Sentinel lymph node biopsy and breast cancer

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Background and method: This paper reviews and discusses the feasibility and accuracy of sentinel lymph node (SLN) biopsy in breast cancer. A standardized method of identifying the SLN and detecting micrometastases is suggested, along with a strategy for the elimination of routine axillary lymph node dissection (ALND).

Results: Although the SLN can be identified successfully by experienced practitioners using either the dye-guided or γ probe-guided method, identification is facilitated when the two techniques are combined. To improve the likelihood of spotting metastases in the SLN, it is desirable to perform step sectioning combined with haematoxylin and eosin staining and immunohistochemistry of permanent and frozen sections. SLN biopsy is as accurate for T2 tumours as it is for T1 tumours. However, it is highly unlikely that all false-negative cases can be eliminated, even by detailed histological examination. Nevertheless, patients with T1 tumours with micrometastases in the SLN have shown no evidence of tumour in the non-sentinel nodes. In other words, ALND can be avoided in these patients, even if histological examination of the SLN fails to detect micrometastasis.

Conclusion: In practice, routine ALND can be avoided in patients with T1 tumours when the identified SLN proves to be histologically negative. However, investigation of long-term regional controls and of survival in a prospective randomized trial is necessary before SLN biopsy can replace routine ALND, particularly for patients with T2 tumours.

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Introduction

Sentinel lymph node (SLN) biopsy has been developed to assess accurately the axillary nodal status without removing most of the axillary contents1-6. The SLN is defined as the first node in the lymphatic basin that receives the primary lymphatic flow. A tumour-negative SLN virtually excludes lymphatic involvement of the entire regional lymphatic basin7, rendering axillary lymph node dissection (ALND) unnecessary in patients with node-negative breast cancer. SLN biopsy is likely to be one of the most exciting advances in the surgical management of breast cancer since the advent of breast-conserving treatment. It results in fewer ALND-related complications8, shorter hospital stay, cost reduction9 and a more accurate indication for treatment with adjuvant therapy10.

Because SLN technology has been evolving rapidly, variations in technique have become widespread and no standard procedure has yet been established. Numerous controversial issues concerning the optimal method for identifying the SLN, detecting micrometastases and obviating routine ALND remain unresolved. Specific issues include (1) pros and cons of the dye-guided, γ probe-guided, and dye- and γ probe-guided methods; (2) optimal particle size of radioisotopes, and dose and volume of radioisotopes; (3) advantages of peritumoural versus subdermal or subareolar injection; (4) efficacy of preoperative lymphoscintigraphy; (5) relevance of internal mammary SLN biopsy; (6) most suitable method for histological SLN examination; (7) intraoperative diagnosis of SLN (frozen section versus cytological examination); (8) clinical significance of micrometastases; and (9) patient selection for avoiding ALND in clinical practice. So SLN biopsy must still be considered as experimental; it has not been introduced as a routine procedure in most institutions. The growing demand from patients for less invasive procedures, however, has created a considerable incentive for accepting SLN biopsy without ALND as standard practice. In the light of this, consistency of technique and case selection have attained great significance. The objectives of this article are to review current knowledge of SLN biopsy in breast cancer, to discuss existing controversies, and to suggest a method for
the identification of the SLN and the detection of micrometastases in the SLN in order to eliminate routine ALND.

**Identification of sentinel lymph nodes**

**Blue dye, radioisotopes, or a combination of blue dye and radioisotopes**

SLNs have been identified by using blue dye\(^1\)\(^2\)\(^3\)\(^4\), radioisotopes\(^3\)\(^5\)\(^6\), or a combination of blue dye and radioisotopes (Fig. 1)\(^6\). However, the debate continues as to which method is best\(^11\)\(^12\). In a major series, Cox et al.\(^13\) reported that blue dye helped identify SLNs in 80 per cent and radioisotopes in 89 per cent of patients. Using a combination of blue dye and radioisotopes increased the success rate to 96 per cent. Cody et al.\(^14\) identified SLNs by blue dye in 81 per cent of patients, by radioisotopes in 87 per cent and by the combined technique in 95 per cent. In a multicentre study by the Japanese Breast Cancer Society, SLNs were identified successfully in 94 per cent of patients by using the combined technique, but in only 74 per cent by blue dye alone\(^15\). Similarly, an overview by Cody\(^16\) and a meta-analysis by Miltenburg et al.\(^17\) both described a higher identification rate of SLNs with the combined technique compared with blue dye or radioisotope alone. This improvement in the identification rate may well reflect retrospective learning by surgeons, but it may also be related to a true benefit of the combined technique.

**Dyes**

A number of dyes have been tested for SLN identification. Isosulfan blue is readily available as a blue dye tracer in the USA\(^1\)\(^6\)\(^20\)\(^21\), while patent blue violet is mostly used in Europe\(^22\). Isosulfan blue is the monosodium salt of 2,5-disulphonated triphenyl methane, while patent blue violet is a triphenyl methane similar in structure to isosulfan blue. Biochemically, they are essentially the same agents, and no difference has been observed in their ability to identify SLNs\(^23\).

Other dyes, including methylene blue, indocyanine green and indigocarmine, have also been used by some investigators\(^15\)\(^24\). However, methylene blue should be avoided as one of the known complications of injecting this drug is fat necrosis in patients who have had breast conservation surgery. In Japan, patent blue violet, indocyanine green and indigocarmine are commonly used because isosulfan blue is not commercially available\(^15\). The SLN identification rate was not significantly different between these three dyes in a multicentre study by the Japanese Breast Cancer Society\(^15\). While no prospective clinical study has yet been conducted to compare these dyes, it seems that surgeons using a variety of dyes can expect to achieve a reasonable rate of technical success\(^15\).

**Optimal particle size of radioisotope tracers**

The optimal size of a radioisotope tracer for SLN identification has remained controversial\(^13\). One of the biggest potential difficulties is that small particle tracers
may pass through the SLN and label secondary nodes. A particle size of 3–30 nm is considered effective for lymphoscintigraphy, but the ideal radioisotope tracer for SLN visualization is clearly different from a particulate tracer optimized for the visualization of all lymph nodes by means of scintigraphy. Large particle tracers, on the other hand, appear to pass through to secondary nodes rarely, but they show less uptake at the injection site.

European studies have employed $^{99m}$Tc-radiolabelled colloidal albumin $^{4,28,29}$. Paganelli et al. $^{28}$ demonstrated that the SLN can be identified more easily by using large particle colloidal albumin rather than small particle radioisotopes. Several nodes could be visualized at the axilla by means of lymphoscintigraphy using microcolloids of sulphide (particle size less than 50 nm) or colloids of human serum albumin (particle size less than 80 nm). Conversely, only one node was identified when colloids of human serum albumin (particle size 200–1000 nm) were used. In this last group of patients, the surgeon was able to identify SLNs more easily and in a shorter time by using a $\gamma$ probe. Subsequently, Veronesi et al. $^{29}$ used microcolloidal albumin in a later study, while they used nanocolloidal albumin in their initial study $^{4}$.

American investigators have used $^{99m}$Tc-radiolabelled sulphur colloid $^{1,6,30,31}$ because $^{99m}$Tc-labelled colloidal albumin is not commercially available in the USA. In the initial studies the ideal size of the radioisotope tracer was thought to be between 10 and 200 nm $^{1,6,26,32}$ and so $^{99m}$Tc-radiolabelled filtered sulphur colloid (particle size less than 200 nm) was used. However, some investigators $^{30,31}$ have suggested that unfiltered $^{99m}$Tc-labelled sulphur colloid is preferable. Linehan et al. $^{31}$ identified SLNs in 68 (88 per cent) of 77 cases when unfiltered $^{99m}$Tc-radiolabelled sulphur colloid was used and 42 (74 per cent) of 57 cases with filtered $^{99m}$Tc-labelled sulphur colloid, but the difference did not reach statistical significance ($P = 0.051$).

In Japan, neither $^{99m}$Tc-radiolabelled sulphur colloid nor $^{99m}$Tc-radiolabelled colloidal albumin is commercially available, forcing Japanese investigators to use either $^{99m}$Tc-labelled human serum albumin or $^{99m}$Tc-labelled tin colloid. However, $^{99m}$Tc-tin colloidal human serum albumin (particle size 2–3 nm) is too small, migrates very rapidly and tends to overflow into non-SLNs $^{15,25,33}$, so that most Japanese investigators have used $^{99m}$Tc-labelled tin colloid (particle size 400–5000 nm) in their studies $^{15,34}$. Nevertheless, the size of the latter is too large, as may be appreciated by the relatively low success rate for visualization of SLNs by lymphoscintigraphy $^{15}$. Recently, $^{99m}$Tc-radiolabelled stannous phytate (particle size 200–1000 nm), which is commercially available in Japan, has been used, leading to an improved SLN identification rate (82–100 per cent compared with 67 per cent for $^{99m}$Tc-labelled human serum albumin and 36–63 per cent for $^{99m}$Tc-labelled tin colloid (M. Noguchi, unpublished data)).

The use of medium particle radioisotope tracers (particle size 200–1000 nm) is preferred because the SLN identification rate is relatively high and only one or two SLNs can be identified even the day after injection $^{28}$. This approach avoids the unnecessary removal of non-sentinel nodes.

**Optimal time for radioisotope injection**

The radioisotope tracer flows to and concentrates in the SLN, creating a ‘hot spot’ compared with the surrounding tissues. The time of the injection is not critical, however, as long as there is adequate time to allow the radioisotope to migrate into the SLN. A reasonable window for harvesting SLNs is 2–24 h from the time of radioisotope injection $^{28,36-41}$.

Lymphoscintigraphic studies have shown no axillary uptake in 16–79 per cent of patients 2–0–2–5 h after injection $^{16,38}$, suggesting that radioisotopes should be injected at least 2–4 h before surgery to ensure that all potential SLNs will be detected by the $\gamma$ probe. However, Schneebaum et al. $^{37}$ demonstrated SLN identification by lymphoscintigraphy 24 h after injection of 60 MBq $^{99m}$Tc-radiolabelled rhenium colloid (particle size 50–100 nm). These images were identical to those seen after 2 h. Other authors $^{4,28,29,36,39,41}$ have also reported that the SLN could be identified the day after injection and so it is possible to carry out the SLN biopsy then. This is convenient for surgeons with a tight operating room schedule $^{41}$.

**Dose and volume of radioisotope tracer**

The doses have ranged from 7 to 370 MBq $^{12}$. Krag et al. $^{10,42}$ recommend a standardized activity of 37 MBq, although they initially used a dose of 15 MBq $^{1}$. They suggest that using 37 MBq (1 mCi) results in a technically easier procedure, since counts are higher and the SLN is easier to detect with a $\gamma$ probe $^{10,42}$. However, a dose of 11 MBq (0·3 mCi) was found to be equally effective for most patients receiving a peritumoural isotope injection in another study $^{43}$. In other studies $^{29,44}$ the SLN could be identified with the $\gamma$ probe even 24 h after the injection of 7 MBq (0·2 mCi) of $^{99m}$Tc-radiolabelled colloidal albumin. A smaller radioisotope dose is advantageous because the ‘shine through’ from the high level of radioactivity at the tumour site may obscure a ‘hot spot’ in either the axilla or the internal mammary node $^{45}$, while unnecessary radiation exposure of the surgeon and hospital staff is avoided.

Another variable is the volume of fluid containing the radioisotope that should be injected. Volumes have ranged from 0·2 to 8 ml, a difference of a factor of 40 $^{12}$. 

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Investigators who use a small volume argue that they want to avoid disturbing the physiology of lymphatic flow and the risk of visualizing non-SLNs. Moreover, it has been suggested that injection with a volume of 0·1–0·3 ml reduces the ‘shine through’ effect. Because the lymphatic channels are much richer at the subdermal level than at the peritumoural site, a smaller volume of tracer might be adequate to identify the SLN in the case of subdermal or subareolar injection. On the other hand, investigators using larger volumes actually want to change the physiology by increasing the lymphatic flow to enhance visualization of the SLN. Since large particles migrate poorly within the lymphatics, larger volumes of injection (4–8 ml) facilitate lymphatic uptake via maximal distension of the anchoring filaments, and increase the identification success rate for axillary as well as extra-axillary SLNs. Krag et al. reported a higher identification rate when the volume was increased from less than 3 ml to more than 8 ml for peritumoural injection. A larger volume may well be necessary for peritumoural injection, but this study was not performed in a randomized fashion, and results are likely to improve with learning through experience no matter what technique is used. Although tracer volume remains controversial, detection rates seem to be good with either smaller or larger volumes of tracer fluid. A disadvantage of a large volume, however, is an increase in the diffusion zone at the injection site, which hampers lymphoscintigraphy and probe detection of nearby nodes.

Injection site of dye and radioisotope tracer: peritumoural, subdermal or subareolar

Techniques for the administration of dye and/or radioisotope in breast cancer include intratumoural, peritumoural (parenchymal) and subcutaneous (intradermal, subdermal or subareolar) injection. The optimal injection site of dye or radioisotope tracer for localizing the SLN remains a matter of debate. The goal of SLN biopsy is to identify the node draining directly from the tumour, it makes sense to inject the tracer into the lesion, but there is a potential danger of needle tract seeding by using the intratumoural method. Most of the earlier studies of SLN biopsy for breast cancer involved injection directly into the parenchyma of the breast around the tumour. However, this procedure now appears to be associated with technical problems of inconsistent preoperative or intraoperative SLN identification because of the significant ‘shine effect’ from the peritumoural injection site, especially in the upper outer quadrant. The challenge, therefore, has been to improve the injection site to maximize the identification of the SLN and minimize the ‘shine effect’. Several studies have been performed in which the blue dye or radioisotope tracer was injected either under or into the skin overlying the tumour or into the subareolar tissue.

The subdermal or intradermal approach is based on the idea that the skin overlying the breast parenchyma has the same embryological origin as the underlying tissue and so should share the same lymphatic drainage pattern. Borgstein et al. used intradermal blue dye and radioisotope injection around the lesion to demonstrate complete concordance between the lymphatic drainage of a breast tumour and its overlying skin. Veronesi et al. and Roumen et al. also reported that SLN localization by dermally injected radioisotope can predict axillary nodal status as accurately as can peritumoural injection. Similarly, Linehan et al. showed that the dermal and parenchymal lymphatics of the breast drain into the same SLN in most patients. In their study, SLN localization was successful in 78 per cent with peritumoural injection and 97 per cent with intradermal injection (P < 0·001). They therefore suggested that intradermal injection may simplify and optimize SLN localization because it is easier to perform and more effective. Nevertheless, a warning has been issued that injecting the tracer further away from the tumour increases the risk that a lymphatic watershed is crossed and that the node that is visualized is not the node that drains the tumour (regional mismatch).

The subareolar approach is based on studies indicating that this area provides a central access route to the peripheral lymphatic pathways. In a study by Klimberg et al., subareolar radioisotope injection produced results as accurate as those obtained with peritumoural injection of blue dye. Of the 69 lesions they examined, SLNs were located in 62 (90 per cent) with the blue dye and in 65 (94 per cent) with the radioisotope. All blue SLNs were also radioactive, but the SLN could not be located with either method in the remaining four patients. These findings seem to indicate that there are clear advantages to subareolar injection for SLN localization. It avoids the need for image-guided injection in instances of non-palpable lesions, and of overlap of the diffusion zone in lesions in the upper outer quadrant with that of the SLN in the axilla, and in medial lesions with internal mammary lymph nodes. Subareolar injection may also allow the use of the SLN biopsy technique in patients with multicentric breast cancer.

There is undoubtedly communication between breast and dermal lymphatics, and the lymphatic channels are much richer at the subcutaneous level than at the peritumoural site. Administration of a radioisotope tracer at subdermal or subareolar sites is supposed to enhance the SLN identification rate; subdermal injection over the primary tumour and subareolar injection are becoming...
increasingly popular techniques. However, it remains unclear whether the subdermal, subareolar and parenchymal lymphatics of the breast always drain into the same nodes and which route best simulates the spread of breast cancer.\(^\text{1,12,48,56-59}\). Roumen et al.\(^\text{58}\) pointed out that peritumoural and intradermal injections produce a small number of sequential mismatches for ‘hot spots’ in the axillary region. In particular, a so-called ‘regional mismatch’ was seen in nine (14 per cent) of 66 relating to the internal mammary nodes in their study.

It is important to realize that the intradermal injection route is apparently unable to detect a SLN in the parasternal/internal mammary region\(^\text{4,58,60,61}\), while internal mammary SLNs can be visualized after a peritumoural injection\(^\text{21,46,53}\). In a study by Roumen et al.\(^\text{53}\) parasternal drainage was visualized by means of peritumoural injection in nine (14 per cent) of 66 positive lymphoscintigraphy cases (in two patients the internal mammary chain was the only location). In another study by Roumen et al.\(^\text{58}\), however, internal mammary nodes visualized after peritumoural injection could not be visualized with the intradermal technique. Similarly, in a study by Leong et al.\(^\text{46}\) no internal mammary nodes were seen in any of 50 patients following intradermal injection, whereas six of 55 patients with peritumoural injections had internal mammary lymph nodes identified. Although subdermal and intradermal injections can help to visualize the superficial lymphatic system running towards the axilla, they do not do this for the deep lymphatic system running to the internal mammary, interpectoral or intramammary nodes.\(^\text{59}\).

Peritumoural injection and subdermal, intradermal or subareolar injection may complement each other, resulting in enhanced success rates. If internal mammary, interpectoral or intramammary lymph nodes are not targeted for SLN biopsy, the use of peritumoural injection of blue dye and either subdermal or subareolar injection of radioisotopes can maximize the identification of axillary SLNs.\(^\text{45}\) Intradermal or subareolar injection requires only a small radioisotope dose, which results in lower counts at the injection site and less interference from the ‘blast’ zone, thus allowing a more precise use of radiolocalization in the axilla.\(^\text{62}\) If internal mammary, interpectoral or intramammary lymph nodes are included as biopsy targets, however, the peritumoural injection of a radioisotope combined with the intradermal para-areolar injection of blue dye may be the best choice.\(^\text{28}\) In particular, peritumoural injection maximizes detection of internal mammary SLNs with radioisotope. This cannot be done with blue dye, although intradermal injection of blue dye is effective for identifying axillary SLNs. Further study is required to assess the optimal injection sites for blue dye and radioisotopes.

**Lymphoscintigraphy versus hand-held $\gamma$ probe**

Lymphoscintigraphy may help to identify lymphatic channels and lymph nodes, and to define unpredictable nodal draining basins. It is well established in lymphatic mapping for melanoma, where it identifies anomalous patterns of lymphatic drainage and so has a direct effect on the surgical approach. However, the efficacy of preoperative lymphoscintigraphy remains controversial in breast cancer. Its main advantage is that it allows accurate preoperative localization of the SLN and thereby minimizes the extent of dissection.\(^\text{21,36,39,53}\) Several investigators have been able to visualize SLNs in 75–98 per cent of patients by means of lymphoscintigraphy.\(^\text{4,21,38,39,53}\) In a study by Kollias et al.\(^\text{63}\) the SLN was identified in 113 (97 per cent) of 116 patients with positive lymphoscintigraphic findings, but in only 29 (55 per cent) of 53 patients with negative findings. Noguchi et al.\(^\text{35}\) used a $\gamma$ probe to identify the SLN successfully in all patients with a ‘hot spot’ visualized by lymphoscintigraphy. Thus, a positive lymphoscintigram is an important factor associated with successful intraoperative mapping, while a negative lymphoscintigram does not preclude successful radio-localization at surgery.\(^\text{16,35,64,65}\)

Recently, however, Burak et al.\(^\text{64}\) and McMasters et al.\(^\text{65}\) have demonstrated that preoperative lymphoscintigraphy offers no advantage with respect to SLN localization or false-negative rate compared with management without lymphoscintigraphy. With a hand-held $\gamma$ probe and using intraoperative mapping, axillary SLNs can be spotted in the operating room even though they cannot be visualized with lymphoscintigraphy.\(^\text{16,35,64,65}\) These investigators consider intraoperative scanning with the hand-held $\gamma$ probe to represent the essence of the isotope technique, but in their studies none of the tumours showed drainage to the internal mammary lymph node chain on lymphoscintigraphy.

Probably, then, lymphoscintigraphy is most useful in delineating the lymphatic drainage pattern to internal mammary lymph nodes, allowing appropriate clinical decisions to be made. Although blue dye does not allow visualization of the internal mammary lymph nodes, a SLN may be visualized in the internal mammary chain when preoperative lymphoscintigraphy is performed.\(^\text{16,38,53}\) Noguchi et al.\(^\text{35}\) found that the internal mammary SLN could be visualized by means of lymphoscintigraphy, but not with the $\gamma$ detection probe. These nodes might, therefore, be overlooked if not detected by lymphoscintigraphy.\(^\text{48}\) Arguments have been made in favour of lymphoscintigraphy providing a ‘road map’ for surgeons. So it can be said that most studies suggest that preoperative lymphoscintigraphy yields valuable information on the...
The anatomical site of the SLN and the level at which it is located; in some rare instances it will also show other areas of lymphatic drainage, such as the internal mammary chain. The author’s preference for SLN identification involves a triple technique, comprising preoperative lymphoscintigraphy combined with \( \gamma \) probe-guided and dye-guided methods.

Relevance and identification of internal mammary sentinel lymph nodes

The SLN is most commonly located in the axilla, but it may also be located in the internal mammary chain or elsewhere. The clinical relevance of internal mammary SLN biopsy is a matter of debate. Some investigators do not believe that a lack of internal mammary node imaging is of clinical relevance, because internal mammary dissection has largely been abandoned, but others believe that internal mammary SLNs are clinically relevant because they have the same prognostic significance as axillary nodal metastases. Internal mammary lymph node metastases occur in 7–15 per cent of axillary node-positive patients, and this may have important implications for decision making about internal mammary lymph node dissection. Noguchi et al. reported that the dissection of blue-stained lymphatic vessels and nodes in the internal mammary chain could be difficult after resection of the primary tumour because the blue dye travels rapidly through the lymphatic vessels, and may not remain in the internal mammary lymph nodes long enough for surgical identification and excision.

Internal mammary SLNs can be identified by lymphoscintigraphy and/or the \( \gamma \) probe-guided method. Using lymphoscintigraphy, Uren et al. reported unexpected drainage across the centre line of the breast to either the axillary lymph nodes or internal mammary lymph nodes in 32 per cent of patients with inner or outer quadrant lesions, with nine of the 34 patients having a SLN in the internal mammary chain. Other authors have also used lymphoscintigraphy to identify internal mammary SLNs. A hand-held \( \gamma \) probe, on the other hand, has enabled several investigators to find and excise internal mammary SLNs. Harlow et al. found extra-axillary ‘hot spots’ in 44 (6 per cent) of 680 patients. These extra-axillary SLNs were located in the internal mammary lymph nodes in 34 of the 44 patients, while extra-axillary involvement was confirmed histologically in only three of them.

Internal mammary SLN biopsy, therefore, seems to be relevant and a small but increasing number of institutions remove internal mammary SLNs for analysis. However, identification of internal mammary SLNs is still in the experimental stage. The metastatic rate of internal mammary SLNs reported in some studies is still lower than expected. Further study is thus required to assess the role of SLN biopsy of the internal mammary nodes. As described previously, the peritumoural injection of a radioisotope combined with the intradermal para-areolar injection of blue dye would be indicated. It has been recommended that surgeons in the process of learning internal mammary SLN biopsy should also perform a back-up internal mammary lymph node biopsy. A biopsy of the first and second intercostal spaces would provide accurate information on internal mammary nodal status. A technique of internal mammary lymph node biopsy has been described in detail elsewhere.

Diagnosis of sentinel lymph node metastases

Histological examination: step sectioning with haematoxylin and eosin staining combined with immunohistochemical staining

The gold standard for the assessment of axillary lymph nodes in patients with breast cancer is represented by haematoxylin and eosin (HE) staining of paraffin sections. However, the likelihood of detection of micrometastases by routine histological examination of the SLN is limited. To avoid false-negative evaluations, step sectioning with the addition of immunohistochemical (IHC) staining makes a more intensive pathological examination feasible. With these techniques, SLN biopsy allows the pathologist to focus on a small number of lymph nodes most likely to contain tumours.
The incidence of micrometastases detected by serial or step sections and cytokeratin IHC staining is reported to be approximately 20 per cent in dissected axillary lymph node specimens considered tumour negative at standard pathological examination. Giuliano et al.10 reported that 26 of 68 tumour-involved SLNs had micrometastases only, 11 of which were identified by IHC staining for cytokeratin, but not by HE examination. They also clearly demonstrated the efficacy of enhanced pathological examination of the SLN in breast cancer. Their yield of positive axillary nodes increased from 29 per cent (in 134 patients who underwent ALND with conventional pathology) to 42 per cent (in 162 patients of comparable stage who underwent SLN biopsy with enhanced pathology). There is, therefore, no question that the influence of upstaging by Rogers effect.

However, the optimal number of HE and immunohistochemically stained sections to be examined in practice has not yet been determined. Controversy remains as to the minimal amount of histopathological evidence that needs to be obtained from the SLN. Turner et al.82 have recommended obtaining permanent sections from at least two levels of the tissue block at 0.04-mm intervals for HE staining and cytokeratin IHC staining. This represents eight sections of a dissected lymph node. The additional step sectioning with IHC staining did not significantly increase the number of patients identified with tumour-positive SLNs in their study. However, Jannink et al.81 considered that serial sectioning at 0.5-mm intervals with HE and IHC staining is appropriate and cost effective; in this series, the average number of SLNs examined per case was 2-6 and the average number of sections per node was 14, resulting in 36 sections (2.6 × 14) per case for review.

In a study by Dowlatshahi et al.86, on the other hand, tumour metastases were found in only six patients (12 per cent) when the SLNs were sectioned at 2-mm intervals and stained with HE, compared with 30 patients (58 per cent) when the same lymph nodes were serially sectioned at 0.25-mm intervals and stained with cytokeratin. Of 24 patients whose metastases were detected by means of serial sectioning and cytokeratin staining, 12 had isolated tumour cells and 12 had colonies of malignant cells. The authors suggested that most significant metastases will be found with cytokeratin staining at intervals of 0.25 mm thickness. Because the average maximum dimension of a malignant ductal cell is 0.02 mm, a colony of over 20–30 cells is likely to be detected on sections made at these intervals. This should be proposed as a minimum standard. To section the SLN in this fashion, however, requires 40 sections for an SLN measuring 1 cm in diameter.

Currently, an increasing number of investigators performing SLN biopsy for breast cancer rely on serial sectioning of the SLN, with both HE and IHC staining of each section. However, the large number of sections cut, sectioned and reviewed for SLN requires greater involvement of the technologist and the pathologist, and IHC staining is more expensive than HE staining. In a study by Noguchi et al.33 the addition of IHC staining did not significantly improve the detection of axillary lymph node metastases. Further work is therefore needed to enable a consensus to be reached on the most sensitive and cost-effective technique for pathological examination of the SLN for the staging of breast carcinoma.

**Intraoperative examination using frozen section and/or imprint cytology**

Immediate and reliable intraoperative information regarding the status of the SLN would be useful as it would enable a decision to be made whether or not to perform complete ALND at the time of the initial operation. Several investigators have examined the value of frozen section and imprint cytology for the intraoperative assessment of SLNs.

**Haematoxylin and eosin staining of frozen section**

The accuracy of SLN diagnosis using frozen section has been questioned in several studies. The sensitivity range for frozen-section examination of the SLN is only 64–74 per cent. Veronesi et al.4 reported a sensitivity of 64 per cent, with a false-negative intraoperative diagnosis in 17 per cent of cases. In a large series, the same authors reported a false-negative rate of 32 per cent for frozen-section evaluation of the SLN, which makes the approach inappropriate for deciding whether or not to perform a complete ALND.

Recently, however, the reliability of intraoperative examination of SLN using frozen sections has improved. Viale et al.89 sectioned frozen SLNs subserially at 0.05-mm intervals. For each SLN, one section was stained with HE and the other was immunostained for cytokeratins using a rapid IHC assay, resulting in an overall accuracy of 97 per cent, with a sensitivity of 93 per cent and a specificity of 100 per cent. It was concluded that extensive intraoperative examination of SLNs by frozen-section examination could attain a sensitivity comparable to that obtained by routine histological examination without intraoperative frozen section.

**Cytological examination (imprint cytology)**

Examination by imprint cytology may be useful for determining the status of the SLNs during surgery, because
multiple cut surfaces can be examined quickly with this technique\textsuperscript{87,90}. It would be of interest to evaluate whether imprint cytology can be used as an alternative technique when it is not possible to use frozen section, or whether it has enhancement value when combined with frozen section\textsuperscript{79,91}.

Van Diest et al.\textsuperscript{91} halved SLNs less than 10 mm in diameter and laminated SLNs of 10 mm or more in diameter into pieces 5 mm in size. Imprints were then made of all cut surfaces and stained. This resulted in a sensitivity of 62 per cent and a specificity of 100 per cent. The diagnostic accuracy of imprint cytology of SLNs did not exceed that of frozen-section examination in this study. Ratanawichitrasin et al.\textsuperscript{92}, however, divided SLNs in two if the diameter was less than 8 mm, or cut them into multiple sections 3 mm thick if the diameter was greater than 8 mm. They reported a sensitivity of 82 per cent and a specificity of 100 per cent for determining the status of the SLN by imprint cytology. Motomura et al.\textsuperscript{93} serially sectioned SLNs at 2-mm intervals if they were 4 mm or greater in diameter, or bisected if they were smaller than 4 mm. They reported a sensitivity of 91 per cent and a specificity of 99 per cent, although the results of imprint cytology were compared only with those of IHC examination of SLNs. However, imprint cytology requires an experienced surgical cytopathologist to evaluate the specimen and interpret the findings. In most institutions, therefore, imprint cytology of SLNs may not be suitable as a final staging procedure and must be complemented by histology\textsuperscript{94}. Evidence to date suggests that intraoperative cytological examination should be employed in conjunction with frozen-section examination.

The accuracy of SLN diagnosis by frozen section\textsuperscript{35,89} as well as by imprint cytology\textsuperscript{90,92,93} improves with an increase in the number of sections. This allows the 25–30 per cent of patients who have positive nodes to undergo a completion ALND under the same anaesthetic and thus avoid a second procedure\textsuperscript{95}. However, it is highly likely that even such intraoperative examination of frozen sections and/or imprint cytology would fail to detect micrometastases consisting of a single cell or a small group of malignant cells in SLNs. An ongoing randomized prospective trial\textsuperscript{96} will answer the question whether residual disease will increase axillary relapse.

**Reverse transcriptase–polymerase chain reaction analysis**

Reverse transcriptase polymerase chain reaction (RT–PCR) analysis is receiving increasing attention as an ultrasensitive technique for the detection of occult metastases\textsuperscript{97}. Recently, some investigators have examined SLNs by using RT–PCR for carcinoembryonic antigen (CEA) and mammoglobin analysis, and have reported an improvement in the sensitivity of SLN biopsy\textsuperscript{98}. Kataoka et al.\textsuperscript{98} found CEA to be expressed in 25 per cent of 48 patients with HE-negative SLNs, and mammoglobin expressed in 21 per cent. While HE staining of SLNs could predict axillary lymph node status with an accuracy of 95 per cent and a false-negative rate of 6 per cent, RT–PCR improved these to 99 and 3 per cent respectively. They concluded that SLN diagnosis using RT–PCR is a powerful and sensitive method.

Unlike melanoma, for which the tyrosinase marker has clearly been identified as specific and reliable for the detection of micrometastases, there is as yet no clearly defined RT–PCR marker or set of markers for breast cancer\textsuperscript{99}. Moreover, the prognostic significance of micrometastases detected with this new technique and of tumour markers has not been determined\textsuperscript{99,100}. The usefulness of this technique, which can detect one malignant cell in one million lymphocytes\textsuperscript{96}, two orders of magnitude greater than that obtained with HE staining, requires further evaluation because the test may be too sensitive to be clinically useful\textsuperscript{79,81,101}. It seems unlikely that the majority of such malignant cells would escape immune surveillance and acquire the traits necessary to become clinically important. The RT–PCR method, therefore, has the potential to improve the sensitivity of SLN biopsy, but requires further study\textsuperscript{102}.

**Significance of micrometastasis in sentinel lymph nodes**

The clinical significance of micrometastatic disease has remained controversial. Some authors question the significance of these metastatic deposits, but most series (especially those with longer follow-up) indicate that the presence of these tiny metastases has implications for disease-free and overall survival\textsuperscript{71,103}. In a review of the literature, Dowlatshahi et al.\textsuperscript{71} found that all eight studies, using serial sectioning and HE and/or IHC staining for more than 100 patients and published after 1982, demonstrated an association between micrometastases and poor outcome.

Nevertheless, it is unlikely that all tumour cells detected in lymph nodes are clinically significant. Although the distinction between micrometastases (smaller than 2 mm) and macrometastases (larger than 2 mm) in axillary nodes is a quantitative rather than a qualitative difference, the survival of patients depends more on the quantity of lymph node metastases than on their quality. For example, prognosis is directly related to the number of positive axillary lymph nodes. It has been suggested that colonies of
cells are clinically significant and that individual cells generally are not so that subclassification of micrometastasis in breast cancer may be required.

On the other hand, an overview study has shown that 38-67 per cent of patients with breast cancer and positive SLNs have no disease in other (non-sentinel) nodes. This finding not only strongly supports the SLN concept, but also suggests that ALND can be avoided in such patients. Several investigators have sought to identify factors that might predict which patients with positive SLNs are at a low risk of having disease-harbouring non-sentinel nodes. They found that tumour cells are unlikely to be found in the latter if the primary breast tumour is small and SLN involvement is micrometastatic.

So it may be possible to treat patients with T1 tumours and micrometastasis in the SLN with SLN biopsy only, making full ALND unnecessary for these patients. In other words ALND can be avoided in these patients, even assuming that histological examination of the SLN may fail to detect micrometastases. It is, therefore, more important to diagnose a colony of malignant cells in the SLN rather than a cell or a small group (less than ten) of malignant cells in a lymph node.

**Elimination of axillary lymph node dissection as a result of sentinel lymph node biopsy**

The growing demand from patients for less invasive procedures has created considerable incentives for accepting SLN biopsy without ALND as standard practice. In a trial sponsored by the American College of Surgeons, each institution’s principal investigator is required to document a 90 per cent accuracy rate and a 90 per cent staging accuracy in at least 30 consecutive cases of SLN biopsy followed by complete ALND. When planning the elimination of ALND as a result of SLN biopsy in clinical practice, however, one must estimate the actual risks and consequences of allowing residual disease to remain in the axilla. Several studies have demonstrated that SLN biopsy is highly accurate and sensitive in patients with small tumours, and there have been no reports of false-negative SLN biopsy for breast cancer less than 1-0-1-5 cm in size. The high accuracy of SLN biopsy for patients with small tumours is in part due to the low probability of axillary metastases. It is, therefore, reasonable in actual practice to offer the option of no ALND to patients with small breast cancers (tumour size smaller than 1-5 cm or T1) and a negative SLN. This strategy is supported by findings, already described, that tumour cells are unlikely to occur in non-sentinel nodes if the primary breast tumour is small and SLN involvement is micrometastatic. SLN biopsy without ALND may be also applied to cases of extensive and/or high-grade (comedo subtype) duct carcinoma in situ.

However, recent studies have demonstrated that SLN biopsy is as accurate for the assessment of the axilla in patients with T1 tumours as it is for those with T2 tumours. Bedrosian et al. reported that SLN biopsy can be applied to large tumours (larger than 2 cm but smaller than 5 cm) with a 99 per cent SLN identification rate and a false-negative rate of 3 per cent. Of 30 SLN-positive patients with tumours larger than 3 cm, only three of eight patients with micrometastasis (less than 2 mm) to the SLN had positive non-sentinel nodes compared with 21 of 22 with macrometastasis (larger than 2 mm). This excellent result was achieved by extensive histological examination with IHC staining. A false-negative rate of no more than 5 per cent seems reasonable for performing SLN biopsy without ALND.

Because histological examination of the SLN still has its limitations, it is highly unlikely that all false-negative cases can be eliminated. In particular, intraoperative diagnosis by either frozen-section examination or imprint cytology is unlikely to identify micrometastases. Moreover, it is not clear whether the residual nodal disease, particularly in patients with larger invasive tumours (larger than 2 cm but smaller than 5 cm) undergoing SLN biopsy without complete ALND, will significantly increase the axillary lymph node recurrence rate. This risk is all the more a matter of concern because of the therapeutic benefits of axillary radiation therapy and systemic chemotherapy that may eradicate most micrometastases and some macrometastases. These important questions can only be answered by a randomized prospective trial. Such a trial addressing these issues is being conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP B-32). SLN biopsy alone will be compared with SLN biopsy followed by ALND.

Currently, several surgeons on the basis of their experience with SLN biopsy have already abandoned routine ALND for patients with T1,2N0 disease and a negative SLN biopsy result. However, subsequent relapse in the regional nodal basin should be similar to that after a conventional ALND (less than 1 per cent), while it has not yet been reported in the published literature. In practice, ALND can be avoided in patients with small tumours (T1) who have a negative SLN. However, elimination of ALND in those with large tumours (T2) who have a negative SLN should be considered only by surgeons highly experienced in SLN biopsy who have access to a pathologist experienced in performing an intensive histological examination of SLNs. In most institutions, therefore, it is recommended that SLN biopsy with back-up...
ALND should be used for most T2 cancers until the results of the aforementioned clinical trial are available.

**Conclusion**

1 Although the SLN can be identified successfully by experienced practitioners using either the dye-guided or γ probe-guided method, identification is facilitated when both techniques are used together.

2 The use of medium particle radioisotope tracers (particle size 200–1000 nm) is preferable, because the SLN identification rate is relatively high and only one or two SLNs can be identified even the day after injection.

3 A smaller radioisotope dose (0.2–0.3 mCi) is more effective because it reduces the ‘shine through’ effect from the high level of radioactivity at the tumour site. On the other hand, while a smaller volume of tracer might be adequate to identify SLNs when administered with subdermal or subareolar injection, a larger volume may be necessary for peritumoural injection.

4 The subdermal or subareolar injection technique has attractive practical features, but there is currently insufficient evidence that drainage of tracer injected anywhere in or underneath the skin of the breast reflects drainage from the cancer. Peritumoural injection and subdermal, intradermal or subareolar injection may complement each other, thereby increasing success rates. If internal mammary lymph nodes are targeted for SLN biopsy, peritumoural injection maximizes detection with radioisotopes of internal mammary nodes.

5 Preoperative lymphoscintigraphy yields valuable information on the anatomical site of the SLN and the level at which it is located; in some rare instances it will also show other areas of lymphatic drainage such as the internal mammary chain.

6 Identification of internal mammary SLNs remains controversial. Further study is required to assess the role of SLN biopsy of the internal mammary nodes. It is recommended that surgeons who are learning internal mammary SLN biopsy should perform a back-up internal mammary lymph node biopsy.

7 To increase the chance of finding metastases in the SLN, step sectioning combined with HE and IHC staining of permanent sections is desirable. However, further studies are needed to reach a consensus on the most sensitive and cost-effective technique for pathological examination of the SLN for the staging of breast carcinoma.

8 Extensive intraoperative examination of SLNs using frozen section and imprint cytology could attain a sensitivity similar to that obtained by histological examination of permanent sections. However, imprint cytology requires an experienced surgical cytopathologist to evaluate the specimen and interpret the findings.

9 It is highly unlikely that all false-negative cases can be eliminated even by detailed histological and cytological examination of the SLN. Nevertheless, patients with T1 tumours with micrometastases in the SLN have shown no evidence of tumour in the non-sentinel nodes. In other words, ALND can be avoided for these patients, even assuming that the histological examination of the SLN may fail to detect micrometastases.

10 The role of RT–PCR for SLN analysis in breast cancer remains under investigation; it may be too sensitive to be clinically useful.

11 In practice, routine ALND can be avoided in patients with T1 tumours when the identified SLN proves to be histologically negative. However, investigation of long-term regional controls and of survival in a prospective randomized trial is necessary before SLN biopsy can be expected to replace routine ALND, particularly for patients with T2 tumours.

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