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Factors that affect the false negative rate of sentinel lymph node mapping with methylene blue dye alone in breast cancer

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Abstract

Objective: This study aimed to investigate the clinicopathological factors of the false negative rate (FNR) and accuracy of sentinel lymph node biopsy (SLNB) mapping with 1% methylene blue dye (MBD) alone, and to examine how to reduce the FNR in patients with breast cancer.

Methods: A total of 365 patients with invasive breast carcinoma who received axillary lymph node dissection after SLNB were retrospectively analyzed. SLNB was performed with 2 to 5 mL of 1% MBD. We studied the clinicopathological factors that could affect the FNR of SLNB.

Results: The identification rate of sentinel lymph nodes (SLNs) was 98.3% (359/365) and the FNR of SLNB was 10.4% (16/154). Multivariate analysis showed that the number of dissected SLNs and metastatic lymph nodes were independent predictive factors for the FNR of SLNB. The FNR in patients with 1, 2, 3, and $\geq$4 SLNs was 23.53%, 15.79%, 3.85%, and 1.79%, respectively.

Conclusions: SLNB mapping with MBD alone in patients with breast cancer can produce favorable identification rates. The FNR of SLNB decreases as the number of SLNs rises. Because of side effects of searching for additional SLNs and the FNR, removal of three or four SLNs may be appropriate.

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Breast neoplasm, false negative rate (FNR), sentinel lymph node biopsy (SLNB), methylene blue, metastasis, axillary lymph node dissection

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Background

Breast cancer is listed is the most common malignant tumor worldwide. The incidence of breast cancer has increased over the last 30 years. The age standardized incidence rate of breast cancer in China has reached 23.2/100,000, and the age standardized death rate of breast cancer in China has approached 4.9/100,000.[1] Axillary lymph node (ALN) metastasis is an important indicator for deciding on the prognosis and treatment for patients with breast cancer. When a patient with invasive breast cancer is treated surgically, axillary lymph node dissection (ALND) is a critical procedure of the treatment and it is also the most accurate technique for assessing the metastasis status of axillary lymph nodes (ALNs). However, ALND can lead to post-operative morbidities, such as sensory loss, lymphedema, seroma formation, and limited mobility. This worsens the patients' quality of life and results in pain. Higher awareness of breast cancer prevention and new methods of treatment lead to a higher proportion of early-stage patients. The tumor burden of regional lymph nodes has also been reduced,[2,3] which reduces the number of patients who require axillary surgery. In the 1990s, ALND was replaced by a new technique termed sentinel lymph node biopsy (SLNB) in breast cancer, and the guidance measure of "axillary-conserving" was taken.

SLNs are the first lymph nodes that receive lymphatic drainage from a tumor. Theoretically, they are also the first lymphatic sites of metastasis. SLN-negative patients can avoid ALND, which can reduce the incidence of complications of ALND and does not increase the risk of axillary recurrence.[4,5] However, surgeons and patients are still concerned about the false negative results when performing SLNB instead of ALND. A higher false negative rate (FNR) leads to a physical and psychological burden for patients, which greatly restricts the popularity of SLNB. Consequently, an increasing amount of studies are being undertaken to minimize the FNR of SLNB as much as possible.[6,7]

There are three common mapping methods for SLNB in breast cancer, including the use of blue dye tracer (e.g., isosulfan blue, methylene blue, and patent blue dye), use of radioisotope tracer, and dual staining of blue dye combined with a radioisotope.[8,9] Previous studies have successfully applied 1% methylene blue dye (MBD) in SLNB for breast cancer.[10–18] However, several studies have reported that the combination method of blue dye with radioisotopes is much better than blue dye alone for identification of SLNs.[19–21] Unfortunately, this method is costly and rarely adopted in China because many hospitals currently have no qualifications to provide radionuclide drugs and instruments.[22] MBD overcomes the restriction of radionuclide imaging with specialized equipment in primary hospitals, and also overcomes the disadvantages of the expensive price and difficult source of patent blue and isosulfan

blue. At present, a hierarchical medical system has been set up to improve services at county and township-level health centers, especially in less developed areas in China. Because of this hierarchical medical system, an increasing number of patients with early-stage breast cancer are confined to primary hospitals for treatment in less developed areas. Based on the advantages of MBD, it has been the only option for localizing SLNs in primary hospitals in China. This study aimed to investigate the identification rate and the FNR by applying 1% MBD alone to map SLNs, and to examine the clinicopathological factors affecting the FNR of SLNB.

Methods

Patients

In our study, female patients with early breast cancer were retrospectively enrolled during January 2009 to December 2012 in Beijing Friendship Hospital. Four surgeons (ZCG, XQ, JZ, and LH) participated in this research. GZC, QX, and ZJ have more than 10 years and LH has more than 8 years of experience in performing breast cancer surgery.

The inclusion criteria were as follows: (i) the patient had primary breast cancer; (ii) cN0 cases were confirmed through axillary palpation and imaging examinations, such as mammography or a breast ultrasound examination; (iii) the patient had no history of axillary surgery or radiotherapy; (iv) the patient had no preoperative chemotherapy; and (v) the patient agreed to undergo ALND after SLNB. The exclusion criteria were as follows: (i) the patient had a history of axillary surgery; (ii) the patient had breast cancer in her pregnancy or lactation; (iii) the patient had a history of neoadjuvant chemotherapy or radiotherapy; and (iv) the pathological type was ductal carcinoma *in situ*.

This study was approved by the Ethics Committee of Beijing Friendship Hospital and all patients signed an informed consent form.

Surgical procedure and pathological evaluation

Lymphatic mapping for tracing SLNs was performed using 1% MBD. After successful anesthesia, 2 to 5 mL of MBD was injected into the subareolar area, after which the breast was massaged for 10 minutes. Blue nodes or lymph nodes with lymphatic blue channels were identified as sentinel nodes. The procedure of ALND was performed at levels I to II. When suspicious lymph node metastases were found at level II, then ALND was continued at level III. Slices of nodes were parallel to the longitudinal axis and were not thicker than 2 mm. Intraoperative frozen section analyses was routinely performed for every harvested SLN, which was classified into negative or positive for metastases. Furthermore, all nodes were formalin fixed and embedded in paraffin. Sections were stained with hematoxylin–eosin postoperatively. Sentinel node metastases were classified according to the American Joint Committee on Cancer[23] as isolated tumor cells (cell clusters or a single cell no larger than 0.2 mm), micro-metastasis (tumor deposits between 0.2 and 2.0 mm), and macro-metastasis (tumor deposits >2 mm). The tumors were classified according to the World Health Organization Histological Classification of Breast Cancers, and grading was defined by reference to Elston and Ellis modification.[24] Invasive breast cancer was classified by the 2013 St. Gallen Consensus,[25] and was divided into molecular subtypes as follows. A luminal A-like tumor was estrogen receptor (ER)-positive and human epidermal growth factor receptor 2 (HER2)-negative, with Ki-67 expression $< 14\%$ and high progesterone

receptor (PR) levels (≥20%). A luminal B-like HER2-negative tumor was ER-positive and HER2-negative, with Ki-67 expression ≥14% and low PR levels (<20%). A luminal B-like HER2-positive tumor was ER-positive and HER2-positive. An HER2-positive tumor was HER2-positive, PR-negative, and ER-negative. A triple-negative tumor was HER2-negative, PR-negative, and ER-negative.

Adjuvant therapy

All participants were treated in compliance with National Comprehensive Cancer Network Guidelines. Patients with breast conservation surgery or positive lymph nodes were treated by radiotherapy. Taxanes and anthracyclines were used in chemotherapy regimens. Aromatase inhibitors and tamoxifen were used in hormone therapy. HER-2-positive patients were treated with adjuvant trastuzumab therapy.

Statistical analysis

SPSS 19.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Fisher's exact test and the chi square test were used for group comparisons. Risk factors for the FNR and accuracy rate in SLNB were evaluated with logistic regression analysis. A P value < 0.05 was considered statistically significant. Evaluation of results was performed by the SLNB technical standard of Louisville University.[26] The degree of metastasis was calculated as the number of metastatic lymph nodes divided by the number of lymph nodes and then multiplied by 100%. The identification rate was calculated as the number of identified SLNs divided by the number of patients with the treatment of SLNB and then multiplied by 100%. The FNR was calculated as the number of false negative cases divided by the number of false negative plus true positive cases and then multiplied by 100%. Sensitivity was calculated as the number of true positive cases divided by the number of false negative plus true positive cases and then multiplied by 100%. The accuracy rate was calculated as the number of true positive plus true negative cases divided by the number of patients with the treatment of SLNB and then multiplied by 100%. The negative predictive value was calculated as the number of true negative cases divided by the number of false negative plus true negative cases and then multiplied by 100%.

Results

Patients' characteristics

We reviewed 365 patients from January 2009 and December 2012, but SLNs could not be found in six patients. The final analysis included 359 patients. The patients' clinicopathological characteristics are shown in Table 1. The mean (±standard deviation) age of the patients was 54.97 ± 11.09 years old (range, 30–83 years old). The mean size of pathological tumors was 2.53 ± 1.28 cm (range, 0.6–5.0 cm).The diagnoses included 333 patients with invasive ductal carcinoma, 14 with invasive lobular carcinoma, six with invasive papillary carcinoma, and six with mucinous carcinoma. The molecular subtypes are shown in Table 1. A total of 250 (69.6%) patients received breast-conserving surgery and 109 (30.4%) received mastectomy. No skin necrosis was observed, but blue tattooing of the skin was found in 30 patients. No patients had systemic anaphylactic reactions.

Identification of SLNs using 1% MBD and mapping of axillary SLNs

Of the 365 patients, 359 had SLNs, with an identification rate of 98.3% (359/365).

Table 1. Tumor pathology and patients' characteristics.

Characteristics	Number of patients (%)
Age, years	
≤40	33 (9.2)
>40	326 (90.8)
Menstruation status	
Premenopause	135 (37.6)
Postmenopause	224 (62.4)
Body mass index, kg/m^2	
<30	321 (89.4)
≥30	38 (10.6)
Tumor location	
Outer upper quadrant	173 (48.2)
Inner upper quadrant	95 (26.5)
Outer lower quadrant	50 (13.9)
Inner lower quadrant	32 (8.9)
Central region	9 (2.5)
Side of the tumor	
Left	183 (50.9)
Right	176 (49.1)
Pathological type	
IDC	333 (92.7)
ILC	14 (3.9)
Mucous carcinoma	6 (1.7)
Other	6 (1.7)
Tumor size (mm)	
<20	195 (54.3)
≥20 and ≤ 50	162 (45.1)
>50	2 (0.6)
Estrogen receptor	
Positive	270 (75.2)
Negative	89 (24.8)
Progesterone receptor	
Positive	232 (64.6)
Negative	127 (35.4)
Ki-67 expression levels	
<14%	57 (15.9)
≥14%	302 (84.1)
HER2 state	
Positive	143 (39.8)
Negative	216 (60.2)
Histological grade	
I	46 (12.8)
II	249 (69.4)
III	64 (17.8)
Lymphovascular invasion	
Present	28 (7.8)
Absent	331 (92.2)
Multifocality	
Multifocal	19 (5.3)
Unifocal	340 (94.7)
Molecular subtypes	
Luminal A	49 (13.6)
Luminal B	220 (61.3)
HER2 positive	51 (14.2)
Triple negative	39 (10.9)
Number of dissected SLNs	
1	80 (22.2)
2	88 (24.5)
3	62 (17.4)
≥4	129 (35.9)
Number of metastatic lymph nodes	
0	205 (57.2)
≤3	119 (33.1)
≥4 and ≤9	26 (7.2)
≥10	9 (2.5)

ILC, invasive lobular carcinoma; IDC, invasive ductal carcinoma; HER2, human epidermal growth factor 2; SLNs, sentinel lymph nodes.

Of these 359 patients, 154 (42.9%) had ALN metastasis and 205 (57.1%) were without ALN metastasis. Among 154 patients with ALN metastases, SN metastases were negative in 16 patients, with an FNR of 10.4% (16/154). The accuracy rate was 95.6% (343/359), the negative predictive value was 92.8% (205/221), and the specificity was 100% (205/205). The number of dissected SLNs for 359 patients was 1125, and the mean number of SLNs was 3.13 ± 1.98 (range, 1–10) (Table 2). The number of SLN metastases was 196 and the degree of metastasis was 17.42% (196/1125). Only one sentinel lymph node was dissected in 80 patients. A total of 34 of these patients developed ALN metastasis, of whom 12 patients developed SLN

Table 2. ALN metastasis state of 359 patients who underwent SLNB.

	Non-SLN status		
SLN status	(+)	(−)	Total
(+)	50	88	138
(−)	16	205	221
Total	66	293	359

SLN, sentinel lymph node; ALN, axillary lymph node; non-SLN, non-sentinel lymph node; SLNB, sentinel lymph node biopsy.

and non-SLN metastasis, eight patients developed non-SLN metastasis with SLN-negative, and 14 developed SLN metastasis with non-SLN negative. A total of 46 of these patients did not develop SLN and non-SLN metastasis. Two sentinel lymph nodes were dissected in 88 patients. Among these patients, 38 developed ALN metastasis. Furthermore, 15 patients developed SLN and non-SLN metastasis, six developed non-SLN metastasis with SLN-negative, and 17 developed SLN metastasis with non-SLN negative. A total of 50 of these patients did not develop SLN and non-SLN metastasis. Three sentinel lymph nodes were dissected in 62 patients. A total of 26 of these patients developed ALN metastasis. Among these patients, 10 developed SLN and non-SLN metastasis, one developed non-SLN metastasis with SLN-negative, and 15 developed SLN metastasis with non-SLN negative. A total of 36 of these patients did not develop SLN and non-SLN metastasis. Four sentinel lymph nodes were dissected in 129 patients. A total of 56 of these patients developed ALN metastasis. Among these patients, 13 developed SLN and non-SLN metastasis, one developed non-SLN metastasis with SLN-negative, and 42 developed SLN metastasis with non-SLN negative, A total of 73 of these patients did not develop SLN and non-SLN metastasis. The number of dissected non-SLNs in all of the 359 patients was 5078, and the mean number of dissected non-SLNs was 14.14 ± 5.64 (range, 6–30). The total number of non-SLN metastasis was 259 and the degree of metastasis was 5.1% (260/5078).

Association between the FNR and accuracy rate of SLNB and clinicopathological features

In univariate analysis, the accuracy rate and FNR of SLNB were not significantly associated with factors, such as body mass index, age, tumor location, pathological type, histological classification, molecular subtype, ER state, PR state, HER2 state, menstruation status, Ki-67 protein levels, or other relevant factors (Table 3). The FNR of SLNB was significantly associated with tumor size ($P = 0.048$), the number of dissected SLNs ($P = 0.005$), and the number of metastatic lymph nodes ($P = 0.001$).

In multivariate analysis, the number of dissected SLNs ($P = 0.005$) and metastatic lymph nodes ($P = 0.022$) still remained significant independent risk factors for the FNR (adjusted odds ratio of 0.461 [95% confidence interval, 0.269 to 0.790], 2.801 [95% confidence interval, 1.159 to 6.771], respectively) (Table 4).

Discussion

Management of early breast cancer has shifted over time to conservative treatment. ALND has been replaced by SLNB for judging axillary staging.[27,28] Large-sample, prospective, randomized, controlled trials, such as the ALMANAC, SNB-185, and NSABP B-32 studies, showed that SLNB could accurately predict ALN staging.[29–31] Previous studies showed that differences in overall survival, disease-free survival, and recurrence-free survival between the only SLNB group and ALND group for SLN-negative patients with breast cancer were

Table 3. Comparison of clinicopathological characteristics between the FNR and accuracy rate for SLNB.

Characteristic	n	FNR (%)	n	Accuracy rate (%)	χ^2	P
Age, years					0.000	1.000
≤40	1	8.33	32	96.97		
>40	15	10.56	311	95.40		
Menstruation status					0.288	0.591
Premenopause	5	8.62	130	96.30		
Postmenopause	11	11.46	213	95.09		
Body mass index (kg/m^2)					0.000	1.000
<30	14	10.00	307	95.64		
≥30	2	14.29	36	94.74		
Tumor location					0.436	0.509
Outer upper quadrant	9	10.98	164	94.80		
Other	7	9.72	179	96.24		
Side of the tumor					1.217	0.270
Left	6	7.32	177	96.72		
Right	10	13.9	166	94.5		
Pathological type					–	0.325*
IDC	14	10.00	319	95.80		
ILC	1	16.67	13	92.86		
Other	1	12.50	11	91.67		
Tumor size (mm)					3.913	0.048
≤20	14	16.87	217	93.94		
>20	2	2.82	126	98.44		
Estrogen receptor					0.076	0.782
Negative	3	11.11	86	96.63		
Positive	13	10.24	257	95.19		
Progesterone receptor					0.789	0.375
Negative	4	10.00	123	96.85		
Positive	12	10.53	220	94.83		
Ki-67 expression levels					0.530	0.467
<14%	1	3.26	56	98.25		
≥14%	15	12.19	287	95.03		
HER2 state					0.722	0.395
Positive	8	10.26	135	94.41		
Negative	8	10.53	208	96.29		
Histological grade					0.772	0.680
I	1	5.26	45	97.82		
II	12	11.88	237	95.18		
III	3	8.82	61	95.31		
Lymphovascular invasion					1.426	0.232
Present	3	15.79	25	89.29		
Absent	13	9.5	318	96.07		
Multifocality					–	0.589*
Multifocal	1	9.09	18	94.74		
Unifocal	15	10.34	325	95.59		

(continued)

Table 3. Continued.

Characteristic	n	FNR (%)	n	Accuracy rate (%)	χ^2	P
Molecular subtypes					5.095	0.165
Luminal A	1	4.17	48	97.95		
Luminal B	12	11.88	208	94.55		
HER2 positive	3	17.65	48	94.12		
Triple negative	0	0	39	100		
Number of dissected SLNs					13.048	0.005
1	8	23.53	72	90.00		
2	6	15.79	82	93.18		
3	1	3.85	61	98.39		
≥ 4	1	1.79	128	99.22		
Number of metastatic lymph nodes					–	0.001*
≤ 3	10	8.26	316	96.93		
≥ 4 and ≤ 9	6	25.0	19	76.0		
≥ 10	0	0	8	100		

*Fisher's exact test. FNR, false negative rate; SLNB, sentinel lymph node biopsy; ILC, invasive lobular carcinoma; IDC, invasive ductal carcinoma; HER2, human epidermal growth factor 2; SLNs, sentinel lymph nodes.

Table 4. Multivariate analysis for risk factors of the false negative rate in sentinel lymph node biopsy.

Variables	Odds ratio	95% CI	P
Number of metastatic lymph nodes	2.801	1.159–6.771	0.022
Number of dissected SLNs	0.461	0.269–0.790	0.005
Tumor size	0.223	0.047–1.050	0.058

CI, confidence interval; SLNs, sentinel lymph nodes.

significant.[3,32] However, SLNB instead of ALND is based on a lower false negative rate. The method for mapping SLNs in breast cancer is one of the main factors affecting the identification rate and FNR. The technique of dual localization with a radioisotope and blue dye is effective for mapping of SLNs, and the FNR is 5% to 10%.[33,34] After 24 studies[35] were reviewed systematically, the authors found that the combination of a radioisotope with blue dye resulted in a higher identification rate than that for applying radioisotope alone, but the FNR was not significantly reduced. The technique of dual localization with a radioisotope and blue dye is most widely used and is considered as the standard technology for localization of SLNs. Nevertheless, many primary hospitals in China have the obstacle of limited access to patent blue dye, radioisotope tracers, isosulfan blue, and nuclear medicine facilities. Owing to a detection rate of approximately 90.82% to 98.06%,[22] blue dye staining is currently more extensively applied in China. Furthermore, the problem of limited access to patent blue dye was successfully resolved with application of 1% MBD by some researchers.[13,17,36] A previous study showed MBD had the same effects as did isosulfan blue dye when used for identifying SLNs.[37] MBD is cheaper and easier to obtain in many primary hospitals in China and is not related to allergic reactions that are potentially life-threatening.[18] For pregnant patients, applying MBD for mapping

SLNs is safe.[38] Because of the hierarchical medical in China, MBD alone is widely used for localizing SLNs in primary hospitals. A recent meta-analysis by Li et al.[39] concluded that MBD alone for mapping SLNs leads to a better identification rate of 91%, but a higher FNR of 13% according to criteria recommended by the American Society of Breast Surgeons. Therefore, we attempted to only apply 1% MBD for identification of SLNs. To further reduce the FNR of SLNB, related factors need to be comprehensively analyzed.

The American Society of Clinical Oncology reported the results of six trials on SLNB, and their FNRs ranged from 4.6% to 16.7%,[40,41] with an average of 8.4% overall.[30] The American Society of Breast Surgeons formed a team to establish standards for SLNB. This team suggested in 2000 that the SLNB identification rate should be equal to or higher than 85% and that the FNR should be equal to or lower than 5%.[43]

The identification rate of SLNB in our study was 98.3% and the FNR was 10.4%, which are consistent with most previous reports.[10,11] In our study, univariate analysis showed that the FNR and the accuracy of SLNB were related to the size of the tumor, which is consistent with conclusions drawn by many scholars.[43–45] The lymphatic metastasis rate rises in large volume tumors. Lymphatic channels are often clogged by metastatic cancer cells, This leads to a change in the original lymphatic circulation and the imaging agent or radionuclide in the lymphatic vessels is impeded for normal transfer.[33] Moreover, metastatic tumor cells invade SLN for a long time, which leads to a reduction of antigen-induced lymphocyte induction and a decrease in the ability of macrophages to absorb tracers. However, replacement lymph nodes with absorption tracers do not accurately reflect the ALN status.[46] Multivariate analysis in our study showed that the size of the tumor was not related to the FNR of SLNB, which is similar to the findings of Wong et al.[47] This result may be related to the low numbers of participants in this study.

In our study, the number of dissected SLNs was a significant risk factor for the FNR, which is consistent with many studies. Chok et al.[48] found that the FNR of SLNB significantly decreased with an increased number of dissected SLNs ($P < 0.009$). Goyal et al.[33] found that in patients with one dissected SLN and more than two dissected SLNs, the FNR of SLNB was 10.1% and 1.1%, respectively ($P = 0.010$). Yi et al.[49] reported that $> 99\%$ of positive SLNs were discovered when the number of dissected SLNs was up to five. In the present study, the patients were classified according to the number of dissected SLNs into four groups (1, 2, 3, and ≥ 4 SLNs). The FNR of each group was 23.53%, 15.79%, 3.85%, and 1.79%, respectively, with a significant difference among the groups. We found that the FNR of SLNB decreased with an increase in the number of SLNs ($P = 0.005$). When the numbers of dissected SLNs was more than more, the FNR was 1.79%, and the accuracy rate reached 99.2%, which indicated that more than 99% positive SLNs could be found and the FNR of SLN was significantly reduced. Therefore, for patients with a fewer number of detected SLNs, SLNB is a great risk to replace ALND. However, the number of dissected SLNs affects not only FNR results, but also the morbidity of the SLNB procedure.[42,50] For patients with $>$ four dissected SLNs, the status of axillary lymph nodes can be predicted by SLNB. Approximately 99% of nodal-positive patients are confirmed by removing four SLNs, and thus the benefits of searching for additional SLNs are minimal.[49,51] On the basis of a FNR $\leq 5\%$[42] and the side effects of searching for additional SLNs, we suggest that the

appropriate number of removed SLNs is three to four when applying MBD alone.

The differences in the FNR reported worldwide may be related to the proficiency of surgeons. The experience and proficiency level of surgeons greatly affect the accurate localization of SLNs, especially when blue dye is the only choice as the tracer. Cox et al.[52] thought that to ensure high accuracy, surgeons should be asked to perform 20 surgeries at least independently. Snider et al.[53] thought that surgeons should independently perform operations in 45 cases. The surgeons in the present study have approximately 8 to 10 years of experience of SLNB, and have completed hundreds of these procedures every year. Therefore, effective measures for reducing the FNR of SLNB are strictly selecting the indications, standardizing the operation standards, and improving the operation proficiency of the surgeons.

Limitations of this study are that this was a single-center study and a relatively small number of participants were included. Therefore, multicenter studies with large sample sizes are required for further validation.

Conclusions

This study validates the accuracy and feasibility of SLNB mapping with MBD alone. However, the following factors should be taken into account in clinical practice. First, surgeons should take care when only blue nodes are found in patients with large tumors. When applying MBD alone, surgeons should keep searching for suspicious non-blue lymph nodes to eliminate false negative possibilities. Second, the number of removed SLNs needs to be increased to reduce the FNR. The FNR is $\leq$5% when the number of removed SLNs is three to four. However, the number of removed SLNs not only affects FNR results, but also affects the morbidity of the SLNB procedure. Therefore, we suggest that the appropriate number of removed SLNs should be three to four when applying MBD alone in SLNB. Third, the operation level and proficiency of the surgeons should be improved as much as possible to reduce the FNR.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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