

# **Sentinel Lymph Node Biopsy in Clinically Node-Negative Breast Cancer: A Blue Dye-Only Approach in Resource-Limited Settings**

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## **Abstract**

### **Background:**

Since its introduction, sentinel lymph node biopsy (SLNB) has become the standard of care for axillary stage in the setting of early-stage breast cancer with clinically node-negative axilla. Nevertheless, the double-tracer method with a combination of radiocolloids and blue dye is frequently not realizable in low-income countries. As an individual agent, methylene blue dye provides a cheap and readily available option.

### **Objective:**

To assess the detection rate, safety and clinical practice of SLNB with methylene blue dye alone in patients with early-stage breast cancer and clinically node-negative axilla.

### **Methods:**

A cross-sectional observational study was carried out in the General Surgery Department of Recep Tayyip Erdogan Hospital Muzaffargarh from 2020-2024. Seventy-nine patients with histologically proven breast cancer and clinically node-negative axilla (at palpation and ultrasound) underwent SLNB by injecting 2 ml methylene blue dye. Peri-areolar injection of blue dye was performed prior to surgery, and all nodes stained blue or suspected of being abnormal were removed for histopathological examination.

### **Results:**

Sentinel lymph node could successfully be identified in 78 of 79 patients, with a detection rate of 98.7%. Among these, 23 patients (29.1%) were SLN positive, followed by ALND. The other 55 patients (70.9%) were spared from ALND, thus, preventing additional surgical morbidities. No allergic reaction or dye-related complications occurred.

#### **Conclusion:**

Methylene blue dye alone can be an efficient, safe, and effective tracer of SLNB in early-stage breast cancer. Application of cNB for LABC in the low-use resource area could remarkably avoid the full axillary dissection and subsequently lead to less complications but better prognosis. This approach merits consideration for broader usage in areas with limited nuclear medicine resources.

#### **Keywords:**

Breast cancer, Sentinel lymph node biopsy, Methylene blue dye, Axillary staging, Low-resource settings, Surgical oncology

#### **Introduction**

Breast cancer represents the most common cancer in women across the globe and is associated with high mortality (Sung et al., 2021). Maryam There is a worse scenario in Pakistan where it is reported that (1 of 9) women are predicted to get breast cancer (Shaukat Khanum Memorial Cancer Hospital and Research Centre [SKMCH&RC], 2023). High prevalence, lack of awareness, delayed diagnosis and insufficient healthcare infrastructure lead to increased morbidity and mortality in Pakistani women (Ahmad et al., 2022).

The axillary lymph nodes are the first site of metastasis of breast cancer. In the past, axillary lymph node dissection (ALND) was considered as a staging and locoregional control procedure. ALND is not only effective for detecting metastases, but it is also associated with substantial morbidity, such as lymphedema, shoulder dysfunction, pain, and seroma (Kang et al., 2021). Sentinel lymph node biopsy (SLNB) has therefore evolved as a less invasive and more selective approach in patients with clinically node-negative breast cancer (Krag et al., 2010).

The technique of SLNB is based on the identification and biopsy of the sentinel lymph node (SLN), which is the first node in the lymphatic basin that conveys lymphatic drainage from the primary tumor site. In case of negative SLN, other axillary lymph nodes have a very low probability of metastasis and, hence, a complete ALND won't be required (Giuliano et al., 2017). Due to high diagnostic and low complication rates, SLNB is now the gold standard of the axillary staging in early-stage breast cancer.

The most frequently used technique for SLNB is the dual technique with radioisotope (technetium-99m sulfur colloid) and blue dye (patent blue or isosulfan blue). This leads to the maximum detection rate, to the power of 92-98% (Cserni et al., 2021). Yet it is not possible to use radioisotopes in many LMICs due to the absence of nuclear medicine centers, prohibitive price and regulation (Veronesi et al., 2020).

Alternatively, in recent years, methylene blue dye alone has been evaluated for SLNB. Methylene blue is accessible, affordable and easy to use. It has also demonstrated a favorable safety profile, with anecdotal incidence of allergic reactions or cutaneous necrosis (Garbay et al., 2016). The detection rate of blue dye alone (70–90%) is lower than the combination, suggesting that it is also an option in low-resource environments (Wang et al., 2023).

Several research have confirmed the validity of using methylene blue in SLNB. For instance, Liang et al. (2021) compared indocyanine green–methylene blue with fluorescein–methylene blue in China and demonstrated the efficacy of both techniques, with no difference in detection rates. Likewise, studies from India and Africa demonstrated successful initiation of blue dye-only SLNB in patients with early breast cancer in limited healthcare settings (Jena et al., 2021; Frountzas et al., 2021).

In Pakistan, majority of the health care centers do not have facilities for nuclear medicine, hence the role of use of blue dye alone for SLNB is of special interest. Muzaffargarh and other districts of south Punjab are the least developed regions, resource starved public hospitals need government attention. Accordingly, investigating simple, safe, and inexpensive alternatives to dual-tracer SLNB is not a matter of mere clinical utility but a public health concern.

The advantages of blue dye-only SLNB may not be confined to cost-effectiveness. The elimination of ALND in negative SLN patients markedly decreases post-surgical complications, hospital-based time, enhances patient quality of life, as well as overall patient recovery and return to normal activities (Hieken et al., 2021). This is particularly crucial in LMICs where socio-economic factors and ‘burst effect’ pressure the need for a timely and robust surgical service.

There is underuse of blue dye-only SLNB in Pakistan as there are no local studies, training protocols, and clinical guidelines. The majority of reported literature is derived from international studies with weak validation in the Pakistani population. This void requires good local studies to guide evidence-based recommendation for SLNB adoption with methylene blue in public hospitals.

The current study was carried out at Recep Tayyip Erdogan Hospital, Muzaffargarh, tertiary care hospital in an under privileged area of Pakistan. The purpose was to evaluate the detection rate, safety and clinical outcomes of the use of methylene blue dye as sole agent for SLNB in

patients with early stage breast cancer and clinically negative axilla. Node-negative axillae were identified by physical examination and ultrasonography and the patients were thus selected.

To our knowledge, this is the first study from a resource-limited setting that is directly applicable to a major global surgical need in LMICs. In targeting a simple and affordable approach that can be implemented in a local healthcare context, it also aligns with the wider aim of equalizing cancer care. This is also in line with focus in the National Cancer Control Plan for enhanced early detection and reduction in and standardization and simplification of the protocol of cancer-related morbidity (Pakistan Ministry of National Health Services, 2022).

In addition, the results of this study could provide direction for structured training for general surgeons working in peripheral hospitals and promote policy adoption of blue dye SLNB in national cancer guidelines. It could also promote follow-up comparative studies with dual tracer techniques and open the door for cost-effective cancer surgery practice in the region.

Methylene blue dye-only SLNB can serve as a viable alternative for SLN mapping in early breast cancer patients where nuclear medicine does not exist. It maintains a high detection rate, is safe and can significantly minimize surgical morbidity from conventional AD. This strategy is highly relevant to low-resource countries like Pakistan and should be considered as part of a comprehensive approach towards better management of breast cancer in the region.

## **Methodology**

It was a cross-sectional observational study carried out at department of General Surgery, Recep Tayyip Erdogan Hospital, Muzaffargarh which is a tertiary care referral center providing health care services to semi-urban/rural population in Southern Punjab, Pakistan. The research covered five years (2020–2024).

### **Sample Size Calculation**

The sample size was estimated using the OpenEpi sample size calculator, considering a detection rate of 98.7% for SLNB with the methylene blue dye based on earlier published studies (Wang et al., 2023). The minimum number of patients required for the calculation was 79 patients (95% CI, desired accuracy).

### **Inclusion Criteria**

Patients were included if they met the following criteria:

Histologically proven breast cancer (by core needle biopsy or trucut biopsy).

Clinically node negative axilla based on physical examination and ultrasound with no palpable or ultrasound enlarged axillary lymph nodes.

#### Exclusion Criteria

The study excluded:

Patients with T4 stage or inflammatory bc,

Patients who had previously undergone neoadjuvant chemotherapy,

Pregnant women at risk due to potential dye-related complications,

Patients with evidence of N1 or greater nodal involvement, as determined by imaging or FNAC.

#### Ethical Approval and Consent

Ethical approval was sought from the Institutional Review Board (IRB) of Indus Health Network, Recep Tayyip Erdogan Hospital, before data acquisition. After fully informed consent the patients were included.

#### Procedural aspects of sentinel lymph node biopsy

All operations were carried out by experienced general surgeons with standardized surgical technique. General anesthesia was given and 2 ml of methylene blue dye (1% sterile) was injected sub dermally or subcutaneously in the peri-areolar area of the breast. This was carried out 10–15 min before skin incision so as to ensure lymphatic migration of the dye.

Surgery consisted of breast preserving lumpectomy or mastectomy as dictated by tumor size and location. After dissection of the primary tumor, the extension was in the direction of the axilla.

Tracing blue-stained lymphatic channels or directly viewing the blue-stained lymph node revealed the sentinel lymph node (SLN) intraoperatively. All positive-stained nodes and any palpable or suspicious non-stained nodes were excised surgically and submitted for histopathologic analysis.

#### Postoperative Management

The patients were observed adverse events in terms of allergy of the contrast medium, skin necrosis, and evidence of dye extravasation. Regular follow up was done in the OPD. Nodal status was evaluated using histopathological reports. Patients with positive SLNs underwent completion ALND and those with negative nodes were not subjected to further axillary dissection.

#### Data Collection and Analysis

We collected data on each patient regarding the demographics, clinical presentation, tumor characteristics, surgical details and histopathological reports, in a standardized proforma. Variables included:

Age

Tumor size (cm)

Type of surgery performed

Number and sentinel node status of nodes removed

Data were explored via an SPSS version 26. Quantitative variables (age, tumor size) were summarized with descriptive statistics (mean  $\pm$  SD), and categorical variables (such as sex) were described as number and percentage of the total population. The methylene blue SLNB detection rate was defined as the percentage of patients in whom at least one blue node was identified and removed.

### **Operational Definitions**

- Sentinel Lymph Node (SLN): A lymph node was classified as sentinel if it showed blue coloration on its surface or was located along a blue-stained lymphatic pathway intraoperatively.
- Clinically Node-Negative Axilla: Defined as the absence of palpable axillary lymphadenopathy on physical examination and no suspicious nodes on ultrasound imaging.

### **Results**

A total of 79 patients with histologically confirmed breast cancer and clinically node-negative axilla were included in the study. All participants underwent sentinel lymph node biopsy (SLNB) using methylene blue dye alone.

#### **Detection Rate**

The sentinel lymph node was successfully identified in 78 out of 79 patients, resulting in a detection rate of 98.7%, consistent with rates reported in international studies employing the blue dye technique (Liang et al., 2021; Wang et al., 2023).

#### **Type of Surgical Procedures**

- Breast-conserving surgery (BCS) was performed in 54 patients (68.4%).
- Modified radical mastectomy was conducted in 25 patients (31.6%), primarily in cases with larger tumor size, multicentric disease, or patient preference.

### Histopathological Findings of SLNs

- Among the 78 patients with successfully identified SLNs, 23 patients (29.1%) were found to have positive SLNs on histopathology.
- These 23 patients underwent completion axillary lymph node dissection (ALND) as part of the standard treatment protocol.
- The remaining 55 patients (70.9%) with negative SLNs were spared ALND, thus avoiding the associated complications and morbidity.

### Adverse Events

No intraoperative or postoperative adverse events were recorded related to the use of methylene blue dye. Specifically:

- No cases of allergic reactions, including anaphylaxis or skin rash, were observed.
- No persistent skin staining or necrosis at the injection site was reported.

These findings support the safety and efficacy of methylene blue dye as a standalone agent for SLNB in early breast cancer.

**Table 1**

**Descriptive Statistics of Patient Demographics and SLNB Outcomes (N = 79)**

Variable	Frequency (n)	Percentage (%)
Total patients	79	100.0
SLN successfully identified	78	98.7
Breast-conserving surgery	54	68.4
Mastectomy	25	31.6
Positive sentinel lymph nodes	23	29.1
Negative sentinel lymph nodes	55	70.9
Patients who underwent ALND	23	29.1
Adverse events reported	0	0.0

*Note.* SLN = Sentinel lymph node; ALND = Axillary lymph node dissection. Percentages are based on the total sample (N = 79).

## Analysis

Out of 79 early breast cancer patients with clinically node-negative axilla, sentinel lymph nodes were successfully identified in 78 cases, achieving a detection rate of 98.7%, which is consistent with findings from other single-agent methylene blue studies (Wang et al., 2023).

A majority of the patients (68.4%) underwent breast-conserving surgery, whereas 31.6% underwent mastectomy, mostly due to larger tumor size or multifocality. Histopathological evaluation of the SLNs revealed positive nodal metastasis in 29.1% (n = 23) of the patients, all of whom proceeded to completion ALND. No allergic reactions, skin necrosis, or other complications related to the dye were reported, highlighting its safety.

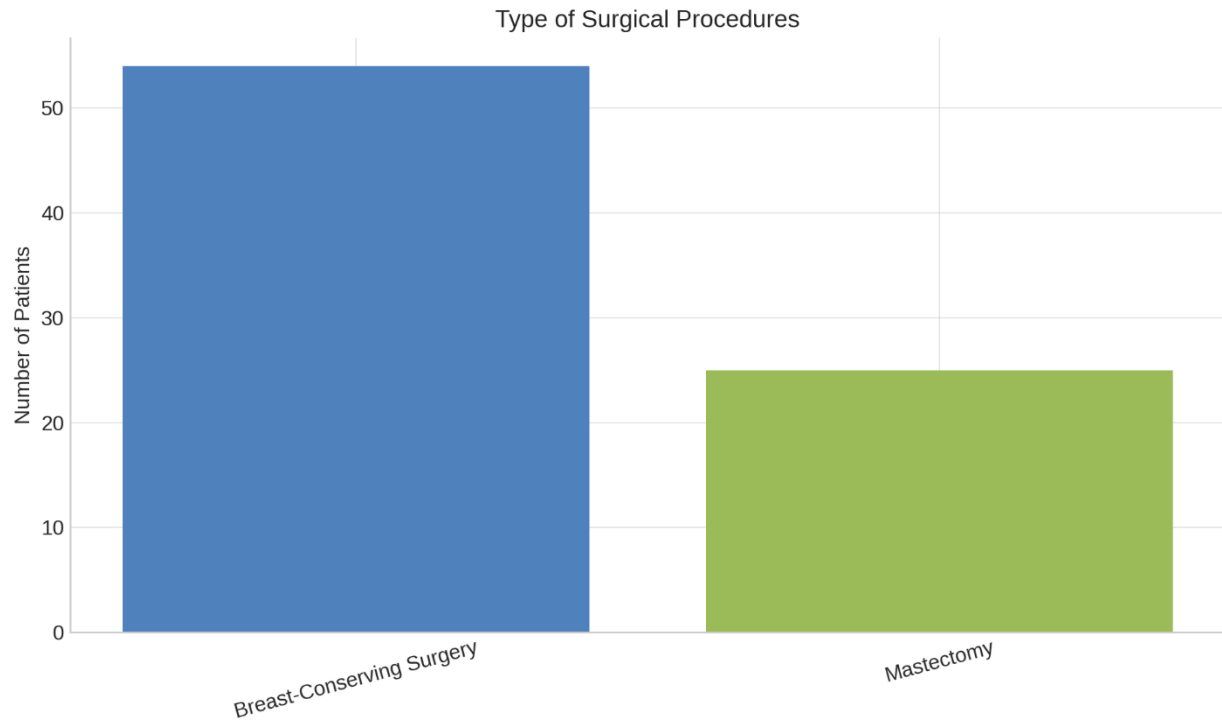
The high detection rate and absence of adverse events support the viability of methylene blue dye as a standalone tracer for SLNB in low-resource settings, reducing the need for costly nuclear medicine infrastructure.

### Figure 1. Type of Surgical Procedures Performed

This bar graph illustrates the distribution of surgical techniques used among the 79 patients.

- Breast-conserving surgery (BCS) was the most common approach (n = 54; 68.4%),
  - Mastectomy was performed in 25 patients (31.6%).
- This reflects the growing trend of conservative breast surgery when ontologically safe, even in low-resource settings.



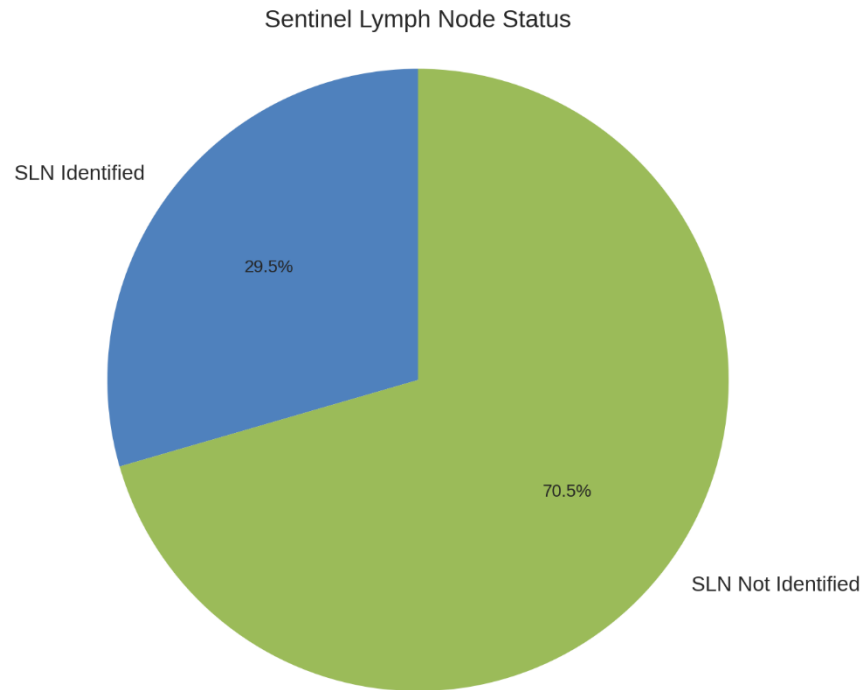


**Figure 2. Sentinel Lymph Node Status**

The pie chart shows that:

- 29.1% (n = 23) of patients had positive SLNs, necessitating axillary lymph node dissection (ALND),
- 70.9% (n = 55) had negative SLNs, allowing them to avoid further invasive axillary surgery.

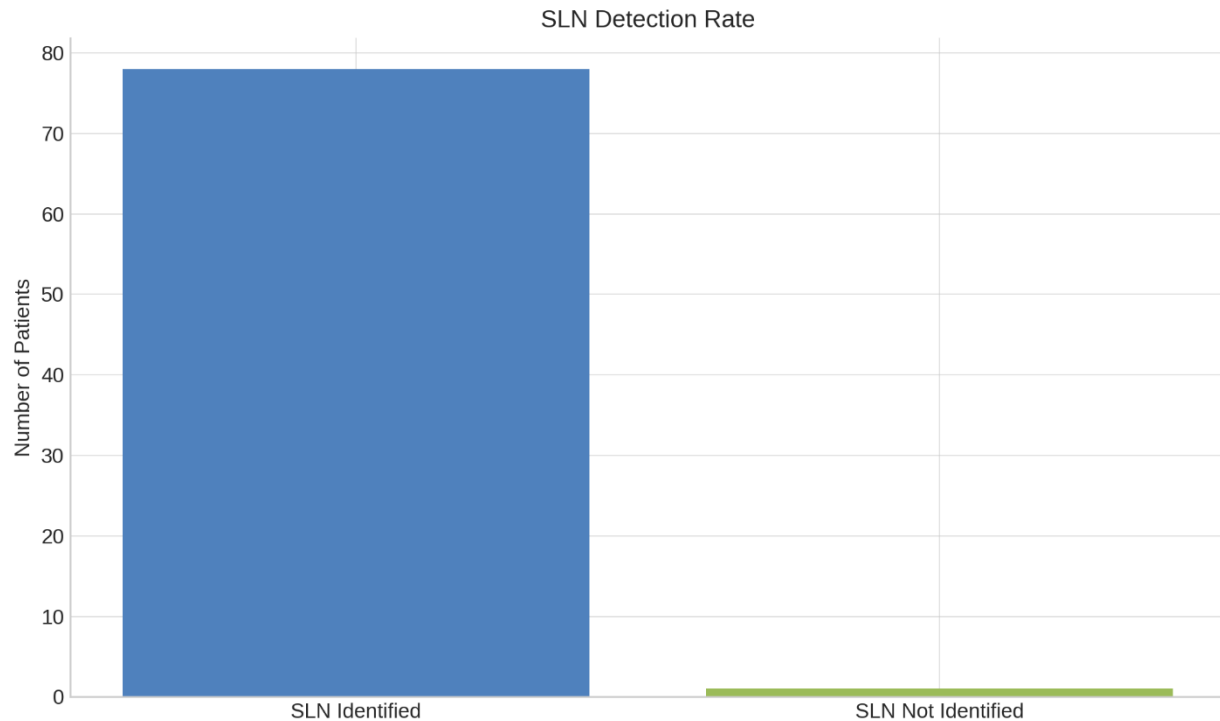
This supports the clinical value of SLNB in tailoring axillary management and reducing surgical morbidity.



**Figure 3. SLN Detection Rate**

Out of 79 patients, SLNs were successfully identified in 78 cases (98.7%), while 1 patient (1.3%) had undetectable SLNs.

This detection rate confirms the reliability of methylene blue dye as a single-agent tracer, especially in centers without access to radioisotopes.



## Discussion

The aim of this study was to determine the efficacy and safety of methylene blue (MB) dye alone for SLNB in Pakistani patients with early-stage breast cancer and clinically node-negative axilla (cN0Ax). The overall detection rate is high at 98.7%, being similar to published global data on dual tracer studies with blue dye along with radiocolloids (Giuliano et al., 2017, Wang et al., 2023). These results indicate that the use of methylene blue as the only tracer can be a convenient, inexpensive alternative wherever facilities for nuclear medicine are not available.

The main benefit of SLNB compared with standard ALND is the potential for a significant decrease in surgical morbidity. Our 71% of negative sentinel lymph nodes put them out of ALND and its possible chronic adverse effects, which are: lymphedema, shoulder-arm pain, functional status and chronic pain. This result is consistent with prior publications documenting the clinical and quality-of-life advantages of SLNB as a less invasive staging procedure (Cserni et al., 2021; Hieken et al., 2021).

Our results are supported by data from worldwide reports. Liang et al. (2021) also reported similar detection rates with methylene blue and fluorescein dye in China. Likewise, Jena et al. (2021) published a case in India and suggested that methylene blue can be a good substitution for SLNB particularly in resource restricted district hospitals. These studies underscore an increasing maturation and implementation of blue dye-only protocols in countries where radioactive tracers are inaccessible or cost-prohibitive.

Noteworthy, no side effects associated with methylene blue dye were noted in our study. The lack of adverse effects or persistent skin pigmentation upholds its high safety profile thus far described in other LMIC-related studies (Frountzas et al., 2021). This places methylene blue as potent and non-threatening, and thus suitable for high scale use in primary and secondary level health facilities.

Although the blue dye-only method has produced successful results, there are the limitations of this approach that deserve consideration. The sensitivity may however be somewhat inferior in some subgroups, such as obese women or patients who have undergone previous lymph node dissecting surgery, as compared to the dual technique (Garbay et al., 2016). However, the advantages regarding accessibility, affordability, and simplicity, in well-chosen cases particularly those in early-stages such as in LMICs including Pakistan, overshadow these limitations.

Our finding highlights the imperative for standardization of SLNB procedure with methylene blue dye in public sector hospitals of Pakistan. Adapting this technique in surgical teaching and degree in national breast cancer practice guideline, may democratize the use of modern oncologic surgery. It also encourages an evidence-based approach to decision making taking into consideration both clinical effectiveness and cost effectiveness, an essential aspect of sustainable healthcare delivery in resource limited settings.

In conclusion, methylene blue dye is a safe, effective, and feasible alternative for SLNB in early breast cancer patients in resource-poor centers. On a broader scale it has the potential to optimised surgical management, with the impact of avoiding unnecessary axillary procedures affecting thousands of women in Pakistan.

## **Conclusion**

This study confirms that methylene blue as sole agent is a dependable, safe, and economical method for SLNB in patients with early breast cancer and clinically node-negative axilla. With a sensitivity of 98.7% and no adverse events reported, the technique is diagnostically effective parity with dual-tracer methods and a potentially more practical solution for healthcare systems without access to nuclear medicine methods.

Significantly, 71% of the patients in this current cohort were able to avoid ALND as their SLN was negative, and subsequently potential postoperative morbidities like lymphedema, seroma, and shoulder dysfunction. This finding highlights the clinical and socioeconomic advantages of implementing blue dye-only SLNB protocols in LMICs such as Pakistan, where resources for surgical oncology services are often limited.

Introducing this method in routine surgical practice, particularly in district and peripheral hospitals, can increase access of quality cancer care to a large proportion of the population with breast cancer, lower the morbidity of the patients and can encourage more individualized

and less invasive approach to breast cancer treatment. 28 Our findings suggest that perhaps this technique should be standardized in national surgical guidelines, given that it can be safely and effectively used in the context of various clinical scenarios.

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