

Comparison of Touch Imprint Cytology and Frozen Section for Evaluating Sentinel Lymph Nodes in Breast Cancer: A Study from a Tertiary Oncology Center in Upper Egypt

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Abstract

Background: Sentinel lymph node (SLN) biopsy is the preferred method for diagnosing axillary nodal involvement in breast cancer. Touch imprint cytology (TIC) and frozen section (FS) are two intraoperative techniques used to assess SLNs. Both methods have their disadvantages, and the superior technique remains uncertain.

Aim: To determine the accuracy of TIC compared to FS in identifying metastases in SLNs in preoperatively node-negative axilla.

Methods: Forty-six females with breast cancer and clinically and radiologically negative axillary lymph nodes were included in the study. For TIC, SLNs were bisected, imprinted onto clean slides, stained, and evaluated. For FS, tissue slices of the same lymph nodes were embedded in an appropriate medium and frozen to -25°C. Tissue sections were prepared using a cryostat, stained, and evaluated.

Results: The patients' ages ranged from 30 to 71 years. There was a strong agreement between TIC and FS results ($\kappa=0.864$; $p<0.0001$). Both TIC and FS findings strongly correlated with the final nodal evaluation of SLNs by paraffin section ($\kappa=0.909$; $p<0.0001$ and $\kappa=0.955$; $p<0.0001$, respectively). The sensitivity, specificity, positive predictive value, and negative predictive value of TIC for detecting axillary lymph node metastasis were 96.4%, 94.4%, 96.4%, and 94.4%, respectively. For FS, these values were 96.4%, 100%, 100%, and 94.7%, respectively.

Conclusion: Touch imprint cytology is a feasible, quick, and cost-effective alternative to frozen section for intraoperative evaluation of SLNs in breast cancer.

Keywords: Breast cancer, Frozen section, Sentinel lymph node, Touch imprint cytology

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Introduction

Breast carcinoma is the most commonly diagnosed malignant tumor worldwide, with over 2.3 million new cases and 685,000 deaths recorded in 2020 ¹. In Egypt, it accounts for 32% of female cancer cases, according to global cancer registry 2020 ², with an annual rate exceeding 22,000 patients ³. Axillary

lymph node dissection (ALND) is essential to assess the disease stage and determine subsequent treatment for breast cancer patients. However, ALND has significant morbidity, including postoperative arm edema. Therefore, ALND is advised with caution, and immediate intraoperative evaluation of axillary lymph nodes is always required to minimize the number of dissected lymph nodes ^{4,5}. Sentinel lymph

node (SLN) biopsy is the gold standard for assessing the status of axillary lymph nodes in breast cancer patients. Frozen section (FS) remains the most popular method of intraoperative SLN evaluation. Touch imprint cytology (TIC) has established accuracy and excellent sensitivity and specificity ⁴. Practically, where FS is not always available, intraoperative TIC could be a valuable and convenient alternative. Touch imprint cytology is a simple, time-efficient and cost-effective method requiring minimal preparation ⁶. In contrast, FS is time-consuming and requires special instruments and several prerequisites for SLN evaluation ⁵.

In this study, the intra-operative evaluation of SLNs by FS and TIC was compared at a tertiary oncology centre in Upper Egypt.

Methods

This prospective study was conducted over one year at the Pathology unit of Sohag Cancer Institute in Sohag, Egypt. It included 46 female patients with breast cancer, all diagnosed preoperatively using image-guided tru-cut needle biopsy (TCNB). Patients included in the study did not have suspicious axillary lymph nodes, either clinically or radiologically. Those

with systemic metastasis or who had received neoadjuvant therapy were excluded.

Tumors were graded using the Bloom and Richardson scheme ⁷, with the tumor size defined as the maximum diameter. Tumor stage was classified according to this diameter: pT1 for tumors up to 2 cm, pT2 for tumors between 2.1 and 5 cm, and pT3 for tumors larger than 5 cm. Estrogen receptor (ER) and progesterone receptor (PR) expression were scored using the Allred scheme ⁸, and HER2 immunoreaction was evaluated in invasive tumor cells according to the 2018 ASCO/CAP recommendations ⁹. The Ki67 mitotic index was calculated as the mean percentage of positive cells in the tumor tissue's hot spots.

For each patient, TIC and FS were performed to evaluate the status of SLNs, and their results were compared to the findings of the standard hematoxylin and eosin (H&E) stained sections of formalin-fixed paraffin-embedded tissue. Intraoperatively, SLNs were identified using a standard protocol involving the injection of patent blue dye. This technique included the subcutaneous administration of 2 mL of patent blue dye into the peri-areolar area, followed by a visual scan of the axilla to localize SLNs ¹⁰. All blue-stained nodes were dissected and evaluated for metastatic deposits using FS and TIC.

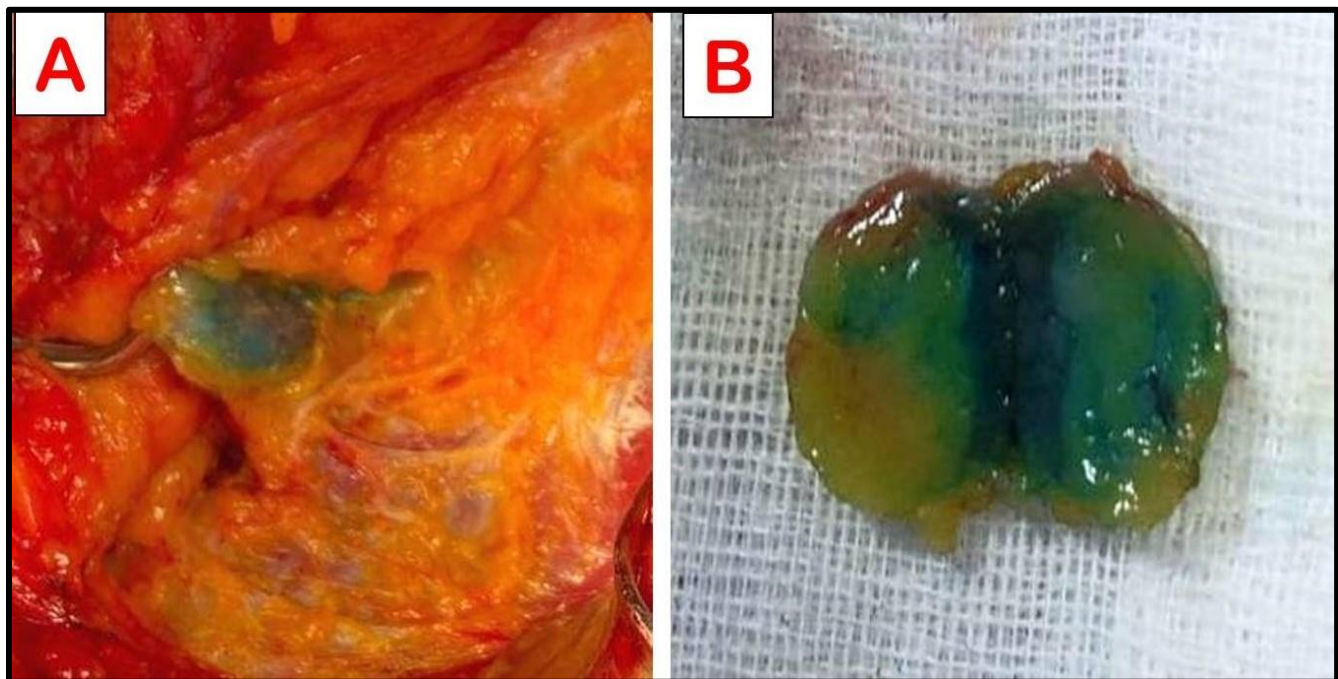


Figure 1: Intraoperative detection of a sentinel lymph node in axillary fat using patent blue dye (A) and a bisected sentinel lymph node after extraction (B)

For evaluation by TIC, the dissected lymph nodes were bisected, and their cut surfaces were imprinted onto clean pre-labeled slides. At least two smears were prepared from each lymph node, and these smears were subsequently stained with H&E using a standard staining protocol. For FS, tissue slices of SLNs were entirely embedded in cryo-embedding medium and frozen to -25°C using a cryostat. Tissue sections of 6 µm thickness were prepared and stained with H&E according to the standard staining protocol. Finally, the remaining tissue slices of SLNs were thawed to room temperature, fixed in 10% neutral buffered formaldehyde, and processed as usual to prepare formalin-fixed paraffin-embedded tissue sections. The findings from TIC, FS, and paraffin sections were examined blindly by two pathologists.

Statistical analysis

Correlation between the findings of TIC and FS with those of the paraffin sections was measured using the Chi-square test (χ^2). The degree of agreement between the findings of TIC, FS, and paraffin sections was evaluated using Cohen's Kappa Coefficient (κ). Sensitivity, specificity, positive predictive value, and negative predictive value of both TIC and FS were calculated using 2x2 tables. The receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was used to assess the accuracy of TIC and FS in comparison to standard paraffin sections. The cut-off for statistical significance was set at $p < 0.05$. Data analysis was performed using the Statistical Package for Social Sciences (Version 22.0, IBM Inc., Chicago, USA).

Results

The study included 46 female patients with breast cancer, confirmed histologically by TCNB at Sohag Cancer Institute. Clinical and pathological parameters of the investigated cases are summarized in Table 1.

The patients' ages ranged from 30 to 71 years, with a mean and median (\pm SD) age of 51.3 and 52 (\pm 11.2) years, respectively. Based on TCNB, the most frequent diagnosis was invasive ductal carcinoma, not otherwise specified, while one tumor was diagnosed as invasive mixed ductal-lobular carcinoma.

Table 1: Descriptive features of 46 breast cancer specimens

Variable	n (%)
Tumor size	
≤ 2cm	17 (37)
> 2cm	29 (63)
Clinical / radiological T stage	
T1	18 (39.1)
T2	26 (56.5)
T3	2 (4.4)
Pathological diagnosis (TCNB)	
Ductal carcinoma in situ	6 (13)
Invasive ductal carcinoma, NOS	34 (73.9)
Invasive lobular carcinoma	2 (4.4)
Mixed duct/lobular carcinoma	1 (2.2)
Mucinous carcinoma	3 (6.5)
Tumor grade	
G1	4 (8.7)
G2	32 (69.6)
G3	7 (15.2)
Ductal carcinoma in situ high grade	3 (6.5)
Estrogen receptor (TCNB)	
Negative (score 0-2)	5 (10.9)
Positive (score 3-8)	41 (89.1)
Progesterone receptor (TCNB)	
Negative (score 0-2)	5 (10.9)
Positive (score 3-8)	41 (89.1)
HER2 expression (TCNB)	
Negative (score 0-1)	34 (73.9)
Positive (score 3)	6 (13.1)
Not evaluated*	6 (13.1)
Ki67 mitotic index	
Mean (SD)	35.5 (29.3)
Median	25
Intrinsic subtype	
Luminal A	17 (37)
Luminal B	19 (41.3)
HER2-rich	1 (2.2)
Triple negative	3 (6.5)
Not specified**	6 (13)

TCNB: Tru-cut needle biopsy, **NOS:** Not otherwise specified, **SD:** Standard deviation, * HER2 was not evaluated in 6 cases; three were in-situ carcinomas and three were inadequate needle biopsies, ** Intrinsic subtypes were not specified in 6 cases due to the absence of HER2 status

Six of the tumors were diagnosed as high-grade ductal carcinoma in situ based on needle biopsy; three of these showed evidence of stromal invasion after evaluation of excisional tissue samples. Pre-operative clinical and radiological evaluation indicated that 27

patients had stage T2 primary tumors, and all patients had no suspicious axillary lymph node metastasis (N0). The intrinsic subtype of the tumors was either luminal A or luminal B in most cases, and HER2 expression was positive in six tumors.

The number of SLNs identified by patent blue dye ranged from 1 to 5 lymph nodes in 42 patients, and it was 6 to 8 lymph nodes in the remaining four cases. Sentinel lymph nodes were evaluated for metastatic deposits using FS and TIC techniques. The average time for preparation and evaluation of imprint smears was 15 minutes, compared to an average time of 28 minutes for the preparation and evaluation of FS.

Upon evaluation of SLNs: TIC (Figure 1A and B) detected positive metastatic deposits in axillary lymph nodes in 18 patients, and FS (Figure 1C) revealed nodal metastatic deposits in 19 patients. Final evaluation by paraffin section (Figure 1D) confirmed metastatic deposits in SLNs of 18 patients.

Statistically, there was a strong correlation between the results of TIC and FS ($\chi^2=34.4$, $p<0.0001$). Additionally, both TIC and FS showed strong correlation with the final nodal evaluation of SLNs by paraffin section ($\chi^2=37.99$, $p<0.0001$ and $\chi^2=42.02$, $p<0.0001$, respectively; see Table 2).

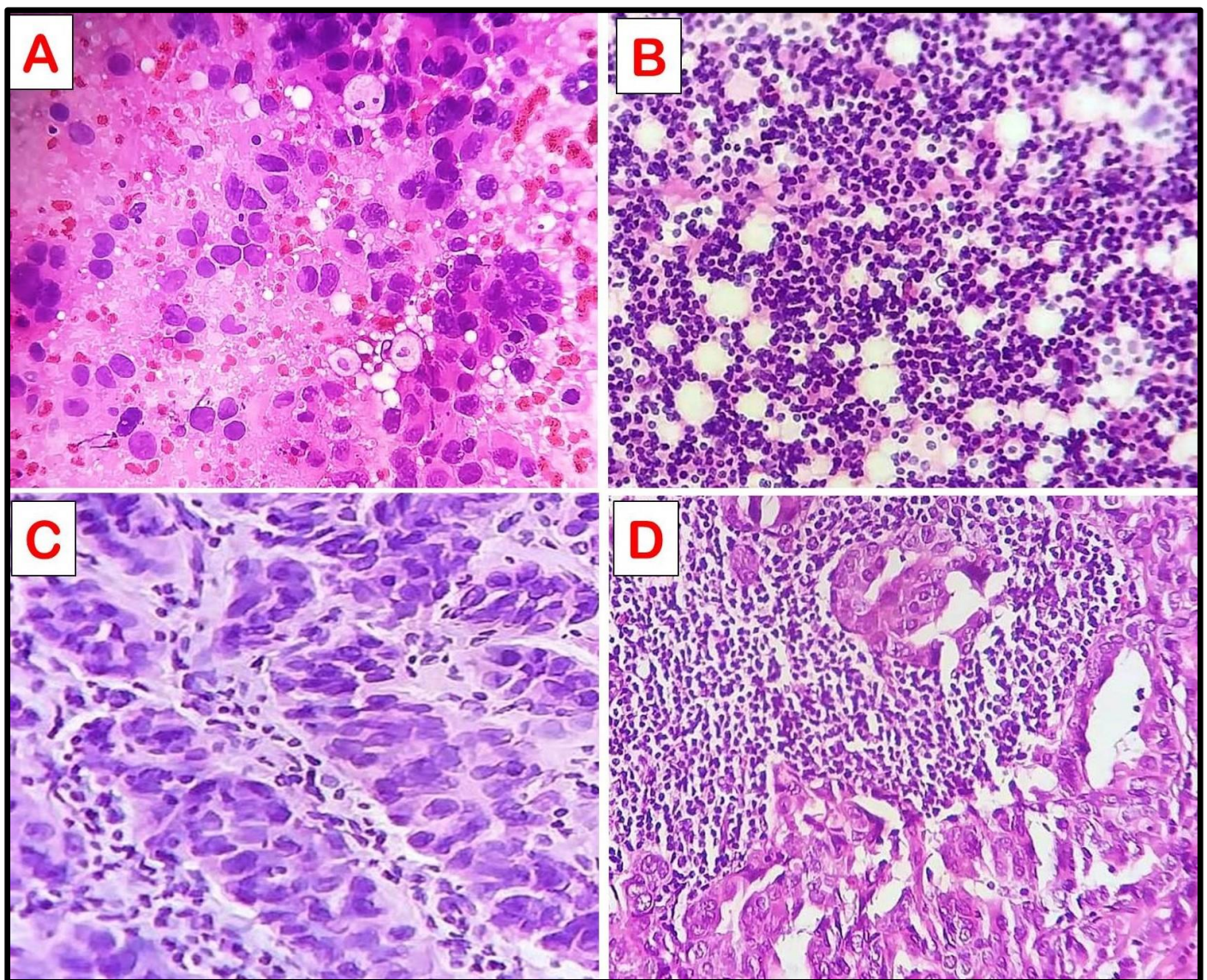


Figure 2: Touch imprint cytology positive (A) or negative (B) for metastatic deposits in sentinel lymph nodes of breast cancer. Frozen section (C) and paraffin section (D) displaying metastatic deposits, stained with H&E. All images are at 400× magnification.

There was a very good agreement between the findings obtained by TIC and those obtained by FS (κ coefficient = 0.864; $p < 0.0001$). Furthermore, findings of both TIC and FS showed strong agreements with the final diagnosis obtained by paraffin section (κ coefficient = 0.909; $p < 0.0001$ and κ coefficient = 0.955; $p < 0.0001$; respectively).

Table 2: Correlation between SLNs evaluation by touch imprint cytology and frozen section with the final evaluation by paraffin section

	Evaluation of SLNs by paraffin section		<i>p</i> value
	Negative	Positive	
Evaluation of SLNs by touch imprint cytology			
Negative	27 (96.4)	1 (5.6)	<0.0001
Positive	1 (3.6)	17 (94.4)	
Evaluation of SLNs by frozen section			
Negative	27 (96.4)	0	<0.0001
Positive	1 (3.6)	18 (100)	

SLNs: Sentinel lymph nodes

The sensitivity and specificity of TIC in detecting axillary lymph node metastasis were 96.4% and specificity 94.4%; respectively and those of FS were 96.4% and 100%; respectively. The positive and negative predictive values of TIC were 96.4% and 94.4%; respectively while those of FS were 100% and 94.7%; respectively (Figure 3).

Discussion

Axillary lymph node dissection is crucial in the surgical management of breast cancer, and assessing lymph node status is vital for determining adjuvant therapy options for this disease ¹¹. Despite its associated morbidities, ALND remains essential in node-positive patients. In cases where the axilla is radiologically and clinically negative, ALND can potentially be minimized with the assistance of intraoperative evaluation using FS. However, many institutions do not utilize FS due to inadequate equipment and infrastructure ¹². Frozen section can give accurate information on lymph node status but limited by frequent tissue loss needed for permanent paraffin sections ¹³.

Touch imprint cytology could be a suitable alternative to FS with a high accuracy rate. A published article concluded that evaluation of SLNs using TIC is a useful technique with excellent specificity and sensitivity rates ¹⁴. Despite its several benefits, TIC cannot distinguish between macro- and micro-metastases ¹⁵. In addition, the procedure requires a trained cyto-pathologist having expertise in breast cytology to obtain optimum and reliable results ¹⁶. In this study, the accuracy of TIC in assessing axillary lymph node status was compared to that of FS in a tertiary oncology centre in Upper Egypt.

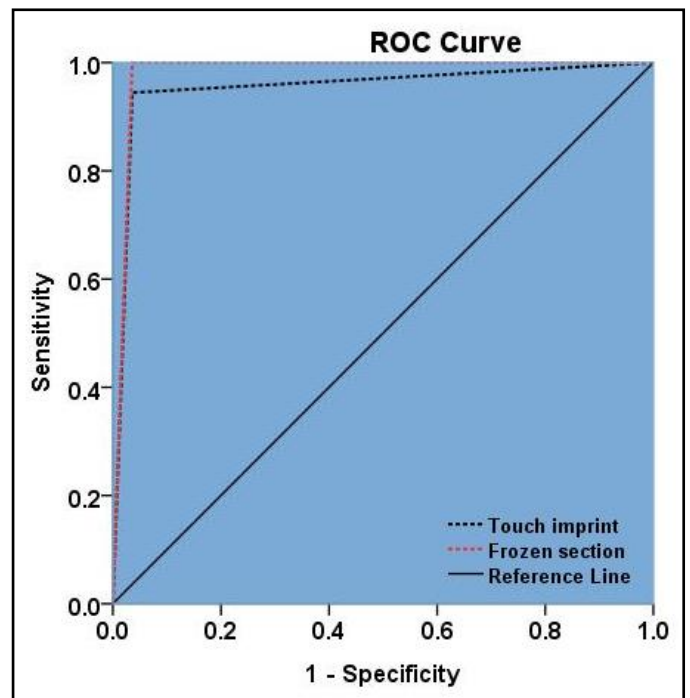


Figure 3: Sensitivity and specificity of touch imprint cytology and frozen section in comparison to paraffin section to evaluate sentinel lymph nodes of breast cancer

The data from this study demonstrated that the sensitivity and specificity of TIC in detecting axillary lymph node metastasis were 96.4% and 94.4%, respectively, while for frozen section (FS), they were 96.4% and 100%, respectively. Previous studies have shown considerable variability in sensitivity, ranging from 44% to 100% for FS and from 34% to 95% for TIC ¹³. Other studies have estimated the sensitivity of TIC to range from 29% to 94%, with accuracy ranging from 78% to 98%, and specificity ranging from 88% to 100%. Combined FS and TIC techniques have been found to

be as effective as FS or TIC alone. However, the detection of metastatic carcinomas and lobular breast carcinomas is less accurate with TIC, and determining the invasive component in cases of in situ carcinoma is challenging with reported cases of false negatives¹⁷.

The explanations for false negative rates of TIC include sampling error or improper sample preparation and evaluation. Another reason for false negative intraoperative diagnoses is the missed imprinting of micrometastases; however, excessive imprinting of the lymph node may resolve this issue¹⁸. In this study, the specificity of TIC and FS was nearly equal, but the false negative rate for TIC was higher than that for FS, consistent with the majority of previous studies¹⁹⁻²². False negative results of TIC in this study were mainly caused by missed micrometastases or low-grade lobular carcinoma, which is consistent with findings from previous research²². In the study conducted by Ivković-Kapicl *et al.* 2020¹², a single case with a false positive imprint was attributed to misinterpretation of epithelioid histiocytes, while germinal center lymphocytes or activated endothelial cells could rarely be mistaken for tumor cells.

Touch imprint cytology demonstrated comparable sensitivity, specificity, and overall accuracy to frozen section and thus can serve as a substitute for it as a rapid and cost-effective test for detecting axillary lymph node metastasis during SLNs biopsy, especially in low-resource settings²³.

In this study, although ductal carcinoma was about three times more frequent than the lobular variant, there was one case of false negative results in TIC for ductal carcinoma in situ, consistent with previous studies^{24, 25}. In summary, the histological type of the tumor affects the accuracy of TIC but not FS¹⁹.

An important limitation of this study is the small number of evaluated patients. Therefore, larger-scale studies are necessary to establish the robust validity of TIC in this context.

Conclusion

Touch imprint cytology offers several advantages over frozen section (FS), including lower cost and rapid evaluation of lymph nodes. Additionally, TIC demonstrates good sensitivity and specificity for detecting macrometastasis, making it an effective method for identifying breast cancer metastases in SLNs when FS is unavailable. In the absence of FS facilities, careful gross examination and meticulous

imprinting of SLNs are crucial to ensure consistent results.

When used in combination, FS and TIC exhibit superior accuracy and lower rates of false negatives compared to when either technique is used alone. Therefore, in settings where both facilities are available, it is recommended to employ both FS and TIC for routine intraoperative evaluation of SLNs in breast cancer patients.

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Authors' contribution

Conception and design: Ahmed ARH, Abdelaal ME, & Mohamed MN; Data Collection: Mohamed MN & Abdelaal ME; Data Analysis and Interpretation: Ahmed ARH & Mohamed MN; Drafting / Revising the manuscript: Ahmed ARH; Approval of the final version of the manuscript: All authors; Agreement to be accountable for all aspects of the work: All authors.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Data availability

Data is available from the corresponding author upon request.

Ethical considerations

The study was approved by the Committee of Medical Ethics, Faculty of Medicine, Al Azhar University - Assiut branch (Registration number: MSR-A.AST./ PAT005/84/218 4/2023). An informed written consent was obtained from participants.

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Study registration

Not applicable.

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