

## **Role of intraoperative frozen section evaluation of sentinel lymph node in breast carcinoma**

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### **Abstract**

**Background** Breast carcinoma is the most common malignancy in women worldwide.

Intraoperative frozen section of SLNs can detect metastatic disease, allowing immediate axillary dissection and avoiding the need for reoperation, at the same time it avoids unnecessary dissection of uninvolved LNs.

### **Aim of the study**

1. To evaluate the current role of frozen section in identifying patients who could benefit from it.
2. To verify the accuracy of intraoperative frozen section examination for SLNs.

**Materials and methods** In this combined retrospective and prospective study, we reviewed the outcome for 33 female patients with breast cancer who underwent intraoperative SLN biopsy, at (Al-Shariqa laboratory) and (Ghazi Al-Hariri Hospital, Medical City) Baghdad, Iraq. and compared it with the permanent H&E sections, covering the period between Jan.2019 and Jan.2021

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**Results:** The patients age was ranging from 32 to 79 years with a mean of 50.7 years and the highest proportion was  $\geq 40$  years (90%).

Only 10 patients (30.3%) of the total cases were diagnosed as having POSITIVE SLNs by intraoperative frozen section examination, while 23 patients (69.7%) had NEGATIVE SLNs. Nine of them (90%) were of ductal type, while only 1 case (10%) was of lobular type. all 10 cases (100%), the primary tumor size measured  $>2\text{cm}$ , (i.e: pT2). Validity of frozen section examination in comparison with permanent sections showed a sensitivity and specificity of 100%.

**Conclusions:** Intraoperative frozen section evaluation of SLN biopsy is a reliable method, it offers a high sensitivity, specificity and diagnostic accuracy when compared with permanent section

## **Introduction**

Breast carcinoma is the second leading cause of cancer related deaths among women worldwide and the presence of axillary LN metastases has been regarded as a predictor of poorer survival.<sup>(1)</sup>

Axillary LN status is one of the most important prognostic indicators in breast cancer, particularly for the selection of adjuvant therapy.

Since the introduction of SLNB in the management of breast cancer, methods for rapid assessment of SLN included frozen section and imprint cytology. Although the gold standard is examining a paraffin-embedded section, intraoperative frozen section at the time of SLNB allows ALND to be avoided if negative.<sup>(2)</sup>

## **Aim of the study**

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2. To verify the accuracy of intraoperative frozen section examination for SLN

## **The Sentinel Lymph Node (SLN)**

-The sentinel lymph node (SLN) is defined as the first regional lymph node to receive lymphatic fluid from a malignant tumor.<sup>(3)</sup> It provides an accurate assessment of histological nodal status and is associated with less acute and chronic morbidity than axillary-lymph-node dissection.<sup>(4)</sup>

Intraoperative evaluation of axillary SLN is standard of care for breast cancer. Intraoperative frozen section or imprint cytology is performed on the SLN to determine need for axillary lymph node dissection.<sup>(5)</sup>

-SLN biopsy is well established as standard care for axillary LN staging in most patients with cN0 breast cancer. Compared to axillary lymph node dissection (ALND), the staging accuracy and oncologic outcomes of SLN biopsy are comparable and the morbidity is less. Current indications for SLN biopsy are as follows:

- T1-2 invasive breast cancer with a clinically negative axilla.
- DCIS sufficient to require mastectomy, or DCIS with suspected/proved microinvasion.
- Patients with clinically negative axillary nodes following neoadjuvant chemotherapy<sup>(6)</sup>

To date, the optimal number of SLNs that should be removed to accurately predict axillary lymph node status remains controversial.<sup>(7)</sup>

Lymphatic mapping techniques have several objectives, but no generally accepted and practiced uniform concept is universally available. So far, three strategies for detection of SLN have been proposed:

- Labeling of the sentinel node(s) with a dye, such as “patent blue” to identify draining lymph vessels and the position(s) of the first regional LNs.
- Labeling of the SLN by <sup>99</sup>mTc-colloid scintigraphy;
- Sophisticated nuclear medicine and radiological methods<sup>(3)</sup>

### **The Frozen Section**

Frozen section represents one of the most important procedures carried out by the pathologist. At the same time, it's one of the most difficult and most stressful tasks in the practice of pathology.<sup>(8)</sup>

Performing a frozen section analysis is a multistep process, beginning with retrieval of the specimen from the patient by the surgeon followed by intraoperative preparation of slides, their microscopic examination and finally rendering the FS diagnosis.<sup>(9)</sup>

The examination is made while the patient is under anesthesia on the operating table and the report will then be conveyed as soon as possible to the operating surgeon via telephone or intercoms and the result will greatly influence the surgeon's intra-operational decision.<sup>(10)</sup>

### **The Principle of Frozen Section**

The rapid freezing of the tissue sample converts the water into ice. The firm ice within the tissue acts as embedding media to cut the tissue. The key instrument for Cryosection is the cryostat, which is essentially a microtome inside a freezer, capable of slicing sections as thin as 1 micrometre.<sup>(11), (12)</sup>

### **Indications for Frozen Section**<sup>(13,14)</sup>

Intraoperative consults, with or without frozen section, should be limited to the following indications:

1. Provide a diagnosis that will allow the surgeon to make an intraoperative decision.
2. Assess margins when additional excision to attain a negative margin is an option.
3. Assess adequacy of diagnostic tissue in a biopsy specimen from an open or a complicated procedure.
4. Plan the workup of the specimen. The need for cytogenetics, flow cytometry, and other special studies should reasonably be evaluated prior to fixation.
5. To do enzyme immunocytochemistry, immunofluorescence study & stain lipid and certain carbohydrate in the tissue

### **Contraindications for Frozen Section**

Frozen section should never be undertaken in cases when:

- Frozen section diagnosis has no immediate implications for decision making
- Tissue is needed for permanent processing (is unique or small or requires extensive study for diagnosis)
- Tissue is heavily ossified / calcified
- Risk of serious infection (HIV, TB, hepatitis B or C)
- The lesion is architecturally fragile, or if the material is very scarce, so that the morphologic attributes might be irreparably damaged by frozen section artifact,
- The specimen consists of a large mass and the necessarily limited sampling at the time of frozen section might lead to high degree of error <sup>(15)</sup>

### **Materials and Methods**

#### **Tissue samples:**

- Data were retrospectively collected from 33 patients who underwent intraoperative SLN biopsy and evaluated with frozen section and definitive section at (Al-Shariqa private laboratory) and (Al-shaheed Ghazi Alhariri teaching hospital)/ Baghdad/ Iraq.
- The study covered the period between January 2019 and January 2021.
- Age of the patients range 32 years to 79 years.
- In all cases, the excised SLNs were submitted for immediate frozen section diagnosis, with the remaining tissue submission for permanent formalin fixed paraffin blocked hematoxylin and eosin sections.
- Patients who received neoadjuvant chemotherapy, patients with carcinoma in situ without invasive component and patients with missing data were excluded.

#### **Method:**

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- First, a careful gross examination, dissection of adipose tissue, and several sampling cuts of tissues were done. Smaller nodes were sliced in two, while bigger nodes were sliced each 3–5 mm. Afterwards, sampling tissues were processed by freezing them with frozen aerosol sprays and put onto the cryostat for sectioning (the temperature inside was about  $-20^{\circ}$  to  $-30^{\circ}$  C). The tissue sections are cut and picked up on glass slides, which are then ready for staining with a modified hematoxylin and eosin (H&E) stains.
- This whole process (from receiving the sample until the delivery of the slides for microscopic examination) takes no more than 12-15 minutes.
- The remaining tissues were sectioned at 2 mm intervals, fixed in formalin, embedded in paraffin, and entirely submitted for definitive diagnosis.
- The results of frozen section and definitive section were compared regarding the pathological diagnosis for the SLNs. The standard was based on the results for the definitive section.
- In addition, we compiled clinicopathological data including patient age, histologic tumor type, tumor size and SLN size.

### **Statistical Analysis:**

Statistical package for social sciences version 24 (SPSS v24) used to analyze data. Continuous variables presented as means with standard deviation and discrete variables presented as numbers and percentages.

Chi-square test for independence used to test the significance of association between discrete variables. Kappa statistics used to estimate the degree of agreement with the exclusion of chance between the results of frozen and permanent sections.

Level of significance was set at P value  $< 0.05$ .

**Results**

The total number of study cases was 33. All of them were females who underwent intraoperative SLN biopsy and evaluated with immediate frozen section and standard definitive hematoxylin and eosin section.

**Age:** Study patients age was ranging from 32 to 79 years with a mean of 50.7 years and a standard deviation (SD) of  $\pm 9.9$  years.

The highest proportion of study patients was aged  $\geq 40$  years (90%).

**Size and Number of Sentinel Lymph Nodes:**

The size of all examined sentinel lymph nodes ranged from 1 to 3 cm, with a mean of 1.5 cm and a standard deviation (SD) of  $\pm 0.6$  cm.

While the number of sentinel lymph nodes per patient in the study ranged from 1 to 5 lymph nodes, with a mean of 2.2 lymph nodes and a standard deviation (SD) of  $\pm 1.1$ .

**Table (4.1): Descriptive statistics for age of patient, size and number of SLN:**

*\*No.SLN: number of sentinel lymph nodes*

Variables	Min-Max	Mean $\pm$ SD
Age (y)	32-79	50.7 $\pm$ 9.9
Size (cm)	1-3	1.5 $\pm$ 0.6
No.SLN	1-5	2.2 $\pm$ 1.1

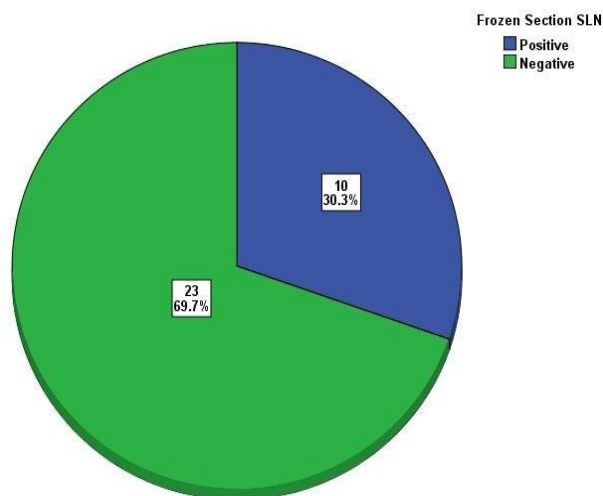
**Characteristics of Studied SLN:**

- **Side:** From the total number of 33 patients in the study, 18 of them (54.5%) were right sided SLNB, while 15 of them (45.5%) were left sided SLNB.

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- **Involvement by metastatic breast carcinoma:** Only 10 patients (30.3%) of the total 33 cases of the study were diagnosed as having positive sentinel lymph nodes by intraoperative frozen section examination, while 23 patients (69.7%) had negative sentinel lymph nodes (free of involvement by malignant cells). (as shown in figure 4.1)

The permanent sections by standard H&E microscopic examination confirmed the same results



obtained from the frozen section examination

**Figure (4.1):** Pie chart showing distribution of sampled specimens according to histology results.

Variable	N=33	100.0%
Side <input type="checkbox"/> Right	18	54.5%
<input type="checkbox"/> Left	15	45.5%



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**Frozen section SLN**

Positive	10	30.3%
☐ Negative	23	69.7%

**Permanent section**

Positive	10	30.3%
☐ Negative	23	69.7%

**Table (4.2): Distribution of total patients according to studied variables:**

**Regarding the total of 10 cases who had positive SLNs (on both frozen and permanent sections):** (as shown in table 4.3)

- **Side:** 4 of them (40%) were from right side and 6 of them (60%) were from left side. (P= 0.269)
- **Histological type of carcinoma:** 9 of them (90%) were of ductal type, while only 1 case (10%) was of lobular type. (P=675).
- **Size of the primary breast tumor (stage):** in all 10 cases (100%), the primary tumor size measured >2cm, (i.e: pT2). (P=0.020)

**Table (4.3): Certain characteristics and their association with the positive SLN:**

Variables	Frozen section & permanent SLN				P value
	Positive		Negative		
	N=10	100.0%	N=23	100.0%	
<b>Side</b>					0.269
☐ Right	4	40.0%	14	60.9%	
☐ Left	6	60.0%	9	39.1%	
<b>Type</b>					0.675
☐ Ductal	9	90.0%	21	91.3%	

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□ Lobular	1	10.0%	2	8.7%	
<b>Stage</b>					<b>0.020</b>
□ T1	0	0.0%	9	39.1%	
□ T2	10	100.0%	14	60.9%	

**Table 4.4: Relations between results of frozen and permanent sections of SLN histopathology: A) Distribution of findings, B) Validity of frozen section examination, C) agreement between SLN & permanent histology results.**

A)

		Permanent section		Total
		Positive	Negative	
Frozen section	Positive	10	0	10
	Negative	0	23	23
Total		10	23	33

B)

Screening Indicators	Value	[95% CI]
Sensitivity	100%	66% - 99%
Specificity	100%	82% - 100%
Accuracy	100%	87% - 100%

C)

Measures of Agreement		
Crude agreement	Kappa	
value	Value	P value
100.0%	1.000	<0.001

**NB) Kappa (Agreement ratio after excluding chance)**

**4.5. The following photomicrographs are from some of the current study cases showing frozen and permanent sections of positive and negative sentinel lymph nodes.**

**4.4 Relations between results of frozen section and permanent section microscopic examination of SLN.**

- As shown in table (4.4 A), the results of permanent sections were completely corresponding to the findings of intraoperative frozen section results.
- Validity of frozen section examination (as shown in table 4.4B), showed a sensitivity and specificity of 100%, with an accuracy CI value of (87%- 100%).

Finally, the crude agreement between the results from frozen section examination and permanent sections was 100% and with a P value of <0.001. (as shown in table 4.4C)

## **Discussion**

Intraoperative detection of metastatic carcinoma in SLNs leads to immediate ALND, avoiding the need for a delayed second surgical procedure.

As there are concerns about the accuracy of intraoperative assessment by frozen section analysis, this review evaluated the role of intraoperative lymph node assessment. <sup>(16)</sup>

This study was the first of its type in this country, so no local studies were available for comparing our results with them. It is performed with the aim of studying the effectiveness of frozen section examination of SLN in breast cancer.

Overall, our findings are in keeping with previously obtained diagnostic conclusions drawn by means of a more extended studies.

The median age of the patients in this study was 50 years with the vast majority of cases being aged  $\geq 40$  years (90%).

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**Almarzooq R. (2018)** in his study in Al-Bahrain showed agreement with a median age of their study patients being 50 years.<sup>(2)</sup>

**Poling, Justin S., et al. (2014)**, agreed in their study on American patients at Johns Hopkins Hospital and estimated a median age of 52 years old.<sup>(5)</sup>

So there is a great agreement with the known fact that peri & postmenopausal women are the main age group to be involved by breast carcinoma.

Intraoperatively, the number of SLNs harvested by the surgeon ranged from one to five (1-5) axillary lymph nodes, with a median of 2.2 lymph nodes.

**Almarzooq R. (2018)**, showed a median number of SLNs of 2 LN<sup>(2)</sup>.

**Poling, Justin S., et al. (2014)**, stated a median number of SLNs of 2.42 lymph nodes.<sup>(5)</sup>

**Kwang Hyun Yoon, et al. (2019)**, in a study from Korea, also showed similar results with a median number of SLNs of 2.73 lymph nodes.<sup>(17)</sup>

This difference is mainly due to random patient selection and different procedural methods applied, per surgeon criteria. Our surgeon depends upon role of 10% in LN excision, and removed any suspected LN.

Regarding the side of involved breast in the 10 patients with positive SLNs, 4 of them (40%) were right sided, while 6 of them (60%) left sided.

**Rutter, Charles E, et al (2014)**, in their study for the American Cancer Society, agreed with this, and showed that a slight majority of cases (50.7%) were left-sided, while (49.3%) were right sided.<sup>(18)</sup> while **Almarzooq R. (2018)** in his study in Al-Bahrain showed a different result with 58 cases (55.7%) being right sided and 46 cases (44.3%) left sided.<sup>(2)</sup>

So results of this study was in keeping with the fact that, there is a slightly higher predilection of malignancy for the left breast.

In the present study, from the total of 33 cases, only 10 cases (30.3%) had positively involved SLNs by malignant cell metastasis, while 23 cases (69.7%) were negative.

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This was in agreement with **Cipolla, Calogero, et al. (2020)**, by a study done at Italy; who detected positive SLN in 239 cases (16.3%), from a total of 1,466 cases, while negative nodes in 1215 cases (82.9%).<sup>(17)</sup>

**Lombardi, Augusto, et al. (2018)**, in an Italian study, agreed to these results by showing that positive SLNs were found in 313 cases (25.6%) of the 1224 patients, and negative in 911 cases (74.4%).<sup>(18)</sup>

These results proved that frozen section examination of the SLNs saved the majority of the patients (in the present and the reviewed studies), from the unneeded complications of axillary lymph node dissection.

The histological type of primary breast carcinoma in our study showed that 9 of the 10 cases with positive SLN (90.0%) were of invasive ductal type, while only one case (10.0%) was of lobular type.

**Godazande, Gholamali, et al. (2020)**, showed comparable results and (94.1%) of their cases were of invasive ductal type, with the lobular type being only (2.9%).<sup>(19)</sup>

**Kwang Hyun Yoon, et al. (2019)**, agreed with our results, from a total of 4046 cases, detected ductal type carcinoma in 3,411 cases (84.3%), while 195 cases (4.8%) was of lobular type, and the remaining (10.9%) was of other histological types.<sup>(17)</sup>

The pathological stage (pT) of all the cases (100%) in the current study which had positively involved SLNs was (pT2).

**Godazande, Gholamali, et al. (2020)**, stated a relatively comparable results and showed that only (21.6%) of their cases were (pT1), while the remaining majority (78.4%) were (pT2).<sup>(19)</sup>

**Hennigs, André, et al. (2019)**, in a German study, also disagreed with our results and stated that (49.5%) of the patients with positive SLNs were (pT1), and only (29.9%) of the patients were staged as (pT2).<sup>(20)</sup>

The present study results showed that fresh frozen section procedure had a sensitivity and specificity of 100% when compared to standard permanent section examination.

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**Ahadi Met al. (2017)** with a study in Iran, showed comparable results with a sensitivity and specificity of 81.8% and 97.8% respectively.<sup>(21)</sup>

**Somashekhar, S. P., et al (2015)**, in a study on Indian patients, showed sensitivity of (92.6%), specificity of (100%).<sup>(22)</sup>

This difference in sensitivity may be attributable to different samples sizes, smaller number of cases and interobserver variability.

Finally, some limitations were observed in this study; a relatively small number of patients (n=33), and the limited sites for sample collection due to the fact of the limited use of frozen section technique in our hospitals.

Also the unavailability of matched resection specimens for some frozen section samples obligated us to ignore these cases and this also can be considered as a limitation to the present study.

## Conclusions

1. SLNB in patients undergoing surgery for breast cancer results in a significant reduction in physical and psychological morbidity.
2. Intraoperative frozen section evaluation of SLN biopsy in patients with breast carcinoma is a reliable method, which can determine the need for immediate axillary dissection in patients with positive results, so it can spare them the need for reoperation. It can also safe patients with negative results from the unnecessary axillary lymph node dissection.
3. Frozen section analysis offer a high sensitivity, specificity and diagnostic accuracy when compared with permanent sections.

## Recommendations

1. Further studies with larger number of cases and clinical follow- up of patients to confirm or reject the results of this study.
2. The routine use of SLN frozen section evaluation in early stage breast cancer patients.

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3. Similar studies to be applied on other tumors and other surgical procedures (for example: assessing surgical margins) to evaluate the important role and assistance of intraoperative frozen section analysis in the surgeon's decision making and, in turn; a great benefit to the patient.

The study is self-funded

There is no conflict of interest between the authors

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