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Comparison of cytopathology and frozen section methods in the intraoperative evaluation of breast sentinel lymph node

Comparação dos métodos de citopatologia e de cortes por congelação na avaliação intraoperatória do linfonodo sentinela de mama

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ABSTRACT

Introduction: Cytopathologic analysis (CP) and frozen section (FS) are available techniques for intraoperative evaluation of sentinel lymph node in surgeries for breast cancer treatment that will define the need for axillary lymphadenectomy. **Objective:** To compare CP and FS of axillary sentinel lymph nodes in metastasis detection of patients with breast cancer. **Materials and methods:** The electronic files from January 2010 and December 2014, from the Centro de Patologia de Curitiba, Parana, Brazil were reviewed, and were included all the cases in which the result of intraoperative exams by both methods, CP and FS, were recorded. The results of intraoperative exams were compared to the histopathology by hematoxylin and eosin (HE) staining. **Results:** A total of 183 sentinel lymph nodes from 94 patients were included. The mean age was 55 years and mean lymph node size was 11.70 mm. There was one false-positive case in both intraoperative methods and four false-negatives in FS, which were micrometastasis. FS's sensibility, specificity, positive predictive value (PPV) and negative predictive value (NPV) and accuracy were respectively 80%, 99.38%, 94.11%, 96.42% and 97.26%. Regarding CP, there were six false-negative in which four were micrometastasis. CP's sensibility, specificity, PPV and NPV and accuracy were respectively 70%, 99.38%, 93.33%, 96.42% and 97.26%. **Conclusion:** The results of the present study shows that both CP and FS are reliable techniques for metastasis detection in breast sentinel lymph nodes, and are equivalent in sensibility, specificity, accuracy, PPV and NPV.

Key words: frozen sections; sentinel lymph node biopsy; breast cancer.

INTRODUCTION

Breast cancer is the second most incident cancer worldwide, with 1.4 million cases (10.9%) and ranks fifth in cancer deaths⁽¹⁾.

The main prognostic factor in invasive breast carcinoma is the axillary lymph node involvement. Traditionally, axillary lymph node dissection was considered the main therapeutic factor for local control of the disease, and an important prognostic factor. However, such procedure is associated with high morbidity, resulting in lymph edema and compromised upper limbs. With the advent of sentinel lymph node biopsy, unnecessary axillary lymph node dissection could be reduced^(2,3).

The frozen sections (FS) and cytopathologic analysis (CP) are intraoperative techniques that evaluate the presence of metastases in sentinel lymph nodes during mastectomy. The result of these techniques prevents the patient to return for a new surgery, immediately assessing the need for further axillary clearance^(4,5). In cases where the intraoperative examination shows false-negative results, there may be a need for axillary lymph node dissection in a second surgical time⁽⁶⁾. However, the histopathological evaluation of the paraffin-embedded material remains the gold standard for the identification of sentinel lymph node metastases. Therefore, after confirmation by biopsy, the efficacy of intraoperative techniques can be evaluated.

OBJECTIVE

To compare the CP and FS techniques in the intraoperative assessment of sentinel lymph nodes in patients with breast cancer.

MATERIALS AND METHODS

This is an observational, descriptive, retrospective study, approved by the Research Ethics Committee of the Health Sciences Sector of the Universidade Federal do Paraná (UFPR), under the opinion number 1.070.833.

The material studied was obtained from the anatomopathological reports of the Centro de Patologia de Curitiba (CPC) of the Hospital Nossa Senhora das Graças, during the period between January 2010 and December 2014. We analyzed the electronic records of patients with breast cancer that underwent both mastectomy and conservative surgery, with intraoperative examination of the sentinel lymph node, which had the samples evaluated by both FS and CP examination. We excluded the cases in which the intraoperative examination result did not specify the findings in both methods concomitantly. The results of intraoperative examinations were compared with the respective definitive examinations of the paraffin-embedded material.

We collected data regarding gender, age, histological type of breast cancer, primary tumor, lymph node and metastasis size, and whether or not metastases were detected in FS and CP analysis.

The histological types were categorized according to the classification of breast tumors of the World Health Organization (WHO)⁽⁷⁾, and were divided into invasive breast carcinoma of no special and special type.

The size of the metastasis was classified according to the tumor-node-metastasis (TNM staging system) classification of malignant tumors⁽⁸⁾, which considers micrometastasis the infiltrations between 0.2 mm and 2 mm, and as macrometastases those greater than 2 mm.

The CP technique employed was by imprint (transfer) or by scraping the cut surface of the lymph node on its longest axis. The slides were fixed in 70% alcohol and stained by toluidine blue (Figure 1).

FS were performed in the Shandon[®] cryostat, in 10 to 15 micron slides and stained with toluidine blue (Figure 2).

The intraoperative examinations were performed by six different CPC pathologists according to daily scale, and the

definitive paraffin examination by a pathologist with specialization in breast pathology.

We calculated the values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), percentage of false-positives and false-negatives, and accuracy of the intraoperative examination by CP technique and by FS in cryostat using the definitive histopathological examination as the standard-gold.

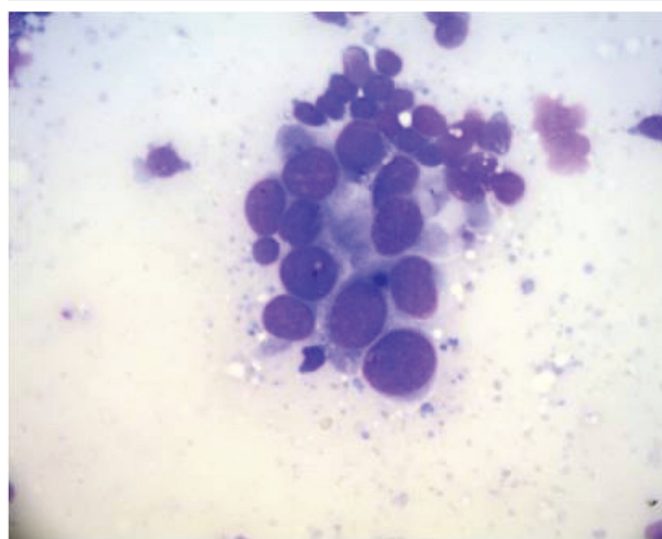


FIGURE 1 – Photomicrography: sentinel lymph node cytopathology showing cohesive clusters of neoplastic cells with nuclear pleomorphism characterizing carcinoma (toluidine blue, 400x)

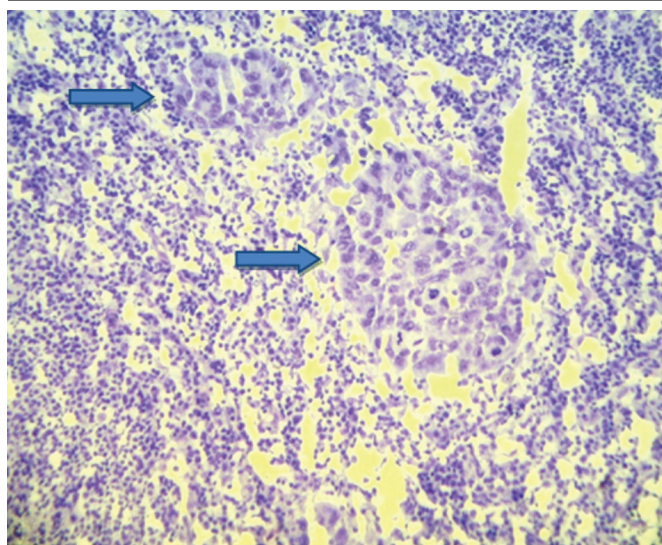


FIGURE 2 – Frozen sections of lymph node with two groups of metastatic ductal carcinoma (arrow) amidst lymphoid tissue (toluidine blue, 100x)

RESULTS

We selected 94 cases, all of which were female patients. The mean age was 55 years, ranging from 27 to 86 years.

From these patients, we analyzed 183 sentinel lymph nodes considered as independent samples. The mean lymph node size was 11.70 mm and the mean primary tumor size was 20.52 mm. We found 69 cases of tumors of the no special histological type, 25 of the special type.

CP (Figure 1) was positive in 15 lymph nodes and negative in 168. There was one false-positive case (6.67%) and six false-negative cases (3.57%). The sensitivity of the test was 70% and the specificity was 99.38%. PPV and NPV were, respectively, 93.33% and 96.42%. The accuracy found in this test was 96.17%.

FS (Figure 2) was positive in 17 lymph nodes and negative in 166. Among the positive ones, there was one false-positive (5.88%) and, among the negative ones, four false-negative (2.4%). The sensitivity of the test found was 80% and the specificity was 99.38%. PPV and NPV were, respectively, 94.11% and 96.42%. The accuracy found in this test was 97.26%.

The gold standard diagnosis by histopathology showed that 19 lymph nodes were affected by metastases, of which seven were micrometastasis, one was suspect, and 163 were negative. In the suspected case, the immunohistochemical evaluation confirmed the presence of metastasis. In this case, considered positive in the definitive examination, both FS and CP were false-negatives (Table 1).

TABLE 1 – Epidemiological index values comparing CP, FS and both methods combined in breast sentinel lymph node

Method	Sensitivity (%)	CI (95%)	Specificity (%)	CI (95%)
CP	70	45.7-88.1	99.4	99.2-99.9
FS	80	56.3-94.3	99.4	99.2-99.9
FS + CP	85	62.1-96.8	99.4	99.2-99.9

CP: cytopathologic analysis; FS: frozen sections; CI: confidence interval.

DISCUSSION

CP and FS are the intraoperative methods most commonly used for breast metastasis screening in breast sentinel lymph node. Cytopathology is a fast and low-cost method whose performance does not cause loss of material for evaluation in the subsequent permanent cutting⁽⁹⁾. The FS method, although more expensive than other techniques and having a possible loss of material, would have the potential advantage of preserving tissue architecture, including the size of the metastasis⁽¹⁰⁾.

In the present study, the accuracy of both methods was high and very close, 96.17% (CP) and 97.26% (FS). In the literature, the sensitivity of these techniques varies from 33%-96% for CP and 44%-100% for FS, while specificity almost does not vary, and is always close to 100% for both techniques⁽¹¹⁾. However, although many studies analyze the efficacy of CP and FS, only a few compare the two methods. The sensitivity and specificity in this study were, respectively, 70% and 99.36% for CP, 80% and 99.38% for FS. These values separately correspond to the range of variation of each technique. In a meta-analysis from 2005, Tew *et al.* calculated the sensitivity and group specificity of four studies comparing CP and FS with sensitivity and specificity values, respectively, of 62% and 99% for CP, 76% and 99% for FS⁽¹¹⁾. In our study, although FS has a greater sensitivity, there was no statistically significant difference when compared to CP. The same occurs in most other studies that compared CP and FS, which can be observed in Table 2.

TABLE 2 – Comparison between the clinical epidemiological indexes obtained by the researched authors and from this study

Studies	CP sensitivity (%)	CP specificity (%)	FS sensitivity (%)	FS specificity (%)
Arlicot (2013) ⁽¹²⁾	33.3	-	59.3	-
Azarpina (2012) ⁽⁶⁾	90	100	90	100
Wang (2012) ⁽¹³⁾	76.2	97.3	69	99.5
Lumachi (2012) ⁽¹⁴⁾	70.3	98.6	75.7	100
Francz (2011) ⁽⁵⁾	69.44	97.8	58.3	100
Memar (2010) ⁽¹⁵⁾	80	100	91	100
Krishnamurthy (2009) ⁽¹⁶⁾	50	100	72.2	97.5
Vanderveen (2008) ⁽¹⁷⁾	66	99.5	67	99.5
Calebioglu (2006) ⁽¹⁸⁾	51	-	73.5	-
Brogi (2005) ⁽¹⁹⁾	57	-	59	-
Tew (2005) ⁽¹¹⁾	62	99	76	99
Aihara (2004) ⁽²⁰⁾	86	-	89	-
Authors (2017)	70	99.4	80	99.4

CP: cytopathologic analysis; FS: frozen sections.

The variation of sensitivity and specificity results of the two methods in the studies is mainly related to the different techniques used⁽²¹⁾. In CP, the preparation technique and the staining used differ from study to study. In FS, the reduction of sections thickness guarantees a greater diagnostic sensitivity⁽²²⁾. However, intraoperative examination is not always within the reach of all centers, since it depends on the technical ability and experience of the pathologist to perform the procedure within the adequate surgical time.

Our study also detected the presence of four false-negatives in FS and six in CP. One of these cases of cytology occurred due to an error in the interpretation of metastatic lobular carcinoma. In this type of neoplasia, metastatic cells present scarce cytoplasm

and low atypia, making this error relatively frequent^(5, 16, 22). All other cases were associated with micrometastasis. In one of the micrometastasis cases, neither detected by CP nor FS, the diagnosis in histology was only suspect and confirmed by immunohistochemistry, which highlights the diagnostic challenge imposed by micrometastasis. Both techniques demonstrated in other studies lower sensitivity in the detection of micrometastasis⁽²²⁻²⁴⁾. Our study determined sensitivity for FS of 100% for macrometastasis and 42.8% for micrometastasis. For CP, the same occurred: sensitivity for macrometastasis was 92.3% and 28% for micrometastasis. However, the role of micrometastasis diagnosis is not defined in the literature⁽²⁵⁾.

The occurrence of false-positive is an extremely undesirable fact in the intraoperative examinations as it leads to unnecessary treatment, which occurred only once in our study. In this case, both CP and FS demonstrated an atypical pattern, which could be related to the neoadjuvant chemotherapy to which the patient was submitted. This treatment induces changes in the cells and lymph node architecture, and may have contributed to the diagnostic misunderstanding; however, a study with a greater number of cases would demonstrate this relationship.

The association of the two methods is a routine in CPC, however, it was demonstrated that the sensitivity gain with FS was not relevant. Although the association embodies a greater sensitivity (85%), it is also not statistically superior to the methods isolated.

CONCLUSION

The results of the present study demonstrated that CP and FS are reliable techniques for the detection of macrometastasis in sentinel lymph nodes of the breast. There was no superiority of one method over the other in diagnostic performance, and the association of the two techniques did not prove to be superior to any of them alone.

SUPPORT

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RESUMO

Introdução: A análise citopatológica (CP) e o método de corte por congelamento (CC) são técnicas disponíveis de avaliação intraoperatória do linfonodo sentinela nas cirurgias para tratamento de câncer de mama que vão definir a necessidade de linfadenectomia axilar. **Objetivo:** Comparar a avaliação CP e os CCs de linfonodos sentinela axilares na pesquisa de metástases em pacientes com câncer mamário. **Materiais e método:** Foram revisados os arquivos eletrônicos do Centro de Patologia de Curitiba, Paraná, Brasil, de janeiro de 2010 a dezembro de 2014, sendo incluídos os casos em que constavam o resultado do exame intraoperatório por ambos os métodos, CP e CC. Os resultados dos exames intraoperatórios foram comparados com a histopatologia, corada por hematoxilina e eosina (HE). **Resultados:** Foram incluídos 183 linfonodos sentinelas de 94 pacientes. A idade média foi de 55 anos, e o tamanho médio dos linfonodos, de 11,70 mm. Houve um caso falso positivo nos dois métodos intraoperatórios e quatro falso negativos na CC, entre os quais todos eram micrometástases. Sensibilidade, especificidade, valor preditivo positivo (VPP), valor preditivo negativo (VPN) e acurácia do CC foram, respectivamente, 80%, 99,38%, 94,11%, 96,42% e 97,26%. Já na CP, houve seis falso negativos, entre esses, quatro eram micrometástases. Sensibilidade, especificidade, VPP, VPN e acurácia da CP foram, respectivamente, 70%, 99,38%, 93,33%, 96,42% e 97,26%. **Conclusão:** Os resultados do presente estudo demonstraram que CP e CC são técnicas confiáveis para detecção de metástase em linfonodos sentinela de mama e se equivalem em sensibilidade, especificidade, acurácia, VPP e VPN.

Unitermos: secções congeladas; biópsia de linfonodo sentinela; neoplasias da mama.

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