Real-time in situ navigation system with indocyanine green fluorescence for sentinel lymph node biopsy in patients with breast cancer

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Research

Keywords: breast cancer, sentinel lymph node biopsy, indocyanine green, real-time in situ navigation

Posted Date: October 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-70840/v2

Version of Record: A version of this preprint was published at Frontiers in Oncology on May 5th, 2021. See the published version at https://doi.org/10.3389/fonc.2021.621914.

Abstract

Background: The naked-eye invisibility of indocyanine green fluorescence limits the application of near-infrared fluorescence imaging (NIR) systems for real-time navigation during sentinel lymph node biopsy (SLNB) in patients with breast cancer undergoing surgery. This study aims to evaluate the effectiveness and safety of a novel NIR system in visualizing indocyanine green fluorescence images in the surgical field and the application value of combined methylene blue (MB) and the novel NIR system in SLNB.

Methods: Sixty patients with clinical node-negative breast cancer received indocyanine green (ICG) and MB as tracers. Two NIR system instruments, namely, lymphatic fluorescence imaging system (LFIS) designed by the University of Science and Technology of China and vascular imager by Langfang Mingde Medical Biotechnology Co., Ltd. (Langfang vascular imager), were used as navigation assistance to locate sentinel lymph nodes (SLNs). Excising the lymph nodes developed by both MB and ICG by two NIR systems or palpably suspicious as SLNs and undergoing rapid pathological examination.

Results: Both instruments exhibited 95% (57/60) success for real-time lymphatic fluorescent images. A total of 186 SLNs were identified, of which two were pathologically confirmed as lacking any lymph node tissue. SLN identification rate was 100% (184/184) for MB plus LFIS and 86.96% (160/184) for MB alone. The average number of SLNs identified by LFIS combined with MB was 3 (range of 1–8), which was significantly higher than that by MB alone at 2 (range 1–7) ($P<0.05$).

Conclusion: LFIS effectively detects SLNs in breast cancer, projects the fluorescence signals during surgery, and provides a continuous surgical navigation system without the need for a remote monitor. The ICG method navigated by combined LFIS and MB may be a promising alternative tracer for radioisotope in SLN mapping.

Background

Sentinel lymph node biopsy (SLNB) is the standard treatment for clinical axillary lymph node-negative breast cancer [1]. Clinical studies and meta-analysis showed that the exemption from axillary lymph node dissection for sentinel lymph node (SLN)-negative patients does not affect their overall survival and saves them from its complications, such as upper limb lymphedema, numbness, and pain [2]. Radioisotope (RI) and blue dye are SLNB tracers that are globally used but have some limitations. Blue dye has a long learning curve, requires practice to achieve high accuracy, has the risk of anaphylactic reactions, and is widely used in China but has unsatisfactory detection rate. Meanwhile, RI has a high detection rate. Dual localization with both tracers is considered the standard method [3, 4] with high detection rate and low false negative rate of 5%–10% [5, 6]. However, RI requires the assistance of nuclear medicine department, and its widespread use is limited by exposure to RIs and high preservation.

Indocyanine green (ICG) has been introduced as a new SLNB tracer since 2005 [7]. The NIR system can visualize the lymphatic flow and provide navigation for the surgeon to find and remove the SLNs. ICG has higher detection rate than traditional tracers RI and blue dye [8, 9]. Recent studies and meta-analysis indicated that ICG and RI tracers show no statistically significant difference in SLN detection rate [10, 11]. Although ICG has a high detection rate and short learning curve, the conventional NIR system need to be improved. Surgeons have to look at the remote monitors to identify the location of the fluorescent image because the fluorescent signal is invisible to the naked eye. The shadowless operating lamp must also be turned off to decrease the white-light contamination of images. This study introduced a novel NIR system that provides real-time operation navigation by producing fluorescent images that are directly visible in the operation field. SLNB was assisted by two NIR systems to verify the feasibility and effectiveness of lymphatic fluorescence imaging system (LFIS). In addition, the fluorescence localization effectiveness of ICG combined with MB was evaluated.

Patients And Methods

Patients

Sixty female patients with early breast cancer and clinically confirmed negative axilla were recruited from the Department of Breast Surgery of the First Affiliated Hospital of Anhui Medical University of China Department of Tumor Surgery, The First Affiliated Hospital of Bengbu Medical College of China, and Department of Breast Surgery of Nantong Cancer Hospital of China between March 2018 and June 2018.

Inclusion criteria were as follows: 1) primary breast cancer confirmed by core needle biopsy or Mammotome biopsy; 2) no enlarged axillary lymph nodes as verified by palpation, mammography, or breast ultrasound examination; and 3) no distant metastasis.

Exclusion criteria were as follows: 1) pregnant or lactating, 2) primary breast cancer confirmed by open biopsy, 3) preoperative radiotherapy at the breast area or neoadjuvant chemotherapy, 4) history of the axillary surgery, and 5) allergy to iodine.

This study was approved by the Institutional Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University, The First Affiliated Hospital of Bengbu Medical College of China and Nantong Cancer Hospital of China. And written informed consent was obtained from each subject.

Imaging System and Reagents

The two kinds of NIR system used in this study were LFIS by the University of Science and Technology of China and Jiangsu Xinmei Medical Engineering Technology Co., Ltd. (Fig 1a) and vascular imager by Langfang Mingde Medical Biotechnology Co., Ltd. (Langfang vascular imager) (Fig 1b). The fluorescence emission from the surgical site is acquired by the LFIS device, calibrated based on the detected working distance, and projected back to the surgical site for surgical guidance. This instrument has the updated version of handheld projective imaging device [12]. Meanwhile, the vascular imager relies on the additional screen. The tracers used were MB (JUMPCAN PHARMACEUTICAL GROUP CO., LTG.) and ICG (Eisai, Liaoning Pharmaceutical Co., Ltd.).

SLNB Procedure

After general anesthesia was administered, the four sites of periareolar region was subcutaneously injected with 1 ml of 1% MB (Fig 2a), followed by 1 ml of ICG after 5 minutes. The areola area was then massaged. Real-time imaging of lymphatic drainage imagine in the outer upper quadrant of the breast was performed using Langfang vascular imager and LFIS, and images of lymphatic drainage were captured (Figs 2b and 2c). SLNB incision was selected 2 cm away from where the fluorescence disappeared, and the consistency of incision location was evaluated. If the subcutaneous lymphatic flow is invisible or is discontinuous, then conventional incision (the external margin of the pectoralis major and the anterior margin of the latissimus dorsi) is chosen.

The fluorescent (ICG-positive) (Fig 3) and/or blue (MB-positive) lymph nodes were localized and excised similarly to the SLNs. The axilla was inspected for residual fluorescent or blue nodes. Lymph node development was recorded, particularly whether the lymph node is MB-, LFIS+, and Langfang+ (Fig 4). All excised nodes underwent immediate and postoperative pathological examinations. Axillary lymph node dissection was conducted only on patients with positive SLNs.

Statistical Analysis

SPSS statistical package version 21.0 was used for statistical analyses. Non-parametric Wilcoxon signed rank test was employed to compare the median number of SLNs between groups. A P-value<0.05 was considered statistically significant.

Results

1 Patient Characteristics

Patient and tumor characteristics are shown in Table 1.

2 ICG Mapping

Among the 60 patients with SLN detected using ICG combined with MB, real-time lymphatic fluorescent images were observed in the skin of 57 patients by both instruments in the same position. Hence, the rate of real-time observation of skin lymphatic streams was 95%. The three patients with no substantial lymphatic streams on the skin underwent SLNB with conventional incision, and their fluorescent lymph nodes were found successfully. In one case, 2 SLNs were ICG positive and MB negative, and pathology revealed no lymph node tissues.

With the exclusion of the above case, 184 SLNs were obtained. Table 2 shows that the detection rate of LFIS combined with MB sentinel node was 100% (184/184), and that of MB alone was 86.96% (160/184). The median number of SLNs identified by LFIS combined with MB was significantly higher (3, range 1–8) than by MB (2, range 1–7) (P<0.05).

Among the 60 patients, 10 has metastatic SLNs, and 15 out of 74 lymph nodes were positive. The trace situation of positive lymph nodes is shown in Table 3. The use of MB alone would have missed 20% of the positive axillas. Given the small number of cases, further clinical trials must be conducted for validation.

3 Adverse effects

No allergic reaction was observed from surgery to discharge. All 60 patients were followed up for 24 months and showed no adverse reactions such as skin lesions, necrosis, and infection at the ICG injection site.

Discussion

This prospective and self-matching study aimed to compare LFIS with conventional NIR system and blue dye method to assess whether the former can be used as a reliable alternative and whether it is superior over blue dye method for early breast cancer.

ICG has a short half-life in plasma. After injection, it can bind tightly to plasma proteins and immediately enter lymphatic vessels to flow to SLNs [13]. Thus, ICG serves as a marker for a specific molecule or cell. SLNs in breast cancer regularly occur in specific areas. Thus, the precise location of incision can be readily chosen, and SLNs are easy to find under fluorescence guidance. The NIR fluorescence band of ICG is 700–900 nm, which is invisible to the naked eye. Therefore, the position and movement of these molecules and cells must be assisted by a NIR fluorescence imaging system to obtain the accurate location of lymphatic vessels and sentinel lymph nodes. Although the ICG fluorescence method is unique in surgical navigation and has high sensitivity, its application to current NIR systems encounters several technical issues that must be addressed. The current NIR system displays the fluorescence image on the mobile monitor, and the surgeon must alternately and repeatedly look at the surgical field and the remote monitor to confirm the site of the lymph nodes. This phenomenon destroys the consistency and increases the complexity of the surgical procedure. A Google-enhanced imaging system was developed in collaboration with the University of Science and Technology of China. When the surgeon wears Google Glasses, the fluorescent signal is projected onto the Google Glasses to achieve real-time display of approximate surgical field [14, 15]. This study presents a novel NIR system called LFIS that can continuously and accurately project the fluorescence image on the surgical field to allow for a focused vision and shorten the operation time. The LFIS provides real-time navigation for SLNB and has two modes: projection mode and lighting mode, which shifts by pressing one button, thus limiting the need to switch the shadowless lamp. LFIS is comparable to conventional NIR systems in locating sentinel nodes. Owing to self-matching, quantitative comparison under short surgery duration is unavailable.

The detection rate of LFIS-guided ICG combined with MB (100%, 184/184) was better than that of MB alone (86.96% (160/184), and this finding was consistent with previous studies. The total number of LFIS positive (176) was higher than that of MB positive (160) and is possibly related to the affinity for the lymphatic system. The affinity of ICG is stronger than that of MB because of the molecular structure and diameter; the molecular mass of ICG (774.9) is larger than that of MB (319.9) [16].

ICG fluorescence imaging has been favored by researchers as a new SLN tracer method since 2005. Recent meta-analysis showed that ICG has SLN detection rate from 81.9% to 100% and sensitivity from 65.2% to 100%. No statistical difference in detection rate and sensitivity was found between ICG combined with RI tracer and ICG alone [17]. The key factor in evaluating the quality of SLNB is the false-negative rate. False negative means that metastatic lymph nodes are not detected, and the tumor stage is underestimated. This phenomenon leads to inadequate systemic treatment and increases the risk of local recurrence and distant metastasis. Another meta-analysis based on six studies reported 8% false negative rate when using ICG as a tracer [18]. In the National Surgical Adjuvant Breast and Bowel Project B-32 trial including 5611 patients with clinically negative axillary lymph nodes, a false negative rate of 9.8% is found when using combined blue dye and RI double tracer [4]; this value was comparable to that of ICG. Findings about the comparability of false negative rate (FNR) between ICG and dual tracer of RI and blue dye are inadequate and thus require additional clinical trials for validation. On the basis of the above data, MB combined with ICG can be a new dual-tracer method to replace RI plus blue dye, especially for institutions without access to RI. This novel method have the following advantages over the gold standard: a) projection real-time navigation with advanced image processing for lymphatic visualization, b) no involvement of physicians from the nuclear medicine department prior to the operation, and c) easy transportation and preservation.

The mean number of removed SLNs is 1.5–3.4 under the guidance of conventional NIR system with ICG fluorescence [11] and 3 under guidance of individual LFIS and conventional NIR system. The median number of SLNs excised by blue dye is 2, which is significantly lower than that by ICG methods ($P<0.05$). Compared with blue dye, the NIR fluorescence imaging system shows sensitivity even at low concentrations that are visible to the naked eye [19] and detects more SLNs. The increase in the number of SLNs detected within the appropriate range can avoid excessive interference in the axillary tissue and enable an accurate and full evaluation of lymph node condition. Extracting only one SLN has a high risk of false negative. To date, 3–4 SLNs are needed to identify more than 97% positive lymph nodes. In combination with postoperative complications, the extraction of no less than 4 SLNs is currently recommended [20].

In a case in this study, two SLNs were detected by the two fluorescence imaging systems but not by MB. These two lymph nodes were re moved and pathologically indicated as lacking lymph node tissues. This finding revealed the limitations of ICG as a tracer. The leakage of ICG caused by intraoperative lymphatic vessel damage and its high sensitivity resulted in the occurrence of non-lymph node fluorescence images in the operative field. This phenomenon is called “contamination”, which may cause the difficulty of lymph node localization.

In this study, three patients had no percutaneous fluorescence signal and superficial lymphangiography but showed ICG lymph nodes as revealed by conventional incision.Body Mass Index (BMI) is negatively correlated with lymphatic vessels, and injection depth and fat thickness are the main factors affecting ICG sensitivity [21]. However, no correlation was found between lymphatic vessels and the detection rate of fluorescent lymph nodes. In the three cases without lymphatic vessel images, fluorescent lymph nodes were observed through conventional incision. The surgeon’s intuitive feeling is that the difficulty of SLNB is relatively high for obese patients. Hence, the difficulty of surgery must be quantified afterward, and the operation time should be measured.

In this study, the number of ICG lymph nodes was negatively and weakly correlated with BMI. In a similar study, patients with high BMI ($\geq$22 Kg/m^2) have fewer removed SLNs than those with lower BMI, but no statistical difference was observed [22].

Conclusion

The novel real-time in situ navigation system is a promising instrument for SLNB in breast cancer. The lymphangiography and SLN development of LFIS are consistent with those of the conventional NIR system. The combination of fluorescence by LFIS and MB may be alternative to the standard method of combined RI and blue dye because of its high detection rate, radiation-free, and operation fluency. This technique satisfies the surgeons’ demand of navigation operation and can be used in various surgical fields.

Abbreviations

near-infrared fluorescence imaging systems: NIR system

sentinel lymph node biopsy: SLNB

methylene blue ：MB

Radioisotope：RI

Body Mass Index ：BMI

false negative rate : FNR

Declarations

Competing interests

The authors declare that they have no conflicts of interest

Consent for Publication

Written informed consent for publication was obtained from all participants and the consent form was kept in 3 clinical trial centers.

Ethics approval and consent to participate

This study was approved by the Institutional Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University, The First Affiliated Hospital of Bengbu Medical College of China and Nantong Cancer Hospital of China. And written informed consent was obtained from each subject.

Ethical code:

PJ2017-11-04 (First Affiliated Hospital of Anhui Medical University), 2018-025 (The First Affiliated Hospital of Bengbu Medical College of China)

2018-001 (Nantong Cancer Hospital of China)

Funding

Anhui Province Science and Technology Major Project (No. 17030801004) and Natural Foundation of Anhui Province (No.2008085MH295).

Authors' contributions

Zhaorui Wang, Xiaowei Yang and Peng Liu designed the experiment ; Zhaorui Wang and Jing Pei analyzed the patient data and writed papers.

Chunguang Han and Yubo Pan recorded data.

Availability of data and materials

The datasets used and analysis during the current study are available from the corresponding author on reasonable request.

Acknowledgment

Not applicable

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Tables

Table 1 Tumor and patients characteristics

Factors	N	%
Total patients	60	
Age, median (range)	50(31-67)	
Body Mass Index	23.93(18.32-30.12)	
<24	31	51.67
≥24	22	36.67
≥28	6	10.00
≥30	1	1.65
Tumor stage		
Tis	6	10
T1	24	40
T2	28	46.67
T3	2	3.33
Histological grade		
1	9	15
2	34	56.67
3	12	20
Unknown	5	8.33

Table 2 Detection rate of various methods

	N	Detection rate (%)
MB+ and LFIS+	184	100
MB+	160	86.96
LFIS+	177	96.20
Langfang Vascular imager+	174	94.57

Table 3 Positive SLNs and patients detected by different methods

		Detected positive nodes Number(%)	Detected positive axillas (patients) Number(%)
LFIS(+)	MB(+)	12(8)	8(80)
LFIS(+)	MB(-)	3(2)	2(20)
LFIS(-)	MB(+)	0(0)	0(0)
Total		15(100)	10(100)

Figures

Figure 1

Two NIR system a. Left one: Lymphatic Fluorescence Imaging System(LFIS) designed by university of Science and Technology of China b. Right one: Vascular imager by Langfang Mingde Medical Biotechnology Co.LTD (Langfang Vascular imager)

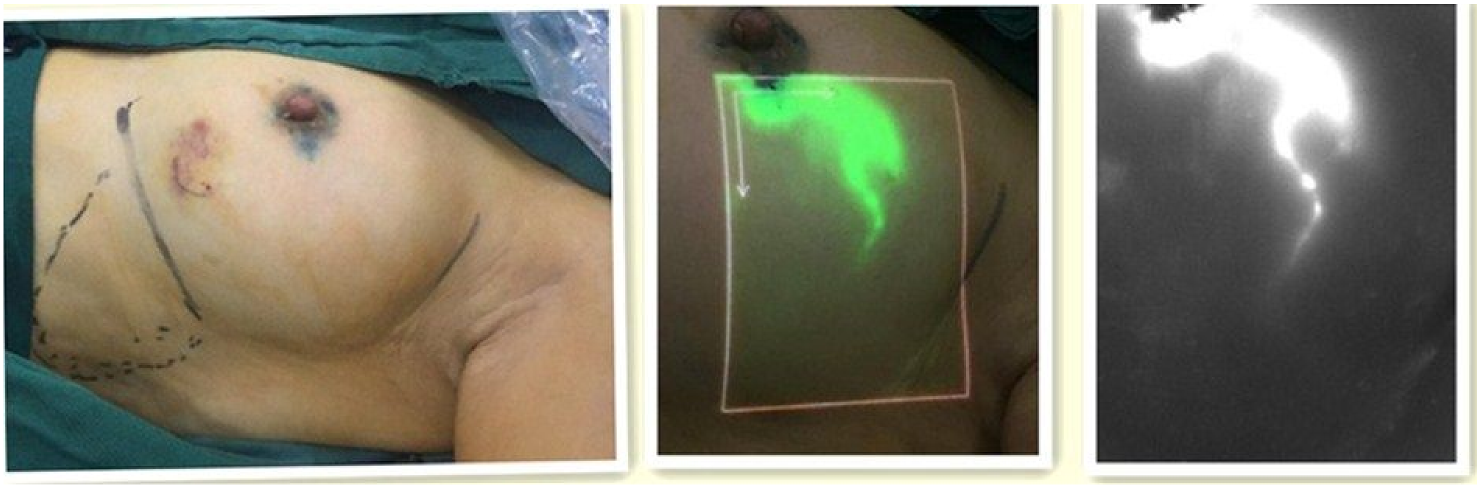

Figure 2

The images of lymphatic drainage a. Left one: ICG was injected around the areola b. Middle one: The image of lymphatic drainage on the skin with the LFIS c. Right one: The image of lymphatic drainage on the monitor with the Langfang Vascular imager

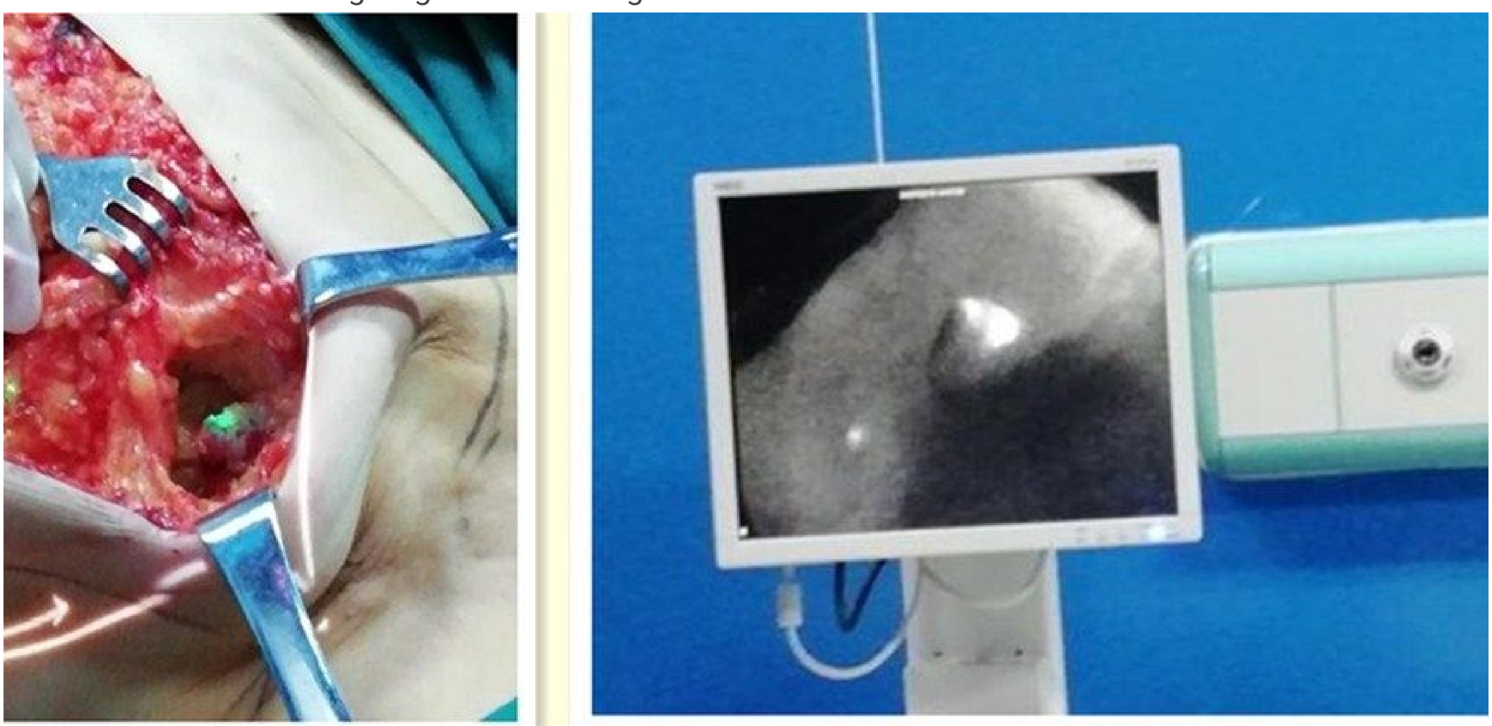

Figure 3

Intraoperative fluorescence imaging of lymph nodes a. Left one: Intraoperative fluorescence imaging of lymph nodes with the LFIS b. Right one:Intraoperative fluorescence imaging of lymph nodes with the Langfang Vascular imager

Figure 4

Image of resected lymph nodes a. Left one: The MB lymph node b. Middle one: The ICG lymph nodes with the LFIS c. Right one: The ICG lymph nodes with the Langfang Vascular imager