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Title: Image-guided cancer surgery : the value of near-infrared fluorescence imaging during oncologic and gastrointestinal procedures

Issue Date: 2015-06-03

Chapter 7

Sentinel lymph node biopsy in melanoma patients using combined radioactive and fluorescence guidance

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Submitted.

ABSTRACT

Background

Recently, near-infrared (NIR) fluorescence has been introduced for sentinel lymph node (SLN) biopsy. NIR fluorescence imaging outperforms blue dye, however radioactive guidance remains essential for preoperative planning and deeply located SLNs. The main aim of this study is to validate the use of a dual-modality fluorescence and radioactive tracer for SLN detection in melanoma patients using a “hands free” imaging system.

Methods

This prospective study included patients planning to undergo SLN mapping for cutaneous melanoma. The day before or on the day of surgery, the dual-modality radioactive and NIR fluorescence tracer ICG-^{99m}Tc-Nanocolloid was injected around the primary excision scar. Preoperative lymphoscintigraphy was acquired. Directly before surgery, blue dye was injected. Intraoperative SLN localization was performed using a handheld gamma probe and the Mini-FLARETM imaging system.

Results

Fourteen patients with cutaneous melanoma undergoing SLN biopsy were included. Preoperative lymphoscintigraphy allowed detection of the SLN in all patients, with a total of 19 nodes. Moreover, in 9 out of 14 patients lymphatic channels draining from the injection site to the SLN could be observed percutaneously using NIR fluorescence allowing real time surgical guidance. Intraoperatively, all SLNs could be identified using both radioactive and fluorescence guidance, whereas only 13 out of 19 stained blue.

Conclusions

This study is the first to demonstrate the added value of continuous visualisation of lymphatic vessels and SLNs in relation to the surgical anatomy using a dual modality fluorescent and radioactive probe.

INTRODUCTION

Sentinel lymph node (SLN) biopsy is currently regarded as standard-of-care in nodal staging of cutaneous melanoma patients¹. SLN biopsy in melanoma patients is conventionally performed using a combination of radioactive colloids and blue dye². The use of this combination facilitates high detection rates (>95%).

Over the last few years, near-infrared (NIR) fluorescence imaging has been introduced for SLN biopsy³. NIR fluorescence imaging has several characteristics that are advantageous in the SLN procedure compared to blue dye. NIR fluorescence imaging has a relatively high penetration into living tissue (several millimetres) and there is no interference with the surgical field as NIR light is invisible to the human eye⁴. Recent studies showed that NIR fluorescence imaging outperformed blue dye staining for SLN identification⁵⁻¹⁰. However, radioactive colloids are still essential for preoperative planning and to identify aberrant drainage profiles and deeply located SLNs¹¹.

To combine both radio guidance and NIR fluorescence, a dual-modality radioactive and NIR fluorescence tracer has been developed. Both the fluorescent label indocyanine green (ICG) and radioactive label ^{99m}Tc-Technecium (^{99m}Tc) are bound by nanocolloid, which provides optimal retention of both signatures within the SLN. The aim of this study was to evaluate this tracer using a “hands free” fluorescence imaging setup for the visualisation of lymphatic vessels and SLNs in melanoma patients.

MATERIALS AND METHODS

The trial was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed in concordance with the ethical standards of the Helsinki Declaration of 1975. Patients planned for therapeutic re-excision and SLN biopsy for cutaneous melanoma were included in this prospective study. Exclusion criteria were pregnancy, lactation, or an allergy to iodine, shellfish, or ICG. The trial has been registered in the Dutch trial registry as NTR3850. All patients gave informed consent and were anonymized.

Tracer preparation

^{99m}Tc-nanocolloid was prepared by adding sodium pertechnetate (approximately 1000 MBq) in 2 mL saline to a vial of 0.5 mg human serum albumin nanocolloid (Nanocall, GE Healthcare, Eindhoven, the Netherlands). After 30 min of incubation at room temperature, 50 µL of 6.4 mM (0.25 mg) ICG (Pulsion Medical Systems,

Munich, Germany) dissolved in water for injection was added to obtain ICG- ^{99m}Tc -nanocolloid at a final ICG concentration of 160 μM and a final pH of 6.0 – 7.0 (fig. 1A)^{12,13}. All procedures were performed under current good manufacturing practice (cGMP) and under supervision of the institution's pharmacist.

Clinical trial

The day before surgery ($n = 12$) or the day of surgery ($n = 2$), 60-100 MBq ICG- ^{99m}Tc -nanocolloid was injected at 4 quadrants around the primary excision scar (fig. 1B). Dynamic images were obtained in the first 15 minutes with subsequent static planar

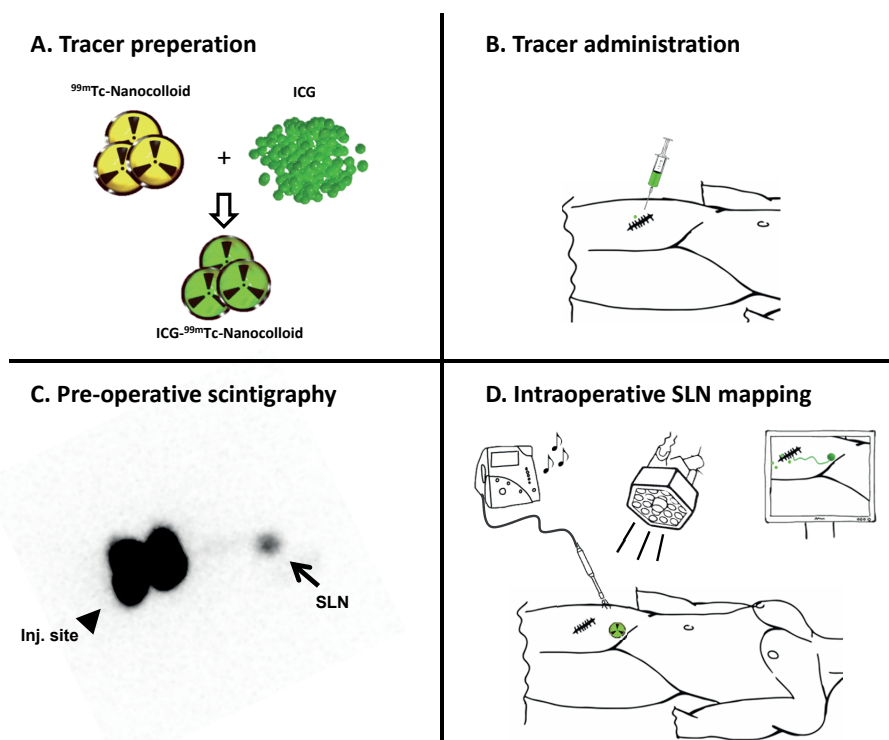


Figure 1 – Clinical Trial Protocol:

A: After preparation of ^{99m}Tc -nanocolloid, ICG dissolved in water for injection is added to obtain ICG- ^{99m}Tc -nanocolloid at a final ICG concentration of 160 μM . B: The tracer ICG- ^{99m}Tc -nanocolloid is administered at 4 quadrants around the primary excision scar. C: Preoperative lymphoscintigraphy is acquired at the department of Nuclear Medicine to determine the position of the injection site (arrowhead) and SLN (arrow). D: Surgical resection of the SLNs is performed using a combination of radioactivity and fluorescence. The surgeon is continuously provided with real-time NIR fluorescence image guidance. When desired, an acoustic gamma probe can be used as well. The mini-FLARE imaging system allows “hands free” visualisation of lymphatic vessels and lymph nodes in relation to surgical anatomy.

images at 15 minutes and 3 hours post injection, using one detector of a single or two-headed gamma camera (Symbia T6, Siemens, Erlangen, Germany or Toshiba GCA-7200PI/7200DI/7100UI, Toshiba, Tokyo, Japan). Before the start of the operation, 1 ml Patent Blue V (Guerbet, Brussels, Belgium) was injected in multiple deposits surrounding the primary excision scar. After surgical scrub, the Mini-FLARE imaging system, as described earlier, was positioned at approximately 30 cm above the surgical field¹⁴. The NIR fluorescence signal was measured percutaneous prior to skin incision. Subsequently, during surgical exploration, the surgeon was continuously provided with real-time NIR fluorescence image guidance and radioactive guidance using a hand-held gamma probe (Europrobe, Euromedical Instruments, Le Chesnay, France) (fig. 2). Lymphatic vessels draining the injection site towards the SLN were visualized using fluorescence. When visible, the blue dye was also used to provide optical guidance.

SLNs were fixed in formalin and embedded in paraffin for haematoxylin, eosin, and immunohistopathological staining using the S-100 and MART-1 markers at six levels, with an interval of 50-150 μm .

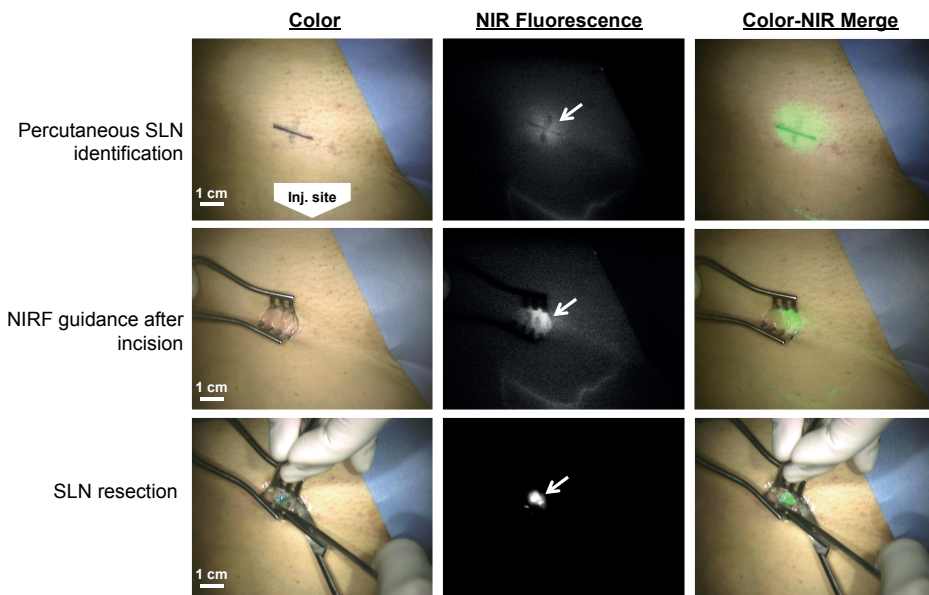


Figure 2 – NIR fluorescence imaging during sentinel lymph node mapping:

Top row, percutaneous near-infrared identification of afferent lymphatic channels and the SLN. The off-page connector symbol indicates the position of the injection site (Inj. site; off-screen) and the marked cross indicates the presumed position of the SLN (arrow) as marked by the nuclear medicine physician. Middle row, real-time fluorescence guidance of the SLN (arrow) directly after incision. Bottom row, resection of the SLN (arrow) under NIR fluorescence guidance, two minutes after incision. Blue dye staining becomes also visible. Scale bars = 1 cm. Camera exposure times were: 200 ms (upper row), 100 ms (middle row) and 20 ms (bottom row).

RESULTS

Patient and Tumour Characteristics

Fourteen patients with cutaneous melanoma undergoing SLN biopsy were included. Seven patients were male. Median body mass index was 25 kg/m² (range 19 - 29), median age was 51 years (range 27 - 74 years), and median Breslow's depth was 1.8 mm (range 0.3 - 4.5 mm). In 6 patients, the melanoma was located at the upper extremities, in 5 patients on the trunk and in 3 patients at the lower extremities.

Table 1 – Sentinel Lymph Node Identification Results

Characteristic	N	%
SLN Detection		
- Number of SLNs Identified	19	
- Median Number of SLNs Identified (Range)	1	(1 - 3)
Method of Detection		
- Radioactive	19	100
- Fluorescent	19	100
- Blue	13	68
Median Time between tracer Injection and Skin Incision (hours), (Range).	23	(5 - 29)
Median Time between Skin Incision and SLN Resection (minutes), (Range)	12	(2 - 24)
Percutaneous identification		
- Lymphatic vessels (no. of patients)	9	64
- SLNs (no. of patients)	4	2
SLN localization		
- Axilla	13	68
- Groin	6	32
Histology		
- Negative	17	89
- Micrometastases	2	11
- Macrometastases	0	0
Adjuvant treatment (no. of patients)		
- None	12	86
- Axillary Lymph Node Dissection	2	14

SLN, sentinel lymph node

Sentinel Lymph Node Detection

Preoperative lymphoscintigraphy allowed detection of at least one SLN in all patients with a total of 19 SLNs. The median time of ICG-^{99m}Tc-nanocolloid injection to surgery was 23 hours (range, 5 – 29). In 9 out of 14 patients (64%) lymphatics draining the injection site towards the SLN were observed percutaneously. Moreover, in 4 patients SLNs could be observed percutaneously (fig. 2).

Surgical resection of the SLNs was performed using a combination of radioactivity, continuous fluorescence-imaging, and blue guidance. All 19 resected SLNs were both radioactive and fluorescent (Table 1). However, only 13 out of 19 SLNs (68%) were stained blue. Lymphatic vessels draining the SLN were still fluorescent up to 29 hours after tracer injection (fig. 2). Median SLN fluorescence signal-to-background ratio was 5.6 (range, 2.5 – 13.8). Median time between skin incision and resection of the first SLN was 12 minutes (range, 2 – 24). In 2 patients one resected SLN contained micrometastases. No adverse reactions or complications occurred.

COMMENT

The used dual-modality tracer allowed both preoperative planning and real-time intraoperative radioactive and fluorescence guidance for SLN detection, and aided in the intraoperative detection of fluorescent lymphatic vessels up to 29 h after injection.

Recently, our group have demonstrated feasibility of accurate SLN mapping using NIR fluorescence and ICG in melanoma patients¹⁵. However, radioactive guidance appeared to be still obligatory for preoperative planning and for the identification of deeply located SLNs. ICG-^{99m}Tc-nanocolloid permits both fluorescence and radioactivity guidance after a single injection and has already been tested in various cancer types^{16,17}. And, unlike patent blue, ICG does not alter the look of the surgical field or tattoo the skin of the patient. In addition, this tracer maintains the properties of ^{99m}Tc-nanocolloid, which is a commonly used lymphatic tracer in Europe and only needs addition of a small amount (0.025 mg) of ICG.

An advantage of NIR imaging is that it has the ability to provide real-time guidance. However, to enable the surgeon to apply this guidance efficiently, navigation in relation to the surgical anatomy is obligatory. The camera system used in this study is capable of displaying NIR fluorescence signal in relation to the surgical anatomy, and especially designed for hands free operation. Moreover, this allowed clear detection of fluorescent lymphatic vessels, which especially aided in the detection of the SLN.

In conclusion, this study demonstrates the added value of direct lymphatic guidance towards the SLN in relation to the surgical anatomy by combining the use of

a “hands free” camera system and a dual modal NIR probe. This technique has the potential to shorten time of surgery and to reduce surgical manipulation.

ACKNOWLEDGMENTS

This study was performed within the framework of the Centre for Translational Molecular Medicine, project MUSIS (grant 03O-202), the Dutch Cancer Society grant UL2010-4732 and PGF 2009–4344, and the EUROSTARS grant (E7555), Research reported in this publication was also supported by National Institutes of Health grant R01-CA-115296. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors thank the Departments of Pharmacy and Radiology for their active participation and David Burrington Jr. for editing.

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