Bio-Optical Devices in Indocyanine Green Fluorescence Guided Sentinel Node Biopsy for Breast Cancer

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Abstract: *Background:* The indocyanine green (ICG) fluorescence method is a newly developed technology to facilitate the sentinel node biopsy with the guidance of ICG fluorescence images. However, the surgical technique is still difficult, since the sentinel node cannot be indentified before skin incision and careful dissection of the subcutaneous lymphatic vessels is necessary. In this paper, an axillary pressing technique is introduced.

Materials and methods: The subcutaneous lymphatic vessels just under the skin were easily detectable, but the detection of the sentinel nodes deep under the skin was difficult by ordinary observation of the fluorescence images. By pressing the axillary skin against the chest wall, the sentinel node is close to the skin and the fluorescence signal becomes intense enough to be detected. The skin incision was made on the pressure-induced fluorescent spot, and the sentinel node was directly approached with the guidance of fluorescence without dissecting the subcutaneous lymphatic vessels.

Results: Pressure-induced fluorescence signal in the axilla was observed in all of 36 cases. Direct approach to the axillary sentinel nodes without tracing the subcutaneous lymphatic vessels was successful in 33 cases.

Conclusions: The ICG fluorescence method is reliable and little training is required. The axillary pressing technique is useful to make it more popular.

Keywords: ICG fluorescence imaging, breast cancer, sentinel node biopsy, axillary pressing, near infrared.

INTRODUCTION

Sentinel node biopsy is an established procedure to access the lymph node status in early breast cancer to avoid unnecessary axillary dissection [1-4]. There are two methods used in sentinel node biopsy: radioisotope guided method [5] and dye guided method [6]. The radioisotope method is the worldwide standard, because little training is required until satisfactory results can be obtained. The dye method, although certain training is necessary, is also advantageous, because it is free from radiation exposure, and does not require radioisotope facilities.

The indocyanine green (ICG) fluorescence method is a newly developed technology [7-8]. It is a modification of the dye method [9-10], in which the detection of sentinel nodes is facilitated by fluorescence navigation. Tissue penetration of near infrared (NIR) light is an important point of this method. Under visible light, subcutaneous lymphatic vessels and the lymph nodes in the fatty tissue are not visible to the naked eye, but fluorescence imaging makes these structures visible. High sensitivity is also beneficial.

Even in cases where one can hardly tell which nodes are stained by ICG green color, fluorescence imaging clearly shows which are sentinel. However, the surgical procedures are still not easy as compared with the radioisotope method, because the sentinel node cannot be detected from the skin. The sentinel node is usually at a depth of 2cm or more in the axilla, and the fluorescence signal from the sentinel node is mostly lost by scattering. In this paper, we describe the clinical presentation of the ICG fluorescence method, and introduce bio-optical devices to overcome the difficulties involved in the ICG fluorescence method.

MATERIALS AND METHODS

ICG Fluorescence Method

Original procedures of ICG fluorescence method, which were reported elsewhere [7], are described briefly. An infrared fluorescence imaging system (photodynamic eye, Hamamatsu Photonics, Japan), which consists of light emitting diodes at 760nm as a light source, and a charge coupled device camera with a cut filter below 820 nm as a detector, was used to measure NIR fluorescence images.

Surgical procedures of the ICG fluorescence method are principally the same as those of the dye method [9]. After induction of general anesthesia and sterilization of the operating site, 5mg/1ml of ICG is injected into the areolar skin [11]. After a few seconds, lymphatic drainage was observed with fluorescence images. Subcutaneous lymphatic vessels were detected over the skin, usually in one or two minutes, towards the axilla, and disappeared beyond the lateral edge of the pectral major muscle. This is the point where the subcutaneous lymphatic vessel enters the axillary space. After a small skin incision is made at this point, the

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lymphatic vessel is dissected until the sentinel nodes in the axilla are reached under the guidance of fluorescence images. The sentinel nodes can be differentiated from lymphatic vessels, because the lymph nodes have a more intense fluorescence signal and a round shape. It is important not to injure the lymphatic vessels during the dissection. Otherwise, due to the fluorescence signal of ICG spilling into the surgical field, further dissection becomes difficult. It is recommended that the lymphatic vessel is not exposed but dissected with surrounding fatty tissue. Since several fluorescent spots are usually observed around the first nodes, those fluorescent nodes are dissected *en bloc* with the surrounding fatty tissue. Lymph nodes in the dissected specimen are isolated and investigated under the infrared camera. All fluorescent nodes were regarded as sentinel nodes and examined by frozen section (Fig. **1**).

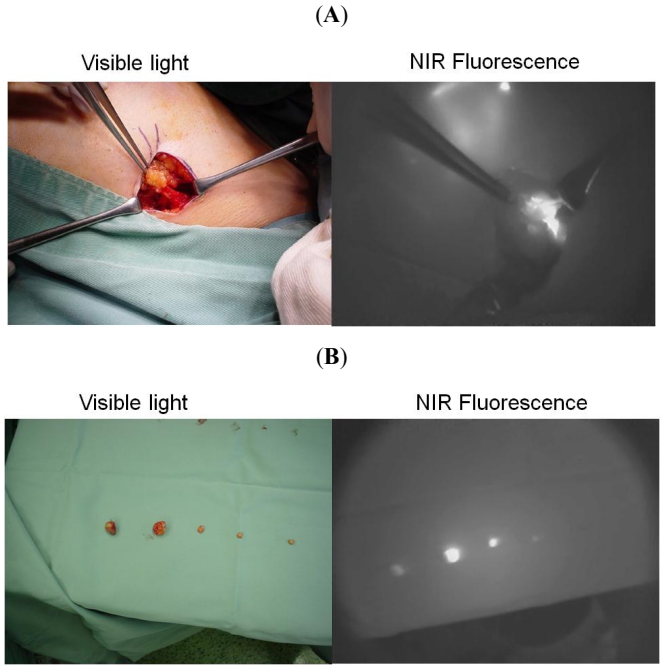

Fig. (1). (**A**) After injection of ICG around the areola, the subcutaneous lymphatic vessels were dissected toward the axillary sentinel nodes by the guidance of fluorescence images. (**B**) Lymph nodes stained by ICG can be judged with high sensitivity.

A validation study of 38 cases was undertaken from 2004 to 2005 [7]. The detection rate was 96.5 %, and the number of sentinel nodes was 2.7 on average. In this method, the number of sentinel nodes is greater than other methods, because ICG tends to drain to farther lymph nodes than radioactive colloid, and the detection by fluorescence is more sensitive than ordinary inspection. One false negative case was encountered. Negative predictive value was 96 %, and the false negative rate was 7.1 %.

Axillary Pressing Technique

In January 2008, we began using the axillary pressing technique in order to identify the location of sentinel nodes before skin incision. Fig. (**2**) shows a schematic drawing of the axillary pressing technique. The subcutaneous lymphatic vessels just under the skin are easily detectable. However, the detection of the sentinel nodes deep under the skin was difficult by the original method. By pressing the axillary skin against the chest wall, the sentinel node is close to the skin and the fluorescence signal becomes intense enough to be detected. We used a small transparent plastic bowl for this purpose. There are four small holes on the top for the purpose of skin marking (Fig. **3**). Ordinary observation of the ICG fluorescence image shows that the subcutaneous lymphatic drainage disappeared beyond the lateral edge of the pectral major muscle, with no signal in the axilla. The axillary skin is pressed against the chest wall by a transparent hemisphere to search for a point where a distinct fluorescent signal appeared by pressing. The sentinel nodes are supposed to be under the pressure-induced fluorescent point. After skin incision was made, a weak and ill-defined fluorescent signal was observed. While dissecting the underlying fascias, the fluorescent signal became intense and well localized, and finally, the shining sentinel node could be pulled out of the axillary space with surrounding fatty tissue (Fig. **4**). Since direct approach to the sentinel nodes is possible by axillary pressing technique, the subcutaneous lymphatic vessels are not dissected as the original procedures.

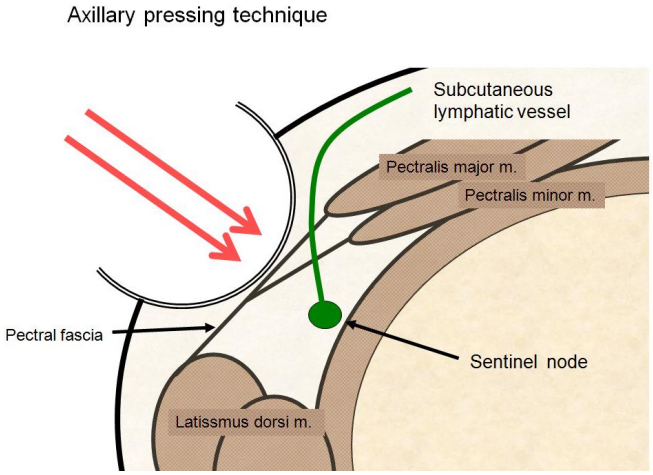

Fig. (2). Schematic drawing of axillary pressing. By reducing the depth of the lymph node, the light scattering is greatly reduced.

Patients

The axillary pressing technique was used in 36 patients with node-negative breast cancer from January 2008 to August 2009 in Nara Social Insurance Hospital. The mean age was 65.4 (41~93), and the body mass index (BMI) was 23.3 (18.5~36.4). The mean tumor size was 1.6 (0.5~3.5) cm. Five cases were operated on by an attending physician, and the other 31 cases were done by a surgical resident under supervision. This study was approved by the institutional review board of Nara Social Insurance Hospital. Informed consent was obtained from the study participants.

RESULTS

Pressure-induced fluorescent signal in the axilla was observed in all cases. Direct approach to the axillary sentinel nodes without tracing the subcutaneous lymphatic vessels was successful in 33 cases (Table **1**). In three cases, however, the fluorescent signal obtained by pressing was proven to come from the axillary lymphatic vessels, and

(**A**)

(**B**)

Fig. (3). (**A**) Two types of transparent hemispheres for pressing the axilla. Round one for general use, and sharp one for obese patients. (**B**) Axillary pressing using a transparent hemisphere.

subsequent dissection towards the sentinel nodes was necessary. Sentinel node was successfully identified in all cases, and the number of sentinel nodes was 3.9 (1~8). It took 25.7 (10~47) minutes to remove the sentinel nodes after ICG injection. Direct approach was successful in all 15 patients younger than 65, but not successful in three of 21 patients with older than 65. The axillary pressing technique was effective in eight obese patients with BMI greater than 25 as well, although the duration of the procedure was slightly longer than that of the patients with BMI lower than 25. The duration of the procedure of the attending physician was significantly shorter than that of the surgical resident. There was no difference between the early cases and the late cases in this series.

DISCUSSION

Superior characteristics of the ICG fluorescence method over the conventional dye method are as follows: 1) the site of skin incision can be precisely identified. 2) tracing the lymphatic vessels into the axillary lymph nodes is facilitated by fluorescence images. 3) lymph nodes stained by ICG can be judged with high sensitivity. ICG has a characteristic fluorescence spectra in the near infrared wavelengths, ranging from 700 to 900 nm, which is called “an optical window.” This is advantageous to clinical application, because the near infrared light can penetrate deep into the tissue without being absorbed by hemoglobin or water [12-13].

However, when applied to living tissue, light scattering becomes an important issue [14-15]. The fluorescent excitation and emission are attenuated by scattering during traveling in the tissue. Fat droplets in the axilla are the main scatterers [16-17]. In the preliminary experiment, ICG fluorescence signal at the depth of 1 cm in the phantom was detectable, but the detection becomes more difficult as the depth increases.

Among criticisms against the ICG fluorescent method, the main difficulty is that the sentinel nodes cannot be detected from the skin. Since the excitation or emission light is scattered out in the axillary fatty tissue, the fluorescence signal from the sentinel nodes is too weak to be detected. The limit of detection depth is around 1 cm. The dissection of the subcutaneous lymphatic vessels towards the axillary sentinel nodes requires certain training, which is not necessary with the radioisotope-guided method.

There are two possible solutions: the improvement of hardware and the reduction of light scattering. The first solution is technically difficult at present. Even if the fluorescence signal becomes strong, scattered light only illuminates the whole axilla and the sentinel nodes cannot be recognized. The second solution can be achieved by shortening the distance between the skin and the lymph node using a pressing technique. The attenuation of the fluorescence intensity is determined mainly by the scattering coefficient of the tissue and the distance between the detector and the sentinel node. By pressing the axillary skin against the chest wall, the sentinel node is close to the skin and the fluorescence signal becomes detectable. Even after pressing, the scattering coefficient of the tissue does not increase. Among several pressing devices that we tried in the preliminary study, a transparent hemisphere with a diameter of 4 cm was the best to detect the fluorescent signal from the sentinel nodes.

In our series, pressure-induced fluorescent signal from the deep lymphatic vessels was mimicked as that from the sentinel nodes in three cases. Generally, the signal from the lymphatic vessel is weak and linear-shaped, and that from the sentinel nodes is intense and round-shaped. However, it is not always easy to differentiate them. Our results suggested that the axillary pressing technique was also effective with obese patients as well, but that it may not work well in some elderly cases. The difference in the duration of procedure between the attending physician and the surgical resident may indicate that there is a certain learning curve. However, there was no difference in the early cases and the late cases, suggesting that the axillary pressing technique is feasible from the start.

The axillary pressing technique is a convenient and effective way to improve the results with little training. However, caution must be paid not to miss the sentinel nodes proximal to the dissected nodes. It is mandatory to ensure that the subcutaneous lymphatic drainage enters the dissected area.

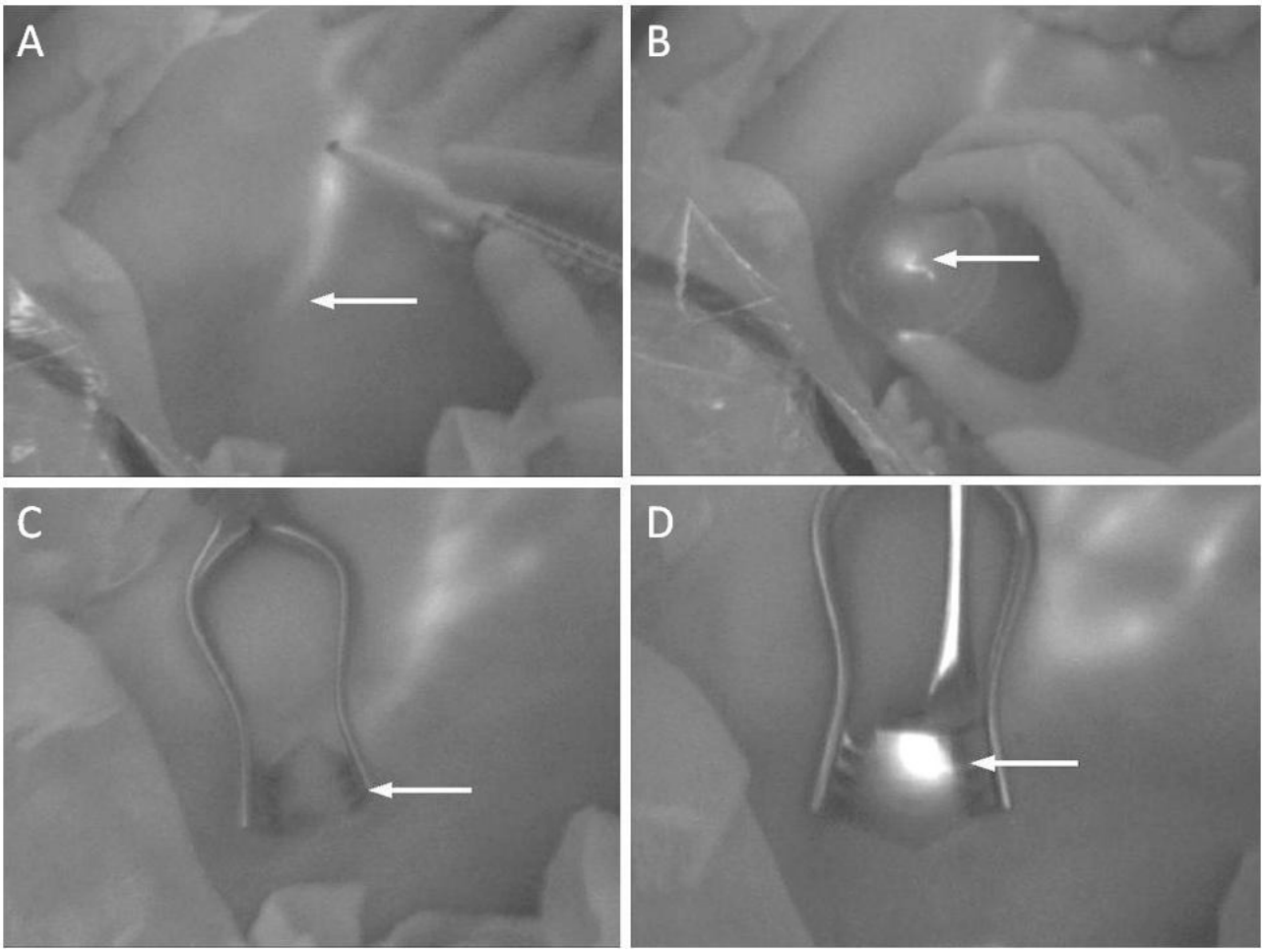

Fig. (4). Axillary pressing technique in fluorescence images. (**A**) Subcutaneous lymphatic drainage is marked. (**B**) Sentinel node can be detected from the skin by pressing the axilla. (**C**) After skin incision, obscure fluorescence signals from the sentinel node can be recognized. (**D**) After direct dissection towards the axilla, the sentinel node can be detected.

Table 1. Success Rate of Direct Approach and Duration of Procedures

		Success Rate of Direct Approach		Duration of Procedures
All		33/36	(91.6%)	25.7±8.0
Age	**<65**	15/15	(100%)	22.9±6.6
	>65	18/21	(85.7%)	27.7±8.4
BMI	**<25**	25/28	(89.3%)	24.7±8.3
	>25	8/8	(100%)	29.1±6.1
Surgeon*	**A**	5/5	(100%)	18.8±7.3**
	B	28/31	(90.3%)	26.8±7.6**
Series	**Early**	16/18	(88.9%)	24.2±8.9
	Late	17/18	(94.4%)	27.2±6.8

*Surgeon a: attending physician, b: surgical resident.
**Statistically significant, $p<0.05$.

CONCLUSIONS

The ICG fluorescence method is more reliable and less training is required as compared with the conventional dye guided method. The axillary pressing technique is useful to make sentinel node biopsy more popular, especially in a small volume hospital where no radioisotope facility is available.

None of the authors have any conflict of interest on this work.

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