



Indocyanine green detects sentinel lymph nodes in early breast cancer

Jun Liu, Linping Huang, Ning Wang and Ping Chen

Abstract

Objective: To explore the clinical value of indocyanine green (ICG) for the fluorescence-guided detection of sentinel lymph nodes (SLNs) during sentinel lymph node biopsy (SLNB) in patients with early breast cancer.

Methods: This retrospective study included female patients with breast cancer. Patients were administered methylene blue and ICG using standard techniques. All SLNs that were collected during surgery were submitted for pathological examination. SLNs were defined as those that were either fluorescent, blue, fluorescent and blue or palpably suspicious. Surgical complications, axillary recurrence, distant metastasis and overall survival rates were observed postoperatively.

Results: A total of 60 patients were enrolled in the study. The fluorescence detection rate of SLNs was 100% ($n = 177$), with a mean of 2.95 SLNs per patient. The methylene blue staining rate was 88.3% ($n = 106$), with a mean of 1.77 SLNs per patient. Pathological assessment of intraoperative frozen specimens revealed SLN metastases in 10 patients, who immediately underwent axillary lymph node dissection. No patient had axillary recurrence or distant metastases, with a survival rate of 100%. Patients who underwent SLNB showed good appearance in the axillary wound, with no limited shoulder joint abduction and upper limb oedema.

Conclusion: Fluorescence-guided SLNB has several advantages and is suitable for clinical application.

Keywords

Breast cancer, sentinel lymph node biopsy (SLNB), fluorescence imaging, indocyanine green, methylene blue

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Introduction

Breast cancer is the most prevalent cancer in females worldwide, with about 1.7 million cases occurring annually.¹ It is equally the leading cause of death among female cancer patients.² The presence or absence of

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axillary lymph node involvement represents one of the most important prognostic indicators of long-term patient outcome for breast cancer.^{3–5} This demonstrates the importance of sentinel lymph node (SLN) identification during surgery in breast cancer patients. Currently, sentinel lymph node biopsy (SLNB) is considered the first option for breast cancer axillary lymph node staging evaluation because of its reduced morbidity compared with axillary lymph node dissection (ALND).³ However, a false negative rate of 5–10% was reported for SLNB.^{6,7} Multiple techniques proposed for detecting SLNs use a dye or a gamma probe, alone or in combination.⁸ The dye and gamma probe combination is more effective in detecting SLNs compared with the dye used alone, but exposes the patients to radioisotopes,⁹ which are costly and potentially harmful to the patients, requires a license and specialized instruments; as a result, this method is rarely applied in China. Indeed, dye staining is more widely used in China, but has a detection rate of only 70–80%;¹⁰ it also requires a longer learning curve, thereby limiting its application.

Developing safe SLNB methods with high efficiency has attracted increasing attention. Indeed, combination of fluorescence with a visible dye was shown to be a highly sensitive method for SLN identification.⁷ Optical imaging employing near-infrared fluorescence provides novel options for general and oncological surgery.¹¹ Indocyanine green (ICG), a near-infrared tracer, is currently used for intraoperative identification of solid tumours and SLN mapping;¹² it is radiation-free with high tissue penetration, clearly revealing the lymph nodes.¹³ However, there is still a lack of a standardized operational procedure for SLNB, and the value of ICG in SLN mapping in breast cancer is not completely understood. Therefore, this study aimed to validate the clinical usefulness of ICG in

guiding SLNB for patients with early breast cancer.

Patients and methods

Patient population

This retrospective study included consecutive female patients with early breast cancer who underwent surgery between March 2012 and October 2013 at the Breast Cancer Centre, China-Japan Friendship Hospital, Beijing, China. All surgeries were performed by the same surgeon (L.H.).

Inclusion criteria were: (i) primary breast cancer; (ii) no enlarged lymph nodes found either by axillary palpation, mammography, or breast ultrasound examination; (iii) no preoperative chemotherapy; (iv) no history of axillary surgery or radiotherapy; (v) agreement to undergo SLNB, without ALND if no metastases were found in intraoperative frozen tissues. Exclusion criteria were: (i) previous surgery at the axillary area; (ii) multi-site breast cancer; (iii) previous chemotherapy or radiotherapy at the breast area.

This study was approved by the Ethics Committee of China-Japan Friendship Hospital (no. 2012-4). All study participants provided written informed consent.

SLN detection during surgical treatment

After successful anaesthesia, 1 ml (5 mg/ml) of methylene blue was injected into peritumoral breast tissues using a 1 ml syringe, at the same site for all patients. Routine disinfection and drape placement were carried out, and 1 ml of ICG (1 mg/ml) (Yichuang Pharmaceutical, Dandong, China) was injected subcutaneously and intradermally at the areola.

During SLN positioning and biopsy, surgical lights were turned off. The areolar area was locally massaged and explored using an infrared observation PDE C9830 camera (Hamamatsu Photonics, Hamamatsu, Japan), which showed real-time imaging of

the lymphatics in the areolar area and drainage to the axilla, with the SLN location marked at 1–2 cm from the distal end of lymphatic disappearance. Then, a 3-cm incision was made at the marked location, and gradually extended to fluoroscopically-developed lymph nodes through fluorescence guidance with sharp dissection by electric knife cutting. SLNs were defined as those that were either fluorescent, blue, fluorescent and blue or palpably suspicious. Afterwards, the fluoroscopically-developed, blue-stained, and peripheral palpable enlarged lymph nodes were harvested and immediately sent for pathological examination. Patients showing SLN metastases immediately underwent ALND; those without SLN metastasis detected in intraoperative biopsy samples were not submitted to ALND. However, if metastasis was later confirmed after paraffin section evaluation, a second round of ALND was suggested. Operations were performed according to preoperative planning and surgical approaches included: mastectomy, breast-conservation surgery, and nipple-sparing mastectomy. Two patients submitted to nipple-sparing mastectomy were simultaneously implanted with prostheses.

Subsequent treatment and follow-up

As directed by the National Comprehensive Cancer Network guidelines,¹⁴ subsequent treatment approaches were selected according to the postoperative pathological results. Axillary surgery complications, axillary recurrence rate, distant metastasis rate, and overall survival rate were evaluated by outpatient visit and telephone follow-up. In addition to wound infection and effusion, other axillary surgery complications were assessed by follow-up in the outpatient clinic at 6 and 12 postoperative months: (i) upper limb pain (persistent, intermittent, and no pain, with persistent pain referring to one lasting > 50% of the day); (ii) upper limb

numbness; (iii) limited shoulder joint abduction (bilateral angles between the lateral chest wall and humerus were measured, and the variations between the affected and contralateral sides, i.e. preoperative angles, were divided into <5%, 5–10% and >10%); (iv) appearance of axillary wound; (v) upper limb oedema (the circumferences at the transverse crease of the wrist, 10 cm above the wrist, elbow, and 10 cm above the elbow of the affected limb or contralateral limb were measured; a 2 cm circumference increase of the affected arm compared to that of the healthy arm or that before operation was considered the basis for lymphoedema diagnosis).

Statistical analyses

All statistical analyses were performed using the SPSS[®] statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows[®]. Group comparisons were performed using Student's *t*-test, χ^2 -test and one-way analysis of variance as appropriate. A *P*-value < 0.05 was considered statistically significant.

Results

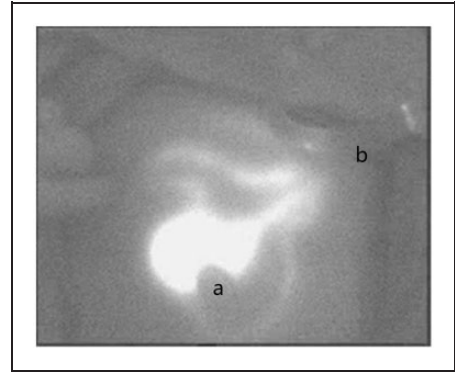
Of the 60 patients with early breast cancer who were enrolled in this study, 41 were diagnosed with preoperative puncture and 19 were diagnosed with excision biopsy. The diagnoses included intraductal carcinoma ($n=9$), intracystic papillary carcinoma ($n=2$), apocrine carcinoma *in situ* ($n=1$), invasive lobular carcinoma ($n=4$), mucinous carcinoma ($n=1$), cribriform carcinoma ($n=1$), and infiltrating ductal carcinoma ($n=42$). The mean \pm SD age of the patients was 57.8 ± 12.8 years (range, 34–86 years). Other patient baseline demographic and clinical characteristics are presented in Table 1.

The SLNB procedure was successfully implemented in all 60 patients (Figures 1 and 2). A total of 10 patients showed SLN

Table 1. Baseline demographic and clinical characteristics of patients with primary breast cancer who were included in this study ($n = 60$).

Characteristic	<i>n</i> of patients (%)
Tumour location	
Outer upper quadrant	25 (41.7)
Outer lower quadrant	14 (23.3)
Inner upper quadrant	13 (21.7)
Inner lower quadrant	5 (8.3)
Central region	3 (5.0)
Tumour size, cm	
≤1	18 (30.0)
>1 – ≤2	25 (41.7)
>2 – ≤3	9 (15.0)
>3	8 (13.3)
Body mass index, kg/m²	
≤25	47 (78.3)
>25	13 (21.7)
Age, years	
≤35	3 (5.0)
>35 – ≤60	30 (50.0)
>60	27 (45.0)
Menstruation status	
Premenopause	22 (36.7)
Postmenopause	38 (63.3)
Oestrogen receptor	
Positive	48 (80.0)
Negative	12 (20.0)
Progesterone receptor	
Positive	46 (76.7)
Negative	14 (23.3)
Ki67 protein, %	
≤15	33 (55.0)
>15 – ≤30	15 (25.0)
>30	12 (20.0)

metastases in intraoperative frozen sections and immediately underwent ALND. Meanwhile, lymph node micrometastases were found in postoperative paraffin embedded tissues from two patients, who rejected the recommended surgery and are now followed-up, with no recurrence to date. No metastases were found in intraoperative frozen sections and postoperative paraffin embedded specimens from the remaining 48 patients.

**Figure 1.** Representative image of the fluorescent lymphatic network within the breast of a patient with primary breast cancer detected using indocyanine green. a: nipple; b: axilla.

Development was completed at 1–3 min after ICG injection into the areolar area, which showed drainage of hypodermic lymphatics towards the axilla, and then disappeared at the SLN in the axilla. In addition to direct injection into the axilla through the areola, the lymphatics often flowed to the mammary gland edge and subsequently into the axilla. Meanwhile, lymphatics in the areolar area were also found to move towards the internal mammary in a few patients.

Operation time from the beginning of ICG injection to SLNB completion ranged from 5 to 32 min. All lymph nodes were fluorescently labelled; a total of 177 SLNs were detected, with 1–6 (mean ± SD 2.95 ± 1.41) SLNs per patient. A total of 106 SLNs were stained blue, with 0–4 (mean ± SD 1.77 ± 0.91) SLNs per patient; blue-stained SLNs were not detected in seven patients. Therefore, fluorescence and blue-staining rates for SLNs were 100.0% and 88.3%, respectively, representing a statistically significant difference ($P = 0.006$). The mean ± SD number of SLNs detected by the fluorescence method was 2.95 ± 1.41, which was significantly higher than the 1.77 ± 0.91 SLNs obtained with the blue-staining method ($P < 0.001$).

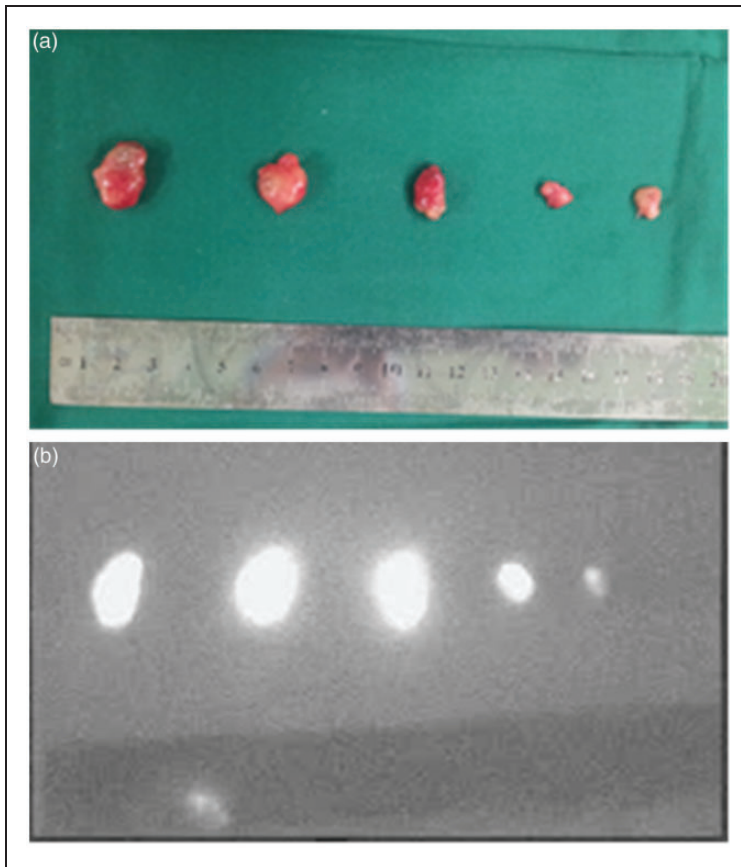


Figure 2. Five representative sentinel lymph nodes (SLNs) with pathological confirmation that were detected by indocyanine green or methylene blue-staining: (a) A total of two of five SLNs were blue-stained; (b) fluorescence was found in all five SLNs. The colour version of this figure is available at: <http://imr.sagepub.com>.

As shown in Table 2, the mean number of detected SLNs was not significantly associated with age, body mass index (BMI), tumour location, tumour size, Ki-67 protein levels, menstruation status or other relevant characteristics.

Operation times differed with the numbers of patients handled: the mean \pm SD operation time for the first 10 patients was 18.8 ± 5.6 min, and was significantly longer than the mean times obtained for patients 11–20, 21–30, 31–40, 41–50 and 51–60 (13 ± 2.8 , 12.4 ± 4.7 , 12.9 ± 3.8 , 11.6 ± 3.7 , and 11.7 ± 4.0 , respectively; ($P = 0.003$, $P = 0.001$, $P = 0.003$, $P < 0.001$, and $P < 0.001$, respectively). In the

first 10 patients, a mean of 2.4 SLNs were detected, which was increased to a mean of 3.1, 3.1, 2.9, 3.4, and 2.8 SLNs obtained for cases 11–20, 21–30, 31–40, 41–50 and 51–60, respectively; however, the differences were not statistically significant.

All patients were followed-up for a mean of 20.4 months (range, 15–34 months). No case suffered from axillary wound infection and effusion, or displayed axillary recurrence and distant metastases; a survival rate of 100% was obtained. Meanwhile, 50 patients had no lymph node dissection. Follow-up was successfully carried out for 6 and 12 months for 48 patients.

Table 2. Associations between clinical data and the mean number of detected sentinel lymph nodes (SLNs) in patients with primary breast cancer ($n = 60$).

Characteristic	Number of detected SLNs
Tumour location	
Outer upper quadrant	3.00 ± 1.50
Outer lower quadrant	2.93 ± 1.39
Inner upper quadrant	3.15 ± 1.52
Inner lower quadrant	2.60 ± 1.34
Central region	2.33 ± 0.58
Tumour size, cm	
≤1	2.72 ± 1.49
>1 – ≤2	3.04 ± 1.37
>2 – ≤3	3.11 ± 1.27
>3	3.00 ± 1.69
Body mass index, kg/m ²	
≤25	2.98 ± 1.47
>25	2.85 ± 1.21
Age, years	
≤60	3.06 ± 1.44
>60	2.81 ± 1.39
Menstruation status	
Premenopause	3.23 ± 1.51
Postmenopause	2.79 ± 1.34
Ki67 protein, %	
≤15	3.06 ± 1.58
>15 – ≤30	2.80 ± 1.21
>30	2.83 ± 1.19
Oestrogen receptor	
Negative	3.17 ± 1.03
Positive	2.90 ± 1.49
Progesterone receptor	
Negative	3.29 ± 1.27
Positive	2.85 ± 1.45

Data presented as mean ± SD.

No significant associations found between any of the characteristics and the number of SLNs ($P \geq 0.05$); group comparisons were performed using χ^2 -test.

Patients who underwent SLNB showed good appearance in the axillary wound, with no limited shoulder joint abduction and upper limb oedema. Meanwhile, incidence rates of upper limb pain and numbness were 10.4% (five of 48) and 4.2% (two of 48), respectively, at 6 postoperative months;

Table 3. Axillary surgery complications in patients with primary breast cancer ($n = 48$) who underwent sentinel lymph node biopsy (SLNB) and were followed-up for 12 months.

Surgical complications	SLNB	
	6 months	12 months
Pain of upper limb		
None	43	46
Intermittent	5	2
Persistent	0	0
Numbness of upper limb		
No	46	48
Yes	2	0
Limited abduction of shoulder joint		
<5%	48	48
5–10%	0	0
>10%	0	0
Appearance of axillary wound		
Good	48	48
Poor	0	0
Oedema of upper limb		
<1 cm	2	1
1–2 cm	1	0
>2 cm	0	0

Data presented as n of patients.

these values decreased to 4.2% (two of 48) and 0, respectively, at 12 months postoperatively. The remaining recorded surgical complications are shown in Table 3.

Discussion

In 2009, the St Gallen International Expert Consensus on the primary therapy of early breast cancer agreed to consider breast cancer with no axillary lymph nodes, except for inflammatory breast cancer, as an indication for SLNB.¹³ Currently, tracing the breast cancer SLN mainly includes staining and radionuclide methods. Dual localization techniques with both dye and isotope are effective in detecting SLNs, and the false negative rate is 5–10%.^{6,7} However,

because of exposure to radioisotopes,⁹ the radionuclide method is rarely applied in China. Dye staining is more widely used in China, but has a detection rate of only 70–80%.¹⁰

To optimize SLN tracing, the near-infrared fluorescence tracer ICG has been adapted to visualize the lymphatics and lymph nodes, and applied in guiding SLNB for breast cancer.^{15–24} Near-infrared fluorescence-guided SLNB requires a special charge-coupled device camera, which generally contains three parts: a near-infrared sensitive image intensifier, a dynamic charge-coupled device camera, and a light emitting diode. After injection, ICG tightly binds to serum proteins, enters into the lymphatics, and flows towards the SLNs.

In this present retrospective study, the utility of fluorescence-guided SLNB was evaluated in 60 clinically and sonographically node-negative patients with early breast cancer measuring ≤ 3 cm in the majority of cases (eight of 60 patients [13.3%] had a primary tumour > 3 cm). Because recent reports have suggested that the fluorescence technique is more effective in detecting SLNs compared with the dye used alone,^{15–24} this present study did not use the dye method alone as a control.

In this present study, SLNs were detected in all 60 patients, indicating a detection rate of 100%; a mean of 2.95 SLNs were detected per patient, corroborating previous studies.^{15–24} SLNB was first performed in melanoma.²⁵ Combined use of radioisotope and dye is highly recommended in melanoma because there can be multiple lymphatic channels from the primary site.²⁶ In recent years, research has suggested that the ICG fluorescence technique is a feasible method.²⁶ Unlike in melanoma, the site of SLNs in breast cancer does not vary. Thus, it is easy to find the location of incision, and after skin incision, it is easy to find SLNs with fluorescence guidance. A study published in 2005 first considered ICG as a

lymph node tracer and found an SLN detection rate of 94.4% (17 of 18), with no false negatives.¹⁶ Subsequently, similar results were reported by others, who obtained SLN detection rates of 96.7–100% and false negative rates of 5.6–9.5%.^{17–19} In recent years, preliminary studies in China indicated SLN detection rates of 95.3–100% and a false negative rate of approximately 4.9%.^{21–24} Statistical analysis in the present study demonstrated that ICG fluorescence was significantly more sensitive than blue dye for the detection of SLNs (100% versus 88.3%, respectively; $P=0.006$). The mean number of SLNs detected by the fluorescence method was significantly higher than by the blue-staining method (2.95 versus 1.77; $P<0.001$). These additional nodes might contain metastases and ICG did not detect excessive numbers of non-sentinel lymph nodes. The palpably suspicious nodes that are neither fluorescent nor blue have been termed ‘para-sentinel’ in some studies,^{27,28} but in this present study, all SLNs were either fluorescent or fluorescent and blue.

The following advantages of ICG may explain the superiority of the fluorescence method in SLN detection. There are abundant lymphatics in the areolar area, and injecting the tracer in this area may result in a higher SLN detection rate.²⁴ In addition, ICG can be safely injected intradermally or subcutaneously into the region surrounding the areola in patients receiving breast conservative treatment;^{19–23} in this case, it is absorbed quickly, and the phenomenon observed with methylene blue, which tends to cause tattoo-like effects or skin necrosis, cannot occur. After injection into the areola, ICG can move to the SLNs through subcutaneous lymphatics in 1–3 min. Furthermore, since fluorescence can penetrate 1 cm thick tissues, it is possible to observe the whole process of the tracer moving into the axilla through subcutaneous lymphatics using an infrared camera, thereby accurately locating the SLNs.¹³

Moreover, after the skin and subcutaneous layers are incised, and the axillary fascia open, it is easy to observe SLNs through the fat with fluorescence guidance.

Sentinel lymph node detection can be affected by a variety of factors. Evidence suggests that lymph node pimeiosis in elderly patients might weaken the retention of radioactive colloid, thus affecting SLN detection.²⁹ In addition, hydrostatic pressure of lymphatics in elderly patients is decreased, which leads to slow passage of the tracer in the lymphatics and may also affect SLN detection using the staining method.²⁴ Currently, evidence suggests that age is unlikely to significantly affect fluorescence-guided SLNB.³⁰ In this present study, SLNs were detected in all patients; mean numbers of SLNs detected in patients > 60 years old and those \leq 60 years were 2.81 and 3.06, respectively, suggesting that aging is unlikely to significantly affect the detection rate when using the fluorescence method, in agreement with previous findings.³⁰

It was proposed that obesity may also reduce SLNB detection when using radionuclide and staining methods,³¹ because lymph node drainage may be hindered by a thick layer of fat in the axillary region, with a fatty envelope formed around the axillary lymph nodes. Moreover, obesity may increase the operational difficulty for surgeons. Because near infrared light can penetrate only 1 cm thick tissues, the detection rate of ICG prior to skin incision in melanoma was low especially in patients with a high BMI, and the detection rate was high after skin incision.²⁶ However, in this present study, mean numbers of SLNs detected in patients with BMI \leq 25 kg/m² and those with a BMI > 25 kg/m² were 2.98 and 2.85, respectively, suggesting that the fluorescence method can effectively overcome the effects of obesity on SLN detection rate.

Complications and outcomes of ICG-guided SLNB have been rarely reported. In this present study, no patients suffered from

axillary wound infection and effusion. Furthermore, patients thought the wound was in good shape after 12 postoperative months, with motor function of the upper limb restored to its normal level; there was no upper limb numbness, and only 4.2% (two of 48) of patients occasionally had upper limb pain, with 2.1% (one of 48) of patients showing mild upper limb oedema (<1 cm). The NSABP B-32 trial showed that 15.8% of patients suffered from limited upper limb movement at 6 postoperative months, with the incidence of upper limb pain and numbness at 12 postoperative months being 9.2% and 12.6%, respectively, after SLNB guided by radionuclide combined with staining; and the incidence of upper limb oedema was 21.2%.³² The complications of SLNB guided by fluorescence and staining were also reduced in the present study compared with those obtained previously for SLNB guided by radionuclide combined with staining in the NSABP B-32 trial.³² This difference in findings might be explained by the fact that it is possible to observe SLNs through the axillary fat using ICG, thereby avoiding blind exploration and reducing site injury. In addition, all patients in this present study were followed-up for a mean of 20.4 months (range, 15–34 months) and none of the patients showed axillary recurrence or distant metastasis, with a survival rate of 100%. The present findings showed that SLNB guided by fluorescence and staining can achieve a satisfactory outcome, providing both precise treatment and reduced trauma in patients.

Since traditional SLNB is a difficult procedure with a long learning curve, surgeons are required to implement operations in 60 cases in order to ensure high accuracy.¹¹ Meanwhile, fluorescence-guided SLNB can achieve real-time imaging as well as direct visualization of the lymphatics and lymph nodes through the skin and soft tissues; this enables easy learning and accumulation of

experience. In this present study, the operation time was relatively long for the first surgeries (a mean of 18.8 min for the first 10 patients), which was significantly longer than values obtained for the subsequent 50 patients. This present study observed that the surgeon could conduct operations skilfully after implementing operations in 10 patients, completing SLNB in 10–15 min thereafter. In the first 10 patients, a mean of 2.4 SLNs were detected per patient, but this number was not significantly different from those obtained for the remaining 50 patients.

Although fluorescence-guided SLNB is superior to the staining method in many aspects,^{13,19–24} it still has some deficiencies. Indeed, damaged small lymphatic vessels may cause ICG leakage, which results in highlighted fluorescence in a wide surgical field, thereby rendering SLN detection difficult when guided by fluorescence. In our experience, the SLN location can be determined according to the region with highlighted fluorescence where blue-stained SLNs are found, when the region with highlighted fluorescence is slightly separated.

This present study had several limitations. First, its retrospective nature was associated with inherent shortcomings. Secondly, this was a single-centre study with a relatively small sample size. Therefore, well-designed multi-centre, prospective studies with large sample sizes are warranted to confirm these preliminary findings.

In summary, fluorescence combined with dye staining provides a high detection rate and limited trauma; it is radiation-free and easy to implement, and can spare early breast cancer patients with no lymph node metastases from ALND. These findings suggest that this method is clinically valuable.

Authors' contributions

Linping Huang conceived and designed the study; Jun Liu, Ning Wang and Ping Chen collected the data; Jun Liu analysed and

interpreted the data; Jun Liu wrote the article; Linping Huang provided critical revisions that were important for the intellectual content; Linping Huang approved the final version of the manuscript. All authors read and approved the final version of the manuscript.

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Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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