

Sentinel Lymph Node Detection by Indocyanine Green Fluorescence Imaging in Skin Cancer Patients: Technical Refinement

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Abstract: *Background:* Sentinel lymph node (SLN) biopsy has become the standard method for assessing the regional lymph node status of patients with skin cancers such as malignant melanoma, squamous cell carcinoma, or extramammary Paget's disease. Recently, we reported a novel method for detection of SLN in skin cancer patients by real-time fluorescence navigation with indocyanine green (ICG).

Objective: To describe our latest experience and the technical details of this method.

Methods: SLN biopsy was performed with ICG navigation in 24 skin cancer patients (17 with melanoma, 4 with squamous cell carcinoma, 2 with extramammary Paget's disease, and 1 with eccrine porocarcinoma). After ICG was injected intradermally around the primary tumor, the subcutaneous lymphatic drainage was traced and SLNs were detected by real-time fluorescence imaging.

Results: Subcutaneous lymphatics and SLNs were identified in all patients with the primary tumor located in the upper extremity (4/4), lower extremity (12/12), genital region (2/2), and trunk (4/4), as well as in 1/2 patients with head and neck tumors.

Conclusion: SLN biopsy guided by ICG fluorescence imaging is a simple technique with a high detection rate, especially for tumors of the extremities and genital region. This method seems to be a useful option for SLN biopsy in skin cancer patients with a primary tumor located on the upper or lower extremity or in the genital region.

Keywords: Sentinel node biopsy, indocyanine green, fluorescence, skin cancer, melanoma.

INTRODUCTION

The sentinel lymph node (SLN) is the first lymph node which cancer cells reach after traveling through lymphatic vessels from the primary tumor, so cancer cells are thought to appear in the SLN before spreading to other lymph nodes. The concept behind SLN biopsy is that if the SLN is negative for tumor cells, the cancer has not spread to involve the lymph nodes. On the other hand, if the SLN is positive for tumor cells, the tumor may also have spread to the regional lymph nodes. This concept was initially developed for primary cutaneous malignant melanoma (MM) [1], but it has since been widely accepted for other malignancies such as squamous cell carcinoma of the skin, extramammary Paget's disease, breast cancer, and gastrointestinal cancer [2, 3].

Two methods have conventionally been available for detecting the SLN, which are injection of dye or injection of a radioisotope. At the time of surgery, dyes such as patent blue fade earlier compared with ICG fluorescence. In addition, it is impossible to view the lymphatics in real-time by the dye or radioisotope methods.

ICG has not been used to detect lymph nodes in patients with skin tumors because its color fades more rapidly

compared to isosulfan blue or patent blue. However, ICG fluorescence is so strong that it can be clearly observed even in lymph nodes that cannot be detected macroscopically with blue dye [4]. Kitai *et al.* reported a new method for SLN detection by imaging of ICG fluorescence in patients with breast cancer [5]. It has been reported that the ICG fluorescence method shows higher sensitivity than the dye-guided method in breast cancer patients [4]. Recently, we applied the ICG fluorescence method to skin cancer patients [6]. Here, we describe our latest experience and the technical details of this method.

MATERIALS AND METHODOLOGY

Patients

Twenty-four patients with skin cancer were enrolled in this study. Among them, 17 patients had a pathological diagnosis of malignant melanoma (MM), 4 had squamous cell carcinoma, 2 had extramammary Paget's disease, and one had eccrine porocarcinoma. This study was approved by the institutional review board of our hospital, and written informed consent was obtained from all of the patients.

Instruments

ICG fluorescence images were obtained with a portable near-infrared camera system (PDE, Hamamatsu Photonics K.K. Hamamatsu, Japan). The light source was a light emitting diode (LED) array that activated ICG fluorescence by emitting light at the central wavelength of 760 nm, while the detector was a charge-coupled device (CCD) camera

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equipped with a filter to cut out light having wavelength below 820 nm. Thirty-six LEDs were arranged in a circle with a diameter of 7 cm and the CCD camera was set at its center.

The fluorescence signals were fed into a digital video processor and displayed continuously on the monitor of a laptop computer (Vostro1000, DELL inc. Texas, USA) (Fig. 1). Video images of the lymphatic drainage were digitized into the AVI or MPEG2 format and recorded on the hard disk on the computer. The movie files were subsequently processed to obtain still images.



Fig. (1). The PDE system.

Procedure

A 27-gauge needle was used to intradermally inject 0.1 ml (0.5 mg) of ICG (0.5% Diagnogreen; Daiichi Pharmaceutical Co., Tokyo, Japan) at various sites along the excision margin around the tumor. For larger tumors, more injection sites were selected (range: 6-20). Fluorescence images of subcutaneous lymphatic drainage were obtained after intradermal injection of ICG by using the above-mentioned near-infrared camera system. Subcutaneous lymphatics could usually be detected under the skin within a couple of minutes, and lymph nodes were revealed as well. The lymph nodes were marked on the skin, and an incision was made at the site of each SLN. For use during surgery, the CCD camera was wrapped in a sterilized vinyl cover. The room light (which does not emit light at infrared wavelengths) was left on, but the operating light (which emits infrared light) was turned off.

All of the resected SLNs were examined histopathologically.

RESULTS

The entire procedure could be monitored in real-time under near-infrared fluorescent light. All of the lymphatic channels draining from the primary tumor were identified within three minutes after injection. SLNs were identified in all of the patients with the primary tumor located in the upper extremity (4/4), lower extremity (12/12), genital region (2/2), and trunk (4/4), as well as in 1 of the 2 patients with head and neck tumors. In the other patient with a primary tumor of the head and neck region, the subcutaneous lymphatics could not be traced to the preauricular area and the SLN could not be detected.

Among the 4 patients with tumors of the trunk, SLNs were detected in the axilla of 2 patients and in the groin of the other 2 patients. In both patients with extramammary Paget's disease of the genital region, lymphatic channels draining to the bilateral inguinal nodes were identified and bilateral SLNs were resected. The detection rates of SLNs was high in the patients with limb tumors and these nodes were found in the regional nodal basin in all cases. However, detection of SLNs was less reliable in the head and neck region.

Fluorescence was detected in the SLNs by this system for at least three hours after injection of ICG. There were no side effects of ICG injection in any of the patients. Representative cases are presented below.

CASE PRESENTATION

Case 1

An 83-year-old woman was admitted to our hospital with a black tumor on her left heel that was gradually growing larger. Physical examination revealed that the black tumor was 1.0 cm in diameter and irregular. No lymph nodes were palpable in the left popliteal fossa or left inguinal region. She underwent excisional biopsy and the lesion was pathologically diagnosed as MM (Breslow's tumor thickness: 3.5 mm). Contrast enhanced computed tomography (CE-CT) showed no abnormal findings in the popliteal fossa, inguinal region, or lungs. One week after excisional biopsy, extended excision with a 3 cm margin and SLN biopsy were performed. A total dose of 5 mg of ICG was injected intradermally around the scar from the initial biopsy, after which fluorescence images of subcutaneous lymphatics were observed from these injection sites to the medial thigh by using PDE system (near infrared camera). In the left inguinal region, strong fluorescence was observed and was defined as the site of the SLN. No fluorescent spots were detected in the popliteal fossa. After making a skin incision, the SLN was resected along with adjacent connective tissue under fluorescent imaging guidance. ICG fluorescence imaging was subsequently used to confirm complete removal of the SLN (Fig. 2). Histological examination of the SLN revealed no metastasis.

Case 2

A 70-year-old man was referred to our hospital. He had a gradually growing red tumor (3.0 cm in diameter) on the left lower abdomen, which was excised with a 3.0 cm margin at the previous hospital. The lesion was diagnosed as an eccrine porocarcinoma. No lymph nodes were palpable in the bilateral axillary and inguinal regions. CE-CT showed no abnormal findings that indicated metastasis of the tumor. One month after excision of the primary tumor, he underwent SLN biopsy. A total dose of 5 mg of ICG was injected intradermally around the operative scar and ICG fluorescence imaging was performed. In the left inguinal region, a spot of strong fluorescence was observed and was concluded to represent an SLN. No SLNs were detected in the bilateral axillae or the right inguinal region. After making a skin incision, the SLN was resected under fluorescent imaging guidance and imaging was also used to confirm complete removal (Fig. 3). Histological examination of the SLN revealed no metastasis.

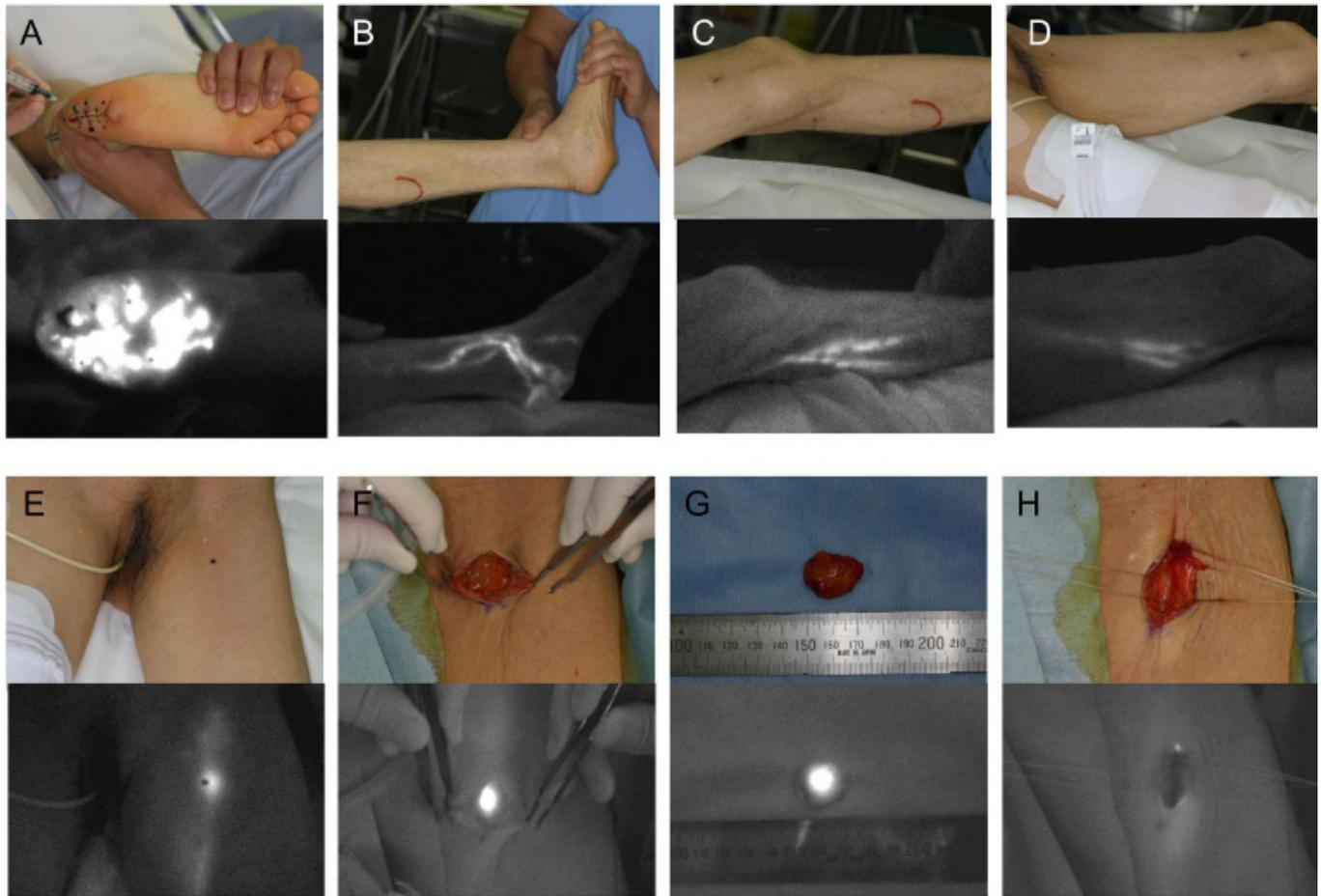


Fig. (2). (A) Injection of ICG around the incision scar. (B) Lymphatic drainage of the left leg. (C) Lymphatic drainage of the left popliteal fossa. No strongly fluorescent spots were observed. (D) Lymphatic drainage of the left medial thigh. Two fluorescent lymphatic channels were observed and these joined together at the left inguinal lymphatic basin. (E) A spot of strong fluorescence was observed in the left inguinal lymphatic basin. This spot was marked on the skin (black dot). (F) After the skin incision was made, the fluorescent SLN was more clearly observed. (G) The resected SLN showed strong fluorescence. (H) There was no residual fluorescence after resection of the SLN.

DISCUSSION

Using this Novel Method for Skin Cancer

The technical details and pitfalls of the procedure are described below.

Intradermal Injection of ICG

If the primary tumor has already been removed by excisional biopsy, ICG is injected around the incisional scar. The number of injection sites should be varied according to the tumor size. If the number of injection sites is too few relative to the tumor size, the lymphatics may be partly missed and not be traced precisely.

During ICG injection, care should be taken not to contaminate the hands with dye. If the surrounding skin is touched and ICG is spread over it, fluorescence will become extensive and the detection of lymphatics will be difficult.

Real-Time Navigation

Fluorescence images of subcutaneous lymphatic drainage from the injection sites to the SLN were observed by the PDE system. Subcutaneous lymphatics could usually be detected under the skin within a couple of minutes.

Promotion of Lymph Flow

Lymph flow is increased by heat, massage, inflammation, movement of a limb, and an increase of pressure within the lymphatic vessel. It is decreased by cold, lack of movement, and external compression [7].

Massage toward the predicted lymph node and elevation of the affected limb can be done to promote lymph flow. It is also useful to keep the patient warm.

Discrimination of Lymphatic Lake from Lymph Node

If a strongly fluorescent spot is observed distal to the predicted lymphatic basin, it is necessary to decide whether it is a lymphatic lake or lymph node. A lymphatic lake is a focal dilatation of the lymphatic collecting vessels, so it emits linear fluorescence when the light source is narrowed by adjusting the PDE system. On the other hand, the fluorescence emitted from a lymph node remains round and not linear when the light source is narrowed.

Detection of SLNs

The lymphatics are traced and the point at which they converge and strong fluorescence is observed is regarded as the site where the SLNs exist.

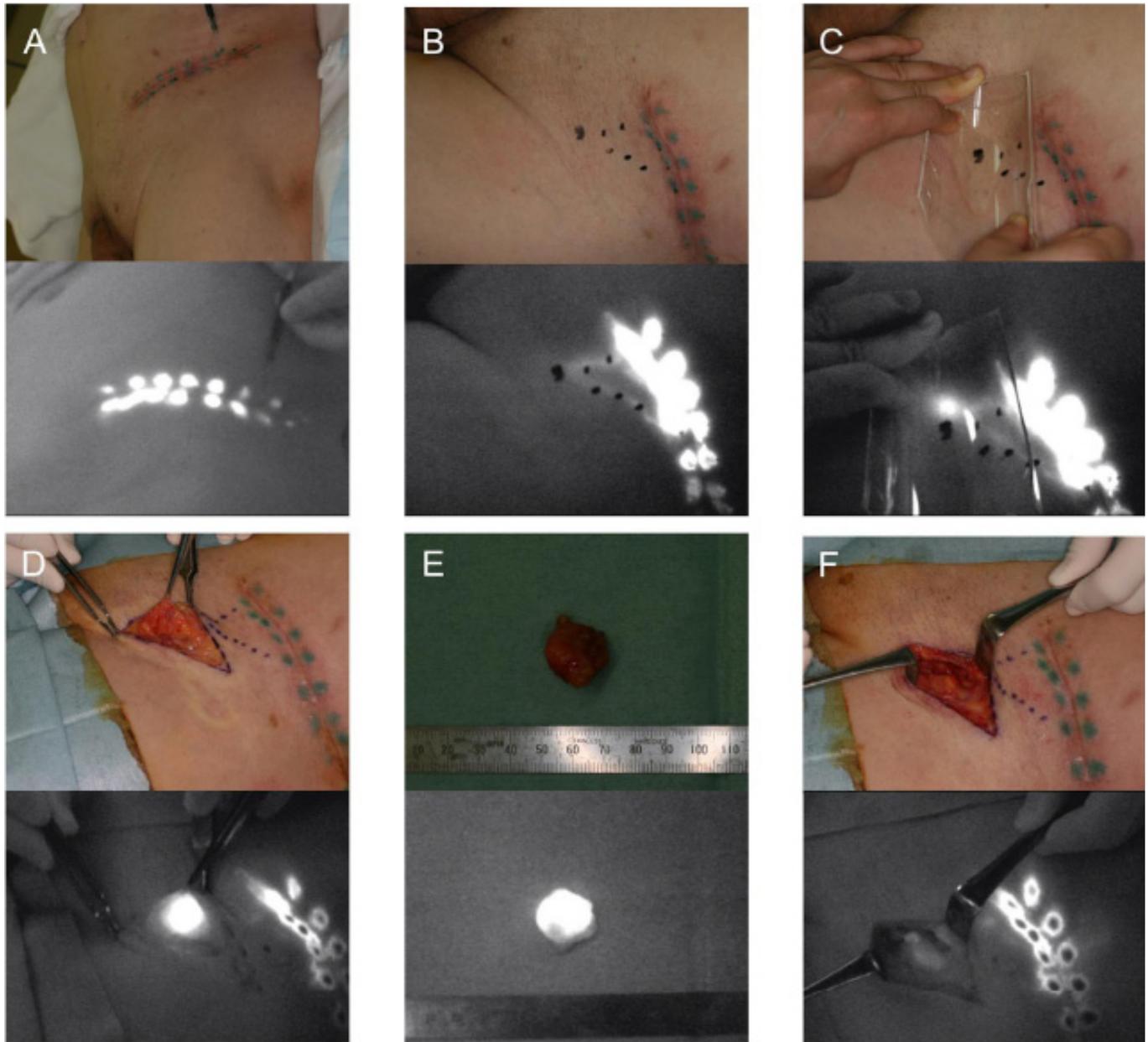


Fig. (3). (A) Injection of ICG around the incision scar. (B) The fluorescent streams of two lymphatics emerged from the injection sites and join together at the left inguinal lymph node. (C) When the skin was compressed with a transparent perspex sheet, the fluorescent spot of the SLN was more clearly observed. (D) After the skin was incised, strong fluorescence of the SLN was observed. (E) The resected SLN showed strong fluorescence. (F) There is no fluorescence emitted after the resection SLN.

The site of the skin incision can be precisely localized because we can easily map subcutaneous lymphatic drainage on the skin by following the fluorescence, except in obese patients. Unfortunately, it is often difficult to detect lymph nodes in obese patients. In these patients, compression of the skin with a transparent convex perspex sheet allows light to reach the lymph nodes more easily so that fluorescence is emitted.

The location of the lymph nodes is marked on the skin, and a skin incision is made along a line which continues that for regional lymph node dissection. After the skin incision has been made, the lymphatic channels and SLNs are more clearly observed as fluorescent lines and spots. If the lymph

node with fluorescence cannot be seen under the skin, an incision is made at the point where fluorescence of lymphatic flow fades. We can usually identify a lymph node near this point.

After the skin incision is made, lymphatic vessels draining into the SLN should not be damaged, because lymph that leaks out from damaged lymphatics, gives off fluorescence and makes detection of the SLN by the PDE system more difficult.

SLN Biopsy

The lymphatic channels are carefully ligated and the SLNs are resected. All lymph nodes that emit fluorescence

should be regarded as SLNs and should be resected. If there is no residual fluorescence in the operating field after SLN resection, it can be confirmed that there are no remnant SLNs and no lymphorrhea.

Limitations and Pitfalls of this Method

In the head and neck region, clinical prediction of lymphatic drainage is unreliable. The SLN is often located deeply within the parotid lymph nodes [7, 8], which cannot be detected under the skin by this method. In one patient, fluorescence disappeared in the preauricular region and the SLN could not be detected, suggesting that it was located in the parotid lymph nodes. Lymphatic drainage often runs from the base of the neck up to nodes in the occipital or upper cervical regions, or from the scalp down to nodes at the base of the neck, bypassing many node groups [7, 9].

In the trunk, the SLNs are often located at unexpected sites. Interval nodes, which are nodes that lie along the course of a lymphatic vessel between a primary site and a draining nodal field, are more commonly SLNs in patients with tumors on the trunk than elsewhere [7]. These interval nodes may be at sites such as the triangular intermuscular space (TIS) lateral to the scapular in the paraaortic, paravertebral, and retroperitoneal regions [7]. In our four cases, SLNs were detected successfully, but there is a possibility that other interval lymph nodes might also have existed as SLNs.

Preoperative lymphoscintigraphy may be essential to detect SLNs in patients with tumors of the head and neck or trunk.

An SLN that was occupied by tumor cells emitted little fluorescence in one case [6]. Recently, Lam *et al.* reported three other such cases [10]. Attention should be paid to possible obstruction of lymph nodes or lymphatics by tumor cells, which disturbs fluorescence from the lymph nodes or lymphatic vessels. Preoperative imaging by positron emission CT or magnetic resonance image may be useful to detect such obstruction.

Indications for Detection of SLNs by ICG Fluorescence Imaging in Skin Cancer Patients

SLNs are rarely interval nodes, such as popliteal or epitrochlear nodes [7], and all the SLNs detected by using this method were in the predicted regional lymph node basin when lesions were on the upper and lower extremities. In patients with tumors of the genital region, all the SLNs were detected among the inguinal nodes.

CONCLUSION

This method is a simple technique and may be a useful option for SLN biopsy in patients with skin cancer, especially when the primary tumor is located on the upper or lower extremity or in the genital region.

The authors have no conflict of interest.

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