Intraoperative detection of sentinel lymph nodes in breast cancer patients using ultrasonography-guided direct indocyanine green dye-marking by real-time virtual sonography constructed with three-dimensional computed tomography-lymphography

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Abstract

Purpose: This study aims to determine the utility of ultrasonography (US)-guided direct dye-marking of sentinel lymph nodes (SLNs) by real-time virtual sonography (RVS) constructed with three-dimensional (3D) computed tomography (CT)-lymphography (LG).

Patients and methods: We identified SLNs in 258 clinically node-negative breast cancer patients using an RVS system to display in real time a virtual multiplanar reconstruction CT image obtained from CT volume data corresponding to the same cross-sectional image from US. CT volume data were obtained using our original 3D CT-LG, which accurately detects SLNs in breast cancer. We then perform US-guided dye-marking close to SLNs using indocyanine green (ICG). Subsequently, indigo carmine blue dye was injected into the subareolar and peritumoral areas around each primary tumor. All patients underwent SLN biopsy and SLN metastases were examined pathologically.

Results: In all 258 patients, we were able to detect the same SLNs visualized on 3D CT-LG, using the RVS system. We detected ICG close to SLNs in 257 of 258 patients (99.6%) during SLN biopsy. In 25 patients (9%), we failed to follow the blue lymphatic route stained by indigo carmine and SLNs were not stained by indigo carmine, but easily detected SLNs by ICG marking.

Conclusion: US-guided direct ICG dye-marking of SLNs using this RVS system seems useful for the detection of SLNs, allowing easy detection of SLNs even when the stained lymphatic route is not followed.

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Introduction

Sentinel lymph node (SLN) biopsy can accurately identify nodal metastasis of early breast cancer. Krag et al. reported that axillary node dissection after negative SLN resection increases morbidity with no survival benefit in clinically node-negative breast cancer patients. SLN biopsy for breast cancer is usually undertaken using blue dye and/or radioisotope labeling. The dye-only method has been widely performed in Japanese general hospitals without departments of nuclear medicine. However, the route to the SLN is occasionally lost in SLN biopsy using this dye method, resulting in failure to detect SLN in breast cancer patients. New methods for the intraoperative detection of SLNs are thus required.

The dye method requires skill to follow the stained lymphatic route toward the SLN and can be hard to learn for beginners. SLNs are defined as the primary site of lymph node metastasis from breast cancer. Whether lymph nodes stained by dye represent the true SLN remains unclear if the lymphatic route through to the SLN cannot be followed to confirm that the route connects with the SLN.

We have developed a new technique for detecting SLNs by interstitial multidetector-row computed tomography (CT)-lymphography (LG) using iopamidol, a widely available water-soluble nonionic monometric contrast agent. We routinely perform SLN biopsy using both the images of 3D CT-LG for guidance and the blue dye method. The surgeons can look at the images of 3D CT-LG displayed on the monitor in the operating room.

A real-time virtual sonography (RVS) system has recently been developed that shows in real-time virtual multiplanar reconstruction (MPR) CT images corresponding to the images from...
ultrasonography (US). We have applied RVS conducted using 3D CT-LG to preoperative detection of SLNs in breast cancer patients.\textsuperscript{9,10}

We have recently developed ultrasonography (US)-guided direct dye-marking of SLNs using RVS constructed with 3D CT-LG, allowing anyone with basic experience in SLN biopsy to detect SLN. The present study attempted to detect SLN by direct SLN marking using RVS constructed with 3D CT-LG.

**Patients and methods**

We performed preoperative examinations for SLNs in 258 clinically node-negative breast cancer patients using the RVS system to display in real time a virtual multiplanar reconstruction obtained from CT volume data corresponding to the same cross-sectional image from ultrasonography (US) (258 women; T1, \( n = 145 \); T2, \( n = 113 \); median age, 57 years; range, 28–85 years). CT volume data were obtained from our original 3D CT-LG.

In all 258 patients, we were able to preoperatively detect the same SLNs visualized by 3D CT-LG, using the RVS system. No patients received any previous treatment. The present study protocols were approved by the institutional review board at Yamaguchi University Hospital. Written informed consent was obtained from all patients prior to the procedures.

**3D CT-LG**

Initial CT-LG was performed using a 64-detector row CT scanner (Somatom Sensation 64; Siemens Medical Solutions, Munich, Germany), as previously described.\textsuperscript{6} Briefly, under local anesthesia, 4 ml of undiluted iopamidol (Iopamiron 370; Bayer, Osaka, Japan) was injected into the periareolar and peritumoral areas (2 ml at each site), followed by gentle massage for 60 s.\textsuperscript{4–6}

Contiguous CT images from the upper thorax to the axillary region were obtained before and immediately after administration of the contrast agent and massage. Transaxial CT images were reconstructed at intervals of 1.25 mm, and digital imaging and communication in medicine (DICOM) data of these images were transferred electronically to a workstation (ZIOSOFT M900, QUADRA; Zaio, Osaka, Japan). Locations of primary SLNs draining directly from the contrast injection sites were determined on these 3D images (Fig. 1a,b).

SLN spots of each patient were marked on the skin surface with an oil painting pen under 3D CT-LG guidance, when the patient was having their CT.

**RVS system**

The RVS system consists of a magnetic motion-tracking device and US system (Preirus; Hitachi Medical, Tokyo, Japan), containing the image-processing workstation and RVS software. A magnetic sensor is fastened to the US probe (5–13 MHz). RVS can display in real time a virtual MPR image corresponding to the image on the US screen. After loading the RVS software and 3D CT-LG volume data (slice width, 2 mm) for the patient, the scanner is positioned at the upper margin of the manubrium sternum and scanning is started. RVS provides a real-time display of the reconstructed CT image corresponding to the US cross-sectional image on the screen (Fig. 1c). We detected SLNs using the RVS system only a few hours before surgery.

![Fig. 1. a) A 3D CT lymphogram. A single route/multiple SLN pattern in a 43-year-old woman with a 30 × 25-mm tumor in the left upper outer quadrant area, where only a single common lymph vessel from the periareolar and peritumoral areas drains into two common SLNs. b) A 3D CT lymphogram (CT axial image). c) Real-time virtual sonography. SLN detected by 3D CT lymphography in the RVS image (left side). The ultrasound image (right side) corresponds to the RVS image of the SLN. This sonographic image shows two SLNs. Histological findings for the SLN are positive. → sentinel lymph node (SLN).](image-url)
Breast surgery

At surgery under general anesthesia, we reproduced the US images created earlier to determine which node is the SLN in the operating room. Then, we dissolved 25 mg of indocyanine green (ICG) (Daichi Sankyo, Tokyo, Japan) in 5 ml saline and performed US-guided injection of the ICG solution (0.1 ml) close to each SLN using a 22-gauge needle. (Fig. 2).

Subsequently, a total of 5 ml of indigo carmine blue dye was injected into the subareolar and peritumoral areas around each primary tumor, followed by 1 min of massage to promote lymphatic flow. An incision (2–3 cm) was made along the skin surface marking that had been made during 3D CT-LG, then SLNs were identified and biopsied during breast-conserving surgery. In cases involving mastectomy, we attempted to identify SLNs after making a skin flap. We tried to detect SLNs by following the lymphatic route dyed by indigo carmine and to detect ICG dye-marking close to the SLN at the same time.

In some patients, we initially found the green color indicating ICG in the axillary area under the skin incision and the SLN close to ICG dye-marking (Fig. 3a). We subsequently confirmed a blue lymphatic route dyed by indigo carmine, which had been intra-dermally injected into the areola and tumor, ran into the SLN (Fig. 3b). SLNs marked with ICG dye are expected to be blue nodes and show a blue afferent lymphatic route.

The entirety of the SLN was cut along the longitudinal axis into sections of 2.0-mm thickness. When a positive SLN was found on frozen section, total axillary lymph node dissection was performed. Postoperatively, all slices were fixed in 10% buffered formalin, embedded in paraffin and examined under hematoxylin and eosin staining.

Results

Drainage lymphatic pathways on 3D CT-LG images was classified into 4 patterns6: single route/single SLN (172 patients), single route/multiple SLNs (24 patients), multiple routes/single SLN, multiple routes/multiple SLNs (33 patients), not classifiable (4 patients). A single SLN was identified in 201 patients, 2 SLNs in 55 patients, 3 SLNs in 2 patients, respectively. There was no one who had unusual drainage patterns, such as sentinel lymph nodes in the internal mammary chain or contralateral axilla.

SLNs were preoperatively visualized in all 258 patients using US-guided by the RVS system. The mean number of SLNs visualized per patient was 1.2 (range, 1–3). The 258 consecutive patients underwent breast surgery (breast-conserving surgery, n = 141; mastectomy, n = 117) and SLN biopsy using both direct ICG dye-marking and the indigo carmine dye method. Single SLN was biopsied in 144 patients, 2 SLNs in 98 patients, 3 SLNs in 14 patients, 4 SLNs in 2 patients, respectively. The mean number of SLNs removed per patient was 1.5 (range, 1–4). Approximately 94% of patients had 1 or 2 lymph nodes removed, while 6% had three or more lymph nodes removed.

In 233 of 258 patients (90.3%), we detected both dyed blue SLNs and blue lymphatic routes connecting to SLNs using indigo carmine dye. In the remaining 25 patients (9.7%), we failed to follow the blue lymphatic routes and detect dyed blue SLNs. In contrast, we were able to detect SLNs (a total of 385 SLNs) in 257 of 258 patients (99.6%) using ICG dye. All of SLNs (a total of 38 SLNs) undetected by indigo carmine dye in 25 patients were successfully detected by ICG dyeing method.

SLNs metastasis was diagnosed in 43 of the 258 patients (micrometastasis; 15, macrometastasis; 28).

Discussion

Intraoperative identification of SLN is achieved in 65–88% of cases using dye alone,11–13 81–93% with radioisotope alone14,15 and 92–98% for the combination.13,15–17

Fig. 2. Schematic presentation of RVS-guided direct dye-marking.
In a meta-analysis, rates for intraoperative identification of the SLN using dye alone, radioisotope alone, or the combination were 83%, 89%, and 91%, respectively.

Use of both dye and radioisotope is generally recommended. Nevertheless, dye-only methods have been widely performed in Japanese general hospitals without a department of nuclear medicine, requiring a high level of technical skill to trace the dye-stained lymphatic route to SLNs.

Recently, a few emerging techniques for SLN detection have been reported. Lymphatic imaging after intraparenchymal microbubble injection has recently been reported in patients with breast cancer. Patients receive a periareolar intradermal injection of microbubble contrast agent, then a guidewire is deployed to localize the SLN. In addition, the combination of a standard hand-held gamma-probe and real-time imaging provided by a portable gamma-camera reportedly offers a high intraoperative detection rate in patients with difficult SLN localization as assessed by presurgical lymphoscintigraphy. ICN near-infrared fluorescence navigation is reported to be a useful option in SLN biopsy for breast cancer. However, Kitai T, et al. pointed out that if any lymphatic vessel is injured, further fluorescence navigation will be difficult because of ICG contamination in the surgical field. In addition, the dissecting procedures are often interrupted by turning off the operating light during the fluorescence observation. The present study represents a first attempt at intraoperatively identifying SLNs using RVS-guided direct marking in breast cancer patients. The ICG dye is not dispersed in the axilla for up to 3 h after injection, in our experience. It is feasible to perform this technique outside of the operating room before the operation. Our method appears superior to the dual tracer technique with blue dye and radioisotope in terms of reliability of identifying true SLN. This method could allow SLNs to be identified intraoperatively without exposing surgeons and medical co-workers to radioisotopes.

The SLN is defined as the first lymph node to receive lymphatic drainage from breast cancer. In other words, a blue node does not necessarily indicate an SLN, if the dye-stained lymphatic route from the dye injection site cannot be followed all the way to the node in question. We must therefore carefully follow up dye-stained afferent lymphatic route from the dye injection site to detect SLN accurately. This procedure seems to require considerable skill. With the radioisotope method, definitive criteria are lacking for the level of radioactivity accurately defining primary SLNs. The level of radioactivity necessary to define a lymph node as ‘sentinel’ varies among surgeons. The lymph node with the highest radioactivity count may not always be the SLN. The scintigrams cannot clearly visualize the direct connection between primary SLNs and their afferent lymphatic vessels. A combination method using dye and radioisotopes to identify SLNs will increase both the detection rate and number of SLNs removed per case. This method often results in the removal of multiple SLNs, because some non-sentinel nodes may be removed.

The RVS system, which displays virtual MRI images obtained from 3D CT-LG, significantly improves the preoperative detection of SLNs. The RVS system is a breakthrough diagnostic imaging system, allowing preoperative detection of SLNs and evaluation of SLN status by US. RVS technology is commercially available from major manufacturers’ platform (e.g. Hitachi medical, Japan and GE Healthcare, America). The RVS system is helpful for preoperatively identifying patients with nodal metastases who can proceed directly to axillary dissection. Our previous data suggest that absence of SLN metastasis may be predicted if cortical thickness of the SLN is <2.5 mm. On the other hand, if cortical thickness of the SLN is >2.5 mm, we recommend preoperative US-guided fine-needle aspiration biopsy (FNA) cytology of the SLN for diagnosis of SLN metastases. Patients with positive US-guided FNA cytology results are taken directly to complete axillary dissection.

Comprehensive preoperative imaging of lymphatic drainage pathways and direct dye-marking assisted us in finding blue dye-stained lymphatic vessels and SLNs and allowed 100% identification of targeted SLNs in the 233 patients who underwent surgery. In the future, we are aiming to use only direct ICG dye-marking without the injection of indigo carmine dye into the subareolar and peritumoral areas.

In conclusion, we have developed a direct dye-marking method by injecting ICG close to the SLN using the RVS system and 3D CT-LG. This method might contribute greatly to easy detection of SLNs and accurate SLN biopsy, especially for surgeons without access to radioisotope injection who perform only the dye staining method.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

**References**


