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Clinical study of combined application of indocyanine green and methylene blue for sentinel lymph node biopsy in breast cancer

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Abstract

Objective: This study aims to investigate the feasibility of combined application of indocyanine green (ICG) and methylene blue (MB) for sentinel lymph node biopsy (SLNB) in patients with early breast cancer.

Methods: A total of 415 patients who underwent SLNB and axillary lymph node dissection were enrolled. Sentinel lymph node (SLN) was assessed in 197 patients with ICG and MB combination method, while, the other 218 patients were detected by MB method alone. During surgery, all SLNs were harvested for pathological examination. Then the detection rate and false negative rate of SLNs were comparatively analyzed between the 2 groups.

Results: In the combined ICG and MB group, the detection rate of SLNs was 96.9%, significantly higher than that of MB group, which was 89.7% ($P < .05$). Similarly, in combined group, the average number of SLNs per patient was 3.0, much higher than that of MB group, which was 2.1 ($P < .05$). There was no statistically significant difference in false negative rate between combined group and MB alone group, which was 7.3% and 10.5%, respectively ($P = .791$).

Conclusion: The combined application of ICG and MB for SLNB is much more effective than MB alone in detecting SLNs.

Abbreviations: ALND = axillary lymph node dissection, ICG = indocyanine green, MB = methylene blue, SLNB = sentinel lymph node biopsy.

Keywords: breast cancer, fluorescence imaging, indocyanine green, methylene blue, sentinel lymph node biopsy

1. Introduction

The operation method for breast cancer is continuously updated, from the initial radical operation to the modified-radical operation, and then to the breast-conserving therapy.[1,2] The incision of surgery becomes smaller and smaller.[3] Although the prognosis of breast cancer is still not ideal, the survival quality of patients has been significantly improved.[4,5]

Sentinel lymph node biopsy (SLNB) is used to identify lymph nodes that have not been metastasized, which can decrease the damage to patients by avoiding the axillary lymph node dissection (ALND).[6] SLNB has gradually become a standard operating procedure for patients with breast cancer.[7] SLNB not only narrows the scope of surgery, from the previous "major surgery" to a "minor one," but also improves the survival quality of patients.[8,9] The SLNB ensures the physiological function of the affected limb after operation, which markedly improves patient's life quality.[10,11] Till now, the effectiveness of SLNB has already been verified in many clinical studies.[12–14] Krag et al reported a median of 97-months period follow-up study. The local recurrence and survival rate of patients with negative axillary lymph node, showed no significant difference between SLNB and ALND treatment.[15,16]

The conventional detecting method for sentinel lymph node (SLN) includes methylene blue (MB), radio-colloid tracer, and a combination of both. Though the conventional biopsy method shows its effectiveness for SLN, it still has several deficiencies. For example, the MB method has a low detection rate of SLNs (70%–80%).[17] Although the radio-colloid tracer has a high detection rate of SLNs,[18,19] its detection requires a high-level equipment and a specific detector, which are expensive and radioactive; thus, the radio-colloid method is still in the experimental stage in China. To improve the detecting accuracy for SLNB, an advanced technique for SLNB is urgently required.

Recently, indocyanine green (ICG), a near-infrared fluorescent tracer, has gradually been used in detecting SLNB. ICG is a water-

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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soluble probe with a hydrodynamic diameter of 1.2 nm, and its absorption peak in human body is 800 nm, which shows a green peak. ICG has been used in liver function test,[20,21] cardiac output test,[22] and retina angiography.[23] Once ICG is intravenously administrated, it combines with plasma proteins immediately.[24] Then the complex will be further uptaken by liver cells and excreted to the bile with negligible toxicity.[24] Most of ICG for SLNB detection combines with albumin and flows in the lymphatic vessels without exosmosis.[25] This ICG drainage formed a fluorescent circuit under subcutaneous lymph vessels that can be observed by using the fluorescent vascular imaging system.[25] In this manner, the anatomic position of the lymph vessels in the armpit can be identified.

Now the combined application of ICG and MB for SLNB has not been well studied. Hence, in this study, we combined the ICG and MB methods for SLNB in breast cancer, and comparatively analyzed the detecting accuracy of the combined group and the conventional-MB group.

2. Methods

2.1. Patients

This retrospective study included patients with early breast cancer who underwent SLNB and ALND in 2016 between January 1st and December 31st at the Affiliated Tumor Hospital of Xinjiang Medical University. The clinical manifestations of patients included breast pain and palpable mass. Some patients even appeared nipple inversion or hemorrhagic nipple discharge. All patients were confirmed breast cancer by ultrasound-guided core needle biopsy.

Inclusion criteria were:

(1) early clinical stage $T_{1\text{-}2}N_0M_0$, with the diameter of single mass less than 5 cm;
(2) no swollen or palpable lymph nodes found by ultrasound examination, mammography, or nuclear magnetism;
(3) no metastasis;
(4) no preoperative chemotherapy;
(5) no history of axillary surgery or radiation therapy.

Exclusion criteria: patients with negative SLNs and no ALND.

A total of 415 patients were enrolled and randomly divided into 2 groups of MB + ICG group (n = 197) and MB group (n = 218). In MB + ICG group, the 197 patients were subjected to ICG combined with MB, while in MB group, the 218 patients were assessed by MB alone.

All participants provided written informed consent with agreement to undergo SLNB or ALND if necessary. This study was approved by the Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University. This project has been registered in the Chinese Clinical Trial and the registered ID is ChiCTR-DCD-15006532.

2.2. SLN detection during surgery

After successful anesthesia, 1 mL of 1% MB (Jiangsu Jumpcan Pharmaceutical Co., Ltd., Baota Bay, Daqing West Road, Taixing City, Jiangsu Province, China) and/or 1 mL of 1.25% ICG (Dandong Yichuang Pharmaceutical Co., Ltd., NO.6 Yongxiang Street, Donggang City, Dandong City, Liaoning Province, China) was subcutaneously injected into patients. ICG was injected into the areola area, and MB was injected into the gland of the areola area. The incision of surgery was designed based on the anatomical position of SLNs and tracer development. In MB group, the blue-staining lymph nodes could be seen by naked eyes. In MB + ICG group, the fluorescent vascular imaging system (Mingde Pharmaceutical Company) was used to show the real-time imaging of ICG in the lymphatic vessels of the areola area and its drainage to the axilla. The SLNs anatomical location was marked approximately 2 transverse fingers from the distal end of lymphatic disappearance. The SLNs were continuously detected by fluorescent vascular imaging system and were further confirmed by blue dye. The fluoroscopically-developed or

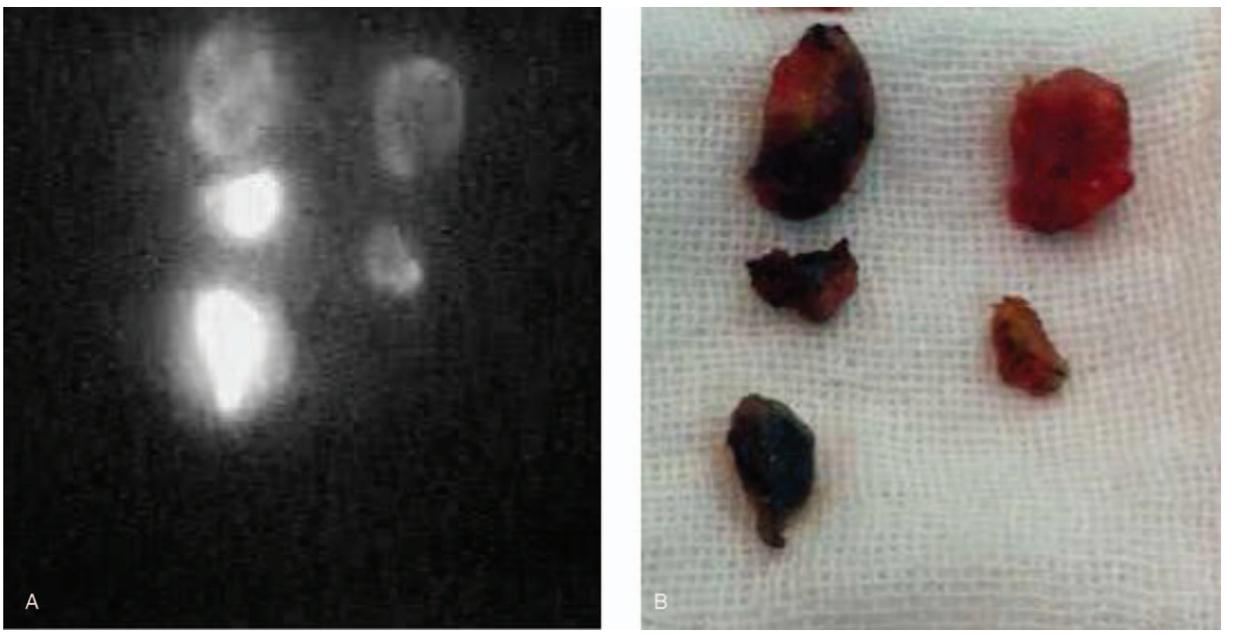

Figure 1. Representative images of 5 harvested sentinel lymph nodes (SLNs) with pathological confirmation. ICG was used for detection. (A) The fluorescent images of the 5 harvested SLNs shown by fluorescent vascular imaging system. (B) The image of SLNs after the resection. ICG = indocyanine green.

Table 1

General clinical information of patients with breast cancer.

Characteristics	Number of patients (%) MB + ICG group (n = 197)	MB group (n = 218)	P
Age		0.368	
≤50	119 (60.31)	141 (64.82)	
>50	78 (39.69)	77 (35.17)	
Menopause		0.471	
Yes	63 (32.06)	77 (35.17)	
No	134 (67.94)	141 (64.83)	
Body mass index (kg/m^2)		0.42	
≤25	147 (74.81)	155 (71.03)	
>25	50 (25.19)	63 (28.97)	
Tumor location		0.584	
Left	102 (51.91)	107 (48.97)	
Right	95 (48.09)	111 (51.03)	
Tumor size (cm)			.502
T1	138 (70.23)	146 (66.90)	
T2	59 (29.77)	72 (33.10)	
Operation type		0.187	
Modified-radical mastectomy	132 (67.18)	159 (73.10)	
Radical mastectomy	65 (32.82)	59 (26.90)	
Estrogen receptor (ER)			.59
+	135 (68.7)	144 (66.21)	
−	62 (31.29)	74 (33.79)	
Progesterone receptor (PR)		0.396	
+	129 (65.65)	134 (61.38)	
−	68 (34.35)	84 (38.62)	
HER-2 expression		0.954	
+	17 (8.4)	20 (8.97)	
−	176 (89.31)	192 (88.28)	
Unknown	4 (2.3)	6 (2.76)	

ICG = indocyanine green, MB = methylene blue.

blue-stained lymph nodes (Fig. 1A and B) were harvested during surgery and sent for pathological examination. Patients with SLN metastases underwent ALND.

All the clinical data were collected and calculated following to the reported formula for SLNB published by the University of Louisville[26]:

Detection rate of SLN% = (n of detected SLN/n of total biopsy) × 100%;
False negative rate of SLN% = (n of false negative SLN/n of total metastatic axillary lymph nodes) × 100%.

2.3. Statistical analysis

All statistical analyses were performed by utilizing SPSS 19.0 statistical package. Group comparisons were analyzed by using Chi-square (χ^2) test. It is considered statistically significant when $P < .05$.

Table 3

Comparison of false negative rate in combined group and MB group.

n of patients	True positive	False negative	False negative rate (%)
MB + ICG group (n = 197)	51	4	7.3
MB group (n = 218)	51	6	10.5

ICG = indocyanine green, MB = methylene blue.

3. Results

3.1. Comparatively analysis of clinical characteristics

To verify the feasibility of the 2 sets of analyses, the clinical characteristics of patients in 2 different test groups were compared. The clinical characteristics of patients were shown in Table 1, which included the age (years), menopausal status, body mass index (kg/m^2), tumor location, tumor size, operating methods, pathological pattern, estrogen receptor, progesterone receptor, and HER-2 expression of HER-2 in the Table 1. Participants involved in this study aged from 30 to 77 years old, with a median age of 46.5 years old. There were no significant differences in the clinical characteristics of patients in 2 treatment groups by using χ^2 test.

3.2. Combined application of ICG and MB reveals SLNB more clearly

To comparatively analyze the detecting accuracy of SLNB by using 2 different methods, their detection rate and average detectable number of SLNs were studied. In combination group, SLNs were successfully detected in 191 patients (n = 197), with a detection rate of 96.9% (191/197) and the average detectable number of 3.0 per patient. By contrast, in MB group, only 196 patients were successfully detected SLNs (n = 218), with a detection rate of 89.7%, much less than that of combination group ($P = .004$). Moreover, the average detectable number of SLNs in MB group was 2.1 per patient, significantly less than that of MB + ICG group ($P = .011$) (Table 2).

3.3. Comparison analysis of the false negative rate

To test the accuracy and feasibility of SLNB, the false negative rate of SLNs was analyzed between the MB + ICG group and MB group. The false negative was considered as follows: there was no metastasis in the SLN pathology, but when the lymph node was removed, the non-SLN showed tumor metastasis. In MB + ICG group, there were 188 patients showed SLNs fluorescence imaging and 176 patients had blue-staining SLNs. About 170 patients showed both fluorescence imaging and MB dye. There

Table 2

Comparison of detection rate and average numbers of SLNs in combined group and MB group.

	ICG + MB (n = 197) ICG	MB	ICG + MB	MB alone (n = 218)	P
n of patients with detected SLNs	188	176	191	196	
Detection rate of SLNs (%)	95.4	89.3	96.9	89.7	.004
n of detected SLNs per patient (range)	3.0 (1–6)	2.1 (1–4)	3.0 (1–6)	2.1 (1–4)	.011

ICG = indocyanine green, MB = methylene blue.

were 4 false negative SLNs in the MB + ICG group (55 patients in MB + ICG group were shown with tumor metastasis), which indicated the false negative rate was 7.3% ($4/55 \times 100\% = 7.3\%$). By contrast, 6 false negative SLNs were detected in the MB group (57 patients in MB group were shown with tumor metastasis), thus its false negative rate was 10.5% ($6/57 \times 100\% = 10.5\%$). However, there was no statistical difference between the MB + ICG group and MB group in the false negative rate by using χ^2 test ($P = .791$) (Table 3).

4. Discussion

SLNB has been widely used in breast cancer treatment. For SLNB, various tracing methods have been developed, especially ICG. Till now, most of the studies focused on the detection rate of ICG. Motomura et al[27] have first reported using ICG to detect SLN. They injected 5 mL of ICG into para-carcinoma tissue of patients, and found the detection rate of SLNs was 73.8% (127/172). Kitai[28] has demonstrated that the detection rate of SLN by ICG was 94.4%. Similarly, Hirche et al[29] have described a higher detection rate (about 97.7%) of SLNs in a total number of 43 breast cancer patients. Due to its high detection rate of SLNs, fluorescence-guided ICG is widely used. In consistent with previous reports, our study also revealed a higher detection rate of SLNs by MB combined with ICG, which was 96.9%. This higher detection rate ensures the successful implementation of SLNB.

The false negative rate is a critical standard to evaluate the effectiveness of SLNB.[30] Standard ALND will be performed if SLNs detection fails.[24] False negative result will lead to incorrect diagnosis of the clinical stage of axillary lymph node, resulting in an insufficient treatment and further contributing to a higher risk of recurrence and metastasis.[30] Straver et al reported a negative correlation between the false negative rate of SLNB and the detected number of SLNs.[31] With more SLNs being detected, a lower false negative rate can be controlled.[32,33] Till now, there are a few of studies reporting the false negative rate of SLNB. For example, Murawa et al[34] have injected ICG subcutaneously into 30 patients with breast cancer, and found that the false negative rate was 9.5%. Guo et al[35] have described that a combination of ICG and MB as a tracer for SLNB revealed a false negative rate of 4%. There were 6 patients with false negative detection results shown in MB group in this study. Interestingly, 5 of them were obese and more than 50 years old, and the other 1 received a lump excision in the upper quadrant before. Due to the obesity of the 5 old patients, their lymph-vessels were blocked by fat, the injected ICG or MB in the lymph nodes cannot be detected. Besides, the patient who received operation in the upper quadrant of breast also revealed false negative. This was because her previous excision blocked the drainage pathways of the lymph vessels, which prevented the ICG or MB flowing to the SLNs. Considering of the 2 conditions, a preoperative biopsy should be performed to decrease the risk of detecting failure.

In addition, ICG had been proved safe via intravenous injection. Grischke[36] had described there was no obvious difference in side effects via different administration way (intradermal or intravenous injection). Consistently, we adopted intradermal injection, and there was no side effect, such as skin inflammation, rupture, or pain. The drug resided in the skin was metabolized within 2 weeks for all participants (data not shown).

Besides safety and effectiveness, Solomayer[37] also demonstrated the economic cost of ICG was a vital factor for its application. By contrast to the nuclide tracing method, ICG and MB combined application significantly reduced the economic cost, which is indeed a "high quality and inexpensive" detection method.

This study has some limitations. First, this study is limited by its retrospective nature. Second, the sample size is relatively small. Further prospective studies with larger sample sizes are warranted.

In conclusion, this study comparatively analyzed the effectiveness of MB alone and combined application of ICG and MB for SLNB. The results indicated the combined application revealed a higher detection rate and average detected number of SLNs. Meanwhile, this combined method also revealed its safe, inexpensive, simple, and practicable advantages, which will make it more popular in breast cancer area.

Author contributions

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Writing – original draft: Chenguang Zhang.
Writing – review & editing: Chenguang Zhang.

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