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Analysis of the Feasibility of Dual-Tracer Sentinel Lymph Node Biopsy After Neoadjuvant Chemotherapy for Breast Cancer

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Abstract: *Objective:* The feasibility and safety of double-tracer sentinel lymph node biopsy (SLNB) for breast cancer patients after neoadjuvant chemotherapy and the possibility of exempting axillary lymph node dissection (ALND). *Methods:* The clinical data of 116 patients admitted to the Second Department of Breast Surgery of Baotou Cancer Hospital from July 2020 to May 2023 were collected. The patients underwent SLNB after neoadjuvant chemotherapy, and the data of the patients were analyzed. *Results:* Among the 116 breast cancer patients who underwent SLNB, sentinel lymph node (SLN) was not detected in 1 case, indicating a detection rate of 99.13% (115/116); 35 cases were positive for ALN and 22 cases were positive for SLN, indicating a sensitivity of 62.86 % (22/35); SLNB was successfully performed in 115 cases, indicating an accuracy of 73.91 % (85/115); 7 cases were false negative, and 35 cases were ALN positive, with a false negative rate of 37.14 % (13/35). *Conclusion*: SLNB cannot wholly replace ALND in breast cancer patients after neoadjuvant chemotherapy. In this experiment, when the number of SLN detected was ≥ 3 or when the breast mass reached pathological complete response, the dual-tracer SLNB could accurately predict the local status of ALN. However, randomized clinical trials with large sample sizes will be needed to consolidate this conclusion.

Keywords: Breast cancer; Neoadjuvant chemotherapy; Sentinel lymph node biopsy; Axillary lymph node

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1. Research background

Breast cancer is one of the most prevalent malignant tumors among women in China, whether it is in urban or rural areas and it is also ranks top four in terms of mortality rate [1]. With the advancement of technology, breast cancer treatment has entered the era of precision medicine. The concepts and methods of treatment have also undergone tremendous changes. Neoadjuvant chemotherapy (NAC) can effectively bring about local downstaging of intermediate and advanced breast cancer. It enhances the advantages of specific drug therapies for breast cancer patients, thereby improving their prospects for retaining their breasts following NAC [2].

Sentinel lymph node (SLN) refers to the first group of lymph nodes that the primary tumor passes when it metastasizes. SLNs serve as the first barrier to preventing the spread of a tumor, and their clinical significance has been valued. SLN biopsy (SLNB) can accurately evaluate the patient's axillary lymph nodes (ALNs) and reduce complications such as postoperative edema caused by total axillary dissection without affecting the patient's survival. Approximately 40% of breast cancer patients who are undergoing NAC have positive ALNs that can be converted into negative lymph nodes through ultrasound [3]. When axillary lymph nodes achieve pathological complete response (pCR), this group of patients may be exempted from ALN dissection (ALND). In recent years, the feasibility of SLN biopsy in evaluating ALN status in early breast cancer has been recognized [4], and the best method has been determined. Most studies believe that the choice of tracer should be either isosulfan blue or the double-tracer method i.e., combining the patent blue-V and nuclide tracer method [5]. However, for most grassroots or Western hospitals, the use of nuclides is subject to many restrictions, so it cannot be used clinically. Therefore, this study used methylene blue-indocyanine green (ICG) fluorescence combined with methylene blue dual-tracer SLN to retrospectively study the clinical utility and impact of dual-tracer SLNB in patients after NAC.

2. Research subjects and methods

2.1. Case selection and general information

The clinical data of breast cancer patients admitted to Baotou Cancer Hospital from July 2020 to May 2023 were collected.

Inclusion criteria: (1) Patients diagnosed with untreated female breast cancer; (3) patients with untreated stage I, II, and III breast cancer; (3) patients diagnosed with invasive breast cancer through preoperative evaluation and puncture; (4) patients who meet the preoperative criteria; (4) patients with NAC indications and had been given targeted therapy using taxanes, anthracyclines, trastuzumab, pertuzumab, platinum, or other drugs; (5) patients who underwent surgery and had their lesions and ALN status evaluated with imaging data before surgery.

Exclusion criteria: (1) Patients who could not tolerate preoperative NAC or surgery after pre-treatment evaluation; (2) patients who could not continue this study due to personal or other reasons; (3) patients with inflammatory breast cancer, bilateral breast cancer, or recurrent breast cancer; (4) patients who had previously undergone breast or axillary surgery; (5) patients who had suffered from other cancers and received chemotherapy and targeted therapy.

One hundred sixteen breast cancer patients were screened, all of whom were female. The patients' ages ranged from 34 to 71 years old, with an average age of 51.65 years. The tumor was located in the right breast in 61 cases (52.58%) and in the left breast in 55 cases (47.42%). As for the molecular classification of breast cancer, there were 37 cases of triple-negative breast cancer (31.90%), 48 cases of HER-2-positive breast cancer (41.38%), 15 cases of Luminal-A breast cancer (12.93%), and 16 cases of Luminal-B breast cancer (13.79%).

2.2. NAC regimen

Before surgery, a suitable NAC plan was formulated based on the patient's pathological type, physical condition, and economic factors. The plan included (1) taxanes, anthracyclines, and platinum drugs; (2) herceptin and/or pertuzumab combined chemotherapy. After each cycle, the efficacy was evaluated, and the dosage and regimen of chemotherapy were adjusted based on the patient's response to the drug, blood routine, blood biochemistry, color ultrasound, and enhanced MRI. After completing chemotherapy, the primary tumor and ALN status were evaluated through clinical examinations and imaging methods.

2.3. Tracer selection

The tracer used was ICG fluorescence combined with methylene blue.

2.4. SLNB

The patient underwent modified radical mastectomy or breast-conserving surgery. Methylene blue was injected into the subcutaneous tissue above the nipple and outside the areola. After massaging for 3–5 minutes, 5% ICG was injected at the upper and outer points of the areola. The infrared searcher could detect a luminous lymphatic vessel that continued until it disappeared in the armpit. It found the blue-stained lymphatic vessels and luminous lymph nodes, which were identified as sentinel lymph nodes. Care was taken to avoid damaging the light-stained lymphatic vessels during the operation. The SLN was removed, and the lymph nodes that had been imaged were confirmed by an infrared searcher and sent for frozen pathology.

2.5. Experimental result evaluation criteria

Clinical evaluation criteria: The breast tumors were evaluated using the RECIST criteria [6], which were divided into complete response, partial response, stable disease, and progressive disease. The ALNs were evaluated based on the American Joint Committee on Cancer (AJCC), 8th Edition standards; cTNM staging and ypTNM staging were used for pre-NAC and post-NAC clinical staging, respectively.

Pathological evaluation standards: pCR is defined as the absence of invasive cancer components in the lesion, with only vascular fibrous tissue stromal components remaining, or only ductal carcinoma *in situ*. The pCR of ALN is defined as the absence of cancer in the ALN upon pathological examinations.

2.6. SLN evaluation criteria

The evaluation standard of Louisville University in the United States was adopted for SLN [8]. The detection rate (DR), accuracy, false negative rate (FNR), and sensitivity of SLNB were calculated.

$$Sensitivity\ (\%) = \frac{Number\ of\ positive\ SLN\ cases}{Number\ of\ positive\ ALN\ cases} \times 100\% \quad (1)$$

$$Accuracy\ (\%) = \frac{True\ positive\ SNL\ cases + number\ of\ true\ negative\ cases}{Total\ number\ of\ SNL\ cases} \times 100\% \quad (2)$$

$$FNR\ (\%) = \frac{Number\ of\ false\ negative\ SLN\ cases}{True\ positive\ SLN\ cases + number\ of\ false\ negative\ cases} \times 100\% \quad (3)$$

$$\mathrm{DR}\ (\%) = \frac{\text{Number of detected cases}}{\text{Total number of cases}} \times 100\% \quad (4)$$

2.7. Statistical analysis

Statistical analysis was performed using SPSS 26.0. Classified data were described by relative numbers, including age, terminal illness, affected breast, BMI, tumor stage before NAC, ALN stage before and after NAC, ALN status before and after NAC, clinical stage, molecular classification, KI-67 expression, mass pCR, medication regimen and cycle, surgical plan, number of SLN detected. Other factors were subjected to a single-factor chi-square test or precise probability test. Factors such as mass pCR, number of detected SLN, and clinical stage that were statistically significant ($P < 0.05$) in the univariate analysis were included in the multivariate logistic regression equation analysis, and OR and CI 95% were used, with $\alpha = 0.05$ (two-tailed).

3. Result

3.1. SLN metastasis

Among the 116 breast cancer patients who underwent SLNB, sentinel lymph node (SLN) was not detected in 1 case, indicating a detection rate of 99.13% (115/116); 35 cases were positive for ALN and 22 cases were positive for SLN, indicating a sensitivity of 62.86 % (22/35); SLNB was successfully performed in 115 cases, indicating an accuracy of 73.91 % (85/115); 7 cases were false negative, and 35 cases were ALN positive, indicating a false negative rate of 37.14 % (13/35).

3.2. Correlation between SLN metastasis and pathological features

There was a significant correlation between whether pCR was achieved and the number of SLN detected ($P < 0.05$). Other factors showed no correlation with the FNR of SLN ($P > 0.05$). There was a significant correlation between the pre-NAC tumor stage, whether PCR was achieved, the number of SLN detected, and the accuracy of SLN detection ($P < 0.05$). There was no correlation between other factors and the accuracy of SLN detection ($P > 0.05$). Further details are shown in **Table 1**.

Table 1. Relationship between clinical and pathological factors in patients with breast cancer after NAC and the efficiency of double-tracer SLNB

Item	Accuracy (% [*n*/*N*])	χ^2	*P*	FNR (% [*n*/*N*])	χ^2	*P*
Age (years)		0.001	0.985		–	0.480*
< 50	74 (37/50)			46.15 (6/13)		
≥ 50	73.85 (48/65)			31.82 (7/22)		
Pre-NAC tumor staging (stage)		–	0.935		–	0.545*
cT1	77.78 (14/18)			33.33 (1/3)		
cT2	72.53 (66/91)			41.38 (12/29)		
cT3	80 (4/5)			0 (0/3)		
cT4	100 (1/1)			-		
Pre-NAC lymph node staging (stage)		4.064	0.131		–	0.065*
cN0	78.82 (67/85)			28 (7/25)		
cN1	56.25 (9/16)			100 (3/3)		
cN2	64.29 (9/14)			42.86 (3/7)		
Post-NAC lymph nodes		2.832	0.243		–	0.249*
ypN1	76.47 (78/102)			32.26 (10/31)		
ypN2	57.14 (4/7)			100 (2/2)		
ypN3	50 (3/6)			50 (1/2)		
Lymph node status before and after NAC		12.171	0.095		–	0.064*
cN0–ypN0	79.76 (67/84)			25 (6/24)		
cN0–ypN1	0 (0/1)			100 (1/1)		
cN1–ypN0	44.44 (4/9)			100 (2/2)		
cN1–ypN1	80 (4/5)			100 (1/1)		
cN1–ypN2	50 (1/2)			-		
cN2–ypN0	77.78 (7/9)			40 (2/5)		
cN2–ypN1	0 (0/1)			-		

Table 1. (Continued)

Item	Accuracy (% [*n*/*N*])	χ^2	*P*	FNR (% [*n*/*N*])	χ^2	*P*
cN2–ypN2	50 (2/4)			50 (1/2)		
Left and right breasts		0.789	0.375		–	0.733*
Left	77.78 (42/54)			33.33 (6/18)		
Right	70.49 (43/61)			41.18 (7/17)		
Menopause		0.746	0.388		–	0.055*
No	71.43 (55/77)			48 (12/25)		
Yes	78.95 (30/38)			10 (1/10)		
Stage		11.565	0.041		–	0.176*
I	80 (12/15)			33.33 (1/3)		
IIA	79.17 (57/72)			28.57 (6/21)		
IIB	36.36 (4/11)			100 (3/3)		
IIIA	61.54 (8/13)			50 (3/6)		
IIIB	100 (1/1)			0 (0/1)		
IIIC	100 (3/3)			0 (0/1)		
Molecular typing		2.472	0.480		–	0.234*
A	72.73 (8/11)			66.67 (2/3)		
B	68.75 (11/16)			37.5 (3/8)		
TNBC	81.25 (39/48)			16.67 (2/12)		
HER-2 overexpression	67.5 (27/40)			50 (6/12)		
HER-2 expression		2.639	0.104		–	0.220*
Negative	68.18 (45/66)			42.86 (12/28)		
Positive	81.63 (40/49)			14.29 (1/7)		
KI-67 expression		0.427	0.514		–	1
Low	69.7 (23/33)			38.46 (5/13)		
High	75.61 (62/82)			36.36 (8/22)		
Mass pCR		13.530	< 0.001		–	0.001*
No	58.18 (32/55)			68.75 (11/16)		
Yes	88.33 (53/60)			10.53 (2/19)		
Plan		1.927	0.382		–	0.302*
TP	70.97 (44/62)			50 (9/18)		
TCBHP	90.91 (10/11)			20 (1/5)		
other	73.81 (31/42)			25 (3/12)		
Number of cycles		0.014	0.904		–	0.519*
< 6	66.67 (6/9)			0 (0/2)		
≥ 6	74.53 (79/106)			39.39 (13/33)		
Surgical plan		0.022	0.882		–	0.164*
Breast conservation	74.55 (41/55)			23.53 (4/17)		
Improvement	73.33 (44/60)			50 (9/18)		
Number of SLN detections		13.638	0.003		–	0.015*

Table 1. (Continued)

Item	Accuracy (% [*n/N*])	χ^2	*P*	FNR (% [*n/N*])	χ^2	*P*
0	33.33 (4/12)			75 (6/8)		
1	60 (6/10)			50 (4/8)		
2	72.41 (21/29)			28.57 (2/7)		
≥ 3	84.38 (54/64)			8.33 (1/12)		
BMI		5.810	0.121		–	1*
Underweight	100 (2/2)			–		
Normal	81.25 (52/64)			35.29 (6/17)		
Overweight	63.64 (21/33)			40 (4/10)		
Obese	62.5 (10/16)			37.5 (3/8)		

Note: *Exact probability test

3.3. The relationship between the FNR of SLN and the number of detections

After stratifying by the number of detected Sentinel Lymph Nodes (SLNs), among 8 patients with zero detected SLNs, the false-negative rate of Sentinel Lymph Node Biopsy (SLNB) was 75% (6/8). For 8 patients with one detected SLN, the false-negative rate of SLNB was 50% (4/8). Among 7 patients with two detected SLNs, the false-negative rate of SLNB was 28.57% (2/7). For 12 patients with three or more detected SLNs, the false-negative rate of SLNB was 8.33% (1/12).

3.4. The relationship between the FNR of SLN and the rate of pCR

After stratifying according to whether the pCR was achieved, the FNR of SLNB among the 19 patients with pCR was 10.53% (2/19), and the false negative rate of SLNB among the 16 patients with non-pCR was 68.75% (11/16).

3.5. Multivariate logistic regression analysis on FNR

The statistically significant factors in the single-factor analysis were included in the multivariate logistic regression analysis. Definition and assignment of variables in the regression equation: The dependent variable was whether there was a false negative in SLN (Yes = 1, No = 0), and the independent variables included the number of SLN detections (0, 1, 2 and ≥ 3) and whether there was a mass pCR (No = 1, Yes = 0). The results revealed that in comparison to mass pCR, mass non-pCR exhibited an OR of 14.257, with a 95% CI ranging from 1.884 to 107.894. Moreover, in contrast to the number of SLN detections (0), the number of SLN detections (≥ 3) displayed an OR of 0.046, with a 95% CI of 0.02 to 0.863. Therefore, this indicates that the above indicators were related to the false negative detection of SLNB after breast cancer NAC (**Table 2**).

Table 2. Multivariate logistic regression analysis of clinical and pathological factors in patients with breast cancer after NAC and false-negative SLNB using double-tracer method

Index	B	SE	Wals	*P-value*	OR	95% CI
Mass PCR	2.657	1.033	6.622	0.010	14.257	1.884–107.894
SLN detections (0)	0.000		4.279	0.233	1.000	
SLN detections (1)	-1.128	1.294	0.760	0.383	0.324	0.026–4.087
SLN detections (2)	-1.285	1.399	0.843	0.358	0.277	0.018–4.295
SLN detections (≥ 3)	-3.072	1.492	4.239	0.040	0.046	0.002–0.863

3.6. Multivariate logistic regression analysis on accuracy

The statistically significant factors in the single-factor analysis were included in the multifactor binary logistic regression equation analysis. Definition and assignment of variables in the regression equation: The dependent variable was whether the diagnosis of SLN was accurate (No = 0, Yes = 1), the independent variables included the number of SLN detected (0, 1, 2, and 3), whether mass pCR was achieved (No = 0, Yes = 1) and stage (I, IIA, IIB, IIIA, IIIB, and IIIC). The results indicated that, compared to non-PCR tumors, the OR for PCR-positive tumors was 7.87495%, with a CI of 1.595 to 13.869. When comparing cases with zero detected SLNs to cases with three or more detected SLNs, the OR was 9.04, with a 95% CI of 1.764 to 46.33. The above indicators are related to the detection accuracy of SLNB after NAC for breast cancer (**Table 3**).

Table 3. Multivariate logistic regression analysis of clinical and pathological factors and the accuracy of SLNB using dual-tracer method in patients with breast cancer after NAC

Index	*B*	*SE*	*Wals*	*P-value*	*OR*	*95%CI*
Mass pCR	1.630	0.536	9.249	0.002	5.103	1.785–14.590
SLN detections (0)	0.000		8.193	0.042	1.000	
SLN detections (1)	1.309	1.051	1.552	0.213	3.701	0.472–29.013
SLN detections (2)	1.165	0.895	1.693	0.193	3.206	0.554–18.542
SLN detections (≥ 3)	2.202	0.834	6.973	0.008	9.04	1.764–46.33
Installment (I)	-20.12	21514.617	0.001	0.999	0.001	0.001–0.001
Stage (IIA)	-20.845	21514.617	0.001	0.999	0.001	0.001–0.001
Installment (IIB)	-19.361	21514.617	0.001	0.999	0.001	0.001–0.001
Stage (IIIA)	-21.407	21514.617	0.001	0.999	0.001	0.001–0.001
Stage (IIIB)	-40.718	45588.964	0.001	0.999	0.001	0.001–0.001
Stage (IIIC)	0.000		2.990	0.701	1.000	

4. Discussion

NAC can reduce the number of tumor cells in breast cancer patients and the size of the primary tumor. Besides, it also lowers the tumor stage, allowing patients to have more surgical opportunities and a higher success rate of breast conservation [1]. NAC can also be used to evaluate the effectiveness of treatment and further improve the pCR. SLN can act as an indicator to assess the status of ALN, and patients with negative results can avoid undergoing ALN dissection, reducing surgical complications and improving patients' postoperative quality of life [9]. An SLNB185 prospective clinical study [10] from Italy confirmed that SLNB can safely replace ALND in SLN-negative patients. This trial prospectively enrolled 516 patients with early breast cancer. The results of a median follow-up of 64.6 months showed no statistically significant difference in breast cancer-related events, including ALN recurrence, distant metastasis, and mortality in the SLNB and ALND groups. Subsequently, a series of prospective, multicenter, large-sample clinical trials such as ALMANAC, NSABP-B32, and ACOSOG Z0010 [11-13] have confirmed that patients with negative SNL have the same local symptoms after SLNB compared with ALND as well as recurrence rate, disease-free survival rate, and overall survival rate. The research results established the standard treatment status of SLNB in patients with clinically negative ALN early breast cancer. However, there is still controversy about whether SLNB using a dual-tracer method after NAC can avoid axillary lymph node dissection.

The SLNB detection rate using the double tracer method was 99.13%. However, the FNR was 37.14 %, which was higher than the acceptable clinical standard of 10% [14]. Some foreign multicenter prospective studies have evaluated the accuracy and feasibility of SLNB after NAC. The ACOSOG Z1071 and SENTINA experiments showed that the accuracy of sentinel lymph nodes after neoadjuvant chemotherapy in patients with positive axillary lymph nodes was low, and the false negative rate was too low [15-16]. The results of ACOSOG Z1071 clinical trial showed that the detection and FNR of SLNB were 92.9% and 12.6%, respectively. In the SNFNAC study of 153 patients, the FNR of SLNB was 13.3%, which was not within the ideal range of ≤ 10% [14]. Therefore, the results of these multicenter prospective trials do not support SLNB as a routine evaluation method for ALN after NAC. However, when the dual-tracer method is used, the SENTINA study results showed that the SLNB detection rate increased to 87.8% and the FNR dropped to 8.6%; the FNRs when detecting 1 and 2 SLNs were 24.3% and 24.3%, respectively; and 18.5% when more than 2 SLNs were obtained, with an FNR of 4.9% [15]. Besides, the ACOSOG Z1071 clinal trial also showed that the detection rate of SLNB using the double-tracer method was 93.8%, while the FNR was 10.8%, which is an acceptable level [17]. These experiments used the dual-tracer method combined with radionuclides, but hospital qualifications and other reasons limited the implementation of the radionuclide tracer method. In addition, using metal marking clips to mark ALN that have been confirmed to have metastases before NAC is beneficial to assessing marked ALN after NAC and improves the accuracy of ALN assessment. ACOSOG Z1071 experiment marked 203 patients with positive ALNs. The results showed that when the marking clip was located in the sentinel lymph node, the false negative rate was 6.8%. When the marking clip was located outside the sentinel lymph node, the false negative rate increased to 19.0% [18]. Therefore, it can be seen that after NAC, many factors are involved to reduce the FNR of SLNB to 10%. Our country is relatively conservative in exempting ALN dissection compared to foreign countries. The “Standardized Operating Guidelines for Sentinel Lymph Node Biopsy in Breast Cancer (2022 Essential Edition)” [19] recommends ALND to be performed on patients who are still cN+ after NAC. For patients initially confirmed with positive axillary lymph node metastasis, who, after neoadjuvant NAC, have downgraded to clinically negative axillary lymph nodes, the performance of SLNB should meet the following conditions: before neoadjuvant therapy, positive lymph nodes need to be marked and identified through biopsy, or SLNB should be conducted using dual tracers (preferably radioactive isotope + dye) with the detection of three or more sentinel lymph nodes. This procedure, after thorough discussion with the patient, is employed to guide subsequent decisions regarding axillary lymph nodes. After full communication with the patient, it will be used to guide subsequent ALN-related issues. This study also drew relevant conclusions about the number of SLN detected.

As the usage of radionuclide combined dye method is restricted in some hospitals due to poor nuclide availability, high cost, and high risk of radiation exposure, this study used ICG fluorescence combined with methylene blue as tracers. Methylene blue is low-cost, safe, and easy to use. However, its success rate is lower than the nuclide method or the combination of the two. ICG has been proven to be a safe tracer [20]. A study by Kitai *et al.* in Japan [21] reported that ICG fluorescence imaging can be used in SLNB technology to allow real-time lymphography and guide the direction of lymphatic vessels, and it causes less adverse reactions and is sensitive and non-radioactive. The report of Vermersch [22] suggested that when the two are combined, the risk of methylene blue allergy will not increase.

Further exploration is necessary regarding the clinical application of dual-tracer SLNB following NAC for breast cancer. This exploration aimed to ascertain if it could potentially serve as a standard treatment method, obviating the need for ALND after NAC in breast cancer patients. However, this study has certain limitations. The surgeries were performed by a single operator with substantial clinical experience, possibly introducing

bias errors. Additionally, the sample size was limited, so multicenter, large-sample randomized and controlled trials will be needed to validate the experimental outcomes observed in this study.

5. Conclusion

In summary, SLNB showed a high FNR based on the results of this study, so it cannot wholly replace ALND after NAC in breast cancer patients. Nevertheless, when three or more SLN were detected or when the breast mass achieved a pCR, SLNB exhibited accuracy in predicting the local status of ALNs. However, the accuracy of these findings must be further validated through randomized clinical trials with larger sample sizes.

6. Prospects

SLN hold significant importance in breast surgery, playing a pivotal role in assessing ALNs. Advancements in imaging, biopsy technology, artificial intelligence, and big data have enabled precise evaluation of the lymph node status. With these advancements, companies can devise comprehensive and personalized plans, ensuring accurate regional treatment tailored to the condition of axillary lymph nodes following NAC.

Disclosure statement

The author declares no conflict of interest.

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