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Original article

Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer

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ABSTRACT

Background: Conservative breast resection with subsequent sentinel lymph node biopsy (SNB) is an increasingly popular initial approach for the treatment of breast cancer due to decreased invasiveness. SNB is a shorter procedure with fewer side effects than more substantial surgical procedures, but it sometimes fails to identify metastatic disease. Therefore, a highly sensitive and convenient method is needed to identify sentinel lymph nodes (SLN) with a high probability of containing disease in SNB. We compared the combination of radioisotope or dye with a fluorescence compound to analyze lymph flow to identify targets for SNB.

Materials and methods: We examined patients with breast cancer lacking metastases in the axillary lymph node (ALN). Two methods for targeted SNB were developed: (1) Indocyanine Green (ICG) and Patent blue were injected into the skin overlying the tumor and sub-areolar region just before the surgical procedure. (2) ICG and radiocolloid were injected into the skin overlying the tumor and sub-areolar region. The draining fluorescent lymphatic duct was visualized using a Photodynamic Eye (PDE). We removed the SLNs that were identified by the dye and fluorescence imaging methods. Method 1 was applied to 113 patients undergoing SNB, and 29 patients were treated with Method 2. In our study, patients were grouped by lymph flow into two types: Type C demonstrated convergence to one lymph duct. Type S demonstrated separate lymph ducts.

Results: Using the fluorescence imaging method, 99.3% of SLNs were identified, and 3.8 SLNs per patient were seen. The SLN identification rates for Patent blue dye and radiocolloid were 92.9% and 100%, respectively, while 1.9 and 2.0 SLNs per patient, respectively, were seen with these methods. We classified two types of lymph flow based on the pattern of lymphatic drainage. Type C converged to a single lymph duct, while Type S drained to separate ducts. Type S lymph drainage was seen in 29/142 patients (20.4%), and Type C drainage was found in 113/141 patients (79.6%). Of the patients with Type S drainage, there were 4.1 SLNs per patient, but only 3.4 SLNs per patient were seen in individuals with Type C drainage. Forty cases had metastases found in the ALNs, and five of these cases were dye-negative and fluorescence-positive. Among these cases, the average number of SLNs identified was one.

Conclusion: The combination of fluorescence with a visible dye is a highly sensitive method for SLN identification. When SNB is guided by only the dye method, there is a risk of missing appropriate SLNs in patients with Type S lymph drainage or weak dye staining. The use of a fluorescence method together with dye could increase sensitivity of detection in these cases. Furthermore, fluorescent methods are ideal for hospitals that cannot use conventional radioactive measures.

Introduction

Recent efforts in the surgical treatment of breast cancer have focused on breast conserving procedures, and ALN resection has become progressively less invasive with the implementation of sentinel lymph node biopsy (SNB). However, SNB can fail to identify lymph node metastases, and it is important to identify the optimal sentinel lymph node (SLN) for biopsy. This is a critical step in the evaluation of ALN status in patients with early breast cancer. Several methods are currently used to identify sentinel nodes including the dye method, the gamma probe-guided method, or a combination of these two, and there are many reports describing the successful use of these methods.[1] The combined use of a dye and gamma probe is more accurate compared to the dye method

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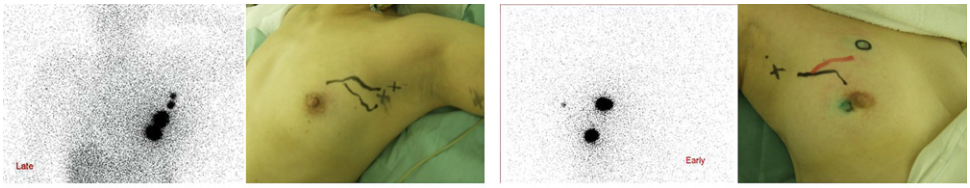

Fig. 1. Lymph flow type.

alone,[13,14] but patients are exposed to a radioisotope. Indocyanine Green (ICG) fluoresces when bound to proteins under physiologic conditions, and it can be used to monitor lymph flow using a charge-coupled device.[6] ICG is currently used clinically in cardiovascular surgery,[8,9] organ transplantation,[10] and stomach and intestine surgery.[11] In this paper, we examined the performance characteristics of ICG in combination with a radioisotope or dye for the selection of targets for SNB in patients with breast cancer, and we further classified the patterns of lymphatic flow in these patients.

Patients and methods

Patients

One hundred and forty one patients with clinically node-negative breast cancer were examined from August 2006 to December 2008 at National Cancer Center Hospital.

Methods

SNB Identification Method 1: A 1% solution of patent blue dye (2 ml) was injected into the sub-areolar region and skin overlying the tumor after the induction of anesthesia. The whole breast was compressed and massaged for about 5 min. ICG (2 ml) was injected into the skin overlying the tumor and the sub-areolar region immediately prior to the surgical procedure. Fluorescence in the draining lymphatic duct was visualized with the PDE, and the incision line was determined. All blue-stained SLNs were harvested. After the initial lymph node resection guided by dye labeling, the remaining nodes were re-evaluated using the PDE and harvested if fluorescence was detected.

SNB identification method 2: lymphatic mapping was performed using a combination of ICG and 30–80 megabecquerels of technetium-99 m-labeled Phytate (Daiichi RI Laboratory, Tokyo, Japan). One day prior to surgery, the radiotracer was intradermally injected into the area overlying the tumor and sub-areolar region, while ICG (2 ml) was injected into the skin overlying the tumor and the sub-areolar region immediately prior to the surgical procedure. Lymph nodes containing radioactivity were identified with a scintillation detector, and harvested. The remaining lymph nodes were then examined with the PDE for fluorescence, and harvested as described above.

Depending on the method used, SLNs were identified as blue stained, radioactive, and/or fluorescent. SNB was then followed by standard level 1/2 axillary lymph node dissection.

All SLNs were histologically evaluated by 3 mm serial sectioning and staining with hematoxylin and eosin (H&E). Lymph nodes as part of the axillary dissection were submitted in their entirety and evaluated using standard H&E staining.

Lymphatic flow was defined as follows: Type C (confluent) lymphatic drainage converged to one lymph duct as assessed by the PDE imaging system, and Type S (separate) lymphatic drainage did NOT converge to one lymph duct (Fig. 1).

Results

The average age of the subjects examined in this study was 59.4 years old (range: 31–83 years). The primary tumor was located in the upper-outer quadrant for 58 cases (41.1%), the upper-inner quadrant for 35 (24.8%), the lower-outer quadrant for 16 (11.3%), the lower-inner for 13 (9.2%), and the central portion for 12 cases (8.5%), while 8 cases had multiple masses (5.7%) (Table 1). All lymph nodes containing dye, radioisotope, or fluorescence were collected, and this was considered the total population of SLNs. In the patients who underwent Method 1, only 92.9% (105/113) of SLNs were dye-positive, but all of the identified nodes were fluorescent (113/113). In patients undergoing Method 2, 100% (29/29) of SLNs were identified by radioisotope detection, and 93.1% of these (27/29) were fluorescent. Overall, 3 SLNs per patient were identified by fluorescence, but only 1.9 and 2.0 were identified by dye or radioisotope, respectively (Median) (Table 2)

We further analyzed the patterns of lymph flow in all 142 patients. In most patients, the lymphatic drainage flowed from the lower-inner quadrant to the upper-outer quadrant, and only one patient had lymphatic drainage to the internal mammary lymph node. Overall, 113 patients had Type C (79.6%) lymph flow while 29 patients had Type S (20.4%). (Table 3) There was no correlation between the number of SLNs identified and the type of lymphatic drainage.

Of those patients examined using Method 1, 31 had metastases detected in the SLN, and, of these patients, 5 were fluorescence-positive and dye-negative (Table 4). Four of these cases were without metastases at non-SLNs that were subsequently resected. The number of SLNs in these 4 cases was one or zero, but there was no relationship to lymph drainage type (Type C or Type S).

Table 1
Patient demographics.

	Number of patients		
	Method 1	Method 2	Total
Age			
Mean	57.6	60	59.4
Range	34–83	31–82	31–83
Tumor classification			
Tis	29 (25.7%)	4 (14.3%)	33 (23.4%)
T1	51 (45.1%)	6 (21.4%)	57 (40.4%)
T2	33 (29.2%)	18 (64.3%)	51 (36.2%)
Tumor location			
A	29 (26%)	6 (21%)	35 (24.8%)
B	11 (10%)	2 (7%)	13 (9.2%)
C	42 (37%)	16 (57%)	58 (41.1%)
D	15 (13%)	1 (4%)	16 (11.3%)
E	10 (9%)	2 (7%)	12 (8.5%)
Other	6 (5%)	2 (7%)	8 (5.7%)
Pathological node status			
Negative	82 (72.6%)	19 (67.9%)	101 (71.6%)
Positive	31 (27.4%)	9 (32.1%)	40 (28.4%)

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Table 2
Detection rate.

State of SLN	Number of SLNs (average)	Detection rate
Flu-positive	3.8	99.3% (140/141)
Dye-positive	1.9	92.9% (105/113)
RI-positive	2.0	100% (28/28)

Flu: Fluorescence, SLN: Sentinel lymph node, RI: Radio isotope

Discussion

Identification of SLNs for biopsy during the initial staging of breast cancer is very important, and highly accurate methods are needed to limit the number of nodes and patients incorrectly identified as metastasis free. This has historically relied upon two methods, the dye- or gamma probe-guided methods, or their combination. The gamma probe-guided method is more accurate than the dye method alone, but requires patient exposure to radioisotope. Thus, we wished to determine if use of the fluorescent dye ICG could improve upon these methods. In the present study, we demonstrated the accuracy of two different combined methods using the fluorescent compound ICG paired with either patent blue dye or a radioisotope tracer. Both methods had high SLN identification rates, but fluorescence was particularly beneficial in difficult cases where SLN were not readily identified using the dye method.

In addition to exposing the patient to radiation, the gamma probe-guided method cannot be used in hospitals that are not equipped to handle radioactivity. However, it is able to identify lymph vessels before incision, and can guide the surgical approach. An ICG-based fluorescent detection method can also identify lymphatic vessels preoperatively with similar sensitivity to gamma probe-guided methods. Moreover, ICG does not require any special licensing, storage, or handling procedures. Therefore, the use of ICG should be particularly attractive to hospitals unable to work with radioactive isotopes. ICG can be a visible dye, and, when used in this capacity, it only identifies 71%[2]–84%[3] of SLNs, and indigo carmine is 94% sensitive. This increases to 94%[6] and 100%[7] when ICG is used as a fluorophore. In this study, ICG fluorescence identified 99% of SLNs, consistent with a previous report, and its use identified 3.8 SLNs per patient. In contrast, use of the dye- or gamma probe-guided method alone identified only 1.9 and 2 SLNs per patient, respectively, consistent with published results.[4,5] The larger number of SLNs identified with ICG could be due to the low molecular weight and high degree of diffusion of ICG allow it to spread beyond the SLN to secondary draining LN. Spread of the radiosotope and patent dye may be more limited. Because SNB by a fluorescence method is sensitive, second or third SLN is detected. As a result, the lymph node which we resected increased. We think that this is a weak point of these methods.

We identified two patterns of lymph flow in our patient group; we labeled these as Type C and Type S. More patients had Type C drainage, and some patients originally identified as Type S were found to have Type C drainage after incision. Type S drainage flows directly into each SLN, and, in patients with Type S drainage, more SLNs were identified (Table 3). The fact that when lymph flow was type S, there is much number of SLN leads to performing biopsy carefully. We think that a thought such as superscription helps prevention of false negative.

Table 3
Type of lymph flow and SLN.

Type of lymph flow	No. of patients	No. of SLNs
Type C	113 Cases (79.6%)	3.4
Type S	29 Cases (20.4%)	4.1

Table 4
Characteristics of the five patients whose SLN metastases were confirmed only by fluorescence.

Case	State of SLN		Number of metastasis LNs	Location of tumor
	Dye positive	Flu positive		
1	1	3	1	A and C
2	0	1	1	B
3	1	6	1	C
4	1	4	1	D
5	1	4	5	C

Forty cases (28.4%), including four cases of micro-metastases, had metastases in the SLN and underwent ALN resection. The lymph nodes containing metastases in five of these 40 cases were missed by the dye method alone but were detected by the fluorescence method (Table 4). Metastases were present in only the SLN in four of these five cases. More thorough analysis could not be performed because of the small number of affected patients, but the inability of the dye method to detect SLNs in these cases is consistent with reports of poor detection by the dye method alone. Thus, it is our view that the dye method should be combined with the fluorescence method in difficult cases.

Suami et al.[12] suggested that nearly all of the lymph drainage of the breast travels to one SLN in the axilla, but some lymph flow drains to more than one SLN in some cases. Thus, there is an anatomic basis to the observed decreased detection of SLNs by the dye method. When considered together, the data suggests that use of a highly sensitive SLN identification technique is indicated, and, for institutions that cannot handle radioactivity, ICG fluorescence offers great promise.

Conflict of interest statement

None declared.

Ethical approval

All patients provided written informed consent to be examined in this study.

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