Review Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.4827165931-048

Evolving Landscape of Intraoperative Evaluation of Sentinel Lymph Node Biopsy in Breast Cancer

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ARTICLE INFO

Received:
25 November 2025
Revised:
8 April 2026
Accepted:
11 April 2026

Keywords:
sentinel lymph node biopsy, breast neoplasms, frozen sections, surgical pathology, nucleotide aptamers

ABSTRACT

Background: Sentinel lymph node (SLN) biopsy has transformed breast cancer management by providing a minimally invasive method for axillary staging and reducing the need for extensive lymph node dissections. Following de-escalation trials such as ACOSOG Z0011, the role of intraoperative SLN evaluation has narrowed, especially for Z0011-eligible patients in whom SLN evaluation now rarely alters axillary management. Nevertheless, intraoperative SLN assessment remains valuable for immediate decision-making, potentially minimizing reoperation rates and improving patient outcomes.

Methods: A literature review was conducted to evaluate intraoperative SLN assessment in breast cancer, with an emphasis on histopathological techniques. English-language articles from 2010 to 2025 were identified in PubMed using the combination terms "intraoperative," "sentinel lymph node(s)," "breast cancer," "histopathology," "pathology," and "frozen section." This work was conceived as a narrative review; no formal systematic review procedures or meta-analyses were performed.

Results: This review outlines the historical development of SLN biopsy, highlights key clinical trials supporting its use, and examines current intraoperative assessment techniques, including frozen section analysis, touch imprint cytology, and one-step nucleic acid amplification. The diagnostic accuracy and clinical relevance of each method are discussed, particularly in the context of low-volume disease such as micrometastases and isolated tumor cells, which rarely influence contemporary surgical or radiotherapy decisions.

Conclusion: SLN biopsy remains central to breast cancer staging, but it can be safely omitted in selected patients with early-stage breast cancer. Emerging molecular-probe-based and artificial intelligence–assisted technologies will improve diagnostic accuracy and consistency in intraoperative assessment.

INTRODUCTION

Breast cancer remains one of the most prevalent cancers among women worldwide, and effective staging is critical for guiding treatment and improving patient outcomes.[1,2] Sentinel lymph node biopsy (SLNB) has become a cornerstone in the staging and

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management of breast cancer, offering a minimally invasive approach to assess the status of axillary lymph nodes.[1] By identifying the sentinel lymph node (SLN)—the first lymph node likely to harbor metastasized cancer cells—SLNB provides vital information on disease spread with significantly lower morbidity than traditional axillary lymph node dissection (ALND).[2] Intraoperative evaluation of SLNs has introduced an additional layer of clinical value by enabling surgeons to make immediate decisions about further axillary interventions during surgery. If metastasis is detected in the SLN intraoperatively, additional axillary treatment may be warranted in the same surgical session, potentially sparing patients from a second operation. This capability is particularly important in early-stage breast cancer, where timely and targeted interventions can lead to improved survival and quality of life.[2]

While intraoperative SLNB evaluation shows great promise, the variability in accuracy and false-negative rates continues to present challenges. A range of intraoperative techniques, including frozen section analysis (FSA), touch imprint cytology (TIC), and one-step nucleic acid amplification (OSNA), ultimately shape clinical decision-making and impact patient outcomes. Moreover, emerging technologies such as optical coherence tomography (OCT) and AI-driven diagnostics show promise in enhancing the precision and efficiency of SLNB assessment.

METHODS

A narrative review framework was employed to evaluate intraoperative SLN assessment in breast cancer, focusing on histopathological techniques and clinical utility. Relevant studies, clinical trials, and guidelines were identified through a PubMed search of English-language articles published between 2010 and 2025 using the terms “intraoperative,” “sentinel lymph node(s),” “breast cancer,” “histopathology,” “pathology,” and “frozen section.” Findings from representative studies were synthesized to compare diagnostic performance, highlight methodological differences, and contextualize their implications for contemporary axillary management. No formal systematic review procedures or meta-analyses were performed. In view of the evolving landscape of SLNB, this review provides a comprehensive analysis of intraoperative evaluation techniques in breast cancer, synthesizing evidence from clinical trials and examining the role of novel technologies. By critically analyzing the strengths and limitations of each approach, we offer insights into optimizing SLNB as a diagnostic tool, reducing the rates of reoperation, and advancing the more effective management of breast cancer.

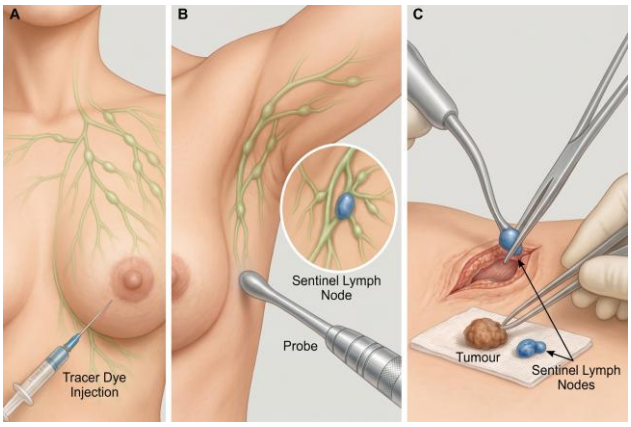

Figure 1. Sentinel Lymph Node Biopsy Procedure in Breast Cancer. A, Localization of the sentinel lymph node: A tracer is injected around the primary breast tumor to identify lymphatic drainage. The tracer travels through lymphatic channels toward the ipsilateral axillary basin, where the first draining (“sentinel”) lymph node in this pathway is identified and visualized. B, Identification and biopsy of the sentinel lymph node: A handheld probe is used intraoperatively to detect tracer uptake and locate the sentinel lymph node in the axilla. Once identified, the node is surgically removed through a limited axillary incision and sent for histopathological examination to assess the presence of cancer cells. C, Tumor and lymph node correlation: The sentinel lymph node, along with any additional lymph nodes if required, is examined to assess metastasis. This approach helps evaluate cancer spread and guide treatment decisions while minimizing unnecessary lymph node removal and tissue damage. Created with BioRender.com.

Historical context

The SLN concept was first introduced by Cabanas in 1977 while studying lymphatic drainage in penile cancer.[3] This groundbreaking idea that the SLN is the primary lymph node to which cancer cells spread from a primary tumor revolutionized the understanding of cancer staging. Building on the work of Cabanas, Morton *et al.*[4] adapted the SLN concept to melanoma in the early 1990s, using lymphoscintigraphy and methylene blue dye to identify and isolate SLNs for accurate staging. This technique quickly showed promise in improving surgical outcomes by offering a less invasive means of lymph node assessment. Recognizing the potential of this approach, researchers soon directed their efforts toward applying SLN mapping to other malignancies. In 1994, Giuliano *et al.*[5] introduced SLNB to breast cancer surgery, where it initially served as an alternative to the conventional ALND. The adaptation of lymphoscintigraphy for breast cancer allowed for targeted removal of SLNs, significantly reducing the risk of lymphedema and other morbidities associated with ALND.

Subsequent multicenter studies, notably by Krag *et al.*[6] in 1998, validated the accuracy of SLNB, showing it could reliably predict axillary node metastasis with a high degree of sensitivity and specificity (Figure 1).

The widespread adoption of SLNB in breast cancer surgery followed these pivotal studies. Since then, SLNB has become a standard procedure in breast cancer surgery, with multiple clinical trials affirming its safety, accuracy, and utility in reducing morbidity compared to ALND. Key studies, such as those conducted by Veronesi *et al.*[7] and Giuliano *et al.*[8], confirmed that SLNB is not only a reliable staging tool but also safe and beneficial in reducing the surgical burden for patients with early-stage breast cancer. Innovations in imaging and molecular technology are further advancing SLNB, strengthening its role in breast cancer staging and management (Figure 2).

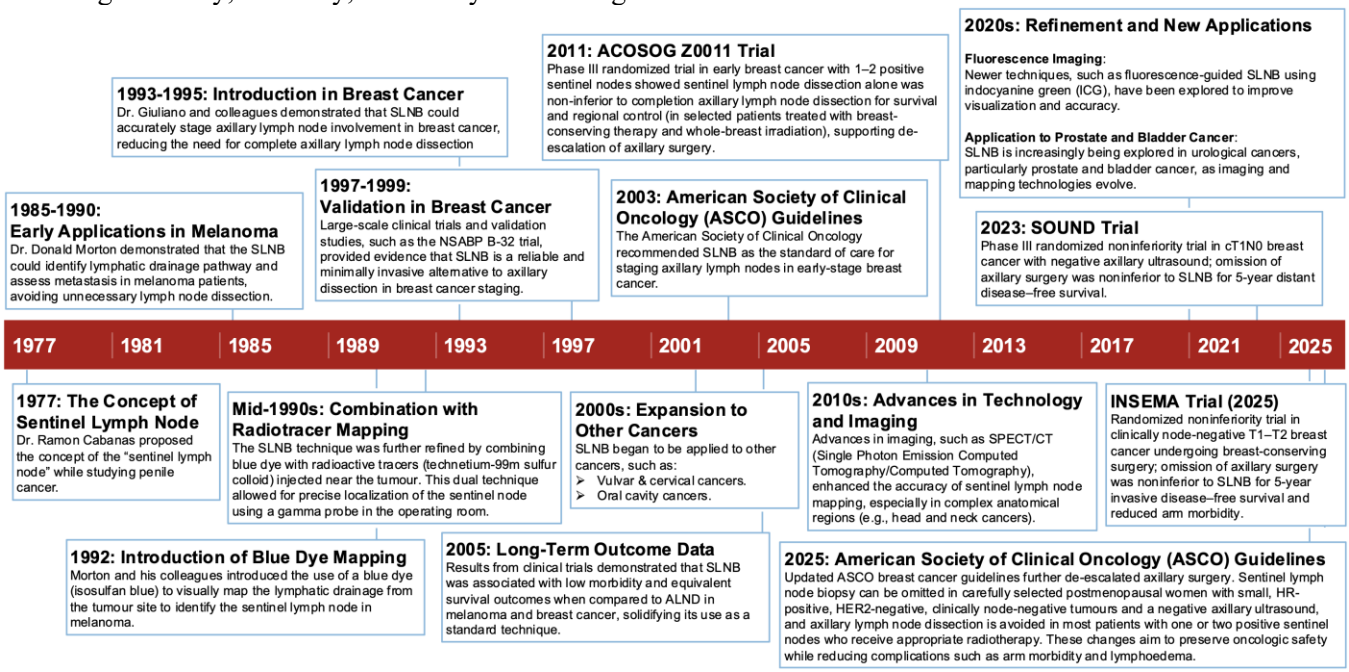

Figure 2. Timeline of the Developments in Sentinel Lymph Node Biopsy Techniques for Breast Cancer.

Clinical trials and evidence on SLNB in breast cancer

Landmark trials establishing SLNB

In the late 1990s, Krag *et al.*[9] conducted a multicenter validation study to evaluate the diagnostic accuracy of SLNB in detecting breast cancer metastases. This seminal study demonstrated that SLNB reliably identifies nodal involvement, thereby establishing its clinical utility and laying the groundwork for its adoption in breast cancer staging. Building on this foundational evidence, Veronesi *et al.*[7] conducted a randomized controlled trial comparing SLNB with conventional ALND. The trial confirmed that SLNB is a safe and effective alternative, offering comparable oncologic outcomes while significantly reducing surgical morbidity. Together, these findings strongly support the wider integration of SLNB into standard breast cancer management protocols. Further clinical trials and evidence on the clinical implications of SLNB in breast cancer are summarized in Table 1.

Trials assessing long-term outcomes

In an update to their earlier work, Veronesi *et al.*[10] provided long-term data from a randomized controlled study comparing SLNB to axillary dissection. The extended follow-up data reaffirmed that SLNB is associated with reduced postoperative morbidity while maintaining equivalent overall and disease-free survival rates. These findings further substantiate the clinical advantages of SLNB over traditional axillary dissection, reinforcing its role as a standard approach in the surgical management of breast cancer.

Building on this evidence, Giuliano *et al.*[8] conducted the Z0011 trial, a phase 3 noninferiority trial, to assess the necessity of axillary dissection in patients with sentinel node metastases. The Z0011 trial found no statistically significant difference in overall survival between patients who underwent axillary dissection and those who did not, suggesting that the omission of axillary dissection may be appropriate in selected cases. These findings contribute to a growing body of evidence supporting less invasive strategies in axillary management.

Further expanding on alternatives to surgical intervention, the AMAROS trial by Donker *et al.*[11] compared axillary radiotherapy with axillary dissection in patients with positive SLNB findings. The study demonstrated that radiotherapy is a viable alternative to surgery, offering similar outcomes with less morbidity, thereby broadening the therapeutic options available for axillary management in breast cancer care. In this review, we focus on the surgical and intraoperative pathological aspects of SLN evaluation and their impact on axillary management. Issues related to breast and regional nodal therapy, including dose, fields, and techniques, are not addressed.

Trials on procedure optimization

Mansel *et al.*[12] conducted the ALMANAC trial, a randomized multicenter study comparing SLNB to standard axillary treatment in patients with breast cancer. The trial demonstrated that SLNB significantly reduces postoperative complications while achieving oncologic outcomes comparable to conventional axillary management, thereby underscoring its advantages in clinical practice. Building upon these findings, the validation phase of the ALMANAC study, conducted by Goyal *et al.*,[13] aimed to investigate the factors influencing the diagnostic accuracy of SLNB. The study identified multiple determinants associated with false-negative results, offering important insights for improving procedural technique and enhancing the overall reliability of SLNB in breast cancer management.

To further consolidate clinical findings in this area, Kim *et al.*[14] conducted a meta-analysis that synthesized data from multiple studies to assess the false-negative rates of SLNB. This comprehensive analysis offered a detailed evaluation of the factors contributing to diagnostic inaccuracies and informed the development of evidence-based strategies aimed at minimizing false-negative outcomes, thereby supporting the continued optimization of the SLNB procedure.

Impact on clinical practice and guidelines

In 2014, Lyman *et al.*[15] published an updated clinical practice guideline for SLNB, incorporating accumulated evidence to reaffirm its pivotal role in breast cancer staging. Developed by the American Society of Clinical Oncology (ASCO) with two subsequent updates, the guidelines emphasized the clinical significance of SLNB and provided evidence-based recommendations for its application in contemporary practice.[16,17] The role of routine SLNB has been further challenged by two recent omission trials in selected patients with early breast cancer. The SOUND and INSEMA phase 3 noninferiority trials randomized clinically node-negative patients with negative axillary ultrasound. The patients were carefully selected, most of whom had small, hormone receptor-positive, and *ERBB2*-negative tumors, and underwent breast-conserving surgery with whole-breast irradiation, either with SLNB or no axillary surgery. Both trials demonstrated noninferior 5-year oncological outcomes and very low axillary recurrence rates (approximately $< 1\%$) in both groups, while patients who omitted SLNB experienced lower rates of lymphedema, shoulder mobility issues, and pain.[18,19] Altogether, these data, with contemporary reviews and guideline updates, support emerging recommendations to omit SLNB in older, low-risk patients with favorable biology and negative axillary imaging, while ongoing debate emphasizes the need for careful patient selection, multidisciplinary discussion, and cautious extrapolation beyond the trial-eligible population.[20] Reflecting this evolving evidence, the 2025 ASCO guidelines have recommended that routine intraoperative SLNB should be omitted in postmenopausal patients (aged > 50 years) with small (< 2 cm), grade 1 to 2, HR-positive/*ERBB2*-negative tumors and negative preoperative ultrasound undergoing breast-conserving surgery. If SLNB is performed, completion ALND is not recommended for 1 to 2 positive nodes in patients receiving breast-conserving surgery with whole-breast radiation. For mastectomy patients with 1 to 2 positive nodes, ALND may be omitted if postmastectomy radiation and regional nodal irradiation are offered. These updates prioritize de-escalating axillary surgery based on tumor biology and planned adjuvant therapies.[16]

De-escalation of intraoperative SLN and its impact on axillary surgery management

The ever-evolving landscape of axillary management has mainly shifted toward the de-escalation of surgery and reoperations. In this context, the American College of Surgeons Oncology Group (ACOSOG) Z0011 prospective randomized trials demonstrated that early-stage clinical T1 to T2 N0 disease and 1 to 2 positive SLNs undergoing breast-conserving surgery with whole-breast irradiation can safely omit completion ALND without compromising locoregional control or overall survival.[8] As a result, the intraoperative identification of 1 to 2 positive SLNs rarely mandates immediate ALND, and many centers have abandoned routine intraoperative SLN assessment for Z0011-eligible patients to reduce costs and operative time. In parallel, in patients treated with neoadjuvant systemic therapy, a positive SLN no longer mandates ALND.[16] However, it is important to note that ALND remains commonly practiced for

residual nodal disease; multiple ongoing studies are actively evaluating axillary radiotherapy as a noninferior, less morbid alternative. Therefore, these developments have substantially diminished the clinical utility of the routine role of intraoperative SLN assessment, suggesting that its use should now be targeted to carefully selected scenarios where intraoperative findings are expected to change axillary surgery management.

However, intraoperative SLNB evaluation remains clinically necessary in selected high-stakes settings when findings directly alter immediate surgical management. Key indications include high nodal burden (> 3 nodes) and detecting macrometastasis in mastectomy patients for whom regional irradiation is not planned, and ALND remains the standard of care. Assessment is also vital for evaluating residual macroscopic disease after neoadjuvant therapy and in high-risk scenarios such as inflammatory breast cancer to avoid a second axillary operation.[16]

Table 1. Clinical Trials and Evidence on SLNB in Breast Cancer.

Year	Trial name/type of study	Objective	Key findings	Impact on axillary management	Ref.
1998	Multicenter validation	Validate accuracy of SLNB	Accurately identifies metastasis	Provided foundational evidence for SLNB as a reliable staging procedure, enabling avoidance of routine ALND for negative SLNs	9
2003	Milan Trial (Randomized trial)	Compare SLNB with routine axillary dissection	Safe and effective	Supported replacing routine ALND with SLNB for cN0 early breast cancer (ALND reserved for select/positive cases at that time).	7
2006	ALMANAC (Randomized multicenter)	Compare SLNB to standard axillary treatment	Reduces complications with similar outcomes	Established SLNB as standard axillary staging in cN0 disease, reducing the morbidity burden of routine ALND/sampling.	12
2006	ALMANAC Validation	Factors affecting SLNB accuracy	Identified false-negative contributors	Informed quality assurance and technique optimization (e.g., robust mapping and retrieval of >1 SLN) to minimize false negatives and reduce inappropriate axillary undertreatment/overtreatment.	13
2006	Meta-analysis	Assess SLNB false-negative rates	Summarized false-negative rates	Supported broad adoption and de-escalation of routine ALND in appropriate patients.	14
2006	Milan Trial Update (Randomized trial)	Update on SLNB vs axillary dissection	Reduced morbidity with comparable survival	Reinforced preference for SLNB; supported ALND omission for negative SLNs	10
2011	ACOSOG Z0011 (Randomized trial)	Axillary dissection vs no axillary dissection	No significant difference in survival	Enabled completion ALND omission in "Z0011-eligible" patients; shifted control to RT/systemic therapy rather than surgery.	8
2014	AMAROS (Randomized multicenter)	Radiotherapy vs surgery after positive SLNB	Radiotherapy is a viable alternative	Established axillary RT as morbidity-sparing alternative to ALND for selected SLN-positive patients.	11
2014	ASCO Guideline Update	Update practice guidelines for SLNB	Updated practice guidelines for SLNB.	Standardized de-escalation criteria and clarified situations where ALND remained indicated (per 2014 guidance).	15
2017	ASCO 2nd Guideline Update	2nd update on practice guidelines for SLNB	Reinforced practice consistency	Omit ALND for negative SLNs and 1–2 positive SLNs with BCS and whole-breast RT	17

2023	SOUND (Phase 3 noninferiority)	Compare SLNB with no axillary surgery	Noninferior 5-year distant disease-free survival; axillary recurrence was very low in both groups.	Supported selective omission of SLNB in carefully selected patients with small, cN0 breast cancer with negative axillary imagingundergoing breast-conserving surgery.	19
2025	INSEMA (Randomized noninferiority)	Compare omission of axillary surgery with SLNB	Noninferior 5-year invasive disease-free survival; associated with lower lymphedema, better arm mobility and less pain.	Strengthened axillary de-escalation for low-risk patientswhile highlighting the need for caution in larger or higher-risk tumors where nodal status may still influence adjuvant treatment decisions.	18
2025	ASCO 3rd Guideline Update	3rd update on practice guidelines for SLNB	Moves toward less invasive surgery overall	Moves toward less axillary surgery overall (select omission of SLNB; expanded omission of ALND), with increased reliance on RT/systemic therapy for regional control where appropriate.	16

ALND, axillary lymph node dissection; ASCO, American Society of Clinical Oncology; BCS, breast-conserving surgery; cN0, clinically node-negative; RT, radiotherapy; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy.

Sentinel lymph node biopsy procedure: techniques and current workflow

A variety of innovative techniques have been well-documented in the literature for performing SLNB, each contributing to enhanced precision and diagnostic confidence. The preoperative phase often incorporates radiologically guided localization to accurately identify SLNs (Table 2).[4,5] This is followed by careful intraoperative evaluation. The diagnostic pathway culminates in a definitive postoperative histopathological examination, which remains widely used for assessing nodal involvement (Figure 3).

Table 2. Different Preoperative Techniques for Sentinel Lymph Node Biopsy Procedure

Preoperative technique	Description
Lymphoscintigraphy	Imaging technique using radiocolloids to map lymphatic drainage preoperatively.
Ultrasound with fine-needle aspiration	Preoperative imaging and biopsy to assess axillary lymph node involvement.
Contrast-enhanced ultrasound	Employing contrast agents with ultrasound to improve visualization of sentinel lymph nodes.
Magnetic resonance imaging	Preoperative imaging technique to identify sentinel lymph nodes and assess tumor extension.
Lymphatic mapping with radiocolloid and blue dye	Combined use of radiocolloid and blue dye for precise localization of sentinel lymph nodes during preoperative mapping.

Intraoperative evaluation techniques for SLNB in breast cancer

Axillary lymph node evaluation and staging are key components in determining the prognosis of operable breast cancers. ALND is often performed because of positive metastasis SLN mapping during breast cancer surgery. However, decisions on ALND are individualized based on the extent of nodal involvement and other clinical factors.

SLN assessment in breast cancer surgery encompasses preoperative, intraoperative, and postoperative phases, each contributing uniquely to staging and therapeutic decision-making (Figure 4). However, the de-escalation of SLNB in the post-Z0011 era has changed the SLNB perioperative workflow in patients undergoing mastectomy and breast-conserving surgery.[15] Preoperative evaluation focuses on the initial detection of nodal metastases, which informs the need for SLN biopsy prior to surgery. Commonly employed imaging modalities include ultrasound-guided needle biopsy, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). While these techniques aid in identifying suspicious lymph nodes, they are limited by moderate spatial resolution, reduced sensitivity for detecting micrometastases, and a lack of detailed morphological information—factors that contribute to relatively high false-negative rates.[21,22] Postoperative histopathological assessment of SLNs employing immunohistochemistry remains the gold standard for definitive evaluation. However, this approach is inherently time-intensive and may necessitate a second operative procedure, such as ALND, if metastatic involvement is identified postoperatively.

Intraoperative diagnostic techniques for SLN assessment help overcome the limitations of preoperative and postoperative evaluations by enabling real-time decisions regarding the need for ALND, potentially avoiding a second surgery.[20]

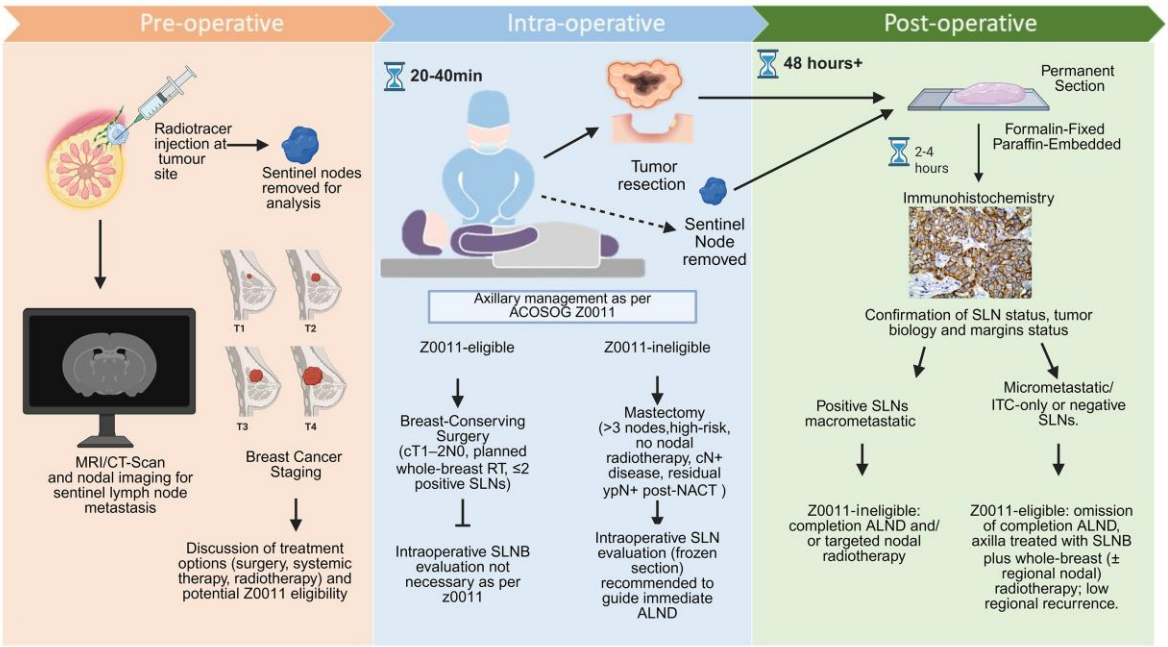

Figure 3. Typical Breast Cancer Perioperative Workflow Illustrating Axillary Management in the Post-Z0011 Era. Preoperative staging and nodal imaging to guide treatment planning and potential Z0011 eligibility. Intraoperative evaluation focuses on either omitting intraoperative SLN for Z0011-eligible patients or selectively performing intraoperative SLN for Z0011-ineligible or high-risk patients to guide axillary management. Postoperative evaluation widely uses permanent histology and immunohistochemistry (IHC) to confirm SLN status, margins, and tumor biology to inform the omission or escalation of axillary treatment. Created with BioRender.com. cN^+ indicates clinically node-positive; cT1–2N0, tumor size up to 2 cm and no clinically enlarged lymph nodes; ITC, isolated tumor cells; LN, lymph node; NACT, neoadjuvant chemotherapy; RT, radiotherapy; SLN, sentinel lymph node; ypN^+, positive axillary LN after neoadjuvant therapy.

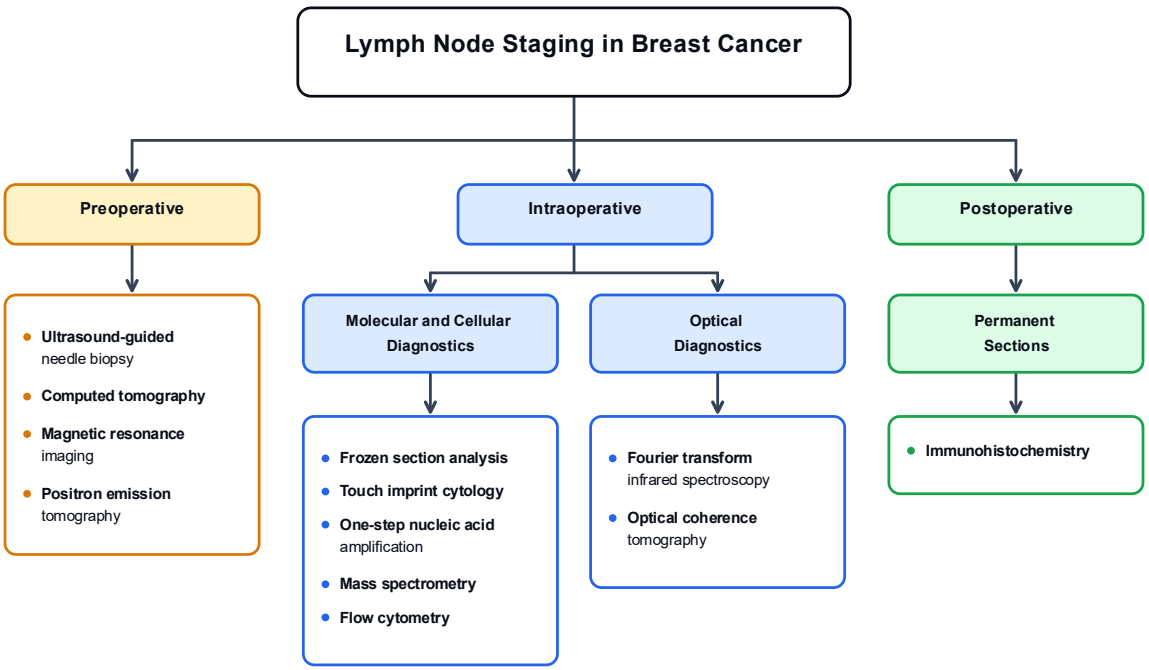

Figure 4. Different Diagnostic Techniques in Lymph Node Staging in the Management of Breast Cancer Surgery.

An ideal intraoperative method should deliver accurate results within the operative timeframe of 30 to 40 minutes, demonstrate high sensitivity and specificity, and be simple to perform without requiring specialized expertise. Current techniques can be broadly classified into cellular/molecular

diagnostics and optical methods. Traditional approaches, such as FSA and TIC, are well-established, while emerging optical and spectroscopic technologies aim to improve diagnostic speed and precision. This section reviews key intraoperative SLN assessment methods, highlighting their strengths and limitations (Table 3).[23,24]

Table 3. Advantages and Disadvantages of Intraoperative Sentinel Lymph Node Biopsy Evaluation Techniques

Intraoperative technique	Advantages	Disadvantages	Sensitivity	Specificity	Clinical utility on axillary management	Ref.
Frozen section analysis	Fast procedure (~30 min) Established intraoperative evaluation protocol Widely used	Varying sensitivity and accuracy due to sampling errors Better at identifying macrometastasis than micrometastasis	~40%–75%	Up to 100%	High relevance: mastectomy, >3 positive nodes expected, Z0011-ineligible, cN+ upfront surgery, positive result still triggers intraoperative ALND. Low/moderate relevance: BCS patients meeting Z0011/IBCSG 23-01 criteria, where most micrometastases/ITCs would not change axillary management.	26, 28, 60, 61
Touch imprint cytology	Fast procedure Minimal tissue preparation/loss Effectively excludes macrometastasis	Detects only ~50% of micrometastases Requires cytologist expertise Lacks tissue architecture	~63%–85%	~99%	High relevance: mastectomy, >3 positive nodes expected, Z0011-ineligible, cN+ upfront surgery, positive result still triggers intraoperative ALND. Low/moderate relevance: BCS patients meeting Z0011/IBCSG 23-01 criteria, where most micrometastases/ITCs would not change axillary management.	29, 30, 62
One-step nucleic acid amplification (OSNA)	High sensitivity Minimal supervision Detects micrometastasis	Inability to preserve tissue architecture Dependent on single molecular marker (CK19) Requires specialized equipment/training	~98%	Highly specific to CK19 target	No increase in adjuvant axillary or systemic treatment despite higher micrometastasis detection. Added sensitivity risks stage migration and potential overtreatment without proven survival benefit	31, 33, 63, 64
Flow cytometry	Highly specific Does not rely on pathologist expertise	Risk of false-positives Needs optimization across breast cancer cell types	93%	~92%	Promising for rapid, whole-node or label-free assessment, but therapeutic relevance is still investigational	65
Mass spectrometry	Specific to tumor biomarkers Real-time results Minimal sample preparation	High system costs Limited sampling coverage Limited clinical validation	~100%	93%	Promising for rapid, whole-node or label-free assessment, but therapeutic relevance is still investigational	50, 51
Fourier transform infrared spectroscopy	Rapid and real-time analysis Nondestructive minimal sample	Inability to evaluate tissue architecture Background interference	82%–99%	84%–95%	Promising for rapid, whole-node or label-free assessment, but therapeutic relevance is	52, 53, 66

	preparation Molecular-level information	Tissue handling artifacts Requires costly equipment			still investigational	
Optical coherence tomography	Fast results (~25 s) Real time Depth penetration suitable for margins Scans up to 100 cm^2	Low sensitivity Requires dedicated hardware High cost Complementary role with histopathology	~58.8%	81.4%	Promising for rapid, whole-node or label-free assessment, but therapeutic relevance is still investigational	57, 59, 67, 68

CK19, cytokeratin 19; OSNA, one-step nucleic acid amplification.
[a]High relevance includes mastectomy, expectation of > 3 positive nodes, and clinically node-positive upfront surgery (positive result triggers intraoperative axillary lymph node dissection). Low/moderate relevance includes breast-conserving surgery patients meeting Z0011/IBCSG 23-01 criteria, where micrometastases/isolated tumor cells do not change management.

Molecular and cellular diagnostic techniques

Frozen section analysis

FSA is a well-established intraoperative pathological technique that enables rapid microscopic evaluation of fresh tissue specimens, providing timely diagnostic guidance during surgery. However, in contemporary practice, intraoperative FSA of SLNs is performed selectively rather than routinely. It is generally reserved for patients who fall under subgroups where completion axillary surgery, nodal irradiation fields, and reconstruction or adjuvant treatment may still depend on real-time nodal status and may meaningfully alter immediate axillary or breast cancer management. First introduced at the turn of the 20th century,[25] FSA remains a benchmark method for intraoperative tumor assessment. In SLN evaluation, either the entire node or a representative portion is submitted to the pathologist, where it is rapidly frozen using a cryostat, sectioned at approximately 5 µm, and stained with hematoxylin–eosin (H&E) for microscopic examination. FSA relies on the assessment of histomorphological features to establish a diagnosis. H&E staining, a cornerstone of histopathology, facilitates visualization of cellular and architectural detail: hematoxylin, a basic dye, stains nuclei and other acidic structures blue to purple, while eosin, an acidic counterstain, highlights cytoplasmic components in varying shades of pink. The entire FSA process is typically completed within 30 minutes, making it well-suited for intraoperative decision-making. However, the sensitivity and accuracy of FSA can be compromised by several factors, particularly when detecting micrometastases or isolated tumor cells (ITCs).[26–28]

These challenges underscore the limitations inherent in intraoperative evaluation techniques, which can be broadly categorized into 3 principal areas: sampling error, technical artifacts, and interpretative error. Sampling errors often result from inadequate specimen selection. For example, tissue samples may be too small or not fully representative of the lesion, particularly in cases involving tumor necrosis or degeneration. In such instances, careful selection of viable tissue is essential for diagnostic accuracy. Technical issues include freezing artifacts, poorly prepared tissue sections, or suboptimal staining quality, which can hinder the pathologist's ability to evaluate histomorphological features reliably. Interpretative errors arise from the inherent difficulty of distinguishing malignant cells from benign tissue components during rapid intraoperative assessment. Tumor heterogeneity further complicates this challenge, leading to potential diagnostic misinterpretations. Consequently, these limitations increase the risk of false-positive or false-negative results, which may ultimately lead to suboptimal intraoperative decisions.[26–28]

Touch imprint cytology

TIC represents a cytopathological alternative to FSA for the intraoperative evaluation of SLNs. The technique involves bisecting the SLN, after which the freshly cut surfaces are gently pressed onto clean glass slides to transfer cellular material. Multiple imprints are typically prepared from each bisected face, followed by air-drying and subsequent H&E staining for cytological evaluation.[29]

Compared to FSA, TIC offers the advantages of minimal tissue processing, rapid turnaround time, and reduced instrumentation requirements, making it particularly suitable for use in resource-limited settings or in institutions lacking cryostat facilities.[30] However, the diagnostic performance of TIC is limited by its reliance on exfoliated cellular material, which may be sparse and lack architectural context. The absence of complete tissue morphology can lead to variability in sensitivity and diagnostic accuracy. Clinical data indicate that TIC demonstrates higher sensitivity for detecting macrometastases, whereas its performance significantly declines in the identification of micrometastases and ITCs.[29]

One-step nucleic acid amplification

The OSNA assay is a fully automated molecular diagnostic method used intraoperatively to detect

SLN metastases in breast cancer. It employs reverse transcription–loop-mediated isothermal amplification (RT-LAMP) to quantify cytokeratin 19 (CK19) mRNA levels in homogenized lymph node tissue. Based on *CK19* mRNA copy number, nodal involvement is categorized as negative (≤ 250 copies/μL), micrometastasis (> 250 to 5000 copies/μL), or macrometastasis (> 5000 copies/μL).[31–33]

Compared to conventional FSA, OSNA offers significantly higher sensitivity (approximately 95%) and specificity (approximately 94%).[34] Moreover, its standardized, automated workflow reduces interobserver variability and mitigates sampling errors, enhancing diagnostic reliability. Despite its advantages, the OSNA assay is limited by its sole reliance on *CK19* mRNA expression, potentially compromising diagnostic utility in breast cancer subtypes with low or absent CK19 expression, particularly triple-negative breast cancer (TNBC), which comprises 15% to 20% of breast cancers with high metastatic potential, necessitating additional methods such as imprint cytology.[35,36] In contrast, FSA can identify metastatic involvement irrespective of CK19 expression. While OSNA offers higher diagnostic sensitivity, it often provides marginal clinical benefit in the era of surgical de-escalation. In OSNA, destruction of nodes is required, wherein critical architectural data and features of anatomical distribution and the pattern of metastases within the lymph node are lost. These morphological features may convey prognostic significance and influence subsequent treatment decisions.[37] Moreover, its increased detection rate of 7% more than histopathology frequently identifies non-SLN–negative disease, risking overdiagnosis and unnecessary ALND.[38] In patients who are Z0011-eligible and aligned with 2025 ASCO criteria, these molecular findings rarely alter management. Thus, OSNA may result in workflow disruptions due to increased operative times and avoidable second surgeries. The broader implementation of OSNA also faces substantial logistical and economic challenges. The need for specialized, nonstandard equipment, often unavailable in routine pathology settings, along with significant alterations to established workflows, poses considerable barriers. These challenges are compounded by the high initial cost of the required instruments, ongoing consumable expenses, and mandatory maintenance. Consequently, despite its technical advantages, OSNA's overall clinical benefit remains modest,[32] and its cost-effectiveness falls short compared to FSA, which continues to serve as the gold standard owing to its lower cost, diagnostic versatility, and broad applicability across breast cancer subtypes.[39] Future developments aimed at incorporating additional targets in CK19-negative metastatic cancer will enhance the clinical utility of the OSNA assay.

Flow cytometry

Rapid intraoperative flow cytometry (iFC), also known as the Ioannina Protocol, is a quick cell-cycle analysis method developed at the University Hospital of Ioannina in Greece for characterizing tumor margins during brain tumor surgery.[40] This technique requires only a small tissue sample (2 to 5 mm^3) and uses propidium iodide–based DNA staining to assess cell cycle phases (G0/G1, S, G2/M) and ploidy status. Results are generated within 5 to 6 minutes, providing a tumor index (percentage of cells in S and G2/M phases) and a DNA index (aneuploidy ratio). iFC is often applied in surgeries for brain tumors, hepatic malignancies, head and neck cancers, and breast cancer. By detecting proliferative and genetically abnormal cells at the resection margins, it facilitates surgeons in optimizing resection planes.[41–43]

However, the application of iFC for detecting cancer metastasis in lymph nodes remains limited. Due to the high heterogeneity of cancer cells, relying solely on proliferation status is insufficient for the detection of metastasis.[44] Current evidence does not support the broad use of iFC for SLN assessment due to several inherent limitations, including suboptimal sensitivity, absence of detailed morphological context, restricted sampling capacity, and operational complexity.[41,45,46]

Mass spectrometry

Ambient ionization mass spectrometry was introduced 15 years ago as a sample preparation-free alternative to conventional mass spectrometry workflows.[47] Building on this advancement, rapid evaporative ionization mass spectrometry (REIMS) was later developed to facilitate intraoperative tissue identification and surgical margin assessment, offering a real-time alternative to frozen section histology by directly integrating electrosurgical devices with mass spectrometry.[48] In REIMS, aerosol particles produced by electrosurgical dissection are subjected to mass spectrometric analysis, providing spectral information on the structural molecular composition of tissues.[49] A wide range of mass spectrometry methods combining both existing surgical instruments and mass spectrometric analysis have been used intraoperatively. For example, the MasSpec Pen and desorption electrospray analyze the surface of tissues without damaging the cells.[50] However, the applicability of these methods is constrained by limited feasibility for larger tissue samples, high costs, and technical challenges associated with mass spectrometry instrumentation

and the sampling area of the device.[51] Ongoing advancements in this technology, particularly those facilitating real-time analysis of molecular signatures in direct correlation with the histological architecture of tissues, are paving the way for REIMS to become a pivotal tool for accurate intraoperative tissue characterization.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a technique that utilizes infrared spectroscopy, relying on the absorbance of electromagnetic waves at infrared wavelengths to determine the molecular structure and composition of the tissue sample. Although FTIR spectroscopy cannot match molecular assays in pinpointing specific molecular identities, it enables simultaneous qualitative and quantitative analysis of broad molecular classes, including nucleic acids, proteins, and lipids. As a result, FTIR spectroscopy offers a comprehensive overview of the biochemical composition and overall status of the analyzed specimen. The vibrational absorption features in FTIR spectral analysis differentiate changes in the intracellular environment.[49] The uncontrolled growth of cancer cells can be identified through various bands in the FTIR spectra, which can then be analyzed through spectroscopic data with AI systems to establish metastasis detection. This method has the potential to serve as a diagnostic tool for detecting and differentiating malignant tissues.[49,52,53] However, this method has not been optimized on large sample sizes, and standardization of sample collection is needed to ensure reproducibility in diagnostic settings.[53]

Optical coherence tomography

Optical coherence tomography (OCT) is a high-resolution microscopic optical imaging technique that yields real-time multidimensional images of subsurface tissue structure. In 1991, Huang *et al.*[54] employed near-infrared light to generate cross-sectional images of tissue microstructure without the need for physical contact. In the early 2000s, this technique was adapted for image-guided breast cancer surgery to assess surgical margins while preserving tissue integrity.[55] In breast tissue, OCT typically penetrates to a depth of 1 to 2 mm, enabling rapid and precise visualization of tissue morphology over relatively large surface areas during surgical margin assessment.[56] Currently, OCT is predominantly employed in ophthalmic surgery, where it is often integrated into surgical microscopes.[57] Despite its utility, several limitations restrict its broader clinical application, including limited penetration in opaque or adipose tissues, high system costs, and the technical expertise required for operation.[58,59] Future advancements in high-resolution full-field OCT and molecular OCT are expected to facilitate the broader clinical adoption of the technology.

DISCUSSION

The false-negative rates of SLNB in breast cancer across various countries

The accuracy of the intraoperative evaluation of SLNs in breast cancer is influenced by a variety of factors. A common issue is the discordance between intraoperative diagnosis and final postoperative assessment, wherein SLNs initially reported as negative are later found to contain metastatic disease. Such discrepancies result in false-negative outcomes, which may require a second surgical intervention. The extent of false-negative rates (FNRs) is affected by both clinicopathological factors and technique-related limitations. Key clinicopathological factors include tumor histological grade, the size of the metastatic deposit within the SLN, the number of SLNs retrieved, and the effects of prior neoadjuvant chemotherapy and/or hormonal therapy.[69] In addition, technique-related errors may arise from the specific method used for intraoperative SLN assessment, as described in previous sections. Currently, the widely used practice routine is FSA, which has a number of limitations as outlined. False-negative results can be minimized by selectively identifying the most important lymph nodes, which can be used to "ultrastage" with multiple serial sectioning intraoperatively or postoperatively via immunohistochemistry or OSNA. Such ultrastaging allows for the accurate detection of low-volume metastatic disease, such as micrometastases and ITCs.[28,69]

Guidelines for the intraoperative evaluation of SLNs in breast cancer surgery vary across countries, reflecting the challenges associated with detecting occult metastases, particularly micrometastases and ITCs, which are frequently missed during FSA. Intraoperative SLN assessment practices differ substantially among surgeons worldwide, primarily due to institutional variability in histopathological techniques for frozen section preparation and analysis. The absence of a standardized protocol for SLN processing further contributes to inconsistencies in diagnostic accuracy. Some retrospective and prospective studies evaluating the efficacy of FSA as a criterion standard for intraoperative SLN assessment in breast cancer surgery across diverse geographical regions are summarized in Table 4. Formal systematic review methodology, including predefined search strategies and quantitative meta-analysis, was not undertaken, as the objective was interpretative and hypothesis generating rather than

definitive. The reliability of FSA for SLN evaluation in breast cancer varies considerably across countries.

The reported FNRs range from 3% to 50% for macrometastases, 42% to 84% for micrometastases, and 11% to 51% for ITCs. Cumulative global clinical evidence indicates that FSA demonstrates limited sensitivity in detecting micrometastases and ITCs, as highlighted in a recent meta-analysis evaluating the diagnostic accuracy of intraoperative frozen section biopsy for SLN metastases in breast cancer.[61] Nevertheless, imprint cytology may offer practical diagnostic utility for detecting ITCs in resource-constrained settings where cryostat facilities are not available.[70] Notably, the frozen section sensitivity and FNRs differ substantially between countries and institutions, reflecting differences in pathology workflows, sectioning protocols, and training. This variability challenges "one-size-fits-all" standardization of intraoperative SLN assessment. While high sensitivity for macrometastasis is desirable, efforts to standardize practice must be guided by the identification of metastases that would actually alter surgical management and treatment, rather than performance targets alone. In this context, although we report sensitivity, specificity, and FNRs, these diagnostic metrics do not necessarily determine clinical value, as high sensitivity for micrometastases and ITCs has increasingly limited therapeutic consequence in the era of de-escalated axillary surgery and broadened indications for regional radiotherapy.

Clinical consequences of micrometastasis and ITCs detection

The detection of micrometastases and ITCs does not often alter surgical or radiotherapy strategies in contemporary practice. The false negative for a single micrometastasis in a Z0011/IBCSG 2301–eligible patient is unlikely to change systemic or radiation treatment, whereas missed macrometastatic disease in mastectomy or higher-risk settings may still be therapeutically relevant.[8,71] Moreover, while ALND remains a common standard for residual disease, the OPBC-05/ICARO study has validated the safety of omitting ALND specifically in patients with residual ITCs (ypN0i$^+$) after chemotherapy.[72] Therefore, intraoperative efforts to achieve higher sensitivity for residual ITCs may lead to overtreatment rather than improved care.

The future of intraoperative sentinel lymph node evaluation: role of next-generation molecular probes

Surgical treatment is administered in approximately 70% to 90% of breast cancer cases; however, long-term data reveal that up to half of these patients experience distant tumor recurrence within 20 years, an outcome that may be partially attributable to diagnostic inaccuracies during the initial evaluation.[85,86] Consequently, the accurate intraoperative diagnosis of SLN involvement during tumor excision is critical for effective oncologic management and reducing the likelihood of reoperation. FSA, a technique introduced in the late 19th century, has long been employed for intraoperative SLN assessment.[87,88] However, despite more than 130 years of refinement, its diagnostic accuracy remains limited due to its exclusive reliance on morphology-based interpretation performed under strict time constraints. Enhancing this intraoperative approach could lead to more precise SLNB staging and a reduction in avoidable repeat surgeries. A promising strategy to enhance the diagnostic sensitivity and accuracy of FSA is the incorporation of molecular probes to complement conventional morphological assessment. Given current de-escalation paradigms, these molecular and rapid immunohistochemistry (IHC) techniques are unlikely to meaningfully change axillary management for the majority of patients with early-stage disease in whom micrometastatic disease or ITCs do not trigger ALND.[16,71] Their main near-term value lies in improving the speed and confidence of pathological assessment and in niche decision points such as complex reconstructions and histologically subtle disease. Therefore, their impact on reoperation rates and the extent of axillary surgery remains hypothetical and will require dedicated prospective evaluation.

Antibodies, as the criterion standard in immunohistochemistry, are ideal candidates for this molecular-based approach. To enable antibody staining within the intraoperative window of approximately 20 minutes, several rapid immunohistochemical techniques have been developed. These methods employ polymer-conjugated horseradish peroxidase (HRP) linked to either primary antibodies[89] or secondary antibodies.[90,91] Alternatively, signal enhancement was facilitated by microwave irradiation[92] or magnetic field exposure.[93] Additionally, high-voltage, low-frequency alternating current fields have been shown to significantly expedite the immune staining process.[94] Although rapid antibody-based immunohistochemistry offers significant potential, it has not yet been integrated into routine clinical workflows. Barriers include the dependence on specialized equipment that is not widely available in diagnostic laboratories and the necessity for multiple fixatives, which necessitates significant modifications to established laboratory workflows.[95]

Furthermore, the inherent limitations of antibodies related to antibody specificity, inconsistent reproducibility, and batch-to-batch variation have intensified efforts to identify and validate alternative molecular probes that can offer greater reliability in molecular pathology.[96,97]

Table 4. Comparative Diagnostic Accuracy of Sentinel Lymph Node Frozen Section Across Countries (Sorted by Year)

Country	Study period	No. of patients	No. of SLNs examined	Positive SLNs by frozen section, No.	True-positive results by FFPE, No.	False-negative rate, %	Ref.
Netherlands	2000–2006	615	615	126	176	28.4	84
USA	2003–2012	1940	1940	305	400	23.7	60
Hong Kong	2005–2007	260	243	53	86	38.4	73
Jordan	2005–2010	440	446	135	161	16.2	76
Singapore	2005–2012	NR	2202	571	660	13.5	77
Italy	2007–2019	2079	1466	250	392	36.2	27
Pakistan	2008–2011	154	154	50	62	19.4	75
China	2008–2016[a]	1272	NR	294	347	15.3	78
France	2011–2012[a]	672	672	86	145	40.7	74
Venezuela	2011–2015	281	281	58	76	23.7	80
Malaysia	2012–2015	82	NR	13	15	13.3	81
New Zealand	2014[a]	80	NR	18	20	10.0	79
India	2014–2018	424	424	117	134	12.7	82
Australia	2017–2022	565	NR	66	174	29.0	83

Abbreviations: FFPE, formalin-fixed paraffin-embedded; NR, not reported; SLN, sentinel lymph node.
[a]Estimated study period.

The development of smaller molecular probes as substitutes for antibodies in FSA holds significant potential to advance and refine intraoperative surgical pathology by addressing current limitations in speed and diagnostic precision. The following approaches are at an early developmental stage and primarily experimental in nature, with no completed clinical trials demonstrating a reduction in axillary surgery. Their current relevance is primarily conceptual and pathological, rather than immediately practice-changing for surgeons. The use of smaller molecular probes confers distinct benefits, such as minimized steric hindrance, improved access to broader molecular surface area, and accelerated diffusion through tissue. These features are largely attributed to Brownian motion, which occurs more rapidly in smaller molecules due to the inverse relationship between molecular size and diffusion rate.[98] Some of the emerging small molecular probes used in immunohistochemistry include nanobodies, affibodies, and nucleic acid aptamers, illustrated in Figure 5.

Nanobodies

The advent of nanobody technology originated from the discovery of heavy-chain-only antibodies (HcAbs) in *Camelidae* species. Unlike conventional antibodies, which rely on the assembly of 2 heavy and 2 light chains for antigen recognition, HcAbs function with just 2 heavy chains, each harboring a single variable domain termed VHH. This VHH region, widely known as a nanobody, independently facilitates antigen binding.[99,100] Nanobodies can be isolated from immune or naïve phage display libraries derived from camelids, such as camels and alpacas, or constructed synthetically *in vitro*. Due to their compact structure, nanobodies exhibit excellent solubility and thermal stability, and they are well-suited for high-yield recombinant expression in prokaryotic hosts such as *E coli*, enabling their expanding use in molecular imaging, targeted therapy, and biosensing platforms.[100]

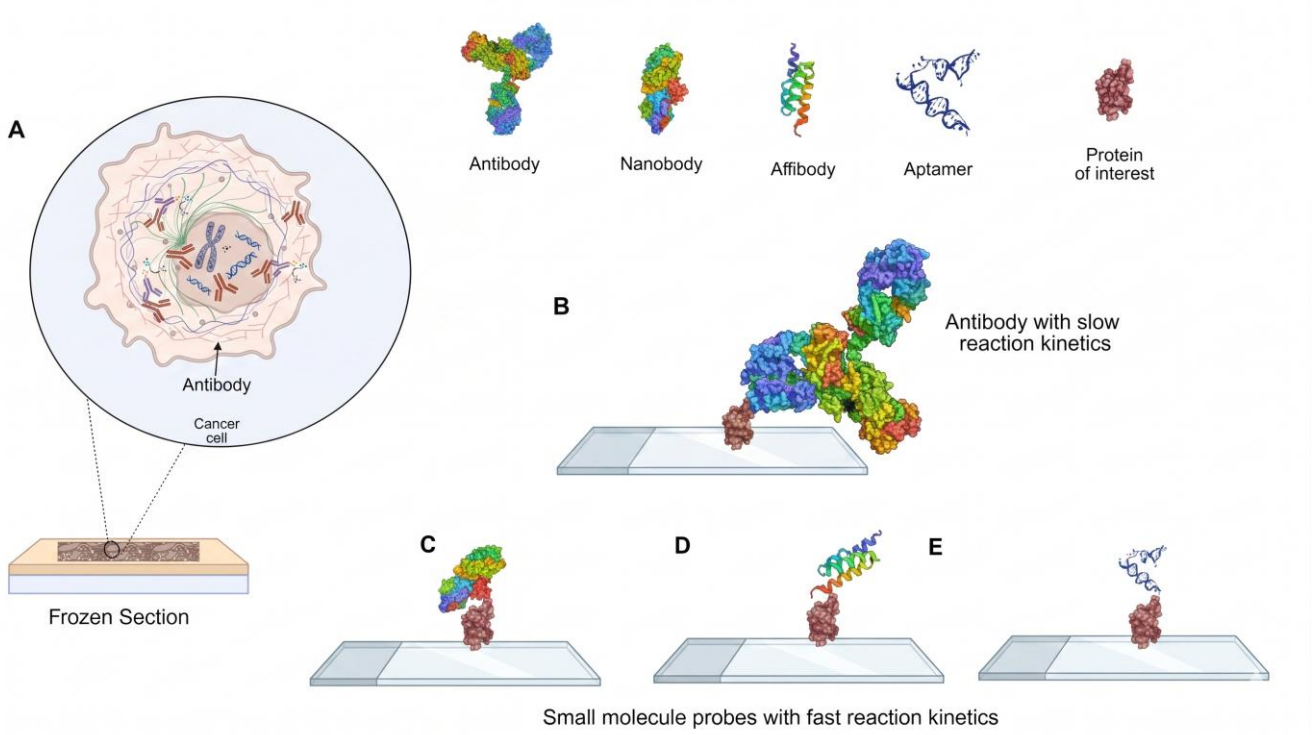

Figure 5. Schematic Representation of Small Molecular Probes for Intraoperative Pathology. A, The standard secondary antibody-based immunohistochemistry for cancer cell detection in a frozen section. B, Schematic illustration of the large size of antibodies compared with that of the target, with slow reaction kinetics. C–E, Emerging small molecular probes with fast reaction kinetics: nanobody-based immunohistochemistry (C); affibody-based immunohistochemistry (D); and aptamer-based immunohistochemistry (E). Created with BioRender.com.

Nanobodies possess distinct structural characteristics compared to conventional antibodies, with a molecular mass of 12 to 15 kDa (approximately 10% of that of an IgG) and compact dimensions of 4.2×2.5 nm. Their small size facilitates superior tissue penetration, allowing access to densely packed epitopes and cryptic binding sites that are typically inaccessible to larger antibodies. This enhanced penetrance arises from their single-domain architecture, which lacks the Fc region and incorporates hydrophilic framework residues that improve solubility and reduce off-target interactions. These attributes are particularly beneficial in intraoperative contexts, such as FSA, where fast and accurate biomarker detection can inform immediate clinical decisions.[99]

While nanobodies present clear structural and functional advantages over traditional antibodies, their clinical application in intraoperative FSA has not yet achieved widespread adoption. Several challenges contribute to this delay in clinical translation, including small-scale production capabilities that are inadequate for broad implementation, limited commercial availability, high production costs, and a lack of established clinical validation and standardized protocols tailored to intraoperative diagnostics.[101] Moreover, efforts to improve nanobody stability are ongoing, and many candidates still require targeted engineering to meet the performance demands of intraoperative diagnostic pathology.

Affibodies

While antibodies and nanobodies originate from natural immune systems, affibodies are entirely synthetic constructs generated through advanced protein engineering. These compact proteins (approximately 6.5 kDa) are based on the Z domain of *Staphylococcus aureus* protein A, which forms a stable, 58–amino acid 3-helix bundle devoid of disulfide bonds—an architecture that supports structural robustness and ease of manipulation.[102] Affibody libraries are produced by randomizing selected residues within the Z domain, followed by *in vitro* selection using display platforms such as phage, ribosome, mRNA, and cell surface display.[103] This combinatorial method enables the rapid development of affibodies with high specificity and affinity for diverse targets.[104]

Affibodies exhibit a wide array of favorable characteristics that make them attractive for therapeutic and diagnostic applications. These include low immunogenicity, efficient folding, robust stability, high tissue penetration, and high expression titers in bacterial hosts. Moreover, they display advantageous pharmacokinetics, strong binding affinities, thermal and chemical stability, and considerable tolerance to structural modifications.[105]

Owing to these attributes, affibodies have found particular utility in in vivo imaging. In one example, a dual-labeled anti-EGFR affibody significantly outperformed the antibody cetuximab in differentiating tumor from normal brain tissue in a glioma xenograft mouse model.[106] Beyond imaging, affibodies have been explored for receptor blockade and targeted drug delivery.[102] However, their kinetic profile, which is advantageous for imaging, is less relevant to static tissue analyses, e.g., those used in anatomical pathology. Adapting affibodies for such applications would require re-engineering of labeling strategies and detection methods to suit FSA. Crucially, these reagents must also undergo rigorous validation for specificity, reproducibility, and adherence to established pathology protocols. Thus, despite their potential, affibodies have yet to make their way into pathology laboratories for intraoperative diagnostics, where established antibodies remain the preferred diagnostic tools.

Aptamers

First developed in 1990 as a novel class of non-protein high-affinity ligands,[107,108] aptamers are short single-stranded DNA or RNA molecules that can fold into unique tertiary structures through intramolecular interactions (Figure 6A). They are selected through an *in vitro* molecular evolution process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment), which does not rely on the use of an animal or a cell. Following isolation, aptamers are chemically synthesized *in vitro* and are therefore termed chemical antibodies, in contrast to their protein-based affinity ligand counterparts.[109] The folding of an aptamer involves the transformation of a single-stranded nucleic acid into a well-defined 3-dimensional (3D) structure. This process is driven by internal base pairing and a suite of intermolecular forces, including hydrogen bonding, van der Waals interactions, hydrophobic effects, and base stacking. These forces promote the development of secondary structural elements, such as hairpins and pseudoknots, which guide the progressive folding into complex 3D shapes, ultimately enabling the aptamer to selectively recognize and bind its cognate target. Aptamer–target binding is driven predominantly by an induced-fit mechanism, wherein structural adjustments facilitate specific molecular recognition.[110] This process is reinforced by auxiliary non-covalent interactions, including hydrogen bonding, electrostatic attraction, base stacking, and van der Waals forces.[111] Although such non-covalent mechanisms are predominant, emerging evidence indicates that covalent bonding may also contribute to aptamer–target complex formation in certain contexts.[112,113]

Compared to traditional antibodies, nucleic acid aptamers offer several distinct advantages, including minimal batch-to-batch variability, high structural and thermal stability in vitro, excellent specificity and reproducibility, and lower production costs. Notably, aptamers can exhibit faster binding kinetics and more rapid diffusion within tissues, which facilitates improved access to epitopes in immunohistochemistry and histological applications (Figure 6B, 6C). For instance, Zeng *et al.* demonstrated the superior specificity of CD30 aptamers compared to antibodies, showing that CD30 aptamers can efficiently and specifically bind to targets in fixed tissues, with reduced background staining in necrotic areas compared to antibodies.[114,115] Similarly, Shigdar *et al.* reported that an RNA aptamer targeting the epithelial cell adhesion molecule (EpCAM) exhibited enhanced specificity and sensitivity in formalin-fixed paraffin-embedded primary breast cancer tissues, outperforming the corresponding monoclonal antibody.[116] Notably, both studies highlighted that aptamers bind to their targets in significantly shorter reaction times than antibodies. In line with these findings, a Cy3-labeled DNA aptamer targeting EpCAM was successfully employed for immunofluorescence staining of frozen tissue sections from colon and rectal cancers, utilizing a protocol that requires approximately 40 minutes.[117] Despite these promising advances, the clinical utility of aptamers in histopathology remains

underdeveloped, highlighting the need for further research to develop aptamers targeting clinically relevant markers.

Rapid immunohistochemistry

Over the past 2 decades, significant efforts have been made to enhance the sensitivity and accuracy of intraoperative surgical pathology through the incorporation of antibody staining in frozen section diagnostics. This has led to the development of various rapid IHC techniques.[118–123]

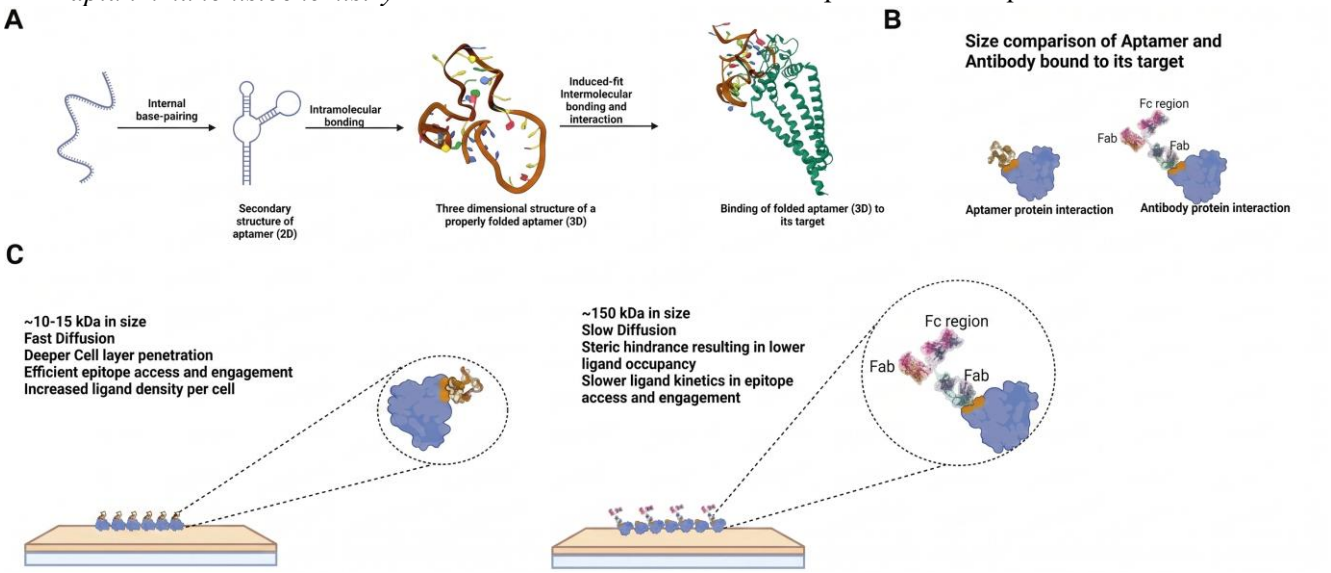

Figure 6. Schematics of Aptamer Technology and Its Advantages in Histochemistry. A, Illustration of the folding of a linear nucleotide to a 3-dimensional structure to enable its specific binding to a target protein. B, Size comparison between an aptamer and an antibody relative to a target protein. C, Advantages of aptamers over antibodies in frozen section histochemistry. Created with BioRender.com.

The overall strategy employed in various rapid IHC procedures involves the use of unconjugated primary antibodies available on the market, with the addition of multiple reporter molecules of poly–horseradish peroxidase (HRP). The poly-HRP consists of multiple HRP molecules conjugated to a linear or branched polymer or other nanoparticle structures, which are then attached to a secondary antibody. This multimeric arrangement amplifies the enzymatic activity, accelerating the catalysis and enhancing the generation of the final clinically useful DAB staining within the required time frame of approximately 30 minutes.

There are at least 7 reported antibody-based rapid IHC systems. Toda *et al.* in Japan developed the Histo-Tek R-IHC, which utilizes Agilent/Dako's EnVision HRP-labeled polymer conjugated with secondary antibodies. This system employs an electric field to accelerate the antibody staining of frozen sections.[123] Microscope slides are placed between electrodes, followed by the application of a high-voltage (4.0 to 4.5 kV, offset 2.25 kV) and low-to-high frequency (5 to 90 Hz) alternating current electric field delivered by a proprietary instrument. In addition, this research team introduced a novel fixation method for frozen sections involving acetic acid, formalin, and ethanol.[124] In contrast, Novodiax Inc developed the Direct Immunohistochemistry Assay, which utilizes a polymeric enzyme-antibody conjugate for frozen section IHC (WO2015171938A1).[125] This procedure involves fixing slides in glacial acetic acid, 37% formaldehyde, and methyl alcohol, and is marketed alongside a proprietary instrument.[126]

Similarly, Guizhou Meixinda Medical Technology Co., Ltd. in China has developed a rapid IHC method using polyenzyme-antibody combinations, some of which are sourced from Novodiax Inc.[127] Additionally, Shanghai Baiying Biotechnology Co., Ltd. has introduced a micropolymer-HRP-nano antibody reporter for primary antibody detection, enabling rapid IHC.[128]

Bio SB Inc. (CA, USA) has developed the SB Mohs PolyDetector technology,[129] which incorporates a proprietary micropolymer backbone conjugated to anti-mouse and anti-rabbit Fab fragments of IgG, along with multiple units of HRP. The elimination of the immunoglobulin Fc region aims to minimize non-specific reactions and background staining.[124]

Finally, a rapid micro-immunohistochemistry system was developed using a microfluidic device consisting of a horizontally oriented microfluidic probe.[130] Despite ongoing efforts, these rapid IHC systems have yet to be widely adopted by anatomical pathologists globally, primarily due to a lack of

rigorous clinical verification and validation, as well as practical challenges in their feasibility and practicality for integration into routine intraoperative surgical pathology workflows. Although the emerging techniques such as molecular probes and rapid immunohistochemistry platforms discussed here remain experimental and investigational, their potential value should be considered in terms of specific intraoperative surgical endpoints rather than diagnostic performance alone. In the current era of de-escalated axillary surgery, these techniques are unlikely to broadly change management for Z0011-eligible patients or for those with low-volume residual nodal disease.[8,16,71,72] However, these techniques may be beneficial for selected scenarios such as mastectomy with immediate reconstruction, wherein more sensitive and rapid intraoperative identification of clinically relevant nodal disease could prevent a second operation. Also, improved techniques may benefit histologically challenging subtypes, such as invasive lobular carcinoma, where rapid immunohistochemistry may increase the detection of macrometastases, thereby influencing axillary management.[11,71,72,131,132] Future improvements are likely to focus on workflows where real-time molecular or optical readouts are necessary to prioritize which nodes require full pathological workup. This will lead to reduced turnaround time of intraoperative evaluation.

CONCLUSION

SLNB has revolutionized the staging and treatment of breast cancer, offering a less invasive alternative to ALND with significantly reduced morbidity. Intraoperative evaluation of SLNB enables immediate decision-making regarding axillary management, potentially sparing patients from additional surgeries and enhancing their quality of life. In the post-Z0011 era, the role of SLN evaluation has become more selective toward the de-escalation of routine SLNB in low-risk patients. FSA of SLNs remains the most established and widely validated intraoperative technique where it is beneficial in high-risk patients or Z0011-ineligible patients. The future of intraoperative SLN evaluation is likely to be highly context-dependent rather than universally applied. Emerging molecular and rapid-immunohistochemical methods must focus on large-scale validation studies aimed at integrating emerging technologies in the intraoperative diagnostic process and must ultimately be judged by their ability to improve therapeutic decisions rather than diagnostic performance alone. Further investigation is also needed to explore how artificial intelligence and predictive modeling can enhance SLNB, improving diagnostic accuracy and consistency. As axillary treatment continues to move toward de-escalation, other validated alternatives via close interdisciplinary collaboration will be essential to refine these methods, ensuring that SLNB is used selectively in situations where it clearly adds clinical value beyond what postoperative pathology and guideline-directed management already provide.

ETHICAL CONSIDERATIONS

Not applicable.

DATA AVAILABILITY

This article is a review. All the data synthesized during the study are included within this published article and the references cited herein. No new primary data were generated or analyzed in this study.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

This work was supported by the Dalian Municipal Guidance Project in the Field of Life and Health Sciences, Grant No. 2024ZDJH01PT105 (to YZ) and the Australian Ministry of Education, Grant No. AE230100237 (to WD).

ACKNOWLEDGMENTS

JC, SM, and RN are supported by Deakin University Postgraduate Research Scholarships.

AI DISCLOSURE

No artificial intelligence was used in the preparation of this work.

AUTHOR CONTRIBUTION

JC and SM: Conceptualization, Visualization, Software, Data Curation, Writing-Original Draft, Writing-Review & Editing; RC, RN, CP, DS, LW, QW, and DX: Formal Analysis, Visualization, Validation, Writing – Original Draft. YZ, WD, and AH: Funding acquisition, Project Administration, Supervision, Writing – Original Draft, Writing – Review & Editing.

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How to Cite This Article

Catague J, McClintock S, Chowdury R, Napit R, Pu CW, Stupart D, et al. Evolving Landscape of Intraoperative Evaluation of Sentinel Lymph Node Biopsy in Breast Cancer. Arch Breast Cancer. 2026; 13(3):252-74.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/1269