Intra-Operative Diagnosis of Breast Mass-Lesions: Comparison of the Validity of Touch Smear Preparation and Frozen section Techniques

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ABSTRACT

Background: A reliable intra-operative pathological diagnosis is a mandatory before proper surgery of breast cancer. So far, The Frozen Section Technique (FS) is the standard technique with a relatively high level of validity. However, when the special facilities of FS are unavailable, Touch Smears Preparation technique (TSP) could be alternatively adopted.

Objective: Our objective is to compare the validity of TSP in the intraoperative diagnosis of breast mass lesions, with that of FS technique. An additional aim is to determine the special advantages of both techniques.

Patients and Methods: The study was conducted on 130 breast mass lesions, obtained from operable cases, requesting intra-operative diagnosis at the Pathology Department at the National Cancer Institute (NCI), Cairo University, during the years 2004-2007. The specimens were subjected to the two diagnostic techniques, namely: FS and TSP. Then both were examined separately, by two different observers. The results were expressed as: positive for malignancy, negative for malignancy or deferred diagnosis. The validity of each FS and TSP is tested by using the validity statistical formulas and then results of both were compared. Paraffin section was the golden standard for the true diagnosis.

Results: The accuracy of FS was found to be higher than TSP (100%). Thus, a sensitivity of 100% and a specificity of 100%. Whereas, TSP had a sensitivity of 92.99%, a specificity of 93.33% and an overall accuracy of 92.5%. TSP had also a negative predictive value of 80% and the positive predictive value was 97.65%. The rate of deferred diagnosis was lower in FS (1.5%) than TSP (6.2%).

Conclusions: The study concluded that The FS is much superior to TSP in intra-operative the diagnosis of breast mass. However, when the special FS equipment is lacking, TSP could be a reliable alternative provided that an experienced cytopathologist is available. Analysis of predictive value rates revealed that a positive cytological report is more reliable than a negative one. Moreover, TSP could also be an adjuvant to FS.

Key Words: Breast cancer – Intra-operative diagnostic techniques – Frozen section technique (FS) – Touch smears preparation technique (TSP).

INTRODUCTION

Breast cancer is a global and national oncol-ogical health problem causing a high female mortality and morbidity rates [1]. So, in a recent intradepartmental registry held at the Surgical Pathology Unit, Pathology Department, National Cancer Institute (NCI), Cairo University, breast malignancy ranked number one in all malignant tumors, contributing 34.3% of all female cancers [2]. The study also noticed an increase in the disease by time trend analysis [2]. This report is in an agreement with other Egyptian private registries [3] in which the disease contributed 49.6% of all female cancers. A comparable high figure 32% was recently reported from USA [4]. In another European study, the disease constituted 24.3% of all female cancers [3].

An accurate pathological diagnosis is an essential step for proper management of breast cancer. This is due to the fact that a diagnosis based only on clinical and mammographic findings, carries a relatively high error of about 20% [5]. In mammographic studies, the sensitivity of suspecting or diagnosing breast cancer...
was only 44% [6]. Moreover, a study conducted by the Physicians Insurers Association of America (PIAA) on 675 breast cancer medico-legal claims concluded that claims for radiodiagnosis were 24, compared to only 2 claims for pathology [7].

There are two pathological diagnostic approaches for breast lesions, namely: the preoperative diagnostic methods and the intraoperative diagnostic techniques [8]. Preoperative diagnosis, includes either histopathological based techniques [9] as core needle biopsy (CNB), image guided CNB or stereotactic biopsy [10,6], as well as, excsional biopsy [9]. Whereas, fine needle aspiration cytology biopsy (FNA) is a cytopathologically based technique [11].

Intra-operative diagnosis includes two histopathological methods, namely: the frozen section technique (FS), first introduced by De Riemer in 1918 [12], and the reflected light technique (RL) [13], introduced by Leroux, 1931 [14]. The latter was then improved by Gerard-Marchant in 1962 [13]. Whereas, there are three intra-operative cytopathological methods [15], namely: the touch smear preparation technique (TSP), intra-operative FNA and scrap preparation (SP) [16]. The TSP is much more superior to the SP, as in benign lesions SP may be misleading, showing small epithelial clusters, single dispersed cells with intact cytoplasm and increased cellularity [16].

It has been established that FS is the most reliable intra-operative diagnostic method with a high sensitivity of about 99% [15]. Unfortunately, FS special equipments & technical support may not be available in many hospitals with limited resources, particularly in developing countries, as well as, rural health centers; hence there is a great need for an alternative simple method.

The aim of the present study is to evaluate the validity of TSP in intra-operative diagnosis of breast mass lesions as compared with FS, as well as, exploring its advantages as an alternative or a complimentary procedure. The results of paraffin section were considered the golden standard (i.e. True test results) for the comparative study.

**PATIENTS AND METHODS**

The study included a prospective consecutive samples of 130 patients from the Surgical Pathology unit, Pathology department, National Cancer Institute (NCI), Cairo University, during the years 2004-2007. The patients presented by a breast lump at the out clinic of the Surgical Department, NCI. All were operable and intra-operative diagnosis was indicated [17]. Despite of the large number of cases with breast cancer only few were subjected to frozen section as most of them were confirmed previously by fine needle aspiration cytology (FNAC) or biopsy. Accordingly, lumpectomy specimens were submitted to the Department of Pathology, NCI, for intra-operative diagnosis. The patients included 129 females and only one male. The final paraffin section diagnosis of the specimens included: 95 malignant tumors and 35 benign and non-neoplastic lesions. The mean age of presentation for malignancy was 50.4 years, whereas, benign conditions had a mean age of 38.4 years. Malignant tumors had a mean size (Largest diameter in cms) of 3.2 cms., while benign tumors and non neoplastic lesions had an average size of 2.9 cms. Carcinomas had a predominant estrogen steroid hormone receptor (ER) positive tissue immunoreactivity (56.7%).

The submitted surgical specimen or specimens were first examined for sampling and in order to locate the lesion or lesions, which is then bisected. The same and most representative part of the bisected lesion or lesions was used for the preparation of both TSP and FS. At first four TSP were made, by applying a gentle pressure on the tissue by the slide [16] and then immediately fixed in 95% ethanol [16]. The same tissue sample was then processed for FS by cryostat, cutting 5 microns sections [8]. For both techniques, rapid Hematoxylin and Eosin stain was used [8,16].

FS material was examined by the first and third authors of this paper (T El-Bolkainy), a staff member of the Surgical Pathology Unit, with ten years of experience. Whereas, TSPs were examined by the second author (H. Shabaan), also a staff member with a ten years of experience in cytopathology. Four cases (2 FS case and 2 TSP cases) intra-operative consultation with senior staff was necessary before reaching a definite diagnosis [18]. The decision for further surgery was totally based.
RESULTS

The histopathological diagnosis of the present series, confirmed by paraffin section, is presented in Table (2). In two cases of FS and 8 cases of TSP the diagnosis was inconclusive and hence deferred for paraffin section. This resulted in 128 evaluable cases in FS and 122 cases in TSP validity study. The histology of the inconclusive cases proved by paraffin section to be atypical ductal hyperplasia: ADH (3 cases), ductal carcinoma in situ: DCIS (2 cases), invasive lobular carcinoma: ILC (2 cases) and sclerosing adenosis (one case).

FS gave correct diagnosis of all malignant and non-malignant specimens examined with an overall accuracy of 100%. Conversely, the overall accuracy of TSP was 92.5% (Table 3). Comparison of the validity and deference rates between TSP and FS is presented in Table (4). FS had a higher accuracy and a lower deference rate. The positive predictive value of TSP is higher (98%) than negative predictive value, denoting that this method is more reliable when positive than when it is negative for malignancy.

IDC appeared in TSP as cellular smears of variably atypical cells, arranged in groups or dissociated forms. The later had intact cytoplasm (Fig. 1-A). ILC showed sparse monotonous cells with an eccentric nuclei and mucinous cytoplasm (Fig. 1-B). Mucoid carcinoma showed a mucinous background, papillary groups, and bland-looking forms with occasional signet ring morphology (Fig. 1-C,D). The medullary carcinoma showed a lymphoid element associated with the broad sheets of tumor cells. NHL smear was cellular, composed of dissociated malignant large rounded lymphocytes with large vesicular dyskaryocytic nuclei showing single or multiple marginated nucleoli and scanty cytoplasm (Fig. 1-E). Cytopathological diagnosis of DCIS (Fig. 1-F) required both mammographic and gross correlation.

All benign lesions were cytologically distinguished from malignant tumors by their relative low cellularity, bland cytomorphology and the presence supportive stromal cell element (Fusiform cells and naked bipolar nuclei) in the smears, with intimate relation to the epithelial cells [21].

Three diagnostic categories of both techniques were adopted, namely: negative for malignancy, positive for malignancy or deferred diagnosis. The later were excluded from the study of validity. So, only positive and negative diagnoses were included in the validity analysis. Validity rates (Table 1) were performed for both techniques, using the formulas previously reported by Dajani [23]. The results of paraffin section were considered the true test results for both techniques.
Intra-Operative Diagnosis of Breast Mass-Lesions

Fig. (1-A): High grade invasive duct carcinoma with loosely cohesive clusters of large cancer cells with marked nuclear abnormalities (H & E x 400).

Fig. (1-B): Lobular carcinoma with dispersed smear pattern of relatively small cancer cells, showing nuclei pushed to one side forming plasmacytoid morphology (H&E x400).

Fig. (1-C): Mucinous carcinoma with typical cohesive clusters of cancer cells having only slight nuclear abnormalities, set in a mucinous background (H&E x400).

Fig. (1-D): Mucinous carcinoma showing signet ring forms, rich in cytoplasmic mucin with eccentric anaplastic nuclei (H & E x 400).

Fig. (1-E): Imprint of a case of NHL, shows predominantly large cells with round vesicular nuclei and prominent occasionally multiple nucleoli (H & E x 400).

Fig. (1-F): DCIS, comedo type shows poorly preserved malignant cells with large pyknotic nuclei in a background of debris and scattered inflammatory cells (H & E x 200).

Fig. (2-A): ADH showing a minor degree of nuclear dysplasia, but free of separate atypical forms (H & E x 400).

Fig. (2-B): UDH are cytologically distinguished from malignant tumors by their relative low cellularity, bland cytology intimately related to stromal cell element (H & E x 200).

Fig. (2-C): Intra-cancicular fibroadenoma with stag-horn sheets (H & E x 200).

Fig. (2-D): A fibroadenoma showing monolayer sheets of bland cells, having an even chromatin distribution (H & E x 400).

Fig. (2-E): Fat necrosis with a foamy histiocyte, having a uniform nucleus (H & E x 400).

Fig. (2-F): A case of chronic breast abscess showing pus balls of polymorphs (H & E x 400).
In the present study, the FS accurately diagnosed all 128 evaluable cases when compared with paraffin section diagnosis, with neither false positive (FP) nor false negative (FN) results. Conversely, 3 cases were misdiagnosed by TSP, including: a single FP and two FN diagnoses out of a total number of 122 evaluable cases. Thus, FS technique showed a higher validity than TSP in intra-operative breast mass-lesion diagnosis with less case deference. However, the diagnostic errors of FS only appear in large series [27]. Thus, in a historical review of FS accuracy of a 17 previously reported studies conducted between the years 1938-1989 by Oneson and his colleagues recorded a low accuracy level of 88.9% with a FP rate of 0.1-0.6% and a FN rate of 1-9% [27]. However, such low accuracy was encountered in a very old series (1938), with old equipments of a low technical capability.

Diagnostic errors of TSP in the present study included: a single FP diagnosis encountered in a case of ADH and two FN diagnoses out of a total number of 122 evaluable cases. ADH was first described by Azzopardi in 1979 as epitheliosis [17], then recently highlighted by the WHO as proliferative breast ductal condition with cytological atypia, carrying a high-risk of subsequent development of IDC ranging from 3.7 up to 22% [1]. It is noteworthy, in both

<table>
<thead>
<tr>
<th>Test to be evaluated</th>
<th>Paraffin section diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (Malignant)</td>
<td>TP (True Positive)</td>
<td>FP (False Positive)</td>
</tr>
<tr>
<td>Negative (Benign)</td>
<td>FN (False Negative)</td>
<td>TN (True Negative)</td>
</tr>
<tr>
<td>Total</td>
<td>TP+FN</td>
<td>FP+TN</td>
</tr>
</tbody>
</table>

*Validity rates formulas included:
(1) Sensitivity rate: Is the percentage of positive results in cancer patients = 100 (TP/TP+FN).
(2) Specificity rate: Is the percentage of negative results in normal individuals = 100 (TN/TN+FP).
(3) Accuracy: Is the percentage of all correct results, including both the positive and the negative results in the total series = 100 (TP+TN/TP+TN+FP+FN).
(4) Positive predictive Value (PV+): The probability of the disease being present in patients with positive report = 100 (TP/TP+FP).
(5) Negative predictive Value: Probability of the disease being absent in individuals with negative results = 100 (TN/TN+FN).

<table>
<thead>
<tr>
<th>Histopathological diagnosis (130 cases)</th>
<th>No. of cases (130 cases)</th>
<th>Sex ratio (M:F)</th>
<th>Mean age (In years)</th>
<th>Mean Size (Largest diameter in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign (35 cases):</td>
<td>35</td>
<td>0.35</td>
<td>38.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Usual ductal hyperplasia (UDH)</td>
<td>18</td>
<td>0.18</td>
<td>39.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Atypical ductal hyperplasia (ADH)</td>
<td>3</td>
<td>0.3</td>
<td>42</td>
<td>1.9</td>
</tr>
<tr>
<td>Fat Necrosis</td>
<td>3</td>
<td>0.3</td>
<td>44</td>
<td>3.1</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>9</td>
<td>0.9</td>
<td>28.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Inflammation</td>
<td>2</td>
<td>0.2</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>Malignant (95 cases):</td>
<td>95</td>
<td>1.94</td>
<td>50.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Invasive duct carcinoma (IDC)</td>
<td>70</td>
<td>1.69</td>
<td>50.9</td>
<td>3</td>
</tr>
<tr>
<td>Duct carcinoma in situ (DCIS)</td>
<td>4</td>
<td>0.4</td>
<td>49.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Invasive lobular carcinoma (ILC)</td>
<td>13</td>
<td>0.13</td>
<td>51.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Mixed duct lobular carcinoma</td>
<td>4</td>
<td>0.4</td>
<td>51.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Mucoid carcinoma</td>
<td>2</td>
<td>0.2</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td>Medulary carcinoma</td>
<td>1</td>
<td>0.1</td>
<td>48</td>
<td>2.5</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1</td>
<td>0.1</td>
<td>49</td>
<td>6</td>
</tr>
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</table>

Table (3): Validity analysis of TSP (122 cases)*.

<table>
<thead>
<tr>
<th>TSP diagnosis</th>
<th>Paraffin section diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (Malignant)</td>
<td>Malignant</td>
<td>Benign</td>
</tr>
<tr>
<td>Negative (Benign)</td>
<td>Malignant</td>
<td>Benign</td>
</tr>
<tr>
<td>Total</td>
<td>Malignant</td>
<td>Benign</td>
</tr>
</tbody>
</table>

* Total TSP evaluable cases after the deference of 8 patients from the total number of 130 cases and all results are expressed as number of patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TSP</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deferred diagnosis</td>
<td>8 (6.15%)</td>
<td>2 (1.54%)</td>
</tr>
<tr>
<td>Evaluable cases</td>
<td>122 (93.85%)</td>
<td>128 (98.46%)</td>
</tr>
<tr>
<td>TP</td>
<td>88 (98.88%)</td>
<td>95 (100%)</td>
</tr>
<tr>
<td>FP</td>
<td>1 (1.12%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>TN</td>
<td>31 (93.94%)</td>
<td>33 (100%)</td>
</tr>
<tr>
<td>FN</td>
<td>2 (6.06%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sensitivity rate</td>
<td>92.22%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity rate</td>
<td>93.33%</td>
<td>100%</td>
</tr>
<tr>
<td>Accuracy rate</td>
<td>92.5%</td>
<td>100%</td>
</tr>
<tr>
<td>The NPV*</td>
<td>80.0%</td>
<td>100%</td>
</tr>
<tr>
<td>The PPV**</td>
<td>97.65%</td>
<td>100%</td>
</tr>
</tbody>
</table>

* The negative predictive value ** The positive predictive value

DISCUSSION

In the present study, the FS accurately diagnosed all 128 evaluable cases when compared with paraffin section diagnosis, with neither false positive (FP) nor false negative (FN) results. Conversely, 3 cases were misdiagnosed by TSP, including: a single FP and two FN diagnoses out of a total number of 122 evaluable cases. Thus, FS technique showed a higher validity than TSP in intra-operative breast mass-lesion diagnosis with less case deference. However, the diagnostic errors of FS only appear in large series [27]. Thus, in a historical review of FS accuracy of a 17 previously reported studies conducted between the years 1938-1989 by Oneson and his colleagues recorded a low accuracy level of 88.9% with a FP rate of 0.1-0.6% and a FN rate of 1-9% [27]. However, such low accuracy was encountered in a very old series (1938), with old equipments of a low technical capability.

Diagnostic errors of TSP in the present study included: a single FP diagnosis encountered in a case of ADH and two FN diagnosis of ILC. ADH was first described by Azzopardi in 1979 as epitheliosis [17], then recently highlighted by the WHO as proliferative breast ductal condition with cytological atypia, carrying a high-risk of subsequent development of IDC ranging from 3.7 up to 22% [1]. It is noteworthy, in both
the cytology and the small tissue biopsy material; ADH is a common diagnostic pitfall, being over diagnosed as low-grade DCIS [10,11]. However, this is not a serious FP error, since the biology of ADH is relatively similar to low-grade DCIS [1]. It is noteworthy; a valid diagnosis of ADH requires an excisional biopsy [10,11]. Whereas, the two FN cases of ILCs were characterized by sparse cellularity by TSP examination. The pronounced desmoplastic stromal element of this tumor contributed to the low cellular yield [15]. Thus, when ILC is suspected during sampling, it is better to be done by the scrap preparation technique rather than TSP [15].

Our TSP results are in agreement with different authors. So, Hiregoudar and his colleagues [24] conducted a study for testing TSP accuracy on 40 cases, 21 malignant & 19 benign. The sensitivity and specificity were 95.24% and 100% respectively. This study had also an accuracy rate of 97.5% with a false negative rate of 2.5%. In another study of comparative analysis between cytologic diagnosis and histopathological findings in intraoperative breast tumors conducted on 34 benign and 31 malignant breast tumors, the cytological and histopathological diagnosis were in accordance in 98.8% of benign breast tumors and in 100 of carcinomas [25]. Scopa and his colleagues [26] studied 230 cases of intraoperative cytopathological diagnosis, achieving an accuracy rate of 94.3% for benign lesions and accuracy for malignancy was 91%.

The hazard to their patients from diagnostic errors varies according to the type of error [30]. A FP diagnosis is more serious than a FN one. A FP error will lead to an unnecessary surgery, but a FN diagnosis will be immediately corrected by paraffin section diagnosis [8,13]. Intraoperative pitfalls in cancer typing and grading are considered minor errors, since they do not alter the subsequent surgical procedure [8,13]. There are four main causes for committing errors in intraoperative diagnosis of breast pathology [18], namely: inexperience (57%), microscopic sampling errors (24%), gross sampling errors (9.5%) and lack of interdepartmental communication (9.5%).

Each of the studied techniques had distinctive benefits, supporting the benefit of their complimentary practice [17]. On such bases, FS had the ability to report on in situ carcinoma component, information difficult to be record by TSP [17]. Moreover, FS informs the surgeon about the histopathological type, grade and adequacy of excision in malignancy with high accordance with paraffin section report [17]. However the advantages of TSP exceed its limitations. Thus, TSP is a rapid, relatively safe, coast effective technique, requiring less financial, human and technical facilities [11]. Moreover it is particularly helpful for examining multiple small specimens requiring a rapid intraoperative diagnostic decision [15]. It is also a rapid method to detect small pathologic foci in large specimens, hence useful for proper sampling [15]. TSP is also valuable in obtaining fresh samples for DNA studies [28]. John & his colleagues reported an additional role for TSP [29], namely, a simple and rapid technique for intraoperative diagnosis of breast cancer sentinel Lymph node (SLN) status, with no false positive results [29]. Thus, TSP could be used as an adjuvant to FS adding a good deal of information [15,17].

Conclusion:
It is concluded from the present study that FS is superior to TSP in intraoperative diagnosis of breast mass lesions. However, the study approved the relatively valid use of TSP as an acceptable alternative in hospital with limited technical, financial and human supplies provided the availability of an experienced cytopathologist. The predictive value analysis indicated that a positive diagnosis by TSP is more reliable than a negative one hence; the later cases must be subjected to lumpectomy to avoid missing malignant tumors. Finally, TSP appeared to be of value when used as an adjuvant to FS.

Recommendations:
We recommend that further future studies of both histopathological and cytopathological breast intraoperative diagnostic techniques require the addition of two more statistical parameters, aiming to strengthen subsequent metaanalysis and achieving accreditation. Such parameters include investigating test precision (i.e. the similarity of values of a test, when performed multiple times by the same observer) and reproducibility (i.e. the similarity of values of a test when performed multiple times by different observers) on larger samples [23].
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