

Non-Invasive Identification of Sentinel Lymph Nodes Using Indocyanine Green Fluorescence Imaging in Patients with Breast Cancer

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Abstract: *Background:* Recently, sentinel lymph node biopsy (SLNB) has been carried out routinely in patients with early breast cancer. Avoidance of axillary lymph node dissection is considerably desirable items of maximizing the quality of life of postoperative patients. Here we report non-invasive identification of SLN using indocyanine green (ICG) fluorescence imaging, which provides a high detection rate and a low false-negativity rate.

Patients and Methods: One hundred and fifty breast cancer patients with tumors less than 3 cm in diameter were enrolled in this study. ICG dye and indigo carmine were injected subdermally at the same time into the areola. Subcutaneous lymphatic channels draining from the areola to the axilla were immediately rendered visible by fluorescence imaging using a Photodynamic Eye (PDE). After incising the axillary skin over the location of the LN identified by ultrasonography, the SLN was dissected under fluorescence imaging guidance with adequate adjustment of sensitivity.

Results: Lymphatic channels and SLN were successfully identified in all patients. The mean number of SLN and the operation time for SLNB were 3.2 and 15.2 min, respectively. Twenty-six patients (17.3%) were found to have lymph node metastases pathologically. Adjustment of the sensitivity of PDE facilitated a reduction in the operation time. There were no intra- or postoperative complications associated with SLN identification.

Conclusions: This method is feasible, safe, and only minimally invasive for intraoperative detection of SLN, allowing real-time observation without any need for training.

Keywords: Image overlay surgery, sentinel lymph node biopsy, breast cancer, navigation surgery, fluorescence imaging.

INTRODUCTION

Recently, sentinel lymph node biopsy (SLNB) has become a routine procedure for patients with early-stage breast cancer. Avoidance of axillary lymph node dissection is considerably desirable in terms of maximizing the quality of life of postoperative patients. In general, the tracers used for SLNB in breast cancer can be divided into two types: radioactive colloids and dyes. Krag *et al.* [1] first introduced SLNB using a radioactive colloid in 1993, and Giuliano *et al.* [2] subsequently reported the use of a blue dye in 1994. Up to now, many multi-center studies have reported the usefulness of SLNB in terms of detection rate, false negativity rate, and avoidance of lymph edema or sensitivity of the upper extremity [3-9]. In fact, a combination of radioactive colloid and dye has been shown to yield high detection and accuracy rates in SLNB [10, 11]. Obtaining better outcomes of SLNB requires the acquisition of procedural skill, the use of radioactive colloid, and suitable equipment housed in nuclear medicine facilities. However, most institutions or hospitals in Japan use dye alone for SLNB, and thus the resulting outcomes are variable.

After 2003, near-infrared fluorescence imaging began to be used for detecting lymphatic flow and lymph nodes in animal models [12-15]. Subsequently this technique was

applied to clinical fields such as cardiovascular surgery, organ transplantation and gastrointestinal surgery [16-21]. Kitai *et al.* [22] reported the successful use of SLNB using indocyanine green (ICG) for patients with breast cancer. We have also performed a feasibility study of sentinel lymph node (SLN) detection using ICG fluorescence imaging [23]. Here we present our results of non-invasive identification of SLNs using ICG fluorescence imaging allowing real-time observation without any need for training.

PATIENTS AND METHODS

Patients

Between May 2005 and July 2009, 150 patients with breast cancer were enrolled in this study. Their ages ranged from 29 to 86 years, with a mean of 55 years. All had breast cancer that had been histologically confirmed by core needle biopsy of lesions smaller than 3 cm without axillary lymph nodes involvement, and were scheduled to receive standard care in accordance with the designated clinical pathway. This study was approved by the Institutional Review Board of Dokkyo Medical University. Informed consent was obtained from all patients. Patients with a history of allergy to iodine or shellfish were excluded from the study.

Surgical Procedure

Initially, intraoperative ultrasonography was performed to detect the axillary lymph nodes. We identified a SLN as the first lymph node (LN) recognized during ultrasonography scanning from the edge of the breast gland in the direction of the axilla and marked its position on the

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axillary skin. This procedure was added when the SLN was not recognized as a blue node upon fluorescence imaging. Subsequently, 0.75 ml (2.5 mg/ml) of ICG (Daiichi-Sankyo Pharmaceutical, Tokyo, Japan) and 0.75 ml of indigo carmine (Daiichi-Seiyaku, Osaka, Japan) were injected subdermally at the same time in the region of the areola. After injection of the dye mixture, near-infrared light produced by a PDE (Photodynamic Eye: Hamamatsu Photonics Co., Hamamatsu, Japan) (Fig. 1) was directed onto the operative field. This apparatus measures 8 x 8 x 18 cm, weighs 0.5 kg, and is equipped with a light-emitting diode that produces light with a wavelength of 760 nm, together with a charged-coupled device (CCD) camera with a filter. The fluorescence of several lymphatic ducts was identified immediately on the monitor connected to the CCD camera, and fluorescence imaging was conducted from the nipple in several directions including the axillary area (Fig. 2). The point of disappearance of the fluorescence was identified on the skin. In most cases, the line previously marked by ultrasonography was located approximately 1 cm towards the axillary side from the point of disappearance of the fluorescence stream towards the axillary area. The marked line was then incised and the subcutaneous connective tissues were dissected. The PDE was then re-applied to the incised area and the fluorescent areas including the SLN were revealed on the monitor with adequate adjustment of sensitivity (Fig. 3). In cases where lymphatic flow to the SLN was visible, the surface of the breast or axillary skin was compressed using a transparent hemispherical device to observe the lymphatic flow (Fig. 4). The SLNs with or without blue-dyed lymph nodes were then resected using the fluorescence image on the monitor as a guide. The PDE was then used to confirm whether or not the resected SLNs were fluorescent. All LNs showing fluorescence were investigated for the presence of metastasis in the pathology department intraoperatively.



Fig. (1). The photodynamic eye apparatus.

RESULTS

Lymphatic channels were successfully identified by PDE in all patients. In 2 patients (1.3%), however, no fluorescence image of the SLNs was obtained. The detection and accuracy rates were 98.7% and 100%, respectively. The latter two patients also showed no blue nodes. The mean number of SLNs detected by fluorescence imaging was 3.7 (range: 0-11). The number of SLNs in the initial 20 patients ranged from 2 to 11, with a mean of 5.5, and in the last 20 patients ranged from one to 5, with a mean of 2.6. One hundred and four patients (69.3%) had LNs stained blue

with the injected dye. The mean number of such LNs was 2.3 (range: 0-8). Twenty-six patients (17.3%) were found to have LN metastases pathologically. All patients with pathologically positive LNs were recognized by fluorescence imaging, but in 11 (42.3%) of them the LN metastases were not identified by staining with vital dye. The sites of skin incision were also identical with the LN that had been demonstrated by ultrasonography in all patients. The mean operation time was 15.2 min (range: 6-28 min). Adjustment of PDE sensitivity for identification of the SLNs ranged from 20% to 25%. Furthermore, compression of the axillary skin using the transparent hemispherical device was effective for identifying the location of SLNs in non-obese patients. However, in 38 patients (25.3%), the SLN was invisible on the axillary skin even after compression. There were no intra- or postoperative complications associated with SLN identification or the use of ICG itself. ICG tattooing around the areola disappeared spontaneously within two weeks.



Fig. (2). Lymphatic flow was visualized from the areola to the axilla under PDE guidance.

DISCUSSION

In SLNB, the detection rates obtained using dye alone, radioactive isotope alone and a combination of both have been reported to be 80-81%, 87-89% and 95-96%, respectively [24, 25]. In Japan, the reported detection rates with the use of dye alone and in combination with a radioactive isotope are 74% and 94%, respectively [26]. The two-mapping procedure has been the method of choice for identification of SLN. However, the introduction of ICG fluorescence imaging heralded a new era of SLNB. ICG is a common diagnostic reagent that can be purchased easily, and can demonstrate the lymphatic stream directly upon naked eye observation after local injection using near-infrared irradiation [27]. There is no requirement for preoperative administration of a radioactive colloid, and thus exposure to radiation is avoided. ICG is a very convenient reagent to use, and its application for fluorescence imaging is gradually increasing in many institutions as it allows easier detection of SLNs.

With regard to SLNB using ICG, Motomura *et al.* [7] reported that the rate of SLN detection using ICG alone was 73.8% and that the mean number of SLN was 1.7. Kitai *et al.*

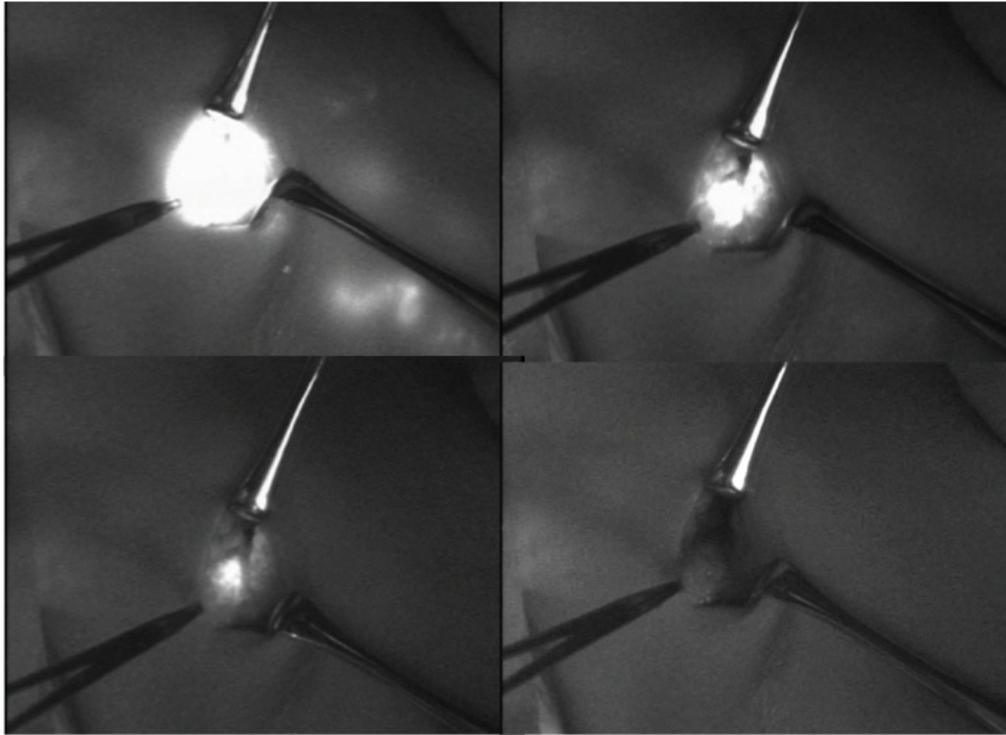


Fig. (3). Differences in PDE sensitivity (100%, 50%, 20%, and nil).

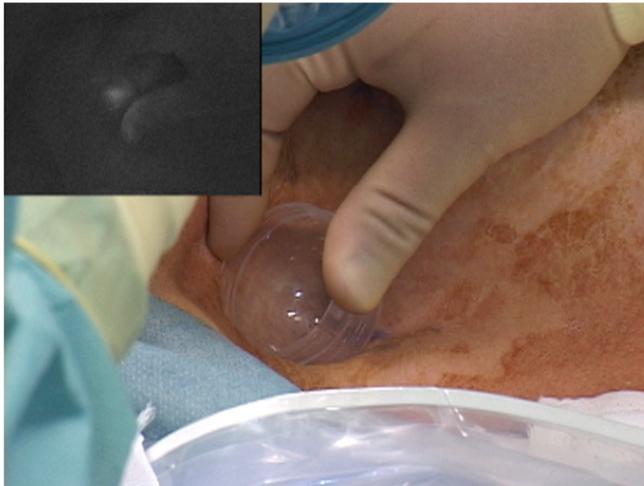


Fig. (4). Compression of the axillary skin using a transparent hemispherical device was effective for identifying the location of SLNs.

[22] reported that the rate of SLN detection using both ICG and fluorescence imaging was 94% and that the mean number of SLNs was 2.8. Our previous study [23] showed that the detection rate achieved with ICG and fluorescence imaging was 100%, and that the mean number of SLNs was 5.4. The present study showed that the detection rate was 98.7% and the mean number of SLNs was 3.7. However, with ICG alone, the detection rate was 69.3% and the mean number of SLNs was 2.3. These results were similar to those of Motomura *et al.* for the use dye alone without fluorescence imaging. In general, the detection rate using radioactive colloid has ranged from 87% to 98%, with a mean of 92.3% [4-6, 8, 9, 11]. The introduction of fluorescence imaging has yielded a detection rate equal or

superior to that of radioactive colloid. Fluorescence imaging using a PDE has high sensitivity for ICG. The combination of fluorescence imaging with a dye is more advantageous for SNNS at institutions that lack suitable facilities for handling radioisotopes. However, a study to compare the performances of fluorescence imaging and radioactive colloid should be performed to determine the optimal indications for each method.

The advantages of this method are that it allows real-time observation of the lymphatic streams on the skin, and superior recognition of the lymphatic vessels and LNs compared with dye staining; there is also no specific need for specialized training in detection of SLNs, and no requirement for a radioisotope facility [22, 23]. These potential advantages may ultimately lead to acceptance of the superiority of this method in comparison to dye alone or radioactive colloid. However, the method also has several problems including the need to turn the shadowless light on and off during the procedure, the time limitation for detection of SLNs due to easy spreading of ICG through the subcutaneous tissue, the fact that it is inapplicable to patients with iodine hypersensitivity, and residual ICG tattooing that persists for 10 to 14 days [22, 23]. However, we think that the advantages of this method outweigh its disadvantages, and that ICG fluorescence imaging provides better outcomes in SLNB.

Recently, it has become relatively easy to reconstruct three-dimensional (3-D) images from computed tomography (CT) with contrast medium. The guidance afforded by 3-D CT image is very helpful for surgeons in confirming the anatomy of the operating field. We have also reconstructed 3-D CT images by volume rendering with Osirix, and this provides high-quality images and immediate 3-D reconstruction. In our patients undergoing breast surgery, the

3-D image was projected onto the operative field with clear visualization of axillary LNs *via* a micro-projector connected to a personal computer. We always utilize image overlay navigation surgery when performing breast resection and SLNB. In particular, this navigation system is very helpful for detecting SLNs intraoperatively in obese patients reducing the operation time and stress for the surgeon. It is anticipated that computer aided surgery will become an extremely useful adjunct in various surgical fields.

The number of SLNs detected using ICG fluorescence imaging depends on the time taken to identify them. In our present series, the number of SLNs in the initial 20 patients was over twice that in the last 20 patients. At the time of introduction of SLNB using ICG fluorescence imaging, identification of SLNs took 20 min or more. However, as the identification time increased, more SLNs were found. Therefore, we think that the optimum time taken for SLN identification is around 10 min, based on the results of recent practice. Furthermore, to minimize the time taken for SLN identification, it is necessary to adjust the sensitivity of the PDE, to compress the axillary skin using the transparent hemispherical device, and to introduce image overlay navigation surgery. At full power (100%), the PDE showed strong fluorescence from all parts of the surgical field, which hindered identification of SLNs. Therefore, the adjustment of PDE sensitivity for identifying SLNs ranged from 20% to 25%. Although compression of the axillary skin using the transparent hemispherical device is effective for identifying the location of SLNs in non-obese patients, there is an absolute need for the image overlay navigation system in obese patients because it is often difficult to detect the fluorescence of SLNs due to the large amount of connective tissue in the axilla.

We conclude that the present method is feasible and safe for intraoperative detection of SLN. It is also minimally invasive, allows real-time observation, and does not require any specialized training. It is anticipated that this method will facilitate high detection rates and low false-negativity rates in SLN surgery and may be practical and applicable to various other surgical fields.

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