

# Evaluation of the accuracy of frozen section in different anatomical sites

## *Avaliação da acurácia diagnóstica do exame intraoperatório por congelamento em diferentes sítios anatômicos*

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### ABSTRACT

**Introduction:** Frozen section is recommended in several situations to: establish the nature of a lesion; establish the presence of a lesion; confirm the presence of a benign lesion; confirm that sufficient tissue is present for diagnosis; establish the grade of the lesion; determine the organ of origin and determine the adequacy of margins. **Objectives:** To evaluate the accuracy of frozen section biopsy in multiple organs and analyze possible factors in discrepancy. **Methods:** A retrospective study was carried out during a five-year period at a teaching hospital of Recife, Pernambuco, Brazil. The diagnoses of frozen section were compared with results obtained in the permanent section and classified as concordant or discordant. The discordant cases were reviewed by a pathologist and subdivided into false positives and false negatives. Possible reasons for discrepancy were indicated. **Results:** A total of 1.226 specimens were analyzed, of which 1.181 (96.33%) were concordant and 45 (3.67%) were discordant. After the review of the discordant cases, 39 remained, six (15.4%) were false positives and 33 (84.6%) were false negatives. The tissue that had most false-positive results was mammary sentinel lymph node (3/1.2%), whereas ovarian showed most false negative outcomes with 17 specimens (51.51% of all false negatives). The possible reasons for discrepancy were sampling error, misunderstanding and complexity of the diagnosis. **Conclusion:** The frozen section accuracy of 96.3% found in our study is similar to specialized literature and does not seem to depend on the tissue analyzed.

**Key words:** frozen sections; biopsy; neoplasms by site.

### INTRODUCTION

Cancer is considered the second major death cause in the Brazilian population, surpassed just by cardiovascular diseases and their complications. Surgery is one of the cornerstones of cancer treatment, and intraoperative histological assessment of specimens, developed in the beginning of the 20<sup>th</sup> century, has been used all over the world<sup>(1)</sup>.

Frozen section biopsy is an intraoperative examination, and according to Sociedade Brasileira de Patologia, aims at histologically and intraoperatively assessing a small fragment of injured tissue or organ in which there is diagnostic doubt<sup>(2)</sup>. Among the indications of frozen section, are determination of nature and extension of a lesion, with the resulting differentiation between benign and malignant lesions, besides analysis of

surgical margins<sup>(3, 4)</sup>. Frozen section has become attractive for surgeons due to its rapid histopathological diagnosis provided during the surgical procedure and consequent decreased need for reoperation<sup>(2, 5, 6)</sup>.

The accuracy of frozen section, that is, how reliable this method is when compared with histopathological diagnosis in paraffin-embedded tissue (gold standard), has been extensively examined in the specialized literature; it is above 90% in most studies, and varies according to the analyzed organ<sup>(3, 6-10)</sup>. Currently, in Brazil there are few works encompassing more than one type of anatomical structure, with priority for specific areas<sup>(3, 11)</sup>.

The present evaluation aims at analyzing the accuracy of intraoperative frozen section examinations in several anatomical sites at a teaching general hospital.

## METHODS

A retrospective study was conducted at Instituto de Medicina Integral Professor Fernando Figueira (Imip), in the city of Recife, Pernambuco, Brazil, during a period of five years. The pathology department of that institution began performing frozen sections systematically in January, 2011. All intraoperative frozen section examinations done between January 2011 and March 2016 were analyzed by means of an active search in the department data bank. Exam results after histological processing in paraffin were obtained by means of the Imip reporting system, while those from frozen sections, by the department internal data bank.

Frozen section begins with a gross evaluation of the referred specimen. Next, representative samples of up to 0.5 cm undergo freezing in cryostat at -17°C, with sections of 5 micrometers being cut. The material is stained with hematoxylin and eosin (HE) for microscopic evaluation by the staff pathologist. The analysis result returns to the surgeon and is recorded in the department internal data bank.

Results were categorized into concordant and discordant; the latter was subdivided into false positive and false negative. Exams were considered concordant when diagnosis of frozen section coincided with the final histopathological report, and discordant when frozen section and paraffin diagnoses were different. Discordant cases were divided into two subgroups: 1. false negative – those in which frozen section was negative and final report was positive for malignancy; 2. false positive – those samples that demonstrated malignancy in frozen section reports and did not present malignancy in paraffin.

All sentinel lymph nodes evaluated at Imip during the collection period were analyzed by imprint or frozen section; however, all lymph nodes evaluated by imprint were excluded from the analysis. Therefore, samples equivalent to 243 cases of sentinel lymph nodes were exclusively evaluated by frozen section and are part of the work sample.

All discordant cases were reviewed by an experienced pathologist for better understanding of the factors contributing to disagreement. The present study was previously approved by the Ethics Research Committee, under approval number 58851116.4.0000.5569.

## RESULTS

In a time period between January 2011 and March 2016, 1,356 frozen sections were carried out; among them 130 were excluded

because of lack of information in the reports. This study, therefore, encompasses a total of 1,226 surgical specimens. Frozen sections and final histopathological reports after paraffin processing were concordant in 1,181 (96.3%) cases, and discordant in 45 (3.6%) samples (**Table 1**).

**TABLE 1 – Accuracy in each of the evaluated anatomical sites (*n* = 1,226)**

Anatomical site	<i>n</i> cases ( <i>n</i> )	Concordant ( <i>n</i> %)	Discordant ( <i>n</i> %)
Ovary	335 (27.32%)	317 (94.6%)	18 (5.4%)
Sentinel lymph node	243 (19.82%)	235 (96.7%)	8 (3.3%)
Breast	101 (8.23%)	100 (99%)	1 (1%)
Peritoneum	87 (7.09%)	79 (90.8%)	8 (9.2%)
Uterus	82 (6.68%)	82 (100%)	0 (0%)
Lymph node	78 (6.36%)	75 (96.1%)	3 (3.9%)
Liver, gallbladder, pancreas	64 (5.22%)	63 (98.4%)	1 (1.6%)
Esophagus, stomach, colon, rectum	61 (4.97%)	59 (96.72%)	2 (3.28%)
Urologic	52 (4.24%)	50 (96.2%)	2 (3.8%)
Lung	34 (2.77%)	34 (100%)	0 (0%)
Skin	25 (2.03%)	25 (100%)	0 (0%)
Others	19 (1.54%)	19 (100%)	0 (0%)
Omentum	17 (1.38%)	17 (100%)	0 (0%)
Central nervous system	13 (1.06%)	13 (100%)	0 (0%)
Retroperitoneum	9 (0.73%)	7 (77.7%)	2 (22.3%)
Vulva, vagina, cervix	6 (0.48%)	6 (100%)	0 (0%)

The anatomical sites where frozen sections were mostly used were ovary (335 cases/27.3%), mammary sentinel lymph node (243 cases/19.8%), breast nodules (101 cases/8.2%) and peritoneal nodules (87 cases/7.1%).

The highest precision was identified in samples from uterus, lung, skin, omentum, central nervous system (CNS), vulva, vagina, and cervix, which presented 100% agreement between frozen sections and final histopathological report.

After review of the 45 discordant cases by the pathologist, four cases were reclassified as concordant, and two as insufficient samples. Considering the four cases changed to concordant, in two that alteration occurred because the purpose of the frozen section was not clarified (the exam objective were the margins, not the lesion characteristic), so agreement was kept when comparing them with the correct objective. In the other two cases, the alteration occurred because the nomenclature used during the collection period was not uniform. The distribution of the 39 final discordant cases is presented in **Table 2**.

Evaluating the 39 cases that remained discordant, six (15.4%) were false positives and 33 (84.6%), false negatives. The organ that mostly presented a false positive diagnosis was sentinel lymph node, with three cases (1.2%), followed by lymph nodes of other

regions, with two cases (2.6%), and ovary, with one case (0.3%). The anatomical site that demonstrated a larger quantity of false negatives was the ovary, with 17 (5.1%) cases, followed by the peritoneum, with seven (8%), and sentinel lymph node, with three (1.23%).

After review of the 39 cases, we concluded that the possible causes for disagreement between the intraoperative frozen section and the final histopathological report after paraffin processing were: sample size, method limitation, diagnosis complexity, and presence of micrometastases. The distribution of these causes is presented in **Table 3** (regarding the cases of ovaries) and **Table 4** (other organs).

## DISCUSSION

The intraoperative frozen section examination, a method developed in the beginning of the 20<sup>th</sup> century<sup>(12)</sup>, became

an important tool used in surgical procedures to guide some intraoperative conducts. Although it can be used in benign pathologies, as in organ identification (for example, parathyroid), it is mostly used in malignant diseases, in which it helps malignancy identification, staging, margin evaluation and determination of the extent of removal.

Given the need of an immediate diagnosis and the consequent morbidity associated to the surgical conduct to be followed, several studies tried to evaluate the accuracy of frozen sections comparing them with definitive histopathological paraffin diagnosis (gold standard)<sup>(3, 6, 13-16)</sup>. We did not find, yet, studies in the Brazilian North-Northeast that assessed this issue.

The present study analyzed a sample of 1,226 cases in a period of five years at the pathology department of Imip. It is a considerable number of cases, larger than the average presented by other studies<sup>(3, 7, 12, 17, 18)</sup>. Besides, our investigation encompasses

**TABLE 2 – Distribution of discordant findings after reassessment in relation of evaluated organs (*n* = 39)**

Anatomical site	<i>n</i> of discordant findings ( <i>n</i> %)	<i>n</i> of false positive ( <i>n</i> %)	<i>n</i> of false negative ( <i>n</i> %)
Ovary	18 (5.3%)	1 (0.3%)	17 (5.1%)
Peritoneum	7 (8%)	0 (0%)	7 (8%)
Sentinel lymph node	6 (2.5%)	3 (1.2%)	3 (1.2%)
Lymph node	3 (3.8%)	2 (2.6%)	1 (1.3%)
Urologic	2 (3.8%)	0 (0%)	2 (3.8%)
Retroperitoneum	2 (22%)	0 (0%)	2 (22%)
Breast	1 (0.9%)	0 (0%)	1 (0.9%)

**TABLE 3 – Causes of discordance found in ovary re-testing**

Frozen section diagnosis	Paraffin diagnosis	Reason for discordance
Serous borderline cystic tumor	Well-differentiated serous carcinoma	Sampling error (sample size: 6 × 4 × 1.3)
Borderline serous papillary tumor	Well-differentiated papillary serous adenocarcinoma	Sampling error (sample size: 12 × 8.5 × 4.5)
Mucinous borderline tumor	Ovarian mucinous carcinoma	Sampling error (sample size: 20 × 19 × 10)
Mucinous papillary borderline tumor	Papillary mucinous cystadenocarcinoma	Sampling error (sample size: 15 × 11 × 8.5)
Serous borderline tumor	Invasive papillary serous adenocarcinoma	Sampling error (sample size: 24 × 16)
Serous borderline tumor	Serous cystadenocarcinoma	Sampling error (sample size: 12 × 9 × 4)
Mucinous papillary borderline tumor	Papillary mucinous cystadenocarcinoma	Sampling error (sample size: 29.5 × 22 × 13)
Mucinous borderline tumor	Invasive mucinous adenocarcinoma	Sampling error (sample size: 26 × 25 × 6)
Borderline serous papillary tumor	Papillary serous adenocarcinoma	Sampling error (sample size: 4.5 × 3.5 × 3.5)
Serous borderline tumor	Endometrioid adenocarcinoma	Sampling error (sample size: 10.5 × 10.5 × 7)
Mucinous tumor of uncertain behavior	Papillary mucinous adenocarcinoma	Sampling error (sample size: 30 × 17 × 13)
Papillary serous tumor	Metastatic adenocarcinoma	Sampling error (sample size: 2.6 × 2 × 1.8)
Mucinous tumor of uncertain malignant potential	Invasive mucinous adenocarcinoma	Interpretation error
Proliferation of atypical squamous epithelium	Epidermoid carcinoma	Sampling error (sample size: 22 × 18 × 18)
Metastatic carcinoma	Atypical mature bone	Diagnostic complexity
Serous cystadenoma	Serous cystadenocarcinoma	Sampling error (sample size: 14 × 12 × 9)
Atypical glandular proliferation	Serous cystadenocarcinoma	Sampling error (sample size: 4 × 4.3)
Spindle-cell neoplasm with atypias	Low-grade leiomyosarcoma	Diagnostic complexity

TABLE 4 – Causes of discordance found in re-testing of other organs

Anatomical site	Frozen section diagnosis	Paraffin diagnosis	Reason for discordance
Peritoneum	Adipose connective tissue infiltrated by atypical cells	Poorly-differentiated adenocarcinoma	Interpretation error
Peritoneum	Atypical cells of undefined nature	Moderately differentiated adenocarcinoma	Interpretation error
Peritoneum	Necrotic tissue interspersed with atypical epithelial cells	Metastatic undifferentiated carcinoma	Interpretation error
Peritoneum	Adipose connective tissue infiltrated by cells of undefined nature with hyperchromatic nuclei	Metastatic poorly-differentiated carcinoma	Interpretation error
Peritoneum	Atypical glandular proliferation	Metastatic adenocarcinoma	Interpretation error
Peritoneum	Fragment of connective tissue comprising inflammatory elements interspersed with large cells of probable epithelial nature	Metastatic signet ring cell carcinoma	Interpretation error + diagnostic complexity
Peritoneum	No evidence of neoplasm	Implantation of poorly-differentiated adenocarcinoma with signet ring cells	Interpretation error + diagnostic complexity
Sentinel lymph node	Presence of metastatic disease	Presence of neoplasm was not verified in the analyzed samples	Micrometastasis
Sentinel lymph node	Absence of neoplasia	Metastatic neoplasia	Interpretation error
Sentinel lymph node	Absence of neoplasia	Foci of metastatic carcinoma	Micrometastasis
Sentinel lymph node	Absence of neoplasia	Area suspicious for micrometastasis	Micrometastasis
Sentinel lymph node	Presence of a small area suspicious for micrometastasis	Absence of metastasis	Micrometastasis
Sentinel lymph node	Presence of carcinoma micrometastasis	Reactive hyperplasia of the lymphoid tissue	Micrometastasis
Lymph node (pelvic)	Neoplasia free	Presence of micrometastasis	Micrometastasis
Lymph node (mediastinal)	Carcinoma metastasis	Free of metastatic neoplasia	Micrometastasis
Lymph node	Focus of metastatic adenocarcinoma	Reactive lymph node	Micrometastasis
Urinary bladder	Not recognized as neoplasm	Urothelial carcinoma	Interpretation error
Urinary bladder	Neoplasia-free muscular layer	Squamous cell carcinoma	Interpretation error
Retroperitoneum	Epithelial proliferation with mild atypia	Neuroendocrine carcinoma	Sampling error (sample size: $0.7 \times 0.6 \times 0.2$ )
Retroperitoneum	Necrotic tissue with inflammatory alteration	Poorly-differentiated malignant neoplasm	Sampling error (sample size: $24 \times 3$ )
Breast	Unclassified epithelial proliferation	Ductal carcinoma	Interpretation error

multiple tissues and organs, differently from several other studies that analyze isolated organs, such as ovary<sup>(18)</sup>, and breast<sup>(17)</sup>.

In the present analysis, the anatomical sites in which frozen sections were most used were ovary (27.3%), mammary sentinel lymph node (19.8%), breast nodules (8.2%), and peritoneal nodules (7.1%). Pretti *et al.* (2016)<sup>(12)</sup>, analyzing 227 cases, identified lymph nodes as being the most common site (34.4%), followed by the oral cavity (24.5%), and gallbladder (6.7%). The breast was the most common site in the study by Kauffman *et al.* (1986)<sup>(7)</sup>, corresponding to 41.2% of 586 cases of frozen sections. At the institution (Imip) where our research was carried out, the gynecologic oncology service treats a great number of patients, what favors a higher frequency of frozen sections in ovarian neoplasms.

In the literature, the accuracy observed for frozen sections ranges from 87% to 97%<sup>(3, 12, 19-21)</sup>. Our study, although being retrospective, including multiple organs and evaluating exams performed by different professionals (important variable that can

hamper the final result), presented an accuracy of 96.3%. Method used in the study places, disagreement in professional training, diversity of organs analyzed, and aspects inherent in difficulties of the method are cited factors that can influence the accuracy of frozen sections<sup>(3, 6, 7, 22)</sup>.

Considering the several analyzed sites, our work demonstrated the highest accuracy (100%) in tumors of lung, skin, CNS, vulva, vagina, and uterine cervix. Sentinel lymph node biopsy had an accuracy of 96.7%, while frozen section of ovarian tumors had an accuracy of 94.6%. These percentages are similar to those observed in the literature<sup>(3, 17, 18, 23)</sup>.

The lowest accuracy (77.7%) in the present analysis was observed for retroperitoneal tumors. A possible reason for this low accuracy is the fact that such tumors are, in their majority, mesenchymal neoplasms (sarcomas) or lymphomas whose histopathological diagnosis is complex, needing a large sample of the lesion and complementary immune histochemical techniques for definition. Other authors<sup>(3, 7)</sup>, on the other hand, do not

associate organs and structures with higher or lower degree of accuracy. They always emphasize the adequate method and the technical training of the professionals who will evaluate the exam.

Among the 1,226 analyzed cases, we found 45 discordant ones, which were reassessed by an experienced pathologist of Imip. After the new analysis, four (8.9%) cases were considered concordant, two (4.4%) became inconclusive, and 39 (86.7%) remained discordant. The organ presenting most discordant results was the ovary (18 cases); tissue sampling of large surgical pieces seem to have been the cause of this discordance in 15 cases, while diagnostic complexity and limitation of the method may have influenced the other cases (Table 3). It is worth emphasizing that the morphological complexity of borderline ovarian tumors decreases frozen section sensitivity, as indicated by Yarandi *et al.* (2008)<sup>(24)</sup>. In the study by Malipatil and Crasta (2013)<sup>(18)</sup>, the rate of false negatives in borderline ovarian tumors was 26%.

Considering the 39 discordant cases in this analysis, 33 (84.6%) were classified as false negatives, and six (15.4%), as false positives. The false negative rate is similar to that of other studies<sup>(6, 18, 25)</sup>. The false positive rate, conversely, is higher, on average, than the reported in the literature<sup>(6, 11, 25)</sup>. In the study by Yarandi *et al.* (2008)<sup>(24)</sup>, analyzing 106 ovarian tumors, a rate of 2.5% (two cases) was found, while Kauffman *et al.* (1986)<sup>(7)</sup> found just one case among the 586 specimens of several organs.

Considering the six false-positive cases in this investigation, one was in the ovary and the others in lymph nodes (three mammary sentinel lymph nodes and two lymph nodes in other

regions). In the ovarian case, the frozen section diagnosis was metastatic carcinoma, while the final diagnosis was atypical mature bone. The rarity and the complexity of the case seem to have been responsible for the result.

The presence of micrometastases in the lymph nodes may have led to the alleged false-positive result. These small lesions are known to be taken in the initial slide confection, and therefore, will not be seen in the definitive evaluation. The clinical management of these patients is still a source of great controversy in the literature<sup>(17, 26)</sup>.

## CONCLUSION

The present investigation confirms the value of intraoperative frozen section for therapeutic definition at an analysis including multiple organs at a general teaching hospital. The high accuracy of this method allows its effective usage, as long as it is performed with adequate technique and standardization, and by trained professionals.

## DECLARATION

We declare that we received financial support from Conselho Nacional de Pesquisa (CNPq), by means of Programa Institucional de Bolsas de Iniciação Científica (Pibic), during a 12-month period.

## RESUMO

**Introdução:** O exame intraoperatório por congelção (EIC) visa avaliar histológica e intraoperatoriamente um pequeno fragmento de tecido ou órgão lesado no qual haja dúvida diagnóstica. Entre as indicações do EIC estão a determinação da natureza e a extensão da lesão, com consequente diferenciação entre lesões benignas e malignas, além da análise das margens cirúrgicas. **Objetivos:** Avaliar a acurácia do EIC em múltiplos órgãos e analisar possíveis fatores de interferência. **Métodos:** Foi realizado um estudo retrospectivo em um período de cinco anos (entre janeiro de 2011 e março de 2016) em um hospital de ensino da cidade do Recife, Pernambuco, Brasil. Os resultados dos EICs foram comparados com os laudos finais após o processamento histopatológico e classificados como concordantes ou discordantes. Os casos discordantes foram revistos por patologista e subdivididos em falso-positivos e falso-negativos. Possíveis causas para a discordância dos exames foram levantadas. **Resultados:** Foram analisadas 1.226 peças cirúrgicas, das quais 1.181 (96,33%) foram concordantes e 45 (3,67%), discordantes. Após reavaliação dos casos discordantes, 39 permaneceram, sendo seis (15,4%) falso-positivos e 33 (84,6%) falso-negativos. A estrutura que mais apresentou resultado falso-positivo foi o linfonodo sentinela mamário (3/1,2%), enquanto o ovário foi o órgão com mais resultados falso-negativos, com 17 amostras, 51,51% de todos os casos negativos. As possíveis causas para a discordância foram tamanho da amostra, limitação do método e complexidade do diagnóstico. **Conclusão:** A acurácia do EIC encontrada neste estudo foi de 96,3% e é semelhante à literatura especializada.

**Unitermos:** secções congeladas; biópsia; neoplasias.



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