ORIGINAL ARTICLE

Sentinel lymph node biopsy in breast cancer patients after overnight migration of radiolabelled sulphur colloid

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Objective: To evaluate the performance and feasibility of sentinel lymph node biopsy in breast cancer patients using technetium-99m (^{99m}Tc) sulphur colloid and gamma probe.
Methods: From May 2000 to March 2001, 70 patients with a tumour less than 5 cm with clinically negative axillary lymph nodes underwent sentinel node biopsy followed by standard axillary dissection. ^{99m}Tc sulphur colloid was injected around the primary tumour the day before surgery and a gamma probe was used to detect the sentinel lymph node during the surgical procedure. Sentinel lymph node biopsy was compared with standard axillary dissection for its ability to accurately reflect the final pathological status of the axillary nodes.
Results: The sentinel lymph node was successfully identified in 67 of 70 patients (95.71%). The number of sentinel lymph nodes ranged from 1–5 (mean 1.5) and non-sentinel nodes ranged from 5–22 (mean 13.3). Of the 67 patients with successfully identified sentinel lymph nodes, 43.28% (29/67) were histologically positive. Sensitivity of the sentinel lymph node to predict axilla was 82.75%; specificity was 100%. Positive and negative predictive values were 100% and 88.3% respectively. The sentinel lymph node was falsely negative in five patients, yielding an accuracy of 92.53%. Sentinel lymph node biopsy was more accurate for T1 tumours than for T2 tumours.
Conclusions: The gamma probe guided method after overnight migration of ^{99m}Tc sulphur colloid is technically feasible for detecting sentinel lymph nodes in most breast cancer patients, accurately predicting the axillary lymph node status, and appears more accurate for T1 lesions than for larger lesions. This minimally invasive axillary staging procedure represents a major advance in the surgical treatment of breast cancer.

The purpose of axillary node dissection in breast cancer patients is to achieve information of disease stage, obtain local control, and assist in the decision making for adjuvant therapy. However, for those patients with pathologically negative nodes, dissection does not increase survival.[1] About 60%–70% of patients with early breast cancer have no metastasis to the regional lymphatic basin,[2 3] so axillary dissection and consequent morbidity can be avoided if there is an accurate alternative for axillary node staging. Sentinel lymph node biopsy may provide physicians with the ability to distinguish negative from positive axillary lymph nodes in a relatively simple fashion.

In 1992, Morton *et al* reported this technique in patients with clinically node negative melanoma.[4] This technique has now been defined for breast carcinoma by Krag *et al*[5] and Giuliano *et al*[6] as a rational and accurate approach to the study of the regional lymphatic basin. Sentinel lymph nodes are physiologically determined as entrance lymph nodes to the lymph node basin and drain a particular tumour and, if negative, predict the status of the remaining axillary nodes.[7 8] During the past eight years, sentinel lymph node biopsy in breast cancer patients has become an exciting research topic. Results from international breast cancer centres show that, with the use of optimal techniques, the accuracy of sentinel lymph node biopsy to predict axillary nodal status is 95% or more. Using radiolabelled sulphur colloid as a tracer, after its overnight migration, we performed sentinel lymph node biopsy in patients with breast cancer and evaluated its feasibility for Chinese patients.

PATIENTS AND METHODS

Patients

Seventy breast cancer patients with clinical TNM status T1–2, N0 admitted to our hospital were included in a prospective study performed between May 2000 and March 2001. Both mastectomy and breast conservation patients were equally eligible. Informed consent for injection of radiotracer around their breast mass and surgical procedure to be performed was taken from each patient undergoing the study. The institutional ethical review board at Fudan University Medical Center had accepted the procedure. The age of the patients ranged from 30 to 86 years with a mean age of 50.9 years; 38.67% were premenopausal and 61.4% were postmenopausal. All patients underwent sentinel lymph node biopsy and consecutive axillary dissection with the preplanned mode of surgery.

Methods

Ten to 16 hours before the operation, 1–2 mCi (1 mCi = 37 MBq) of unfiltered technetium-99m (^{99m}Tc) sulphur colloid (prepared by the department of nuclear medicine, Fudan University Cancer Hospital) in a volume of 3–5 ml was injected in divided aliquots into the breast tissue surrounding the primary tumour or biopsy site at the 3, 6, 9, and 12 o'clock positions. Injections were made at 1–3 mm surrounding the tumour or biopsy cavity. Breasts soft in consistency and bigger in size received the larger volume of injection. Among 70 cases, only a single patient presented with itching and erythema of skin at the injection site, which subsided after antihistaminic administration. A hand held gamma probe (Capintec Gammed IV, Capintec Inc, New Jersey, USA) was used to detect the distribution of radioactivity around the breast and axilla before the operation. Hot spots were detected and the skin marked accordingly. A hot spot was defined as the spot that had the greatest radioactivity counts

Abbreviations: H&E, haematoxylin and eosin; KP-1, *Klebsiella pneumoniae*; ^{99m}Tc, technetium-99m

in the lymphatic basin, which was at least 25 counts per 10 seconds or greater. After resection, the activity of the resection bed should be less than 10% of the most radioactive node.[7 8] In 63 patients, static lymphoscintigraphy was performed before operation to ascertain the overall distribution of the radiotracer. Skin marks were made over the hot spot to facilitate the search during the operation.

For sentinel node biopsy, a 4–5 cm skin incision was made over or near the hot spot, and a hand held gamma probe was used to guide the further dissection until the hot node resection was complete. In 27 cases, sentinel node biopsy was performed after the dissection of skin flaps. In the four most preliminary cases, the sentinel node was dissected from the specimen after radical mastectomy. A standard axillary dissection was then performed. When the sentinel lymph node could not be tracked even in the dissected axillary specimen, the procedure was considered to be a failure.

Pathological examination

Excisional biopsy of the lesion was performed and frozen sections taken for the confirmation of cancer. The sentinel node biopsy specimens were identified as SLN1, SLN2, etc and were separately infiltrated into a 10% solution of formalin before delivery. All lymph nodes in the specimen were identified and dissected from the surrounding tissue by the pathologist. The nodes were then bisected and embedded for sectioning. One or two sections obtained from the central cross section from each block were then stained with routine haematoxylin and eosin (H&E) for the detection of cancer cells.

In 63 sentinel lymph nodes of 43 cases which showed no cancer cells by conventional pathological method, further evaluation was performed by using immunohistochemical stains for cytokeratin (antibodies for CK8 and CK19, DAKO products).[9] Three sections were taken of the paraffin block, two stained for cytokeratin and one with H&E. Staining was performed in the department of pathology, Fudan University Cancer Hospital. A positive finding was defined as a cluster of cells in brown staining in its cell membrane or plasma which had to be further verified in H&E staining of the same level. Scattered anaplastic cells in the subcapsular region of lymph node which was positive for cytokeratin antibody were evaluated with immunohistochemical stains for *Klebsiella pneumoniae* (KP-1) (CD-68) to exclude histiocytes.[10]

Statistical analysis

False negative rate was defined as the percentage of patients who had histologically negative sentinel lymph nodes but other nodes positive. Accuracy was defined as the percentage of patients in which the sentinel node status has accurately represented the lymph node status of patient. Fisher's exact test and χ^2 tests were used to analyse the impact of different factors to this method.

RESULTS

The clinical data of patients and pathological characteristics of the tumour are detailed in tables 1 and 2. Among the 63 patients who underwent lymphoscintigraphy, all had lymphatic drainage towards the axilla, including 12% who had simultaneous internal mammary drainage.

The sentinel lymph node was successfully detected in 67 of the 70 patients—that is, a detection rate of 95.72%. In three cases that were failures in detecting the sentinel lymph node, including one case in which the pathologist could not identify a lymph node-like structure in the surgically sent specimen. These three patients were 58, 62, and 60 years old with a tumour size of 2, 3, and 2.3 cm respectively.

In 67 successful cases, the number of nodes detected ranged from 1–5. A total of 101 sentinel nodes were retrieved and the mean number of sentinel lymph nodes per case was 1.5. The number of dissected non-sentinel nodes ranged from 5–22, a total of 925 nodes, with a mean of 13.8 nodes per case. Except for one case in whom the sentinel lymph node was in the third intercostal space, all the other sentinel nodes were located at level I of the axilla.

A total of 29 (43.3%) patients had cancer cells in their lymph nodes, with sentinel nodes positive in 24 cases

Table 1 Clinical data of patients (n = 70)

Characteristic	No (%)
Age (years)	
Range	30–86
Mean (SD)	50.9 (10.8)
Menopausal status	
Premenopausal	27 (38.6)
Postmenopausal	43 (61.4)
Location of primary tumour	
Lateral quadrants	48 (68.4)
Lateral superior	38 (54.3)
Lateral inferior	10 (14.1)
Medial quadrants	20 (28.6)
Medial superior	13 (18.6)
Medial inferior	7 (10.0)
Subareolar	2 (3.0)
Surgery performed	
Modified radical mastectomy	51 (72.9)
Radical mastectomy	11 (15.7)
Breast conserving surgery	8 (11.4)

Table 2 Pathological features of the tumour (n = 70)

Characteristic	No (%)
Size (cm)	
⩽2	26 (37.1)
2–3	24 (34.3)
>3	11 (15.7)
After excision	9 (12.9)
Pathological subtype	
Infiltrating ductal	58 (82.9)
DCIS with early invasion	4 (5.7)
Infiltrating lobular	3 (4.3)
Mucinous	2 (2.9)
Medullary	2 (2.9)
Paget's disease + IDC	1 (1.4)
Oestrogen receptor	
Positive	41 (65.1)
Negative	22 (34.9)
Progesterone receptor	
Positive	38 (60.3)
Negative	25 (39.7)
Her2/neu	
Positive	38 (60.3)
Negative	25 (39.7)

Data missing in seven patients after excisional biopsy.
DCIS, ductal carcinoma in situ; Her2/neu, human epidermal growth factor receptor 2; IDC, infiltrating ductal carcinoma.

Table 3 Summary of sentinel node biopsy

Parameters	No (%)
Patients with lymph node metastasis	29 (43.28
Patients without nodal metastasis	38 (56.71)
Patients with positive SLN	24 (35.82)
Patients with false negative SLN	5 (7.46)
Patients with SLN and ALN concordance	62 (92.53)
Patients with only SLN metastasis	7 (10.44)

SLN, sentinel lymph node; ALN, axillary lymph node.

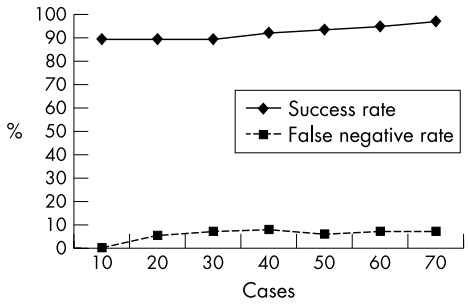

Figure 1 Learning curves for the 70 patients enrolled in the study. They were divided into seven groups with 10 patients in each group. Success rate and false negative rate were calculated for each increment of 10 patients in a cumulative fashion that yielded this curve.

(sensitivity 82.75%). In the remaining 38 patients, the sentinel and non-sentinel nodes were all negative (specificity 100%). Sentinel node biopsy missed metastatic lymph nodes in five patients (false negative rate 7.46%). The positive predictive value was 100%, negative predictive value was 88.37%, with an overall accuracy of 92.53% (see table 3).

Immunohistochemistry for the cytokeratins CK8 and CK19 in 65 H&E staining negative sentinel lymph nodes showed a few single anaplastic cells in five lymph nodes that were considered to be a histiocytes (non-cancer cells) by the pathologist; this was reconfirmed because these cells also stained positive for KP-1.

When the tumour size was stratified, the accuracy was 100% with no false negatives in 25 cases with a tumour size less than 2 cm. In nine cases the patients had undergone excision or incision biopsy; there was also no failure or false negative result. In seven cases the sentinel node was the only positive node, and of these there were four patients who had ductal carcinoma in situ with early invasion (table 4). Our success rate reached 100% after 20 biopsy procedures (fig 1).

DISCUSSION

The sentinel lymph node is the first cancer cell draining node.[4 5] Theoretically, there is a gradual spread of tumour cells from the sentinel nodes to other nodes so further spread of cancer cells may be predicted by the sentinel nodes.[4] In cutaneous melanoma, the accuracy of sentinel node biopsy to predict lymphatic basin involvement is over 95%.[11] In western countries, this is already a part of regular treatment protocol. In the same way, the sentinel lymph node biopsy procedure in breast cancer patients is also a regular procedure in many western countries. Current literature relating to sentinel node biopsy suggests that the accuracy of sentinel nodes to predict axillary status is greater than 95%, with a false negative rate ranging from 0 to 5%.

In this study, our success rate using radiotracer and gamma probe is 95.7%. Results of this preliminary study are similar to the other preliminary studies of Krag *et al* (82%),[5] Pijpers *et al* (92%),[12] and Borgstein *et al* (94%)[13] using similar methods. Giuliano *et al*'s landmark report of a 65% success rate with blue dye shows a success rate of 58.6% in the first half of study and 72.4% in the second half, with a significant increase in success rate in the second half of the study.[6] More recently, these authors reported successful mapping in 93% and 99% of cases.[14 15] We experienced a 91.4% success rate in first half of study and all the failures occurred in first 20 cases. In the second half, the success rate was 100%. This reflects the developmental stage of a new technique, and is shown in the discrete learning curve (fig 1).[16] We also experienced a faster and accurate detection and dissection of sentinel node specimen in the later cases of our study. However, our detection rate was lower compared with the most recent reports of experienced doctors. We believe that we are now in the learning stage and think this is the main reason for the difference. Some reports suggest that blue dye and isotopes are complementary, and that sentinel node identification is facilitated when the techniques are used together, thereby accelerating the learning curve.[17 18] As the blue dye is not commercially available in the Peoples' Republic of China, we have no experience using dye or dual method.

In our study, the observed accuracy of sentinel nodes for the detection of metastatic disease in permanent sections was 92.5%, and false negative cases were 7.5%. The false negative rate is of great concern when this procedure is applied as a treatment protocol. O'hea *et al*, in a series of 60 sentinel node biopsy procedures using dye plus isotope localisation, found that all of their three false negative sentinel nodes occurred in tumours larger than 1.5 cm.[18] Veronesi *et al* have similarly found no single false negative sentinel lymph nodes in tumours less than 1.5 cm in diameter.[19] In this study, we also found that the false negative rate was increased and accuracy diminished with increase in tumour size (table 4). This inaccuracy in larger tumours may be caused by alternate lymphatic drainage pathways or simply by the prevalence of axillary metastasis in patients with larger tumours. In our series, the false negative rate is high compared with that of more experienced doctors. In our trial, T1 lesions only consisted of 37% of all the cases and the high false negative rate might be a function of the greater percentage of larger lesions.

For the methodology, the tracer injection timing is an issue to be debated. The physical half life of ^{99m}Tc sulphur colloid is six hours. Krag *et al* reported the average time between injection and surgery as being 2.9 ± 1.9 hours.[7] This means that surgery is carried out in the afternoon only, which is inconvenient for both patients and surgeons. When the tracer is injected a day before the surgery, the radioactivity in the

Table 4 Stratification of patients (n = 70) and tumour characteristics and respective success rates, accuracy, and false negative rate (FNR)

Parameter	No of cases	Success rate	Accuracy	FNR
Age (years)				
<50	40	97.5	97.4	2.6*
>50	30	93.3	85.7	14.3
Tumour size (cm)				
⩽2	26	96.2	100	0
>2	35	94.3	84.8	15.2
After biopsy	9	100	100	0
Location of lesion				
Outer upper	38	92.1	88.6	11.4
Other quadrants	32	100	96.9	3.1

*p = 0.06.

node, the axillary bed, and the background will certainly be markedly decreased on the day of surgery. But our experience showed the remaining radioactivity in the sentinel lymph nodes, however, was strong enough to be detected by the gamma probe. All the nodes exceeded the criteria of greater than 25 counts per 10 seconds on the day after injection. Static lymphoscintigraphic studies also showed a hot spot at the lymphatic basin after two or more hours and it remained the most active node for the next 24 hours. So the procedure is possible without any problems using overnight migration of ^{99m}Tc sulphur colloid radiotracer. This method gives us enough time to perform lymphoscintigraphic studies of the breast carcinoma, and the low amount of radioactivity is more acceptable to surgeons, anaesthesiologists, nurses, and pathologists.

The multiple injection of radiocolloid around the tumour may give the best diffusion of tracer around the tumour, which in turn is carried to the most accurate sentinel node. Although some authors have used subdermal, subcutaneous,[19] or periareolar[20] injection of radiotracer yielding an excellent sentinel lymph node identification rate, there remained a question as to whether the identified flow was a true representation of the lymphatic flow of the tumour bed.[21] The radiocolloid was injected around the breast tissue surrounding the primary tumour or biopsy site, taking the size and consistency of the breast into consideration. In smaller breasts with a hard texture, the volume injected was smaller, and this is the most frequent situation for our patients.

To determine the factors influencing the success rate and accuracy, McMasters *et al*, in a multicentre study including 806 patients, analysed the age, site of tumour, size of tumour, type of biopsy method, and experience of the surgeon. They found that the success rate was low in patients older than 50 years ($p = 0.03$).[22] The false negative rate was high ($p = 0.004$) for outer quadrant tumours, which is similar to the report of Krag *et al* ($p = 0.05$).[7] In our series, the two real failures were in patients older than 50 years (58 and 62 years) and, in five false negative cases, the lesions were larger than 2 cm at their greatest diameter and all were located in the outer quadrant of the breast. Since the sample size was too small, our data did not show a statistically significant result (table 4). The probable reason for older patients to have a higher failure rate might be a decrease in the capacity of lymph nodes to retain the radioactive colloid, because lymph nodes are replaced by fat in elderly persons.[7] In upper outer quadrant tumours, difficulty in discriminating the signal from background after peritumoural injection of the radiotracer may be the cause of a low detection rate; this is also considered to be a drawback of the radioguided procedure.[15]

So far as radiation hazard to operating room personnel, pathologists, and equipment during a breast sentinel node biopsy is concerned, Stratmann *et al* reported that with 0.7–1.0 mCi of ^{99m}Tc sulphur colloid injected 1.5–3 hours before the sentinel node biopsy, primary surgeon, scrub nurse, and pathologist can perform respectively 2190, 33 333, and 14 705 hours of procedural work before surpassing Occupational Safety and Health Administration limits. Operating instruments, pathology slides, and cryostats do not require special handling.[23] Only the lumpectomy specimens should be quarantined until the dose rate equals background levels. Although we injected 1–2 mCi of ^{99m}Tc sulphur colloid, we performed surgery 10–16 hours after injection. Twice the half life of ^{99m}Tc had elapsed before surgical procedures as its half life is only six hours. Compared with the report of Stratmann *et al*, the exposure to operating room personnel and the pathologist performing frozen section is quite low. Exposure to a patient from a single procedure is of no meaningful concern. In general this procedure requires one 60th the dose of technetium delivered during a routine bone scan. Technetium is a safe radiopharmaceutical because of its relatively low energy gamma ray (140 kEV), short half life, and lack of beta emission.[24]

Our hospital breast cancer database shows that 23% of all the breast cancer patients in our department had T1 tumours and 56% had T2 tumours. Only 19% of cases of T1 lesions were clinically examined to have axillary lymphadenopathy, and 29% of cases were found to have a pathologically positive axilla. Currently, these patient groups are being treated with the traditional method of axillary dissection. The sentinel node biopsy will establish itself as a safe and effective alternative to axillary dissection in this patient subgroup.[25] This modification will decrease the operative morbidity of surgical therapy for breast cancer, providing accurate staging and more individualiesd therapy. This minimally invasive axillary staging procedure is a major advance in the surgical treatment of breast cancer. As radiocolloid can be injected a day before, there will be enough time for lymphoscintigraphy and surgical preparation.

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IMAGES IN MEDICINE

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The embryonic explanation for dermatome distributions

In clinical practice it helps if you can imagine the patient in the "embryonic position". This is easier to do for the upper limbs as these could be placed in such a position. During growth the lower limbs move through 90 degrees to point anteriorly and at the same time rotate so that the plantar surface of the feet face downwards.

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