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Retrospective observational study about reducing the false negative rate of the sentinel lymph node biopsy

Never underestimate the effect of subjective factors

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Abstract

Reducing the false negative rate of sentinel lymph node biopsy (SLNB) for breast cancer patients has always been a focus of clinical research. We aimed to map the sentinel lymph nodes (SLNs) in detail, and analyze the factors related to SLNs located at locations that are often ignored by surgeons, to reduce the rate of false negatives from SLNB. A retrospective analysis involving 545 breast cancer patients who underwent SLNB in west China hospital between August 2010 and February 2016 was performed. Blue dye, radioisotope, or combined methods were used for tracing SLNs. Using blue dye, radioisotope, and a combination of blue dye and radioisotope successfully traced SLNs in 479, 507, and 525 patients, the detection rate was 88.2%, 93.9%, and 97.4%, respectively. Among the 1559 detected SLNs, 139 (9.6%) were located at the latissimus dorsi lateral margin, and 108 (6.9%) were located at level 2. Subcutaneous injection of radioisotope ($P = .004$) and intradermal injection of blue dye ($P = .002$) were independent factors associated with SLNs distributed at level 2 and the latissimus dorsi lateral margin, respectively. It was noteworthy that 2 of 7 patients had skipping metastasis in level 2, so subcutaneous injection of the isotope is strongly recommended for tracing SLNs distributed in level 2 because of the possibility of skipping metastasis. Though intradermal injection of blue dye was superior methods for tracing SLNs located at the latissimus dorsi lateral margin, we surprisingly found those patients with metastasis to the latissimus dorsi lateral margin nodes also could have metastasis to level 1 (expect for the latissimus dorsi lateral margin) nodes, it seemed that maybe there is no need to excise SLNs at the latissimus dorsi lateral margin in SLNB, whether such nodes should be regarded as useful for SLNB still needs to be determined by further large, multicenter clinical studies.

Abbreviations: 99mTc = technetium-99m, ALND = axillary lymph node dissection, ECT = emission computed tomography, ER = estrogen receptor, FISH = fluorescence in situ hybridisation, FNR = false-negative rate, Her2 = human epidermal growth factor receptor 2, IHC = cytokeratin immunohistochemistry, L2 = SLN located at level 2, LDLM = SLN located at latissimus dorsi lateral margin, NL2 = SLN not located at level 2, NLDLM = SLN not located at latissimus dorsi lateral margin, PR = progesterone receptor, SC = sulfur colloid, SLNB = sentinel lymph node biopsy, SLNs = sentinel lymph nodes.

Keywords: breast cancer, distribution of sentinel lymph node, sentinel lymph node biopsy, tracing method

1. Introduction

With the results of multi-institutional randomized studies being made available, sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND) as the standard of care for primary treatment of early axillary lymph node negative breast cancer patients.[1–3] This has greatly reduced ALND-related morbidity such as lymphedema, and sensory and motor dysfunction.[4–6]

Nevertheless, the American Society of Clinical Oncology has reported the results of 6 trials on SLNB, in which the false-negative rate (FNR) ranged from 4.6% to 16.7%.[7,8] Because higher FNR brings a physical and psychological burden for breast cancer patients, this greatly restricts the popularity of SLNB. Consequently, more and more studies are being undertaken to minimize the FNR of SLNB as far as possible.[9,10]

Since the 1990s, with the evolution of the lymph node tracing method, and administration and injection routes, the FNR of SLNB has been reduced to a great extent.[11,12] However, each patient's condition is different, and some subjectivity from the surgeons also affects the accuracy of SLNB. For example, some surgeons are inclined to find sentinel lymph nodes (SLNs) in familiar areas but overlook what they might consider "unimportant" anatomical positions. Thus, the main purpose of our retrospective study was to map SLNs in detail, and explore the factors related to SLNs located at locations that are often overlooked during surgery, so that to reduce the subjective factors that raise the FNR of SLNB, and provide useful guidance to trainee and inexperienced surgeons.

Editor: Yong Liu.

Contributors: ZYT, DZG, ZD, and LQ proposed the study concept and design; performed the research; and acquired the data. ZYT and DZG analyzed and interpreted the data, wrote the first draft, and provided a critical revision of the article with important intellectual content. LQ was the study supervisor. YZ and ZD contributed equally to this study.

The authors declare no conflicts of interest.

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Medicine (2017) 96:34(e7787)

Received: 23 March 2017 / Received in final form: 7 July 2017 / Accepted: 19 July 2017

http://dx.doi.org/10.1097/MD.0000000000007787

2. Patients and methods

2.1. Patients

From August 2010 to February 2016, a total of 545 breast cancer patients underwent SLNB at the Department of Breast Surgery, West China Hospital, Sichuan University, China. Basic clinical data of each patient, including patient age, operation method, tumor location, and pathological characteristics of the tumors, were collected. Hormone receptor positivity was defined as 1% or more of cells staining for estrogen receptor or progesterone receptor. Her2 positivity was defined as 3+ staining by immunohistochemistry or fluorescence in situ hybridization (FISH) amplification with a value greater than 2. The research was in compliance with the Declaration of Helsinki and was approved by the ethics committees of Sichuan University West China Hospital. Informed consent was obtained from each patient.

2.2. SLNB technique

Sulfur colloid (SC) labeled with technetium–99m (99mTc) was injected on the day before surgery, or at least 4 hours before surgery. Methylthioninium (1%; 1 mL) was injected 15 to 20 minutes before surgery. Patients were injected either subcutaneously above the primary tumor, intradermally, or around the areola. After general anesthesia and dye injection, a 3 to 4 cm incision was made in the axillary region. The skin, subcutaneous tissue, and mammary gland tissue were then carefully separated to locate the blue nodes and record their number. Nodes with high radioactivity (hot node) were also identified.

2.3. Identification and evaluation of SLNs

SLNs were identified intraoperatively by use of radioisotope, blue dye, or combined methods. The first lymph nodes with blue lymphatic vessels directly leading to them, and those with a radioactivity count higher than 10% of the highest radioactivity count of the lymph nodes were regarded as SLNs. Cytokeratin immunohistochemistry (IHC) was used to identify whether metastases were present in the lymph node. Metastases were classified according to the 6th criterion of the American Joint Cancer Committee. The presence of macrometastases (≥2 mm), micrometastases (0.2–2 mm), and isolated tumor cells (≤0.2 mm), all resulted in a node-positive classification.

2.4. Statistical analysis

Descriptive statistics included means, ranges, standard deviations, and proportions. Categorical data were presented as percentages, and differences between proportions were compared using the χ^2 test or Fisher exact test. Continuous variables were compared using the unpaired Student *t* test. Multivariate analysis was performed to identify independent determinants for the SLNs located at the latissimus dorsi lateral margin and level 2. All statistical evaluations were performed using SPSS for Windows (SPSS 18.0, Chicago, IL). Results with a $P < .05$ were considered statistically significant.

3. Results

3.1. Tumor clinical pathological characteristics

All breast cancer patients included in this study were females with a mean age of 48.7 ± 11.8 years (range: 19–85 yrs). The average size of the tumors was 2.5 ± 1.5 cm (range: 0.2–12 cm). The majority of patients were treated by mastectomy (83.8%), with the remaining patients treated by breast conserving surgery (16.2%). In a certain number of patients, whose Her2 state was 2 + by IMH testing, FISH tests were not performed for various reasons, resulting in only partial data for these patients. The pathological characteristics of the tumors are shown in Table 1.

Table 1

Tumor pathological characteristics.

Variable	n	%
Histology		
Invasive breast cancer	451	82.8
Ductal carcinoma in situ*	39	7.2
Other†	55	10
Molecular classification		
Luminal A	134	24.6
Luminal B	125	22.9
Her2 positive	45	8.3
Basal-like	40	7.3
Tumor Location		
Upper outer quadrant	259	47.5
Upper inner quadrant	100	18.4
Lower outer quadrant	69	12.7
Lower inner quadrant	29	5.3
Central	88	16.1
T stage		
T1	314	57.6
T2	182	33.3
T3	49	9.1
N stage		
N0‡	371	68.1
N1§	138	25.2
N2	36	6.7
ER state		
Positive	404	74.1
Negative	141	25.9
PR state		
Positive	391	71.7
Negative	154	28.3
Her2 state		
Positive	97	17.8
Negative	247	45.3
Ki-67 expression levels		
≤20%	267	49.0
>20%	278	51.0

* Including Paget's disease (6).
† Including breast mucinous carcinoma (31), breast squamous cell carcinoma (23).
‡ Including 40 N0i+.
§ Including 84 N1mi.

3.2. Identification of SLNs using blue dye and radioisotopes, and the mapping of axillary SLNs

SLNs were successfully found in 543 patients (99.6%). Using blue dye, radioisotope, and a combination of blue dye and radioisotope successfully traced SLNs in 479, 507, and 525 patients, the detection rate was 88.2%, 93.9%, and 97.4%, respectively. Compared with the detection rate of using combined methods to trace SLNs, the detection rate of using blue dye or the radioisotope alone to trace SLNs was significantly different ($P < .05$). Furthermore, we found using combined methods detected 2.42 ± 1.19 SLNs (range: 1–7), using radioisotope detected 2.07 ± 1.16 SLNs (range: 0–6), and using blue dye

Table 2

Axillary sentinel lymph node mapping.

Position of nodes	*n*	%
Level I	1446	92.8
Root of thoracic dorsal blood vessels	580	37.2
Caudate lobe and the surrounding fat	513	32.9
Proximal to the lateral thoracic vessels	140	9.0
Latissimus dorsi lateral margin	139	9.6
Pectoralis major lateral margin	41	2.8
Axillary shallow lymph nodes	33	2.3
Level II	108	6.9
Level III	5	0.3

detected 1.47±1.08 SLNs (range: 0–4). Then we described the location of the removed SLNs in detail (see Table 2 and Figure 1). The total number of removed SLNs was 1559, with the majority of them located at the roots of thoracic dorsal blood vessel or the fat surrounding the caudate lobe of the mammary gland. Of the remainder, a total of 247 (16.5%) SLNs were located at the latissimus dorsi lateral margin (n=139, 9.6%) and level 2 (n=108, 6.9%).

3.3. Factors related to the latissimus dorsi lateral margin and level 2 distribution

Because SLNs located at the latissimus dorsi lateral margin and level 2 were always overlooked by surgeons, then we further explored the factors related to SLNs located at these 2 anatomical positions. Results of the univariate analyses are shown in Table 3. Though univariate analyses results showed age (P=.020) and subcutaneous injection of the isotope (P=.013) were associated with SLNs distributed at level 2, intradermal injection of the isotope (P=.015) and blue dye (P=.032) were associated with SLNs distributed at the latissimus dorsi lateral margin, multivariate analysis results showed that subcutaneous injection of the isotope [P=.004; 95% confidence interval (CI) 0.335, 0.809] and intradermal injection of the blue dye (P=.002; 95% CI 1.185, 2.161) was the only independent factor associated with SLNs distributed at level 2 and the latissimus dorsi lateral margin, respectively. It was noteworthy that 2 of 7 patients had skipping metastasis in level 2. In addition, we found those patients with metastasis to the latissimus dorsi lateral margin nodes also could have metastasis to level 1 (expect for the latissimus dorsi lateral margin) nodes.

4. Discussion

In recent decades, a majority of studies have been devoted for developing novel, consistent SLN tracing methods, to reduce the FNR of SLNB,[12] resulting in the currently used methods of tracing SLNs: blue dye,[13] radioisotope, the combination of blue dye and isotope,[14] fluorescent dye,[15,16] and nanoparticles.[17,18] Though the novel methods of lymphoscintigraphy confer their own unique advantages in reducing the FNR of SLNB in preclinical studies, their application in practice depends upon further prospective clinical studies to identify suitable protocols, and the uptake of such advanced methods might require some time.[19–21]

For now, to better reduce the FNR of SLNB, we should comprehensively analyze the related factors, to determine the most cost-effective and objective lymph node tracing methods.

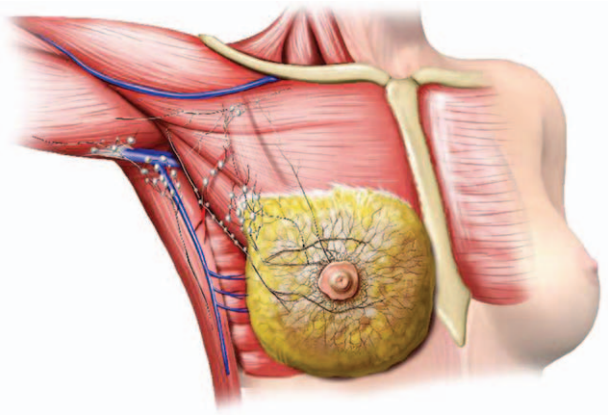

Figure 1. The distribution of sentinel lymph nodes in detail. A large node is equivalent to 10 small nodes.

However, this is far from sufficient. We suspect that if inexperienced surgeons overlooked some SLNs during operations, the FNR of SLNB would increase correspondingly, such that mapping of the SLNs becomes necessary. However, we found only a few studies had described the distribution pattern of SLNs in axillary tissues.[22–24] For example, Clough et al[25] explored the distribution of SLNs in axillary tissues in 242 breast cancer patients, using blue dye or radioisotope to trace the SLNs. They found that the majority of SLNs were located at the medial part of the axilla, alongside the lateral thoracic vein.[25] However, another study showed SLNs were mainly found between the pectoralis major muscle and the lateral thoracic vein, but this study only used the anatomical method, so the false negative rate was up to 21.7%.[26] Though these studies had limitations, they all confirmed one fact that the distribution of SLNs was diversiform, so that if surgeons only gave special attention to certain areas, the FNR of SLNB would increase.[27]

In this study, we found that up to 9.6% and 6.9% of SLNs located at the latissimus dorsi lateral margin and level 2, respectively. Furthermore, by analyzing our SLN distribution map in comparison with other available maps, we found that the latissimus dorsi lateral margin and level 2 were frequently being overlooked by surgeons, so these 2 anatomical positions acquire more attentions from surgeons to reduce the FNR of SLNB. Multivariate analysis results further showed that subcutaneous injection of the isotope and intradermal injection of blue dye were related to SLNs located at level 2 and the latissimus dorsi lateral margin, respectively. To inform trainee and inexperienced surgeons of these positions, we suggest subcutaneous injection of the isotope to trace SLNs located at level 2, and intradermal injection of blue dye to trace SLNs located at the latissimus dorsi lateral margin.

Considering that excision of SLNs at the latissimus dorsi lateral margin may increase lymphedema of patients whose SLNs located at level 1 were negative, we further surprisingly found if a patient has metastasis to the latissimus dorsi lateral margin SLNs, she always has metastasis to SLNs in level 1 (expect for the latissimus dorsi lateral margin) at the same time. This result reminds us that maybe there is no need to excise SLNs at the latissimus dorsi lateral margin in SLNB. Because our study contains a relatively small number of patients, the decision regarding whether SLNs at the latissimus dorsi lateral should be excised still requires further large clinical studies.

Table 3

Univariate analysis of factors associated with SLN located at level 2 and the latissimus dorsi lateral margin.

	Level 2			Latissimus dorsi lateral margin		
Variable	**L2 (n=91)**	**NL2 (n=452)**	***P***	**LDLM (n=138)**	**NLDLM (n=405)**	***P***
Age, y	48.6±11.7	50.2±12.9	.020	48.4±12.0	49.0±11.9	.797
Tumor size, cm	2.2±1.2	2.5±1.5	.306	2.4±1.4	2.5±1.5	.387
Tumor location			.980			.183
Upper outer quadrant	43	215		54	204	
Upper inner quadrant	17	83		32	68	
Lower outer quadrant	13	56		18	51	
Lower inner quadrant	5	24		7	23	
Central	13	74		27	59	
ER state			.793			.823
positive	69	334		105	298	
negative	22	118		33	107	
PR state			.375			.828
positive	69	321		99	291	
negative	22	131		39	114	
HER2 state			.647			.783
positive	20	77		22	76	
negative	45	202		62	185	
Ki-67 state			.568			.378
≤20%	42	224		72	193	
>20%	49	228		66	212	
ECT detection	86	421	.081	136	371	.555
Route of administration of the isotope			.013			.015
intradermal	33	218		72	179	
subcutaneous	52	185		55	182	
periareolar	1	18		10	9	
Route of administration of the blue dye			.128			.032
intradermal	42	167		58	121	
subcutaneous	25	154		54	155	
periareolar	11	80		16	75	

ECT = emission computed tomography, ER = estrogen receptor, L2 = SLN located at level 2, LDLM = SLN located at latissimus dorsi lateral margin, NL2 = SLN not located at level 2, NLDLM = SLN not located at latissimus dorsi lateral margin, PR = progesterone receptor.

In terms of SLNs in level 2, because we found 2 of 7 patients had skipping metastasis in level 2, so this part shouldn't be overlooked. We hope surgeons will carefully consider the SLNs located in level 2 in the future.

5. Conclusion

Subcutaneous injection of the isotope is strongly recommended to trace SLNs distributed at level 2 because of the possibility of skipping metastasis. Intradermal injection of blue dye are superior methods for tracing SLNs located at the latissimus dorsi lateral margin; however, whether such SLNs should be removed still needs to be determined by further large multicentered clinical studies.

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