



Intraoperative Assessment of Sentinel Lymph Nodes in Breast Cancer

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ABSTRACT

Lymph node status has been considered as an important prognostic indicator for survival in breast cancer. Recently the need for axillary clearance and the significance of performing sentinel node biopsy has been questioned.

Despite this, sentinel lymph node biopsy is considered as a standard method of assessment of clinically node-negative breast cancers. Economic implications as well as patient related factors have led to the development a number of intra-operative techniques. Review of the emerging trends of practice in the last 4 years show that although routine histological examination remains the gold standard in most centres, intraoperative assessment remains the most favourable, timely and cost-effective option to analyse sentinel nodes.

Molecular techniques appear to be far more superior to other histological tests such as frozen section or touch imprint cytology. Emerging research suggests that molecular techniques can be used to predict the presence of nonsentinel node metastasis. As a result, this technique may be a reliable surrogate to evaluate axillary tumor burden.

Introduction

Lymph node status remains an important prognostic indicator for survival in breast cancer although other prognostic markers recently gaining importance in directing the adjuvant treatment pathway.¹ Sentinel lymph node biopsy has become a standard method of assessment of clinically node-

negative breast cancers, with delayed axillary clearance as a second procedure, if positive. Economic implications in terms of bed occupancy and duration of hospital stay², technical difficulties, being a more challenging procedure and patient-related factors, including stress and exposure to a second general anaesthesia have led to the development a number of intraoperative techniques for the evaluation of sentinel nodes. Most of these are now clinically implemented with varying degrees of success to reduce second procedure rates. Layfield *et al.* comprehensively discussed the intraoperative methods in use in 2011.³ The following review of the literature examines the intraoperative methods for evaluation of sentinel lymph nodes, emerging trends with comparison of

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**Table 1.** Summary of frozen section and touch imprint cytology

Technique	Sensitivity	Specificity	Advantage	Disadvantage
Frozen section Tissue is frozen and sectioned at 6µm and H&E stained	60.6% (macro-metastases)	100% (macro-metastases)	Established intraoperative assessment tool	Low sensitivity in identification of micro-metastases/isolated tumor cells
Touch imprint cytology Scrape of lymph node surface stained and examined	63%	99%	Less expensive compared to FS with no loss of tissue	Sensitivity varies with technique and low sensitivity to macro-metastases

Abbreviation: FS: frozen section

the national guidelines of the United Kingdom and the United States in the last 4 years for intraoperative evaluation of sentinel lymph nodes.

Current practice

Intraoperative methods of assessment in use include frozen section (FS), touch imprint cytology (TIC), rapid immunocytochemistry and one-step nucleic acid amplification (OSNA) using both whole node and half node analysis methods. One of the commercially available reverse transcriptase based assay (RT-PCR) which was in use, had been withdrawn due to financial problems of the manufacturer.⁴ However a new version, known as Metasin is being developed.^{4,5} In addition, Scanning elastic scattering spectroscopy (ESS), a technology still in development is reviewed. While frozen section has been previously cited as the most commonly used technique, the advent of molecular assays has resulted in increasing numbers of centres now beginning to adopt this process for intraoperative assessment.³ Currently 20 centres in the UK and 180 centres in the European Union are using this technology according to Sysmex®, the commercial manufacturer. However it has not been marketed in the United States.

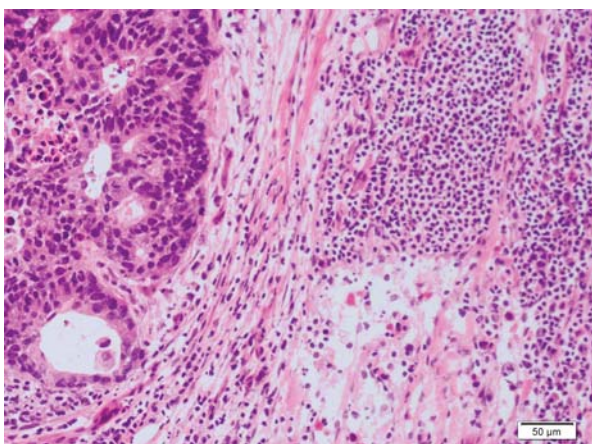


Figure 1. Frozen section histology of a malignant SLN.

Frozen section

This technique involves sending the whole or part of the sentinel lymph node to the pathologist who freezes the tissue. It is then sectioned at 6µm or less and microscopically examined after being subjected to H&E staining. While protocols for this method vary in the published studies, a metaanalysis in 2011 of 47 studies involving 13,062 patients using this method showed that the overall sensitivity was 60.6% with a specificity of 100%.⁶ However, FS had a much lower sensitivity in detecting micro-metastases and isolated tumor cells compared with macro-metastases and the difference was statistically significant (28.9% vs 80.3% $P < 0.001$). The authors concluded that this method lacked the accuracy required to rule out micro-metastases. It has been demonstrated that sentinel lymph node biopsies which only yield micro-metastases and isolated tumor cells are nevertheless associated with a non-sentinel node involvement rate of 20% and 12% respectively when axillary clearance is subsequently performed.^{7,8} Therefore, sole use of this method would result in a small but not insignificant false negative assessment. The FS requires a dedicated team in pathology comprising a biomedical scientist and pathologist available for assessment when required. In addition, it is dependent on the skills and experience of the pathologist interpreting the section. All of these factors make it an expensive option in terms of cost effectiveness. A summary of FS is shown in Table 1 and Figure 1.

Touch imprint cytology

TIC or scrape cytology are performed by pressing or scraping the cut surface of the sentinel lymph node onto a slide. This imprint is then stained and examined. In a meta-analysis of 31 studies involving 4438 patients in 2005, Tew *et al* showed that there was disparity in protocols, as well as intraoperative and histological technique.⁹ Clear comparison and pooled interpretation of the studies therefore should be done with caution. Nevertheless the estimate of overall sensitivity of TIC was 63% and specificity 99%. Subsequently, other

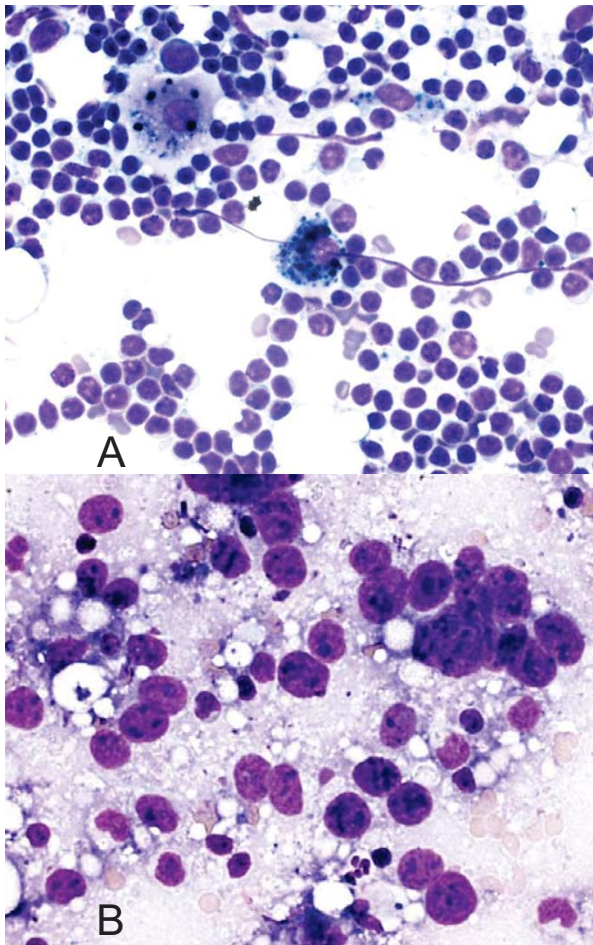


Figure 2. TIC of sentinel lymph node: A) Normal sentinel lymph node with lymphocytes and tangible body macrophages containing blue dye, B) SLN with metastatic deposit on TIC.

authors have also demonstrated that sensitivity varies not just with the specific technique used, but also depends on the size of the metastasis; with low sensitivity in the presence of micro-metastases compared to standard histological examination (22% for micro-metastases compared to 81% for macro-metastases).⁹⁻¹⁴ The advantage of this technique over FS is that it is less expensive and time consuming with no loss of tissue. However interpretation of this technique requires experienced cyto-pathologists and if the result is equivocal there is no clear guidance regarding subsequent management. Moreover, lobular cancers are more difficult to identify using this technique due to overlapping morphological features with lymphoid tissue. The use of immunocytochemical techniques used in conjunction with TIC has been shown to improve the recognition of both of these entities but its use needs to be balanced against the additional time required to perform this test and the cost-effectiveness of implementing this as part of intraoperative diagnosis. A summary of TIC is shown in Table 1 and Figure 2.

Molecular techniques: one-step nucleic acid amplification (OSNA)

The development of molecular assays for intraoperative detection of nodal metastases has helped to address the issue of detection of micrometastases. Two types of molecular assays have been developed. Firstly, an intraoperative real-time quantitative polymerase chain reaction (RT-PCR) assay was developed commercially by GenesearchTM which used dual mRNA markers (CK19 and mammaglobin).¹⁵ For commercial reasons this assay was withdrawn. However, an alternative assay called Metasin using the same mRNA markers has recently been assessed in comparison to the GenesearchTM assay and histology as the gold standard.⁵ The sensitivity and specificity of Metasin compared to the GenesearchTM was 95% and 97%, respectively. Metasin was concordant with GenesearchTM in 148/154 lymph node homogenates and its positive predictive value was 90% and negative predictive value 97% for both histology and GenesearchTM.⁵ This suggests that the Metasin assay is a viable replacement and a valuable assay in intraoperative diagnosis of sentinel nodes. The other molecular assay in use utilises OSNA, developed by Sysmex[®], utilised Ck19 alone as a marker.¹⁶ The result is regarded as "negative" for mRNA copy numbers below 250/ μ l, [1+] for copy numbers between 250- 5000/ μ l (suggestive of micro metastases) and [2+] if greater than 5000/ μ l indicating macrometastases. OSNA is an established technique validated by a number of studies.¹⁶⁻²³ Combined analysis of 9 studies involving 3631 samples by Cserni *et al.* in 2012 showed an overall sensitivity of 91.7%, specificity of 97%, accuracy of 96.1% and positive predictive value (PPV) 85.8% with negative predictive value (NPV) of 98.3%.⁶ There was close concordance among these studies. The high negative predictive value for OSNA compares favourably with other methods of assessment. Direct comparison between OSNA and FS showed that OSNA detected more sentinel lymph node metastases particularly micro-metastases.²⁴ This is because OSNA results reflect the status of the whole processed lymph node, while both FS and TIC are based on the analysis of the cut surface of the lymph node slices. These slices may be 1.5 to 2 mm thick and the unexamined tissue may potentially harbour a metastatic or more commonly, a micro-metastatic deposit. OSNA has the advantage that it does not require purification of mRNA and therefore is a quicker method compared to RT-PCR.²⁵ The additional advantage is that it is an automated method requiring a trained lab technician with a relatively quick turnaround time when the whole node is processed.

Following the publication of these studies, which used half node analysis for validation of their results, most centres where this technology is available carry

**Table 1.** Summary of molecular techniques

Technique	Sensitivity	Specificity	Advantage	Disadvantage
Genesearch™	90%	97%	Dual mRNA markers used. Automated procedure not requiring additional pathologist time. Quick turnaround time	No longer available
Metasin	95%	97%	Dual mRNA markers. Automated procedure not requiring additional pathologist time used. Quick turnaround time	Not yet approved by NICE for other than research
OSNA	91%	97%	Does not require mRNA purification. Automated procedure not requiring additional pathologist time. Quick turnaround time. Approved by NICE	Single mRNA marker CK 19 (which a small minority of breast cancer may not express).

out whole node analysis. While CK19 expression is high in most breast cancers, the prevalence of CK19 negative cancers is estimated by studies to range between 1-3% in unselected series.²⁶ Special subtypes of cancers as well as triple negatives and luminal A have been reported to show a higher prevalence of CK19 negative cancers while one study has suggested that older patients may also show a higher prevalence.^{27,28} The potential risk of whole node analysis is that in a small proportion of patients may be falsely reassured by a negative OSNA result and subsequently not receive appropriate adjuvant therapy. Although lymph node status is not the only prognostic indicator, and its importance has decreased in terms of prognostication and treatment planning, identification of CK19 negative cancers preoperatively may reduce the risk of a false negative result and avoid under-treatment of loco-regional disease.

Cost-benefit analysis comparing OSNA with conventional histopathological evaluation show that although the duration of the first surgery was longer in the OSNA group, the number of admission days overall was reduced and resulted in a lower average cost of surgery with a mean saving of €439.67 per patient.²⁹ However it must be acknowledged that there is an initial cost to set up the service and train staff to appropriately use the technology. In some institutions this could be prohibitive due to limitations of resources and their rationing. In our experience the analysis of up to 2 nodes by OSNA requires 40-60 minutes with additional time for more than 2 nodes. In our practice once the sentinel node biopsy is performed the time required for analysis coincides with the time either breast conserving surgery or mastectomy is occurring. The results often become available as the wounds are being closed so in reality, increase in operative time occurs rather infrequently. If the result is positive additional time will be required for axillary node dissection,

and this needs to be considered in theatre time planning and allocation but this would be required for all methods of intraoperative assessment. Table 2 summarizes the molecular techniques and Figure 3

describes the OSNA process using 50% node analysis.

Elastic scattering spectroscopy (ESS)

This method was developed for rapid detection of metastases within sentinel lymph nodes using a point contact technique that collects broadband optical spectra sensitive to absorption and scattering within the tissue.³⁰ While the full potential of this technology has yet to be realised, the initial assessment of its detection of clinically relevant metastases showed a sensitivity of 69% specificity of 96%.³⁰ This compares well with FS and TIC and does not require a specialized pathologist for interpretation. The proponents of this technology aim to increase its sensitivity without loss of specificity by improved scanning techniques as well as developing more advanced scanning hardware which will reduce scanning time. As no commercial partner has come forward yet the cost effectiveness has not been assessed.

National Institute of Clinical Excellence (NICE guidelines UK) 2013 and NCCN/ASCO recommendations

The NICE guidelines (<http://www.nice.org.uk>), state that pathological methods that can be used intraoperatively include FS and TIC. However, in practice these intraoperative methods may have relatively low accuracy particularly in inexperienced hands. For molecular analysis of sentinel nodes, NICE recommends whole lymph node analysis using the RD-100i OSNA system as an option for detecting sentinel lymph node metastases during breast surgery in people with early invasive breast cancer who require a sentinel lymph node biopsy, and in whom axillary lymph node dissection will be considered. The Metasin test is not yet recommended for detecting sentinel lymph node metastases in patients with early invasive breast cancer in routine clinical NHS practice. However, the guidelines concede that the Metasin test shows promise and the development of robust evidence is recommended to demonstrate its utility in clinical practice.

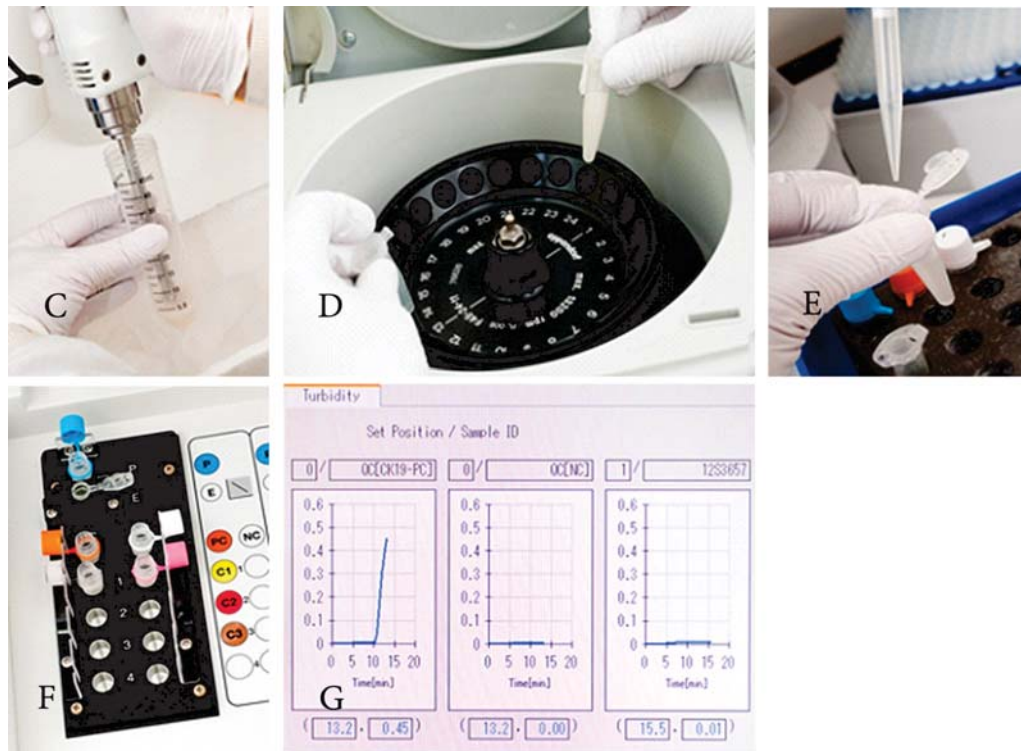


Figure 3. C,D,E) The slices to be assessed by OSNA are homogenized and centrifuged and the supernatant drawn off. F, G) It is added to reagents that facilitate the Loop mediated isothermal amplification (RT LAMP) and then placed in the processor for amplification. The result is displayed as a measure of turbidity, as mRNA copy number value and assessed as positive or negative.

In contrast to these guidelines the National Comprehensive Cancer Network (NCCN) guidelines and the American Society of Clinical Oncology (ASCO) continue to endorse H&E staining with histological evaluation as the gold standard and despite the evidence presented have not amended their recommendations to date to include molecular techniques. These recommendations are

based on ASCOGZ0010a multicentre study over eight years which concluded that since metastases, which were not detectable by H&E stain technique, do not have significant impact on survival, RT-PCR routine use could not be recommended instead of H&E staining. No consideration is given to whole node or half node analysis in this study.

Table 3. Summary of intraoperative techniques in use for evaluation of sentinel lymph nodes

Technique	Sensitivity/ Specificity	Advantages	Disadvantages	NICE (UK) guidelines	NCCN/ASCO guidelines
FS	60.6 % sensitivity; 100% specificity	Established intra-operative technique	Low sensitivity for micro-metastases. expensive	Can be used but Not recommended for routine practice	Not approved for routine practice
TIC	63% sensitivity; 99% specificity	Cheaper than FS	Low sensitivity for micro-metastases. Operator dependent	Can be used but Not recommended for routine practice	Not approved for routine practice
RT-PCR	90-95% sensitivity; 97% specificity	Dual m RNA markers. Quick – turn around time. Automated process with additional pathologist time required.	No commercial availability yet.	Not approved for routine practice	Not approved for routine practice
OSNA	91% sensitivity; 97% specificity	Quick turn-around time no purification required so faster than RT-PCR Automated process without additional pathologist time required.	Single m RNA marker CK 19 not expressed by all breast cancers	Approved for routine practice with whole node analysis recommended while half node analysis with histopathology to be performed according to clinical judgment	Not approved for routine practice

Abbreviations: FS: Frozen section; TIC: Touch imprint cytology; OSNA: One step nucleic Acid Amplification



Discussion

Despite the publication of the results of the ACOSOG Z0011 trial, which questions the need to perform axillary node dissection after positive sentinel node biopsy, investigation of the status of the sentinel node biopsy still remains the most accurate way to predict axillary nodal involvement; an important and critical prognostic indicator of survival. The trial demonstrated that in patients with small primary tumors (T1-T2) and a clinically negative axilla, performing sentinel node biopsy alone did not result in a worse survival outcome compared with patients who had undergone axillary node dissection for limited sentinel node metastases.³¹ However, these results cannot be extrapolated to the treatment of all breast cancers, not only because they do not include patients with higher stage disease but because the protocol showed that there was insufficient blinding of the sentinel node biopsy result from the oncologists, who subsequently treat the patients with chest wall radiotherapy. With the knowledge of positive sentinel node, it is possible that tangential fields may have been used to irradiate the axilla. The implication is that the outcome from this trial should thus be interpreted with caution because of the possibility of treatment of potentially positive axillary nodes with radiotherapy artificially improving the outcome. Retrospective analysis had already shown that 16.4- 20.8% of sentinel node positive patients did not continue to have an axillary node dissection.^{32,33} The trend may be related to the decision to perform breast conserving surgery with adjuvant radiotherapy to the breast with tangential fields covering the axilla.³⁴ The role of axillary nodal radiation following surgery with or without axillary node clearance (ANC) has been explored by the NCIC CTGMA 20 trial in 2011. The results have suggested that it is associated with increased morbidity, therefore its role as adjuvant therapy in node positive patient treated with SNLB alone was uncertain according to this study.³⁵ Following on from this, the IBCSG 23- 01 study also attempted to investigate the outcome for patients who do not undergo ANC in the presence of positive sentinel nodes, but continue to have adjuvant treatment. Unfortunately, this trial has also ended prematurely with poor accrual and is underpowered but the preliminary results supports the Z0011 trial findings.³⁶ However these studies do not address the need to accurately evaluate axillary status in order to assess prognosis and plan appropriate adjuvant treatment in the majority of breast cancers. The most recent multicentre trial comparing the use of axillary radiotherapy with axillary node dissection (EORTC 10981-22023 AMAROS trial) published its results in February 2010.³⁷ The final analysis of outcome of this trial was presented at the American Society of Clinical Oncology 2013 (ASCO 2013).³⁷ Their

results showed that in this trial, axillary radiation was a comparable alternative to axillary node dissection with less morbidity associated with lymphoedema. The authors have suggested that knowledge of the number of lymph node metastases was not necessary in the decision to administer adjuvant systemic treatment, however the results of this trial have not yet been validated. In addition, the trialists acknowledge that it is underpowered to demonstrate non inferiority due to the low number of events with recurrence of axillary dissection at 10 years at 0.43% compared to 1.19% with axillary radiotherapy. In March 2014, ASCO updated its recommendations regarding sentinel node biopsy.³⁸ There is now recommendation for greater selectivity in offering patients axillary surgery. Patients who plan to have breast conserving surgery with whole breast radiotherapy and have 2 or less metastatic nodes are advised not to have axillary clearance. Most patients undergoing mastectomy are still recommended to have sentinel node biopsy followed by axillary clearance if metastases are present. However, patients with large or locally advanced cancers, inflammatory cancers, where breast conserving surgery is planned are recommended not to have a sentinel node biopsy. Upfront axillary dissection is implied for large/locally advanced tumors. Patients with DCIS (even in the case of high grade disease) are advised not undergo any form of operative axillary assessment. These changes have yet to be endorsed by NICE (UK). Our current practice is to offer sentinel node biopsy to patients who do not have evidence of lymph node involvement at triple assessment in the presence of an invasive cancer or high grade ductal carcinoma *in situ* (DCIS). In these cases, OSNA is performed intraoperatively. Axillary clearance is only performed if the OSNA test is positive or is subsequently histopathology shows evidence of metastasis.

However, it is clear that the trend in the future is for performing fewer axillary dissections and perhaps the need to perform sentinel node biopsy maybe called into question. Currently the decision-making tool for axillary dissection is still sentinel node biopsy and intraoperative assessment allows further streamlining of this process.

Although routine histological examination remains the gold standard in most centres in assessing sentinel lymph node status, there are several disadvantages, the most critical is the time needed to produce a result (average 48 hours). In addition, there is considerable variation between centres regarding the method and extent to which these lymph nodes have to be histologically examined. For example, there are differences in relation to the initial slicing of the node; the ASCO guidelines recommend slicing through the long axis whereas NHSBSP Pathology Reporting of Breast



Disease (<http://www.cancerscreening.nhs.uk>) recommends slicing the node perpendicular to the long axis.³⁹

Some centres routinely use immunohistochemistry to assess sentinel nodes and the literature indicates that employing cytokeratins markers can result in upstaging in 2.6–19% of cases.⁴⁰ Other centres do not employ immunohistochemistry routinely.

Intraoperative assessment remains the most favourable, timely and cost-effective option to analyse sentinel nodes. For reasons highlighted above, molecular techniques appear to be far more superior to other histological tests such as FS or TIC and in light of the recent changes in guidelines these techniques may no longer be appropriate in most cases. Although there is agreement that with isolated tumor cells, there is sufficient evidence to support the avoidance of axillary node dissection in cases with ITCs. However, there is no agreement with regard to the further management of the axilla, if micrometastases are identified based on routine histological analysis of the sentinel node alone. Therefore, clinical judgement needs to be used for each case.

Perhaps the most exciting development from the use of OSNA has been the finding that the mRNA copy number can be used to predict the presence of non-sentinel node metastasis. Ohi *et al.* and Osako *et al.* both demonstrated that using whole node analysis by OSNA, non-sentinel node macro-metastatic rate increased in proportion to copy number.^{41,42} Ohi *et al.* have suggested that at a CK 19 mRNA copy number of greater than 1.0×10^5 is the only independent predictor of the presence of 4 or more nonsentinel node metastases ($p=0.014$). In addition to this, a recent study conducted at our institution showed that no nodes with mRNA copy number below 1400 on OSNA had metastases in additional nodes at ANC. These results suggest a relationship between mRNA copy number and the presence of non-sentinel lymph node metastases. Our study allowed us to define a threshold below which no metastases are expected in subsequent analysis of non-sentinel lymph nodes.⁴³ With greater increase and familiarity in using the OSNA technique, ongoing studies will enable refinement of their RNA copy numbers thresholds to become reliable surrogate markers for axillary node tumor burden and staging of axillary disease. Accurate prediction of non-sentinel nodal involvement intraoperatively through molecular analysis, would ultimately allow full assessment of the axillary nodal status without inflicting the potential morbidity of additional axillary surgery. Table 3 summarises the intraoperative techniques in use. In conclusion, intraoperative assessment of the sentinel node is worthwhile, and based on the expertise and resources available in each institution, a choice can be made between imprint cytology,

frozen section or molecular diagnosis.

Conflicts of interests

The authors declare no conflict of interest.

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