign lesions usually demonstrated abundant clusters of normal appearing ductal cells admixed with myoepithelial cells on smears (Fig. 1, panel C). The corresponding histology showed ductal hyperplasia without atypia (Fig. 1, panel D). However, the false negatives (2%, 5 cases) were composed of abundant normal ductal tissue with only small fragments of tumor component. Thus, abundant normal appearing clusters of ductal epithelial cells and myoepithelial cells with only rare neoplastic cells resulted in the malignancy being missed.

Among the 50 indeterminate cases by IC, 22 were judged as probably benign and 28 were ruled as probably malignant. The concordance indices of probably benign and
Fig. 1. Panel A: Imprint cytology of a core needle biopsy of a benign breast lesion demonstrating a cluster of apocrine cells (Diff-Quick, x600). Panel B: The corresponding tissue biopsy of the same case with apocrine metaplasia and cyst formation (H&E, x400). Panel C: Imprint cytology of core needle biopsy of a benign breast lesion showing a cluster of overlapping epithelial/myoepithelial cells and a foamy macrophage (Diff-Quick, x400). Panel D: The corresponding tissue biopsy of the same case demonstrating features of ductal hyperplasia (H&E, x200).

Table 1. Concordance between imprint cytology and histologic diagnosis of breast lesions in 437 cases of core biopsies.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Imprint cytology, # of cases (%)</th>
<th>Benign (%)</th>
<th>Malignant (%)</th>
<th>Inadequate (%)</th>
<th>Atypical hyperplasia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate</td>
<td>39 (9)</td>
<td>33 (85)</td>
<td>2 (5)</td>
<td>4 (10)</td>
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<tr>
<td>Benign</td>
<td>241 (55)</td>
<td>230 (95)</td>
<td>5 (2)</td>
<td>6 (3)</td>
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<tr>
<td>Probably benign</td>
<td>22 (5)</td>
<td>17 (77)</td>
<td>2 (5)</td>
<td>3 (18)</td>
<td></td>
</tr>
<tr>
<td>Probably malignant</td>
<td>28 (6)</td>
<td>2 (7)</td>
<td>21 (75)</td>
<td>5 (18)</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>107 (25)</td>
<td>3 (3)</td>
<td>102 (95)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>437 (100)</td>
<td>285 (65)</td>
<td>132 (30)</td>
<td>4 (1)</td>
<td>16 (4)</td>
</tr>
</tbody>
</table>

Table 2. Concordance between imprint cytology and histologic diagnosis of breast lesions in 437 cases of core biopsies.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Imprint cytology, # of cases (%)</th>
<th>Benign (%)</th>
<th>Malignant (%)</th>
<th>Inadequate (%)</th>
<th>Atypical hyperplasia (%)</th>
</tr>
</thead>
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<tr>
<td>Inadequate</td>
<td>39 (9)</td>
<td>33 (85)</td>
<td>2 (5)</td>
<td>4 (10)</td>
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<tr>
<td>Benign/probably benign</td>
<td>263 (60)</td>
<td>247 (94)</td>
<td>7 (3)</td>
<td>9 (3)</td>
<td></td>
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<tr>
<td>Malignant/probably malignant</td>
<td>135 (31)</td>
<td>5 (4)</td>
<td>123 (91)</td>
<td>7 (5)</td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>437 (100)</td>
<td>285 (65)</td>
<td>132 (30)</td>
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Fig. 2. Panel A: Imprint cytology of core needle biopsy showing a few loosely cohesive epithelial cells with no myoepithelial cells present (Diff-Quick, x400). Panel B: The corresponding tissue biopsy of the same case with a low nuclear grade infiltrating ductal carcinoma (H&E, x400). Panel C: Imprint cytology of a core needle biopsy exhibiting many isolated neoplastic cells with monomorphic appearance. The neoplastic cells are composed of blue cytoplasm and rather evenly sized nuclei with a high nuclear-cytoplasmic ratio and absence of myoepithelial cells. These features are characteristic of a low nuclear grade ductal carcinoma (Diff-Quick, x400). Panel D: The corresponding tissue biopsy of the same case with typical features of low nuclear grade infiltrating ductal carcinoma (H&E, x200). Panel E: Imprint cytology of a core biopsy illustrating a loose cluster of neoplastic cells with pleomorphic and hyperchromatic nuclei, irregular and prominent nucleoli. These features demonstrate morphologic features of high nuclear grade ductal carcinoma (Diff-Quick, x600). Panel F: The corresponding core tissue biopsy of the same case demonstrating large clusters of neoplastic cells with marked variation of nuclear size and shape with a high nuclear-cytoplasmic ratio and characteristics of high grade primary breast carcinoma (H&E, x600).

probably malignant findings by IC with histology were 95% and 75%, respectively. The primary reason for lower concordance in the probably malignant group was that 18% of these cases had a histological diagnosis of atypical hyperplasia. These cases usually presented with a few individual atypical cells on the imprint slides, making it difficult to differentiate atypical hyperplasia from low grade carcinoma. Occasionally, only a small cluster of low nuclear grade malignant cells appeared in the biopsy, and therefore only a few atypical cells with clusters of normal ductal cells were present on the IC. In those circumstances, a diagnosis of malignancy was favored (Fig. 2, panels A and B).

The overall accuracy of IC for malignant breast carcinomas was 95%. There were 5 (5%) false positive cases, including 2 papillary lesions with atypia, 1 granuloma, and 2 atypical hyperplasia diagnosed histologically. All of the false positive cases showed an abundance of atypical individual cells on the smears. The IC of low nuclear grade ductal carcinoma often demonstrated numerous singly distributed neoplastic cells with blue cytoplasm, along with evenly sized and eccentrically placed nuclei with indistinct or distinct nucleoli (Fig. 2, panel C). In comparison, direct smears of high nuclear grade duct cell carcinomas usually demonstrated hypercellularity with marked variation of neoplastic cells and nuclei. These nuclei were hyperchromatic and over-crowded with molding, irregular nuclear membranes, and prominent, irregular nucleoli (Fig. 2, panel E). The core biopsies had the appearance of high grade infiltrating ductal carcinoma, illustrated in Fig. 2, panels D and F.

Discussion

Successful treatment of breast cancer requires early detection, accurate diagnosis, and effective
management [1-7,11]. Imprint cytology is a quick and reliable way to diagnose breast lesions in a clinic setting [9,10,12,13]. The concept of rapid, “one-stop” diagnostic services for breast cancer has emerged over the past decade [3,4,7-13]. Currently, ultrasound-guided core biopsy is a common diagnostic procedure for non-palpable breast lesions [1-7]. However, to render a diagnosis from the core biopsy requires 24-48 hours for fixation and tissue processing. The histopathological diagnosis is typically available within 24-48 hours. Under these circumstances, patients may receive the diagnosis on a follow-up appointment or by a phone call or letter as long as a week after the procedure. In contrast, IC can be prepared within 1-2 minutes by touching a biopsy sample on a glass slide, and the preliminary diagnosis may be given to the patient shortly after the biopsy procedure is completed. With more frequent use of core biopsies by breast health centers, it is important to explore techniques that provide high quality patient care, while reducing patients’ anxiety and expense.

In our department, IC has been used to assess specimen adequacy of ultrasound-guided core biopsies for several years. Beyond the assessment of specimen adequacy, however, IC offers the added benefit of immediate diagnosis, thereby enhancing the quality and timeliness of care the patient receives. The majority of patients (65%) had benign breast lesions, and the high accuracy (98%) of IC for benign breast lesions makes it feasible to render a preliminary diagnosis to patients at the time of the procedure. A benign diagnosis by IC may reduce the stress and anxiety in patients who have been told that they have breast lesions suspicious for breast cancer. On the other hand, the 95% accuracy of IC for the malignant breast lesions makes it a valuable diagnostic tool for breast cancer. The benefits of immediate preliminary IC diagnosis for patients with breast carcinoma may include making an early appointment for surgical and treatment planning, improving the effectiveness of management, and more timely counseling of the patient. For patients suffering from high nuclear grade breast carcinoma, the IC preliminary diagnosis expedites the initiation of effective treatment planning. For borderline cases, although it is difficult to make a diagnosis on IC with only few atypical isolated cells on imprint slides, it is still possible to provide a “probably benign” or “probably malignant” assessment based on cytological features. Sometimes, the malignant cells may be rare among abundant normal appearing ductal cells, or may not appear at all on the IC because a small tumor is mixed with extensive sclerotic tissue in the core sample. In other cases, a few isolated even sized atypical cells may present on the IC, suggestive of low nuclear grade ductal carcinoma, but the lesion cannot be differentiated from atypical hyperplasia. Under this circumstance, it might be reasonable to report that an undetermined lesion is present, qualified with “probably benign” or “probably malignant,” pending histology for a final diagnosis. Nevertheless, if the imprint cytology results are given to the patients at the time of the biopsy procedure—whether they are benign, probably benign, probably malignant, or malignant—the results should be presented as preliminary. It should be explained to patients that a more definitive histology result will be available in two working days and that there is a small possibility that the final core biopsy tissue diagnosis will be different from the preliminary IC diagnosis.

IC has the benefit of improving the efficiency and safety of the core biopsy procedure. When IC reveals an obviously benign or malignant breast lesion, the procedure may be stopped after a minimum of two core samples. Radiologists usually perform 3 to 7 passes to ensure adequate sampling for core biopsy of breast lesions with ultrasound guidance [1-7]. Thus, IC can eliminate unnecessary passes, reducing the patient’s stress and discomfort, and minimizing the complications associated with more passes such as bleeding, infection, hematoma, and swelling. When IC results are inadequate or undetermined, more passes of the core biopsy should be requested in order to ensure the integrity and adequacy of the specimen, reducing the need for re-sampling. In addition, the immediate preliminary diagnosis by the cytopathologist, based on the IC, along with adequacy assessment of the core biopsy, does not increase the cost of the biopsy procedure, but reduces the costs associated with unnecessary passes and repeated procedures due to inadequate specimens.

The diagnostic accuracy of IC results depends on several factors, including the interpretative skills of the cytopathologist, the quality of the core biopsy used to obtain the imprints, the quality of the imprint smears, and the quality of the staining. Over the past 10 years, several studies have shown acceptable agreement between IC diagnoses and corresponding histopathological diagnoses in breast lesions. In a total of 1198 cases from 7 published reports, the sensitivities ranged from 86% to 97%, and the specificities ranged from 78% to 100% [1-7]. The average sensitivity and specificity indices were 93% and 92%, respectively. The results of our study were consistent with these published data, supporting the feasibility of providing patients with a rapid on-site preliminary diagnosis of breast lesions.
A limitation of this study was that IC results were compared only with core biopsy diagnoses, and that clinical outcomes, including results of surgical specimens, were not included in the assessment of accuracy. The study protocol that was approved by the Institutional Review Board did not provide access to clinical information on the subjects. However, the principal intent of this study was to assess the reliability of IC as an alternative to core biopsy, and therefore a direct comparison of IC to biopsy results was appropriate.

In conclusion, IC from ultrasound-guided core needle biopsy of breast lesions helps optimize the procedure in two ways: it can be used to obtain an accurate on-site preliminary diagnosis, and it is helpful for assessing the adequacy of biopsy specimens, minimizing the need for extra specimens and repeated procedures. In addition, we believe that the IC technique reduces anxiety in patients with benign lesions, and expedites assessment and therapy in patients with breast cancer, although these benefits were neither quantified nor proven by our data.

References