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Sentinel Lymph Node Biopsy in Vulvar Cancer using Combined Radioactive and Fluorescence Guidance

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Abstract

Objective—Near-infrared (NIR) fluorescence imaging using Indocyanine Green (ICG) has recently been introduced to improve the SLN procedure. Several optical tracers have been successfully tested. However, the optimal tracer formulation is still unknown. This study evaluates the performance of ICG-^{99m}Tc-nanocolloid in relation to two most commonly used ICG-based formulas during SLN biopsy of vulvar cancer.

Methods/materials—12 women planned to undergo SLN biopsy for stage I vulvar cancer were prospectively included. SLN mapping was performed using the dual-modality radioactive and NIR fluorescence tracer ICG-^{99m}Tc-Nanocolloid. All patients underwent combined SLN localization using NIR fluorescence, and the (current) gold standard using blue dye and radioactive guidance.

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Clinical Trial Registration: NTR3849

CONFLICT OF INTEREST STATEMENT

Floris P.R. Verbeek, M.Sc.: None

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Results—In all 12 patients at least one SLN was detected during surgery. A total of 21 lymph nodes (median 2, range 1 – 3) were resected. Median time between skin incision and first SLN detection was 8 (range 1 – 22) minutes. All resected SLNs were both radioactive and fluorescent, though only 13 of 21 SLN (62%) stained blue. Median brightness of exposed SLNs, expressed as SBR, was 5.4 (range 1.8 – 11.8). Lymph node metastases were found in 3 patients.

Conclusions—NIR fluorescence guided SLN mapping is feasible and outperforms blue dye staining. Premixing ICG with ^{99m}Tc -nanocolloid provides real-time intra-operative imaging of the SN and appears the optimal tracer combination in terms of intraoperative detection rate of the SN (100%). Moreover, ICG- ^{99m}Tc -Nanocolloid allows administration of a 5-times lower injected dose of ICG (compared to ICG and ICG:HSA) and can be injected the up to 20h before surgery.

Keywords

Vulvar cancer; Image-Guided Surgery; Near-Infrared Fluorescence; Sentinel Lymph Node Mapping; Multimodal Imaging

INTRODUCTION

Lymph node involvement remains the most important prognostic factor for survival in women with vulvar cancer ^{1,2}. The introduction of radical wide local excision or radical vulvectomy with inguinofemoral lymphadenectomy using separate groin incisions has significantly reduced morbidity compared to conventional radical vulvectomy with en bloc inguinofemoral lymphadenectomy ³. Nevertheless, up to two third of patients who underwent inguinofemoral lymphadenectomy suffer from lymphedema ⁴⁻⁶. Because < 30% of patients with FIGO (International Federation of Gynecology and Obstetrics, 2009) stage I or II vulvar cancer have positive lymph nodes, lymphadenectomy appears unnecessary afterwards in the majority of patients ⁷⁻⁹. The introduction of the sentinel lymph node (SLN) procedure has provided a less invasive algorithm to assess nodal staging ¹⁰. To date, the use of the SLN procedure has been validated in several large studies and is considered both safe and accurate in a selected group of patients ^{7,11}. Moreover, it appears the most cost-effective strategy for the management of patients with early-stage vulvar cancer due to lower treatment costs and lower costs due to complications ¹².

To locate the SLN, a combination of both radioactive tracers and blue dye is currently the gold standard ¹³. However both modalities have certain disadvantages. Despite the need for radiocolloids during preoperative planning, this technology gives only acoustic feedback intra-operatively and is unable to provide real-time visual guidance. Furthermore, blue dyes cannot be seen through skin and fatty tissue and can result in blue staining of the surgical field at the site of injection. Near-infrared (NIR) fluorescence imaging using indocyanine green (ICG) has recently been introduced to improve optical identification during the SLN procedure ¹⁴⁻¹⁶. NIR fluorescence imaging using Indocyanine green (ICG) has several characteristics that can be advantageous during the SLN procedure, including a relatively high tissue penetration and real-time optical guidance ¹⁷. ICG is approved for clinical use by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) and can be used off-label as a lymphatic tracer ¹⁴.

Feasibility of NIR fluorescence guided SLN biopsy in vulvar cancer has been previously demonstrated using several ICG-based tracer formulas¹⁸⁻²¹. In these studies, multiple methods of tracer administration and dosages of ICG have been investigated. Crane et al. used ICG alone for SLN mapping in vulvar cancer (645 nmol ICG administered in 1 mL) and Hutteman et al. used several concentrations of ICG absorbed to human serum albumin (ICG:HSA) to improve SLN retention (800, 1200 and 1600 nmol ICG administered in 1.6 mL)^{18;19}.

Unfortunately, premixing with HSA, thereby increasing the hydrodynamic diameter to 7nm, did not improve performance compared to ICG alone²⁰. It was recently shown that premixing ICG with ^{99m}Tc-nanocolloid yields the hybrid tracer ICG-^{99m}Tc-nanocolloid with hydrodynamic diameter 20-80 nm, that retains in SLNs similar to the particle which is used as standard-of-care radiotracer ^{99m}Tc-nanocolloid (64 mmol ICG administered in 0.4 mL)^{22;23}. This tracer has recently shown its value during gynecological SN resections in the groin²¹, and during the highly similar procedures related to penile cancer²⁴.

To summarize, several ICG-based formulations and injected volumes have been used, but despite preclinical comparison studies, no comparison between different tracers is available from the clinical setting. This study evaluates the use of ICG-^{99m}Tc-nanocolloid, a dual-modality radioactive and NIR fluorescence tracer. Subsequently results were compared to a previous performed randomized controlled trial in which two other different tracer formulations, ICG alone and ICG:HSA, were used in addition to the standard-of-care procedure using blue dye and radioactive guidance.

MATERIALS AND METHODS

Clinical Trial

This clinical trial was approved by the Medical Ethics Committee of the Leiden University Medical Center and were performed in accordance with the ethical standards of the Helsinki Declaration of 1975. Women planned to undergo sentinel lymph node biopsy for clinically FIGO stage I vulvar cancer and with clinically negative inguinofemoral nodes (determined by palpation and ultrasonography) were eligible for participation in the trials²⁵. Patients were enrolled in the study between January and October of 2013. Exclusion criteria were pregnancy, lactation, or an allergy to iodine or ICG. All patients gave informed consent and were anonymized. The 12 patients included in the present study received ICG-^{99m}Tc-nanocolloid and have not previously been reported.

Tracer Preparation

ICG (25-mg vials) was purchased from Pulsion Medical Systems (Munich, Germany). ICG-^{99m}Tc-nanocolloid was purchased from GE Healthcare (Leiderdorp, the Netherlands). After tracer preparation ICG-^{99m}Tc-nanocolloid had a final activity of 60-100 MBq. The tracer was injected intracutaneously at 3 to 4 depots around the tumor or excision scar. In the ICG-^{99m}Tc-nanocolloid formulation, the ICG concentration was 161 µmol/l, leading to an injected ICG dose of 160 nmol in 1 mL (0.12 mg)^{21;24;26}.

All procedures were performed under current good manufacturing practice and under supervision of the institutional pharmacist.

Study Design

Patients received the standard-of-care SLN procedure using a combination of radioactive (^{99m}Tc -nanocolloid), patent blue, and fluorescence guidance^{11;27}.

The day before or the morning prior to surgery the combined tracer ICG- ^{99m}Tc -nanocolloid was injected at the department of nuclear medicine. In the operating room also 1 mL of patent blue V (Guerbet, France) was injected in all patients. SLN localization was also performed with patent blue V, as it is part of the standard-of-care for SLN mapping in the Netherlands. The tracers (radioactive, fluorescent and blue dye) were all injected intracutaneously at 3 or 4 sites around the tumor or excision scar in case of earlier excision biopsy of the tumor. In all patients, directly after injection of the radioactive tracer, a dynamic lymphoscintigraphy was made at the department of Nuclear Medicine (Symbia T6, Siemens, Erlangen, Germany or Toshiba GCA-7200pi/7200di/7100ui, Toshiba, Tokyo, Japan). The sentinel node in the groin was marked on the skin using a waterproof marker. Directly following injection of the radioactive tracer and approximately 2 hours after injection, planar images were derived. In some patients SPECT/CT was performed on the two headed gamma camera (Symbia T6, Siemens, Erlangen, Germany with 6 slice CT).

SLN mapping was performed using the Mini-Fluorescence-Assisted Resection and Exploration (Mini-FLARETM) image-guided surgery system as described previously^{27;28}. The NIR fluorescence signal was measured percutaneously prior to skin incision, and continuously during the surgical procedure. Relative brightness of the SLNs was determined by measuring signal-to-background ratios (SBR) using customized imaging software²⁹. In all patients the SLN was defined on lymphoscintigraphy as the first node which received lymphatic drainage from the primary tumor³⁰. Intraoperatively, the lymph nodes were identified using acoustic feedback from the gamma probe. When the probe gave a gamma count of 10% or more compared to the most radioactive SLN, these nodes were also considered SLNs.

Excised SLNs were routinely analyzed by histopathological frozen section analysis. SLNs were fixed in formalin and embedded in paraffin for hematoxylin, eosin, and immunohistopathological staining for AE1/AE3 at multiple levels, with an interval of 250 μm , according to the GROningen International Study on Sentinel nodes in Vulvar cancer (GROINSS-V) study protocol¹¹. According to the study protocol a full inguinofemoral lymphadenectomy was performed in case of tumor-positive frozen sections of the SLN showing macrometastases 2 mm or larger or in case the SN could not be found.

Statistical Analysis

For statistical analysis, SPSS statistical software (Version 20.0, Chicago, IL) was used. Graphs were generated using GraphPad Prism Software (Version 5.01, La Jolla, CA). Correlation between Body Mass Index (BMI) and SLN identification time was determined using the Spearman's rank correlation coefficient. To compare patient characteristics the 1-way ANOVA and chi-square tests were used. The 1-way ANOVA was corrected using

Bonferroni correction in case of parametric data. For nonparametric data the Kruskal-Wallis test was used. Data was tested for normal distribution using the Shapiro-Wilk test. $P < 0.05$ was considered significant.

RESULTS

Patient and Tumor Characteristics

Twelve consecutive patients with vulvar cancer who underwent SLN mapping using the combined tracer ICG-^{99m}Tc-nanocolloid were included in the current study. Median age of the patients was 72 years (range: 40 – 90) and median BMI was 24 kg/m² (range: 17 - 30). Tumor characteristics are shown in table 1. The median tumor size and infiltration depth was 9 (range: 4 – 35) mm and 2.3 (range: 0.6 – 9) mm respectively. No adverse reactions associated with lymphatic tracer administration or the use of the Mini-FLARE™ imaging system were observed.

SLN Detection

The position of the SLN could be located during preoperative lymphoscintigraphy in all 12 patients (Figure 1). The SLN was located unilaterally in 4 patients and bilaterally in 8 patients (Table 2). Median time between ICG tracer injection and skin incision was 17 (range: 3 – 21) hours. Before incision, NIR fluorescence imaging enabled visualization of percutaneous lymphatic channels in 6 of 12 patients. A total of 21 SLNs (median of 2 per patient, range: 1 – 3) were resected. Median time between skin incision and first SLN detection was 8 (range: 1 – 22) minutes. During *ex-vivo* analysis, all these 21 nodes were all both radioactive and fluorescent, and 13 (62%) stained blue (Table 2). Fluorescent SLNs could be clearly identified as a bright green signal depicted in the overlay images (Figure 2 and supplementary video). Median brightness of exposed SLNs, expressed as SBR, was 5.4 (range: 1.8 – 11.8). Lymph node metastases were found in 4 out of 20 groins (3 patients), including 2 patients with macrometastases (> 2mm) and 1 patient with isolated tumor cells or micrometastases.

DISCUSSION

The current study evaluates the use of ICG-^{99m}Tc-nanocolloid for combined NIR fluorescence- and radio-guided SLN biopsy in vulvar cancer patients. We argue that the visual intraoperative detection using ICG combined with radioactive guidance (ICG-^{99m}Tc-nanocolloid) may help to optimize the SLN procedure, and will locate the SLN more precisely (Figures 1 and 2). This could reduce the need for an unnecessary full inguinofemoral lymphadenectomy, and thereby reduce anesthesia time and decrease the risk of postoperative and long-term complications such as infection or dehiscence of the wound and lymphedema.

The obtained results were compared to a previous performed and published randomized controlled trial ²⁰. The current trial was performed using the same camera system and identical surgical workup. In the previous trial ICG was prepared using 2 different protocols to obtain ICG or ICG bound to Human serum albumin (HSA). 1.6 mL of ICG alone (N=12) or ICG:HSA (N=12) were injected in the operating room directly before surgery. Hence in

both formulations 0.62 mg of ICG was injected effectively. Overall, a total of 36 patients who underwent SLN mapping using both radioactive, blue, and fluorescence guidance were evaluated in the current analysis. Intraoperative NIR fluorescence based SLN detection rates were 75%, 83%, and 100% for ICG alone, ICG:HSA, and ICG-^{99m}Tc-nanocolloid, respectively ($P = 0.21$). Up to 25% more SLNs were optically identified using ICG:HSA and ICG-^{99m}Tc-nanocolloid compared to ICG alone, showing a trend towards significance ($P = 0.06$). Time between skin incision and first SLN detection were not significantly different between the different patient populations ($P = 0.79$). Time between skin incision and SLN identification increased with BMI ($R = 0.54$; $P < 0.01$) (Figure 3). NIR fluorescence imaging enabled visualization of percutaneous lymphatic channels in 39% of patients which seemed to be inversely correlated to BMI ($R = -0.28$; $P < 0.10$) and could assist the skin incision placement during surgery (Figure 2). A comparison between patient characteristics and SLN identification results can also be found in tables 1 and 2.

All three ICG-based formulations were able to optically detect SLNs during surgery, but, although not significant, ICG-^{99m}Tc-nanocolloid seemed to outperform the other two formulations in terms of the intraoperative detection rate, even though it uses the lowest amount of ICG (only 160 nmol compared to 800 nmol used for ICG alone and ICG:HSA). Moreover, this visualization was achieved with a lower injected volume (1.0 mL vs. 1.6 mL) and a significantly longer period between tracer administration and surgical resection (up to 20h longer). The improved visualization is likely related to the superior retention in the SLN and the direct correlation between pre- and intra-operative findings in which the SLN can be visualized real-time³¹. The 5-times lower injected dose of ICG (ICG-^{99m}Tc-nanocolloid compared to ICG and ICG:HSA) and the up to 20h increase in time between injection and NIR-guided resection, did result in a significantly lower SBR for ICG-^{99m}Tc-nanocolloid. However, the lower SBR of 5.4 did not limit the surgical guidance and did not result in prolongation of the time between skin excision and first SLN identification.

Combining ICG-^{99m}Tc-nanocolloid permits both fluorescence and radioactivity guidance after a single injection. And, unlike patent blue, the use of ICG does not alter the surgical field by dark staining or tattooing the skin of the patient³². In addition, clinical implementation of this hybrid tracer is simple as it is based on ^{99m}Tc-nanocolloid, which is an approved lymphatic tracer in Europe and only needs addition of a small amount (0.05 mg) of ICG, also approved for clinical use (off-label use for SLN identification)²³. In concordance with previous reports where blue dye and NIR fluorescence was compared for drainage sites in the groin^{20;21;24}, NIR fluorescence outperformed blue dye in terms of SLN detection; in the present study only 62% of nodes stained blue. Therefore, blue dye does not appear to add value to the SLN procedure compared to NIR fluorescence imaging, if available. Therefore, we would argue that blue dye staining should not be considered part of the standard anymore in future studies.

A significant advantage of NIR imaging is that it can provide real-time surgical guidance. To date, most centers that are implementing the use of NIR fluorescence-guided SLN biopsy with indocyanine green in an “open” setting are using the Photo Dynamic Eye (PDE; Hamamatsu, Japan) camera system^{19;21}. This system is commercially available and easy to use; however, no color overlay is possible. To enable the surgeon to work under direct

image guidance, navigation in relation to the surgical anatomy is desirable. The camera system used in this study is capable of displaying NIR fluorescence signal in relation to the surgical anatomy by a real-time overlay function between color and NIR fluorescence, using a hands-free setup ²⁸.

It should be pointed out that the hydrodynamic diameter of the tracer compounds used, ICG (1 nm), HSA (7nm) and Nanocolloid (20-80 nm), are considerably different, which could have a major impact on the lymphatic migration and retention in the SLNs ²². A small particle (like ICG) has the potential to travel much faster through the lymphatic vessels and could thereby access second tier nodes. However, average number of SLN identified in the current study did not differ between groups.

During the implementation of a novel technique, it is of major importance to understand its limitations. NIR fluorescence imaging has a maximum penetration depth of approximately 5 mm into (fatty) tissue ¹⁷. Therefore, detectability of SLNs in patients with higher BMI can be challenging. In our study we found that the time between skin incision and SLN identification increases with an increase in BMI. As radioactive guidance has a much higher penetration depth, it could be especially of value in patients with higher BMI. Therefore, radioactive guidance appeared to be still obligatory for preoperative planning and for the identification of deeply located SLNs ³³.

To conclude, 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 radioactive and NIR fluorescence guidance in a single tracer. In vulvar cancer the ICG-^{99m}Tc-nanocolloid based SLN procedure seems to outperform ICG, ICG:HSA and blue dye based SLN procedures in terms of the intraoperative optical detection rate. This improvement is achieved with considerably less dye and is also effective at longer time intervals. Moreover, the ICG-^{99m}Tc-nanocolloid compound is extremely simple to formulate. Therefore we would recommend using ICG-^{99m}Tc-nanocolloid for combined fluorescence and radio-guided SLN mapping in vulvar cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1. Preoperative SLN mapping

Example of SLN mapping using lymphoscintigraphy and SPECT/CT after administration of ICG:^{99m}Tc-nanocolloid. A: lymphoscintigraphy showing the injection spot and one SLN in the left groin. B, C, D and E: fused SPECT/CT images showing the position of the SLN in relation to patient anatomy. SLN= Sentinel Lymph node, inj.: Injection spot.

FIGURE 2. NIR fluorescence-guided sentinel lymph node (SLN) mapping

Intraoperative NIR fluorescence guided SLN mapping performed in the same patient as figure 1, using ICG:^{99m}Tc-nanocolloid. *Top row*, percutaneous NIR identification of afferent lymphatic channels flowing away from the injection site (Inj.). The planned incision site, based on the presumed location of the SLN, is shown as a dashed line. *Middle row*, real-time fluorescence identification of the SLN directly after incision. *Bottom row*, SLN resection under fluorescence guidance. Scale bars = 1 cm. Camera exposure times were: 100 msec (upper and middle row) and 60 msec (bottom row).

Time between incision and SLN detection

FIGURE 3. Time between skin incision and SLN detection

Shown is the effect of Body Mass Index (BMI) (abscissa) on skin incision to SLN identification time (ordinate) in the context of ICG preparation method. Points represent individual patients. Green circles represent patients that received ICG alone; blue circle represents patients that received ICG:HSA; and red circles represents patients that received ICG:^{99m}Tc-nanocolloid. Dotted line represents the median for each group.

FIGURE 4. Signal-to-Background ratios between study protocols

The signal-to-background ratios of the different formulations is shown as mean with range: ICG alone (0.62 mg; 1.6 mL; injected during surgery), ICG:HSA (0.62 mg; 1.6 mL; injected during surgery) and ICG-^{99m}Tc-nanocolloid (0.05 mg; 0.4 mL; injected up to 20h prior to surgery).

TABLE 1**Patient and Tumor Characteristics**

Characteristic	ICG: ^{99m} Tc-Nanocoll (N = 12)	ICG alone* (N = 12)	ICG:HSA* (N = 12)	Total (36 patients)	P
Age (median; range)	72 (40 - 90)	77 (47 - 87)	69 (36 - 83)	71 (36 - 90)	0.23
Body mass index (median; range)	24 (17 - 30)	28 (24 - 40)	27 (21 - 35)	27 (21 - 40)	0.08
Primary tumor size in mm (median; range)	9 (4 - 35)	18 (5 - 48)	20 (5 - 55)	16 (5 - 55)	0.28
Primary tumor infiltration depth in mm (median; range)	2.3 (0.6 - 9)	3.0 (1.5 - 27)	1.9 (1.3 - 14)	2.5 (0.6 - 27)	0.24
Previous groin surgery	1 (8%)	2 (17%)	1 (8%)	4 (11%)	0.77

ICG:HSA, indocyanine green (ICG) adsorbed to human serum albumin (HSA)

* Patients were previously reported in BJOG. 2013 May;120(6):758-64.

SLN Identification Results

TABLE 2

Characteristic	Injected doses of ICG: Timing of injection:		ICG; ^{99m} Tc-Nanocol (12 patients)		ICG Alone * (12 patients)		ICG:HSA * (12 patients)		Total (36 patients)		P
			N	%	N	%	N	%			
SLNs detected by lymphoscintigraphy											
Unilateral	4	42	8	67	6	50	19	53	0.48		
Bilateral	8	58	4	33	6	50	17	47			
Intraoperative detection rate per patient [†]											
	12	100	9	75	10	83	31	86	0.21		
Number of SLNs identified											
	21		12		23		56				
Method of SLN detection											
Radioactive	21	100	12	100	23	100	56	100	0.19		
Fluorescence	21	100	12	100	23	100	56	100			
Blue dye	13	62	11	92	16	70	40	71			
Signal-to-background ratio (median; range)											
	5.4 (1.8 – 11.8)		10.6 (1.3 – 22.1)		10.1 (7.2 – 12.7)		8.4 (1.3 – 22.1)		0.02		
Time between skin incision and first SLN detection in min. (median; range)											
	8 (1 -22)		12 (2 – 24)		7 (2 – 16)		8 (1 – 24)		0.79		
Histology											
Negative	9	75	9	75	8	67	26	72	0.77		
ITC/micrometastases	1	8	2	17	1	8	4	11			
Macrometastases	2	17	1	8	3	25	6	17			

H, Hours; ICG:HSA, indocyanine green (ICG) adsorbed to human serum albumin (HSA); SLN, sentinel lymph node; ITC, isolated tumor cells

* Patients were previously reported in BJOG. 2013 May;120(6):758-64.

[†] Detection rate combining NIR fluorescence imaging, the gamma probe, and blue dye staining