

## Is early dynamic lymphoscintigraphy for detection of sentinel lymph nodes always achievable in breast tumor?

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**Objective:** In this article, we will discuss the achievement of early dynamic lymphoscintigraphic protocol and compare detection of sentinel node between benign and malignant breast tumors, and whether pathologic factor is related or not. **Methods:** During a six-month period, consecutive fifty-nine patients were enrolled into our study. The average age of patients was  $47.6 \pm 9.8$  years and all of them were clinically suspected of having breast cancer. The average tumor was  $2.1 \pm 1.1$  cm in size. First, Tc-99m sulfur colloid was injected around corners of palpable mass or biopsy cavity by the hybrid injection method. Immediately thereafter, dynamic protocol of lymphoscintigraphy, with 10 sec per frame for 60 frames was performed by established simultaneous dual-head vertical angle imaging technique. And delayed two-hour image was also acquired. All patients underwent surgery sixteen to twenty hours later and had a final pathological diagnosis. **Results:** Among 59 patients, 14 of them were diagnosed with fibroadenoma and the other 45 cases with malignant conditions, infiltrating duct carcinoma mostly. The average age of the two groups was similar. From the summation image of dynamic study, identified axillary sentinel nodal activity was found as 80% in the group of benign breast tumor, but only 48% in the group of malignant breast tumor. In more than 88% of patients, sentinel lymph node was detectable on the delayed two-hour image between the two groups. **Conclusions:** Early dynamic protocol of pre-operative lymphoscintigraphy is helpful to clarify the relationship between the local lymphatic drainage basin and sentinel nodal uptake. However, this short period of protocol is not always achievable to detect sentinel node, especially in the group with breast malignant lesions.

**Key words:** early dynamic lymphoscintigraphy, sentinel lymph node, breast cancer

### INTRODUCTION

SENTINEL LYMPH NODE (SLN) biopsy has been developed to accurately assess axillary nodal status without removing most of the axillary content.<sup>1-6</sup> The SLN is defined as the first node in the lymphatic basin that receives the afferent lymphatic flow from a primary tumor. Identification of SLNs has become an important issue in clinical practice.

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Several methods have been used in the past, including blue dye,<sup>1,2</sup> radioisotopes,<sup>3-5</sup> or a combination of blue dye and radioisotopes<sup>6</sup> in helping detect SLNs in clinical settings. In a major series, Cox et al.<sup>7</sup> reported that blue dye helped identify SLNs in 80% and radioisotopes in 89% of patients. Using a combination of blue dye and radioisotopes increased the success rate to 96%. Cody et al.<sup>8</sup> identified SLNs by blue dye in 81%, by radioisotopes in 87%, and by a combined technique in 95% of patients. Based on prior results and our experience, using radioisotopes in the detection of SLNs in breast cancer has provided more sensitive identification and localization. Furthermore, preoperative lymphoscintigraphic imaging has played a role in mapping the direction of the lymphatic drainage basin from the tumor site and has localized the sentinel node versus secondary lymph node.<sup>9</sup> The

imaging information gives surgeons tailored incision planning before intraoperative detection with a gamma probe. Noguchi et al. 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.<sup>10</sup> An early dynamic protocol of preoperative lymphoscintigraphy has been proposed as a helpful method in distinguishing SLNs along the lymphatic basin around the tumor.<sup>9,11</sup> However, is an early dynamic protocol for the detection of SLNs always achievable in breast tumors? In this article, we compare the detection rate of SLNs between benign and malignant breast tumors and related factors using early dynamic lymphoscintigraphy, discussing its merits.

## PATIENTS AND METHODS

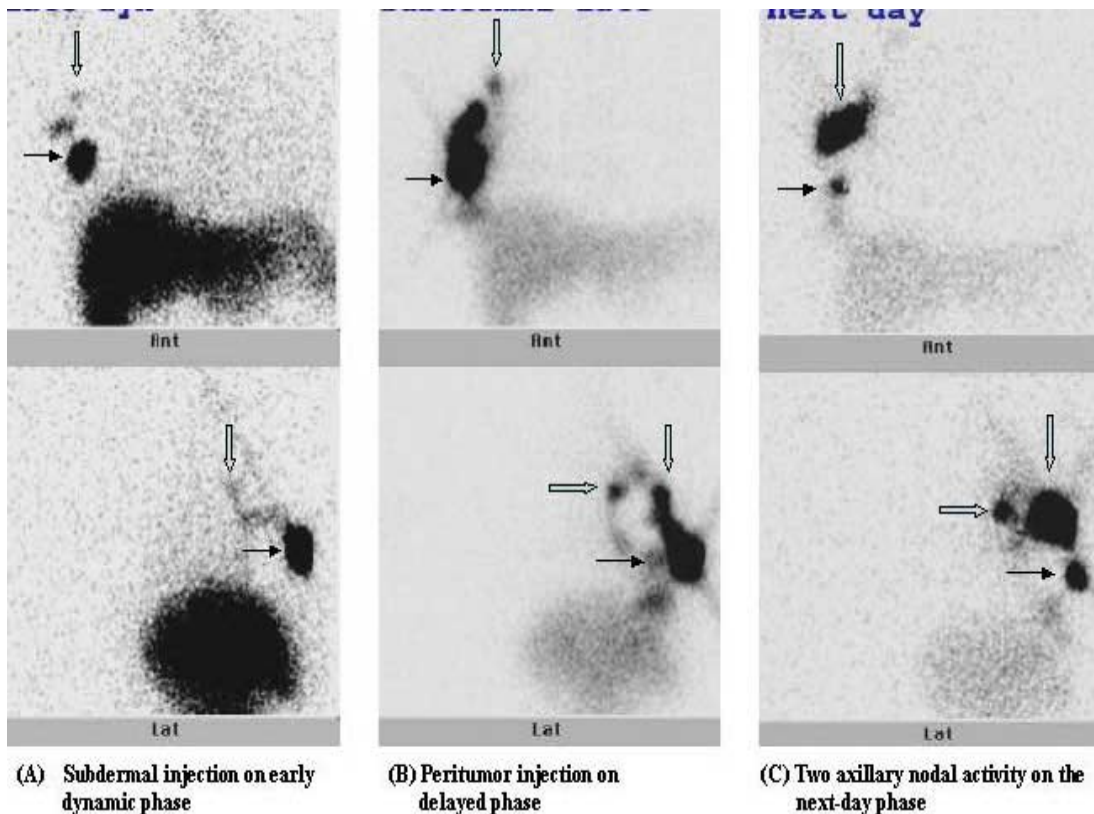
### Patient selection

During a six-month period in 2003, 59 patients, aged 33 to 76 years (average age,  $47.6 \pm 9.8$  years) with an initial clinical stage of T1 or T2, N0MX were enrolled. All

patients were scheduled to undergo curative surgery at our hospital. The average primary tumor size, measured by ultrasonography and based on the longer axis of tumor, was  $2.1 \text{ cm} \pm 1.1 \text{ cm}$  (range, 1.5 to 6.0 cm). Locations of the tumors were categorized into four quadrants of breast and nipple. The ratio of tumor location between the right and left was 2:1. The tumor location in the four quadrants was 64.5% (OUQ), 13.5% (OIQ), 13.5% (IUQ), 6.8% (IIQ) and 1.7% (nipple), respectively. Eighteen percent of enrolled patients had undergone excision biopsy 1 to 2 weeks previously and were proven to have malignant tumors. The remaining patients were diagnosed with malignant tumors according to a cytological result or strong clinical suspicion. Among 59 patients, 14 of them were diagnosed with fibroadenoma, and the other 45 cases were diagnosed with malignant conditions, infiltrating duct carcinoma mostly.

### Lymphoscintigraphy

All enrolled patients underwent preoperative sentinel lymphoscintigraphy 16 or 20 hours before the day of surgery. The standard dose of 11.1–18.5 MBq (0.3–0.5



**Fig. 1** Using a simultaneously vertical angle dual-head dynamic protocol, we recorded three phases lymphodynamics. (A) After subdermal injection around tumor, the summation image of early dynamic protocol showed a lymphatic tract and faint axillary SLN activity. (B) Another larger volume of peritumor injection, visualized different lymphatic tracts and activity of two nodes was demonstrated. (C) Similar two nodal activity, but no further visualized tract was seen on the delayed sixteen-hour image. ( → : injection point; ⇓ : lymph node activity)

**Table 1** Factors related to detection of sentinel lymph nodes on lymphoscintigraphy between early dynamic vs. delayed phases (n = 59)

Total No.	Detectable sentinel lymph nodes on lymphoscintigraphy	
	Early dynamic phase	Delayed two-hour phase
<b>Age</b>		
> 50	24 11/24 (45.8%)	17/23 (73.9%)
< 50	35 19/34 (55.8%)	30/35 (85.7%)
<b>Side</b>		
R	40 20/38 (52.6%)	35/40 (87.5%)
L	19 10/18 (55.5%)	13/19 (68.4%)
<b>Location</b>		
OUQ	38 19/36 (52.8%)	32/38 (84.2%)
OIQ	8 7/8 (87.5%)	-
IIQ	4 1/4 (25.0%)	1/4 (25.0%)
IUQ	8 2/7 (28.8%)	7/8 (87.5%)
Nipple	1 -	-
<b>Size (cm)</b>		
< 2	27 12/26 (46.2%)	24/27 (88.9%)
2-5	21 16/21 (76.1%)	16/21 (76.1%)
Biopsy	11 7/11 (63.6%)	8/11 (72.7%)

mCi) Tc-99m labeled sulfur colloid without infiltration (Syncor, Taiwan), in 1.0 to 2.0 ml of saline, was injected in two to four selected corners of the tumor or adjacent area of the biopsy site, a hybrid combination of subdermal and peritumor injection. The corners of injection were selected upon palpitation and previous ultrasonographic imaging. Dynamic images (Siemens, E.CAM, LEHR collimator) were immediately acquired under 10 sec per frame for 60 frames by well-established techniques in grade shield enhancement and simultaneous vertical angle dual-head imaging at our hospital. For young patients, modified separated dynamic imaging technique for each subdermal and peritumor injection was applied. Delayed images were taken with a dual head technique two hours later. Certain imaging process techniques, such as summation of dynamic images, were helpful in identifying tiny and hypofunctional sentinel nodes. By using a point source matched technique, we marked the location of the sentinel nodes on the skin of the breast, providing the surgeon with a map of the lymphatic drainage basin for guiding the intraoperative gamma probe on the next day.

#### *Intraoperative gamma probe detection*

Our surgeons detected the sentinel nodes through a sterile-packed gamma probe (neo2000™, neoprobe Co., Dublin, OH) based on the previous skin marker indications. Then, the radioactive or hot nodes were removed in the tumor biopsy excision group, first. In the group of cytological diagnosis or clinical suspicion of malignant lesion, both the primary tumor and sentinel node biopsies were performed at the same time. A probe counted fewer than 10% of the most radioactive lymph nodes as back-

**Table 2** Pathologic results and the numbers of axillary lymph nodal metastasis in our patients (n = 45)

N	Pathologic pattern				Number of metastatic nodes		
	D	M	L	Mu	N0	N1-3	N > 4
N	38	3	3	1	29	10	6

D: ductal carcinoma, M: medullar carcinoma, L: lobular carcinoma, Mus: mucinous carcinoma.

ground radiation. Routine axillary lymph node dissection, at levels I-II, was still performed after obtaining definite results of intraoperative frozen section histopathology. Biopsy specimens of sentinel and axillary lymph nodes, stained with hematoxylin and eosin were observed and diagnosed by pathologists.

#### *Statistical analysis*

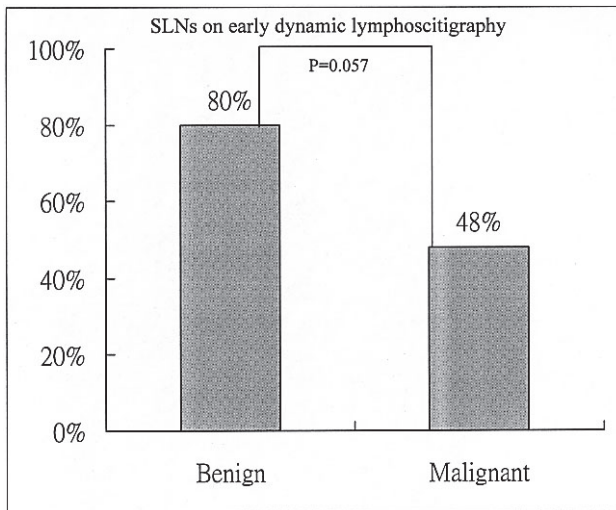
Fisher's exact tests by SPSS for Windows (SPSS Inc., Chicago Ill) were used to evaluate the statistical analysis for proposed factors, including relationship between benign and malignant factors. A value of  $p < 0.5$  was considered statistically significant.

## RESULTS

On lymphoscintigraphy, sentinel nodal activity within the axillary basin was seen predominantly. About 8% to 10% of cases were also visualized with extraaxillary nodal activity (such as internal mammary or supraclavicular areas) and all of them had underlying malignancy. Early dynamic lymphoscintigraphic imaging provided for natural physiologic lymphatic drainage.

In Figure 1, we demonstrate a case that received superficial (subdermal) and deep (peritumor) hybrid injections separately. Consecutive early dynamic lymphoscintigraphies showed different lymphatic tracts and sentinel nodal location between superficial and deep layers. There are young patients under this modified hybrid injection technique with separated dynamic imaging protocol. It was hard to determine the lymphatic drainage tracts in most of the patients in this study. Overall sentinel nodal detection on early dynamic lymphoscintigraphy was only 55% (32/59) with grade shield enhancement. Sentinel nodal detection was improved to 88% (52/59) on delayed two-hour imaging.

From Table 1, we concluded the significant factors resulting in detection of sentinel lymph nodes on early dynamic or delayed phases of lymphoscintigraphy, based on mixed two groups of patients. Tumor on the right side and OUQ have statistical significance for sentinel nodal detection on delayed phase. For detection of sentinel lymph nodal activity on early dynamic phase of lymphoscintigraphy, there are no significant proposed factors, except tumor size over 2 cm ( $p = 0.027$ ). However, other proposed factors, such as age under 50 year-old, and



**Fig. 2** Different ratios of identifying sentinel lymph nodal activity between benign and malignant lesions demonstrated on early lymphoscintigraphy. But, there is borderline statistical significance for sentinel lymph nodal detection in the benign group ( $p = 0.057$  by Fisher's exact test).

tumor size under 2 cm are statistically significant ( $p < 0.05$ ) for detection of sentinel lymph nodes on delayed phase of lymphoscintigraphy. The average age was  $45.8 \pm 9.6$  years for the benign group and  $48.1 \pm 9.7$  years for the malignant group. There was not a significant statistical difference. Except for 11 cases that had received biopsy previously, the average size of the tumors in both groups was  $1.9 \pm 1.4$  cm (benign) and  $2.0 \pm 0.9$  cm (malignant). Under similar, well-established, pre-operative lymphoscintigraphy, a dynamic protocol was performed for each case. From the summation imaging of the dynamic study, sentinel nodal activity, major along the axillary lymphatic basin, was found to be 80% in the benign breast tumor group, but only 48% in the malignant breast tumor group. There was less statistical significance ( $p = 0.057$ ) in the detection of sentinel nodal activity in the benign tumor group on early dynamic lymphoscintigraphy, as is shown in Figure 2.

In Table 2, most of the 45 patients with underlying malignancy were diagnosed with ductal carcinoma, except in seven cases with other pathological entities. There were 16 cases eventually diagnosed with nodal metastasis. The false negative prediction rate of sentinel lymph nodal biopsy was about 10%.

## DISCUSSION

Although the phenomenon of lymph node metastasis has been recognized for over 200 years, the exact mechanisms by which malignant tumors leave the primary tumor site, invade lymphatics, and metastasize to regional lymph nodes have recently been the subject of intense interest. Sentinel lymph node biopsy has rapidly entered the clinical

mainstream for melanoma and breast carcinoma, and this technique has provided confirmation of the orderly anatomic progression of tumor cells from the primary site to the regional lymph nodes through the lymphatic system. Preoperative lymphoscintigraphic imaging not only plays a role in mapping and localizing the sentinel node for surgery, but also provides adjacent lymphodynamic status in a condition of malignant tumor growth for pathophysiologic research. Therefore, early dynamic lymphoscintigraphic protocol provides a unique method of using images to disclose the truth of lymphodynamics. In Figure 1, we demonstrate the pivotal role of early dynamic protocol in a case with an unusual lymphatic path.

Generally speaking, the rates of lymph flow within lymphatic collecting vessels vary in different cutaneous parts of the body, the most rapid flow occurring in the leg and foot (10.2 cm/min), about 2.8 cm/min in the anterior trunk, and slower flow in the head and neck (1.5 cm/min).<sup>12</sup> Using the above estimations, the appearance of sentinel nodal activity from breast lumps to the axillary lymphatic basin should not exceed 30 minutes. However, discrepancies seem evident in clinical practice. From our data, the overall sentinel nodal detection from early dynamic lymphoscintigraphy was only 55% and 88% using two-hour delayed images. Yeung et al. also demonstrated late lymphoscintigraphic images at 2 hours, identifying the SLN in significantly more patients compared with early 30-minute images (86% vs. 68%).<sup>13</sup> Early dynamic protocol for the detection of SLNs is not always achievable in breast tumors. What are the factors resulting in this phenomenon? Based on our patients, we hypothesize that certain parameters disturb lymphodynamics. Several technical issues, such as particle size of the radiotracer and the injection method can result in different dynamic patterns of the lymphatic drainage.<sup>9-11,14</sup> Larger colloid particles show a much slower rate of clearance from the interstitial space and are likely to show a slower accumulation into the sentinel lymph node.<sup>9,10</sup>

Several methods of injection have been proposed, including subdermal, pertumor, periareolar, and intratumor injections in sentinel lymph nodal detection of breast cancer.<sup>9,11,15</sup> Except for intratumor injection that has been proven to provide good results in rare reports, the other three methods are popular in sentinel lymph nodal detection of breast cancer worldwide.<sup>15</sup> Furthermore, the hybrid method of injection, including superficial and deeper layers of the lymphatic tract, has been preferred.<sup>14,15</sup> In this study, we used sulfur colloid particles without infiltration and the hybrid method of subdermal and perilesion injection around breast lumps for early dynamic lymphoscintigraphy. The size of the particle is a factor that disturbs lymphodynamic patterns. We compared larger particles of sulfur colloid without infiltration with smaller particles of phytate in the same patient. In our experience, the sentinel lymph node is always detectable after phytate injection (unpublished). Because of the



application of the two-day protocol, we eventually chose sulfur colloid without infiltration for fixed factor in order to evaluate the suitability for early lymphodynamic image protocol. However, many studies suggest that subareolar or subdermal/intradermal injection maps to the same axillary sentinel nodes as peritumor injection in the vast majority of cases. However, peritumoral injection identifies extra-axillary sentinel nodes, which are important in a minority of patients.<sup>16-18</sup> The hybrid method of injection, including superficial and deeper approaches surrounding the lump demonstrated another constant technical factor in this study. In our prior data, certain relationships between tumor location and the lymphatic drainage basin concluded a greater potential for extra-axillary SLN uptake if the tumor was in the inner quadrants of the breast. In this situation, an early dynamic protocol is necessary to clarify which one is the first node.

The lymphatic system is a component of both the circulatory and immune systems.<sup>14</sup> It is not known whether adjacent regional lymphatic circulation changes in the presence of tumor. The answer is thought to be affirmative, because only half of a patient's SLNs were identified on early dynamic lymphoscintigraphy in this study. Individual physiologic status such as age, tumor size and previous biopsy disturbs lymphatic drainage.<sup>9,19</sup> Based on the younger Asian female cohort (average age,  $47.6 \pm 9.8$  years) in our study, we analyzed the proposed factors that result in the disturbance of adjacent lymphatic drainage around the lump on both early dynamic and delayed phases of lymphoscintigraphy. In Table 1, tumor on the right side and OUQ had statistical significance for sentinel nodal detection on delayed phase. Most people in Taiwan are right-handed. Usually muscular activity results in relative patent lymphatic drainage. But this contention needs to be proven in future. And no statistically significant factors, except tumor size over 2 cm ( $p = 0.027$ ), will interfere with sentinel lymph nodal detection on the early dynamic phase of lymphoscintigraphy. However, tumor size over 2 cm is not of statistical significance when excluding the patients belonging to the benign group. On delayed phase of lymphoscintigraphy, age below 50 years and tumor size smaller than 2 cm had strong correlations with sentinel lymph nodal detection. Borgstein et al. reported a significant correlation between mapping success and invasive tumor size of  $< 5$  cm.<sup>19</sup> However, other investigators have found no such correlation.<sup>20,21</sup> In our experience, smaller breast lump seems to have a good successful rate for sentinel nodal detection, but this is still controversial. In addition, we also demonstrated that the status post biopsy does not result in nonvisualized SLNs.<sup>21</sup>

According to our analysis, axillary SLNs on early dynamic lymphoscintigraphy were found at 80% in the benign breast tumor group, but only 48% in the malignant breast tumor group. There is less statistical significance ( $p = 0.057$ ) in detecting sentinel nodal activity in the

benign tumor group on early dynamic lymphoscintigraphy. Though, no strong statistical significant evidence is proven. We still believe this finding suggests different histological environments around the mass between benign and malignant entities. In prior studies, lymphovascular invasion was the most important factor that influenced the number of positive axillary sentinel lymph nodes.<sup>7,22</sup> Recent reports have concluded that patients with unsuccessful axillary mapping have an increased risk of axillary involvement.<sup>23,24</sup> Due to the small sample size, we did not find any trend as described above. These results may imply a possible difference in the lymphatic microenvironment between benign and malignant groups on the early dynamic protocol of lymphoscintigraphy. Certain histological disturbances of the lymphatic basin surrounding the malignant tumor probably exist and result in delayed detection of sentinel nodes.<sup>7,22-25</sup>

In conclusion, early dynamic lymphoscintigraphy of sentinel lymph nodal detection by subdermal and peritumor injection method, is not always achievable, especially in the malignant group. However, it still has value in younger patients with tumor location in the inner quadrants of the breast, with potential extra-axillary sentinel lymph nodes. In this situation, early dynamic protocol of pre-operative lymphoscintigraphy is helpful in clarifying the true SLNs. Certain disturbances of the lymphatic basin surrounding the malignant tumor probably exist and result in delayed detection of sentinel nodes.

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