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Assessing the benefit of using indocyanine green in addition to methylene blue for breast cancer sentinel lymph node biopsy

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Abstract

In recent years, indocyanine green (ICG) fluorescence has been widely used as a new tracer in sentinel lymph node biopsy (SLNB) for breast cancer. This self-controlled study identified the benefits in SLNB between methylene blue (MB) tracer alone and MB combined with ICG fluorescence imaging (MB + ICG) dual tracing modality in patients with breast cancer. One hundred seventy-nine SLNB with MB + ICG dual tracing in 178 cN0 breast cancer patients were enrolled, and their clinical data were analyzed. The results showed that the identification rate (IR) of sentinel lymph nodes (SLNs) by MB staining alone was 92% (165/179), the mean number of detected SLNs was 2.1 ± 1.2, the rate of ≥3 SLNs was 33% (55/165), and the number of positive (metastatic) lymph nodes was 40. The IR of SLN by MB + ICG dual tracing imaging was 93.9% (168/179), the mean number of detected SLNs was 3.8 ± 1.8, the rate of ≥3 SLNs was 70.8% (119/168), and the number of positive lymph nodes was 40. Compared to MB staining alone, because of the addition of ICG fluorescence imaging, SLN status changed from N0 to N1 or the number of positive SLNs increased in 9 patients, and there was an alteration of axillary management from SLNB to axillary lymph node dissection in 4 patients. Although MB + ICG dual tracing did not significantly improve the IR of SLNs, it increased the average number of SLNs, rate of ≥3 SLNs, number of positive SLNs. This may reduce the false negative rate of SLNB and lead to an alteration in axillary management.

Abbreviations: ALND = axillary lymph node dissection, BD = blue dye, FNR = false negative rate, ICG = indocyanine green, IR = identification rate, MB = methylene blue, RI = radioisotope, SLN = sentinel lymph node, SLNB = sentinel lymph node biopsy.

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

1. Introduction

Sentinel lymph node biopsy (SLNB) is the preferred procedure for axillary treatment of cN0 breast cancer because it can not only accurately assess the status of axillary lymph node metastasis and provide a basis for subsequent treatment, but also significantly reduce the incidence of upper limb lymphedema caused by axillary lymph node dissection (ALND).[1,2] The NSABP B-32 trial was used to verify the efficacy and safety of SLNB for cN0 breast cancer, and the modality of dual tracing with blue dye (BD) and radioisotope (RI) was used in this trial.[3,4] Therefore, BD combined with RI (BD + RI) dual tracing is the gold standard in SLNB for cN0 breast cancer. However, in some countries such as China, RI is not widely used because of its high price and easy nuclear contamination. Most hospitals use only BD, such as methylene blue (MB), for SLNB. However, a meta-analysis of 18 pieces of literature that were retrieved from the databases of PubMed, EMBASE, and Cochrane Library between 1993 and 2018 showed that the false negative rate (FNR) of MB staining alone for SLNB is not below 10%,[5] which is generally unacceptable.

Indocyanine green (ICG) is a water-soluble, small-molecule fluorescent dye that can be drained by lymphatic vessels to sentinel lymph nodes (SLNs) and can be detected by near-infrared fluorescence detectors in vitro through human tissues up to 2 cm in diameter. Lymphatic vessels and lymph nodes can be observed simultaneously, which has good visibility and real-time performance.[6] In 2005, Kitai et al first reported that ICG fluorescence imaging for tracers in SLNB could achieve 94% identification rate (IR) of SLNB.[7] Other studies have also shown that the IR of SLNB or the number of SLNs detected by ICG fluorescence alone was not inferior to that by RI,[8,9] therefore, some surgeons have used ICG fluorescence as a substitute

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

All procedures in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964. The study was approved by the Ethical Committee of Shaoxing People's Hospital. Informed consent was obtained from all individual participants included in the study.

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for RI in SLNB. In recent years, there have been few studies on MB combined with ICG fluorescence imaging (MB + ICG), which can avoid the disadvantages of MB staining alone in SLNB, such as low IR of SLNB, low number of detected SLNs, and high FNR.[10–12] In this prospective, self-controlled study, we aimed to compare the differences between MB tracing alone and MB + ICG dual tracing for SLNB. The results are as follows.

2. Materials and methods

2.1. Study design and patients

Patients with early-stage breast cancer treated in our hospital between January 2020 and May 2024 were consecutively enrolled in this study. The inclusion criteria were as follows: cT_1–$T_2N_0M_0$ patients (cN_0 was defined as no enlarged lymph nodes on physical examination and no suspected lymph node metastasis on imaging), breast cancer confirmed by preoperative biopsy or intraoperative frozen section pathology, no neoadjuvant therapy, and no previous history of breast or axillary surgery. The study was approved by the Ethical Committee of Shaoxing People's Hospital. Informed consent was obtained from all individual participants included in the study.

The trial was self-controlled. All the patients underwent the MB + ICG dual tracing modality for SLNB. According to MB staining or ICG fluorescence imaging status, there were 3 statuses of each excised SLN: MB staining but no ICG fluorescence imaging (MB(+)ICG(-)), ICG fluorescence imaging but no MB staining(MB(-)ICG(+)), and both MB staining and ICG fluorescence imaging (MB(+)ICG(+)). The SLN detection rate, number of detected SLNs, and number of positive SLNs in the single MB staining group (including (MB(+)ICG(-) positive SLNs and (MB(+)ICG(+) positive SLNs), and MB + ICG dual tracing group (including (MB(+)ICG(-) positive SLNs, (MB(+)ICG(+) positive SLNs, and MB(-)ICG(+) positive SLNs), respectively. The differences between the 2 groups were compared to determine whether these differences affected axillary management (Fig. 1).

2.2. Materials and devices

ICG for injection (Tianyi Bio-pharmaceutical, Liaoning, China), MB injection (Jichuan Pharmaceutical, Jiangsu, China), sterile water for injection (Suicheng Pharmaceutical, Henan, China), infrared fluorescence positioning observation camera (Hamamatsu China, Beijing, China).

2.3. Procedure

MB was diluted in saline to a final concentration of 1%, and ICG was dissolved and diluted in sterile water to a final concentration of 0.5 mg/mL before use. One milliliter MB was injected intradermally in the periareolar region 10–15 minutes before SLNB, and 1 mL ICG was injected in the same site 5 minutes before SLNB. ICG fluorescence was stimulated and detected by the infrared fluorescence detector. If the lymphatic drainage was navigated well, the incision of the SLNB was marked 1–3 cm above the site where the lymphatics pointing to the axilla disappeared (Fig. 2). In cases where the lymphatics developed unclearly, the incision was marked 2–3 cm under the axillary fold line along the lateral margin of the pectoralis major. Initially, MB(+) SLNs were localized and excised. Regardless of whether MB(+) SLNs were found or not, the near-infrared fluorescence probe was used to detect whether there were ICG fluorescence imaging lymph nodes in the residual cavity of the axillary, and if there were ICG(+) SLNs remained, they were localized and excised (Fig. 3). Subsequently, a near-infrared fluorescence probe was used to detect whether MB(+) SLNs could be identified by ICG fluorescence imaging. ICG(+) SLNs were also examined with the naked eye to detect whether ICG(+)

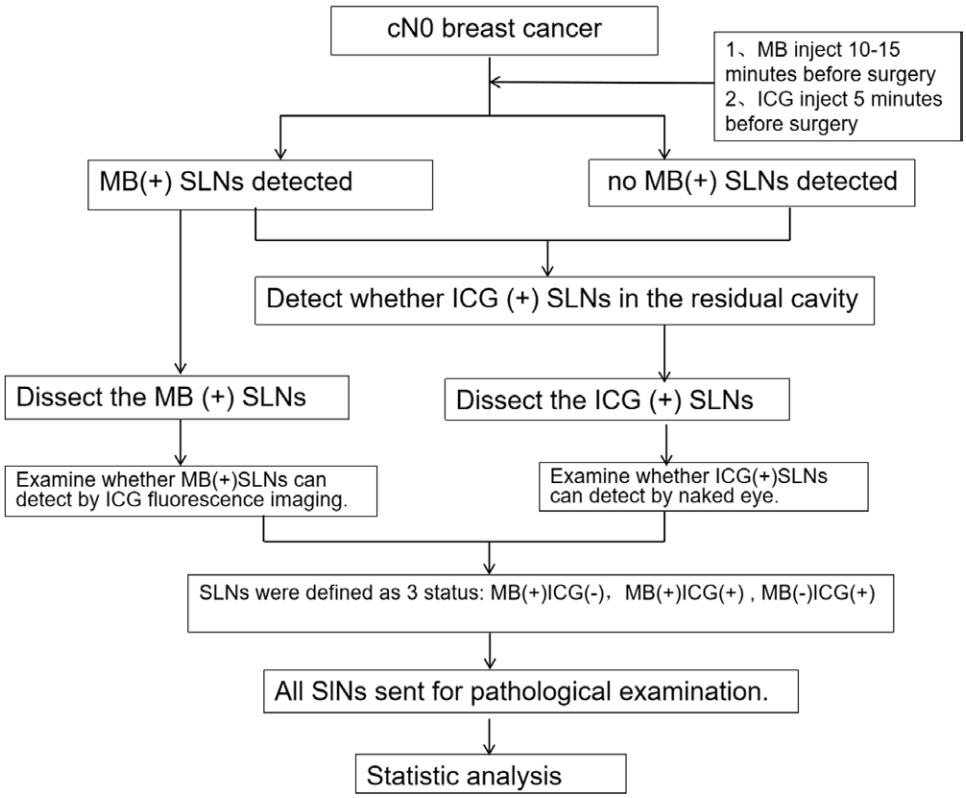

Figure 1. Study design and procedures.

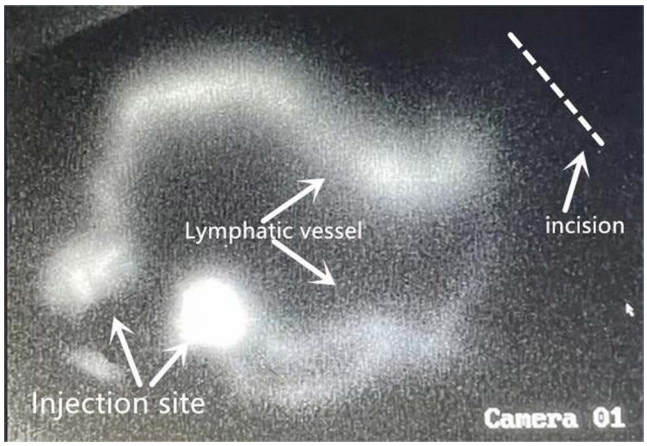

Figure 2. Injection site of ICG, lymphatic vessel detected by ICG visualization and surgery incision. ICG = indocyanine green.

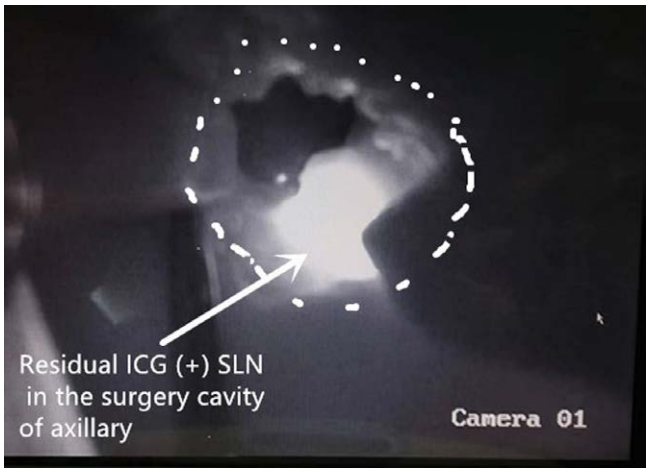

Figure 3. The residual ICG(+) SLN in the surgery cavity of axillary. ICG = indocyanine green, SLN = sentinel lymph node.

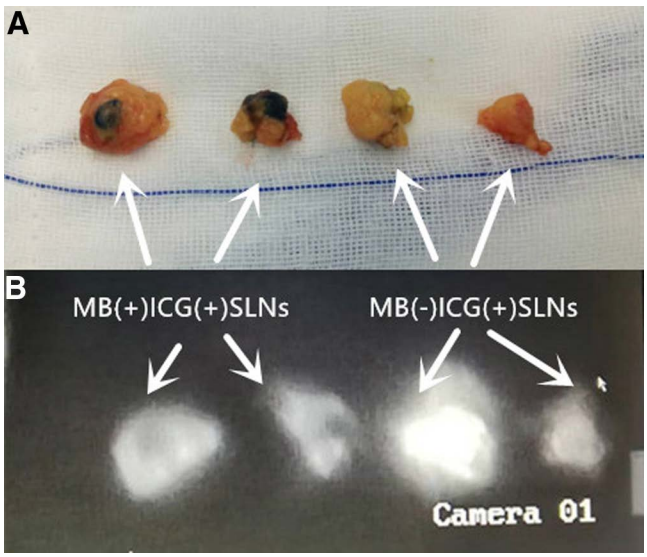

Figure 4. SLNs were obtained through the SLNB procedures. (A) Presentation of SLNs under naked eye. (B) Presentation of SLNs under ICG fluorescence. ICG = indocyanine green, SLNs = sentinel lymph nodes, SLNB = sentinel lymph node biopsy.

SLNs were stained with MB (Fig. 4). Finally, all dissected SLNs were classified, recorded, and sent for pathological examination of frozen sections.

2.4. Statistical analysis

SPSS software (version 23.0) was used for the statistical analysis. The number of SLNs identified between the 2 methods was compared using a T the chi-square test was used to compare differences with respect to the detection rate and the rate of cases with ≥3 SLNs, and statistical significance was set at $P < .05$.

3. Results

3.1. Basic characteristics of the research cohort

1. One hundred seventy-nine SLNB with MB + ICG dual tracing simultaneously in 178 cN0 breast cancer patients (1 patient with bilateral synchronous breast cancer) were enrolled in this study (Fig. 5).
2. The clinical characteristics of the patients are summarized (Table 1).
3. Among 179 cases with cN0 breast cancer who underwent MB + ICG dual tracing, MB(+)SLNs were detected in 165 patients, and MB(+)SLNs or ICG(+)SLNs were detected in 168 patients. The total number of SLNs, mean number of SLNs, and cases with different numbers of SLNs detected (1 SLN, 2 SLNs, and ≥3 SLNs) according to different tracing modalities (MB vs MB + ICG) are presented in Tables 2 and 3, respectively.
4. In total, 348 MB(+) SLNs were identified in 165 patients. A near-infrared fluorescence probe was used to detect whether MB(+) SLNs were visualized by ICG imaging. The results showed that 326 MB(+) SLNs could be visualized by ICG imaging and 22 MB(+) SLNs could not be visualized by ICG imaging.
5. In total, 147 cases of cN0 invasive breast cancer (including microinvasive breast cancer) were included in this study. The number of positive SLNs, number of cases with positive SLNs, and staining or imaging status of positive SLNs are presented in Table 4. Table 5 shows the number of ICG(+)MB(-) positive SLN in the different groups based on the number of MB(+) positive SLN.

4. Discussion

Owing to the low price and easy availability of ICG, BD + ICG dual tracing has replaced BD + RI for SLNB in many countries. In this study, the detection rate of SLNs using MB staining alone was 92.2% (165/179), and the MB + ICG dual tracing detection rate was 93.9% (168/179). The differences were not statistically significant (Tables 2 and 3, respectively). Previous randomized controlled studies have shown that MB + ICG dual tracing has a higher detection rate than MB staining alone. In this study, the relatively consistent detection rate between single MB staining and MB + ICG dual tracing is because this study is self-controlled and uses MB staining and ICG fluorescence imaging simultaneously. The use of MB staining alone is easily affected by the surgeon's learning curve, patient's age, obesity level, and multiple lymphatic drainage pathways.[13,14] In obese patients, MB(+) lymph nodes are more fat-wrapped and difficult to detect by the naked eye. If far-infrared imaging is used simultaneously, the lymph nodes wrapped in fat can be easily detected. In fact, we found that most MB staining SLNs could be simultaneously imaged by ICG fluorescence. In our study, 348 SLNs stained with MB were detected and 326 were simultaneously imaged by ICG fluorescence, with a consistency rate of 93.7% (326/348).

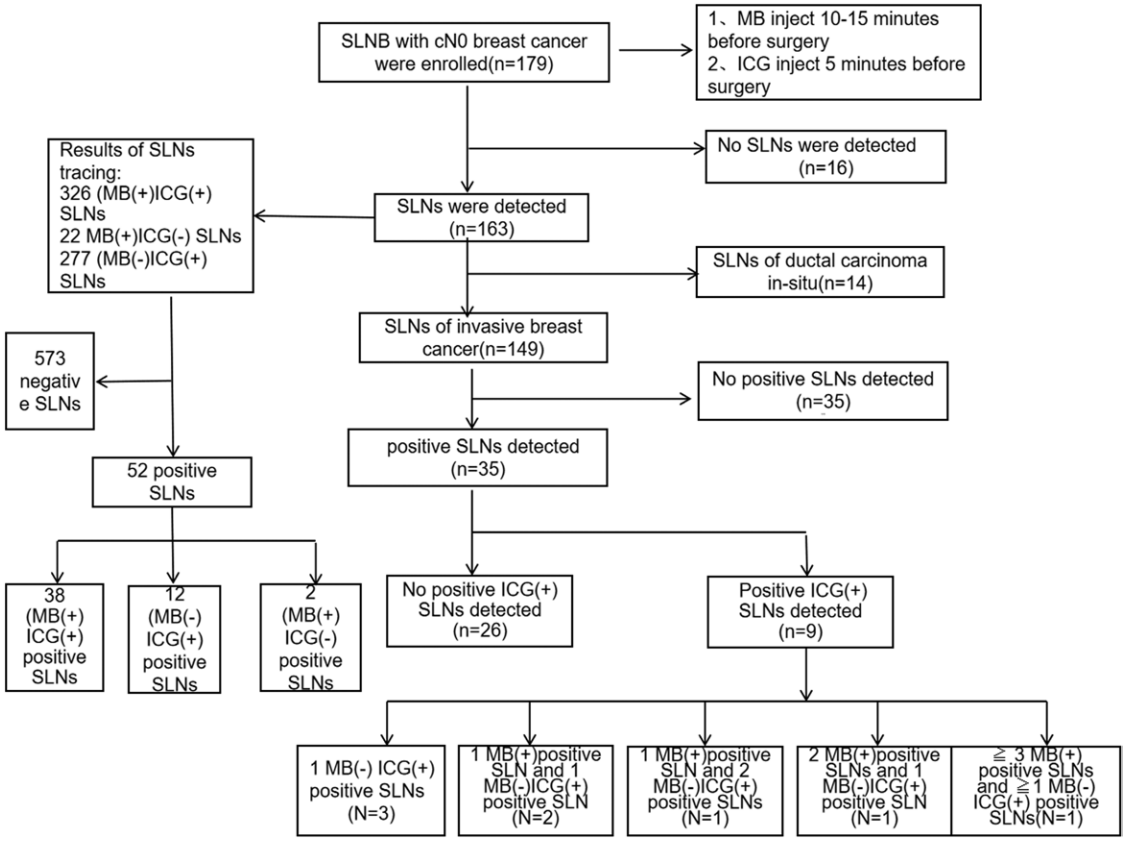

Figure 5. Study profile.

The advantage of MB + ICG dual tracing for SLNB was mainly reflected in the increased number of SLNs detected. The literature reports that the average number of SLNs detected by MB staining alone is 1.7–3.2,[5] with a median of 2, while the average number of SLNs detected by MB + ICG dual tracing is 3.0–3.7,[11,15–17] with a median of 3.0. In our study, a total of 348 SLNs were found using MB staining alone, with an average of 2.1 ± 1.2. The number of SLNs detected by MB + ICG dual tracing was 625, with an average of 3.7 ± 1.8 (Table 2). These results were similar to those reported in the literature and were statistically significant. Adding ICG fluorescence imaging to MB staining can improve the number of detected SLNs, which may be due to the low molecular weight and high degree of diffusion of ICG, allowing it to spread beyond the first SLN to the secondary draining lymph nodes.[15] Detecting more SLNs reduces the FNR. A meta-analysis showed that the average number of SLNs detected by single MB staining was between 1 and 2,[5] and the FNR was 11%. The B-32 trial showed that the FNR was 17.7% when only 1 SLN was detected, and 10% when only 2 SLNs were detected. However, when ≥3 SLNs are detected, The FNR could be <10%.[3,4] Therefore, most experts believe that the number of detected SLNs should be 3 or more, so that the FNR can reach an acceptable range. In this study, 119 cases (119/168, 70.8%) obtained 3 or more SLNs by MB + ICG dual tracing, whereas only 55 cases (55/165, 33.3%) were obtained by MB staining alone. There were significant differences between the 2 modalities (Table 3).

The FNR of SLNB can be reduced only by finding positive SLNs rather than negative (nonmetastatic) SLNs. In this study, 147 patients with cN0 invasive cancer were enrolled, of which 52 were positive SLNs, including 38 MB(+)ICG(+) positive SLNs, 12 MB(-)ICG(+) positive SLNs, and 2 MB(+)ICG(-) positive SLNs (Table 4). This indicated that 23% (12/52) of the positive SLNs were detected by ICG fluorescence imaging. Guo et al also found 58 positive SLNs in 616 SLNs by MB + ICG dual tracing in SLNB, of which 20 positive SLNs were MB(-)ICG(+), 4 were MB(+)ICG(-), and 22.7% of positive SLNs were detected by ICG fluorescence imaging.[12] This study also showed that MB(-)ICG(+)-positive SLNs were detected in 9 patients, including 3 patients without MB(+)-positive SLN, 3 patients with 1 MB(+)-positive SLN, 1 patient with 2 MB(+)-positive SLNs, and 2 patients with ≥3 MB(+)-positive SLNs (Table 5).

In this study, we set the axillary treatment strategy for patients with positive SLNs as follows: if the patient is eligible for the criteria of the Z0011 trial (tumors of clinical stage T1/T2, number of positive SLNs ≤ 2, breast-conserving patients, whole breast irradiation after surgery, and systemic adjuvant therapy after surgery) who do not undergo ALND,[18] the remaining patients will undergo ALND. In these 9 patients with MB(-)ICG(+) positive SLNs, 2 mastectomy patients without MB(+) positive SLNs had an alteration of axillary treatment strategy from SLNB to ALND because of the addition of MB(-)ICG(+) positive SLNs. Meanwhile, another 2 breast-conserving patients with 1–2 MB(+) positive SLNs also had an alteration of axillary treatment strategy because of the addition of MB(-)ICG(+) positive SLNs; the total number of positive SLNs exceeded 2.

In our study involving 147 cN0 invasive breast cancers, 4 patients changed our axillary treatment strategy because the modality of SLNB changed from single MB staining alone to MB + ICG dual tracing. However, can this bring about subsequent clinical benefits? Shen et al performed SLNB with MB staining alone or a combination of MB and ICG in 523 patients, with a median follow-up of 29 months, and found that the dual tracing group had a numerically lower ipsilateral axillary recurrence rate (0.5% vs 1.3%, $P = .322$).[19] Yang et al observed 1221 patients who underwent MB + ICG dual tracing in SLNB, and the regional lymph node recurrence rate was 0.7% after a median follow-up of 5.6 years.[20] Wang et

Table 1

Clinical characteristics of 178 patients.

Clinical characteristics	N	%
Age (yr)		
≥65	39	21.9%
50≤ age <65	78	43.8%
<50	61	34.3%
Gender		
Female	177	99.4%
Male	1	0.6%
Histologic features		
DCIS	32	17.9%
Invasive ductal carcinoma	122	68.2%
Invasive lobular carcinoma	9	5.0%
Other types	16	8.9%
Tumor size		
T1	116	64.8%
T2	60	33.5%
T3	3	1.7%
ER and PR status		
ER and/or PR positive	134	74.9%
ER and PR negative	45	25.1%
HER-2 status		
Positive	42	23.5%
Negative	129	72.1%
Unknown	8	4.5%
Molecular typoes		
HR(+)/HER(-)	101	56.4%
HR(+)/HER(+)	18	10.1%
HR(-)/HER(+)	24	13.4%
HR(-)/HER(-)	26	14.5%
Unknown molecular typoes	10	5.6%
Histology grade		
Grade I	8	4.5%
Grade II	81	45.3%
Grade III	52	29.1%
Unknown	38	21.2%
Ki-67		
≤14%	32	17.9%
15%–30%	38	21.2%
>30%	109	60.9%
Surgical methods		
Breast conservation	62	34.6%
Total mastectomy	117	65.4%

Table 2

SLNs detected by 2 different tracing modality.

SLNB tracer method	The number of SLN cases (n)		The total number of SLNs (n)	The mean number of SLNs (n)
MB staining alone	165	1 SLN 59 2 SLNs 51 ≥3 SLNs 55	348	2.1 ± 1.2
(MB + ICG) dual tracing	168	1 SLN 9 2 SLNs 40 ≥3 SLNs 119	625	3.7 ± 1.8

ICG = indocyanine green, MB = methylene blue, SLN = sentinel lymph node, SLNB = sentinel lymph node biopsy.

Table 3

Efficacy of SLNB in the combined group and the MB group.

Characteristic	Combined group	MB group	*P*
Average number of detected SLNs (total N)	3.7 ± 1.8 (n = 625)	2.1 ± 1.2 (n = 348)	.000
Detection rate	93.9% (168/179)	92.2% (165/179)	.25
The rate of cases with ≥3 SLNs	70.8% (119/168)	33.3% (55/165)	.000

ICG = indocyanine green, MB = methylene blue, SLN = sentinel lymph node, SLNB = sentinel lymph node biopsy.

Table 4

Staining or imaging status of 52 positive SLNs in 35 cases.

Positive SLNs status	Positive SLNs number (%)	Case number (%)
MB(+)ICG(+)	38 (38/52 73.1%)	32 (32/35 91.4%)
MB(-)ICG(+)	12 (12/52 23.1%)	9 (9/35 25.7%)
MB(+)ICG(-)	2 (2/52 3.8%)	2 (2/35 5.7%)
Total	52	35

ICG = indocyanine green, MB = methylene blue, SLN = sentinel lymph node.

Table 5

The ICG(+)MB(-) positive SLN in different MB(+) positive SLN numbers groups.

MB(+) positive SLN patients	The number of additional ICG(+) MB(-) positive SLNs	The cases with additional ICG(+)MB(-) positive SLNs
Patients without MB(+) positive SLN	1	3
Patients with 1 MB(+) positive SLN	1	2
	2	1
Patients with 2 MB(+) positive SLNs	1	1
Patients with ≥3 MB(+) positive SLNs	1	2
Total	12	9

ICG = indocyanine green, MB = methylene blue, SLN = sentinel lymph node.

al followed up 777 patients who underwent MB + ICG dual tracing fluorescence in SLNB for a median follow-up of 5.6 years. Only 0.64% of patients had ipsilateral axillary recurrence.[21] These results show that the ipsilateral axillary recurrence rate was low in patients receive SLNB using MB + ICG. On the other hand, it may be difficult to attain significant differences in controlled trials due to the low rate of axillary recurrence and the small proportion of patients who receive more aggressive axillary surgery on account of adding ICG tracing.

This study was self-controlled, which could eliminate many mixed factors, such as age, body mass index, and pathological type. However, there were also some limitations. Firstly, it was a single-center study, surgical techniques and habits are relatively simplex and the sample size is small. Secondly, because of the time limit, follow-up data on the recurrence rate and long-term survival benefits were missing. Additionally, without a control group, we did not know if there was a statistical local control benefit from a change in surgical approach. Finally, with the continuous improvement of adjuvant therapy, expansion of axillary surgery may not necessarily bring benefits. If this study could combine the postoperative adjuvant treatment program and follow-up data for analysis, it might be able to draw a more reliable conclusion.

5. Conclusion

In conclusion, compared to single MB staining, SLNB using MB + ICG dual tracing can find a higher average number of detected SLNs and positive SLNs. This may lead to an alteration in axillary treatment strategy and potential survival benefits.

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Author contributions

Data curation: Liwei Meng, Ying Yuan, Yufan Li, Hanjin Wu, Wei Zhang.
Investigation: Liwei Meng, Wei Zhang.
Methodology: Liwei Meng, Wei Zhang.
Writing – original draft: Liwei Meng, Mingliao Zhu.
Writing – review & editing: Liwei Meng, Mingliao Zhu.

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