Near-infrared image guidance in cancer surgery

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PART I

Clinical translation of image-guided surgery in sentinel lymph node mapping

Chapter 2

Near-infrared fluorescence sentinel lymph node biopsy in vulvar cancer: a randomized comparison of lymphatic tracers

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British Journal of Obstetrics and Gynaecology 2013;120:758-764

ABSTRACT

This study aims to confirm feasibility of near-infrared (NIR) fluorescence imaging for sentinel lymph node (SLN) biopsy in vulvar cancer and to compare the tracer indocyanine green (ICG) bound to human serum albumin (HSA) versus ICG alone. Patients received ^{99m}Tc-nanocolloid and patent blue for SLN detection. Subsequently, women randomly received ICG:HSA or ICG alone. In 24 women, 35 SLNs were intraoperatively detected. All SLNs detected were radioactive and NIR fluorescent and 27 (77%) were blue. No significant difference was found between ICG:HSA and ICG alone. This trial confirms the feasibility of NIR fluorescence imaging for SLN mapping in vulvar cancer.

INTRODUCTION

Lymph node status is the most significant prognostic factor for survival in women with vulvar cancer.[1,2] Therefore, lymphadenectomy plays a major role in the surgical treatment and staging of vulvar cancer. However, approximately 70% of women undergo unnecessary lymphadenectomy, which is associated with high morbidity and prolonged hospitalization.[3-5] The introduction of sentinel lymph node (SLN) biopsy has provided a less invasive technique for nodal staging.[6-8] SLN biopsy in early-stage vulvar cancer is considered accurate and safe without compromising groin recurrence or survival rates.[3,9]

Currently, radioactive colloids, blue dyes, or a combination of both are used for SLN detection and offer high identification rates and low false-negative rates.[9, 10] Recently, near-infrared (NIR) fluorescence optical imaging for SLN detection has been introduced. This technique uses the clinically available NIR fluorescent tracer indocyanine green (ICG).[11] The use of NIR light (700-900 nm) has several characteristics that can be advantageous in SLN biopsy as it offers relatively high tissue penetration (several millimeters) compared to blue dye and detection of low concentrations of tracer.[12-14] Furthermore, NIR fluorescence imaging outperformed blue dye for SLN detection in multiple clinical studies.[14-16] In vulvar cancer, two pilot studies demonstrated feasibility of NIR fluorescence for SLN biopsy.[17,18] Hutteman et al. used ICG adsorbed to human serum albumin (HSA, complex ICG:HSA) and Crane et al. used ICG alone for SLN mapping in women with vulvar cancer.[17,18] In vitro studies demonstrated that adsorption of ICG to HSA, by simple mixing, increases its fluorescence intensity (by threefold) and hydrodynamic diameter, which possibly results in better retention in the SLN.[19] However, lymphatic vessels contain high concentrations of HSA and other proteins, which would make adsorption of ICG to HSA before injection redundant. Therefore, clinical assessment and comparison of these two lymphatic tracers is essential.

The aim of this double-blind randomized trial was to confirm feasibility of NIR fluorescence imaging for SLN biopsy in vulvar cancer and to assess whether ICG alone could render the same fluorescence intensity in the SLNs as ICG:HSA.

MATERIALS AND METHODS

Tracer Preparation

ICG (25-mg vials, Pulsion Medical Systems ,Munich, Germany) was resuspended in 10 cc of sterile water. To obtain 500 μM, 7.8 mL of the 3.2-mM ICG solution was diluted in 42.8 mL of sterile water or 42.8 ml of Cealb (20% human serum albumin, Sanquin, Amsterdam, The Netherlands) for the preparation of ICG alone or ICG:HSA,

respectively. A dose of 500 μM was chosen based on previous dose optimization studies.[18, 20]

Clinical Trial

This double-blind, randomized, non-inferiority trial comparing ICG:HSA with ICG alone was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975. Inclusion criteria were a clinically FIGO stage I vulvar cancer with an unifocal squamous cell carcinoma measuring less than 4cm in diameter, not encroaching the vagina, anus, or urethra and with negative inguinofemoral nodes as determined by palpation and ultrasonography.[5] However, four participants with a tumour >4cm were scheduled to undergo SLN biopsy of the inguinofemoral nodes outside this protocol, because of other co-morbidity or age > 80. Exclusion criteria were pregnancy, lactation, or an allergy to iodine or ICG. All women gave informed consent and were anonymized. Randomization was performed by the Department of Clinical Pharmacy by block randomization.

Women received the standard-of-care SLN procedure by gynecologic oncologists experienced with SLN biopsy.[3,18] For our institution, this implied peritumoural injections of 60-100 MBq 99mtechnetium-nanocolloid the day before, or the morning prior to surgery. Prior to surgery, 1 mL of patent blue V (Guerbet, France) was injected at 4 sites peritumourally intracutaneously or around the excision scar, in cases of earlier excision biopsy. Subsequently, 1.6 mL total of ICG:HSA or ICG alone was injected as 4 injections at the same location as the blue dye injections. SLN mapping was performed using the Mini-Fluorescence-Assisted Resection and Exploration (Mini-FLARE™) image-guided surgery system as described previously.18, 20 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). Both the surgeon and the assessor of the Mini-FLARE™ data were blinded to the treatment allocation. A SLN was defined as a lymph node on a direct lymphatic drainage pathway from the primary tumour as detected preoperatively by lymphoscintigraphy.[21] Intraoperatively, lymph nodes with a gamma count of 10% or more compared to the most radioactive SLN, were also designated as SLNs. A SLN exhibiting a SBR ≥ 1.1 in situ was considered positive by NIR fluorescence.

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 protocol.[3] A full inguinofemoral

lymphadenectomy was performed in cases of tumour positive frozen sections of the SLN showing macrometastases 2 mm or larger or when the SLN could not be identified intraoperatively.

Power Calculation and Statistical Analysis

The power calculation is based on previous data, in which a SBR of 12.8 ± 4.5 was observed during SLN detection.[18] These data revealed that 24 women are needed to achieve at least 90% power to detect non-inferiority using a one-sided, 2-sample t test ($\alpha = 0.025$) with a margin of equivalence of 6.4 while assuming no difference between the SBR of ICG:HSA and ICG alone. For statistical analysis, SPSS statistical software (Version 16.0, Chicago, IL) was used. To compare the SBR and the number of SLNs identified between ICG:HSA and ICG alone, a 1-sided, 2-sample t test was performed. $P < 0.05$ was considered significant.

RESULTS

Characteristics of participants

Twenty-four consecutive women with vulvar cancer undergoing SLN biopsy were included in this study (Fig. 1). Participant and tumour characteristics and previous treatment are presented in Table 1 and were equally distributed over the treatment groups. Three women underwent previous groin surgery due to varicose veins and SLN biopsy related to a previous vulvar cancer. The two treatment groups included each a total of 12 women.

Table 1. Patient and tumour characteristics

Characteristic	ICG:HSA (n = 12)	ICG alone (n = 12)	P
Age (median, range)	63 (36 - 83)	73 (47 - 87)	.10
Body mass index (median, range)	28 (21 - 35)	30 (24 - 40)	.37
Average tumour size (mm) ± SD	22 ± 15	22 ± 17	.98
Average tumour infiltration depth (mm) ± SD	3.6 ± 4.1	6.2 ± 8.3	.37
Previous groin surgery	1 (8%)	2 (17%)	.54

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

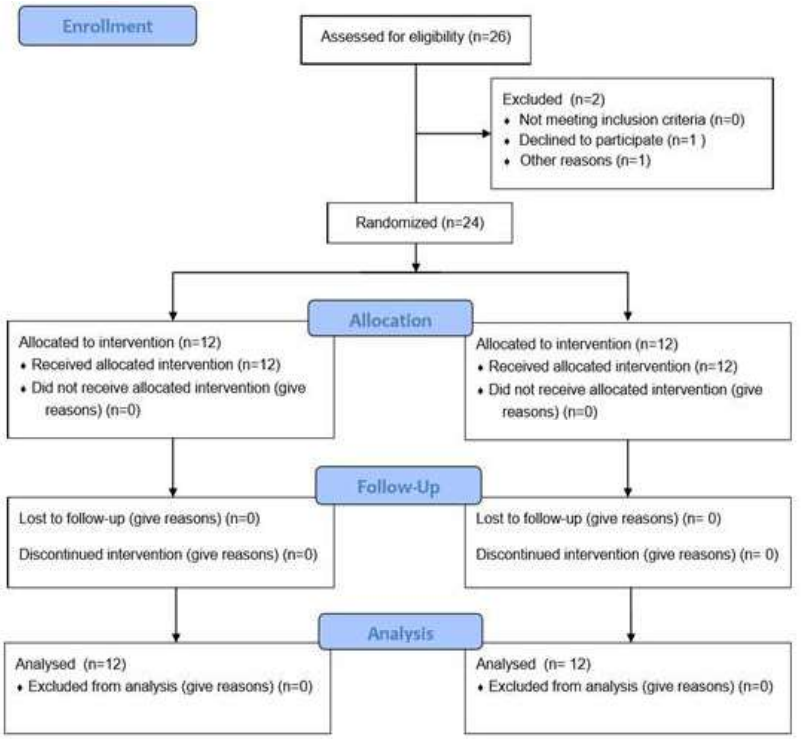

Fig 1. Patient enrollment

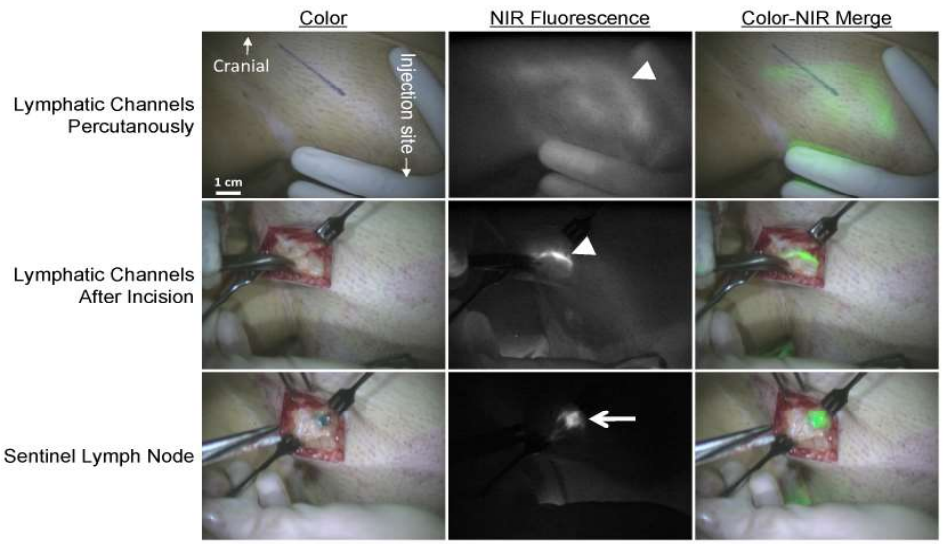

Fig 2. NIR Fluorescence-Based SLN Mapping. NIR fluorescence SLN mapping in a woman with early-stage vulvar cancer. Upper panel: Percutaneous visualization of the lymphatic vessels (arrowhead). Marker line presents planned incision before NIR fluorescence imaging. Middle panel: Identification of the lymphatic vessel (arrowhead) after incision. Lower panel: Identification of the radioactive, NIR fluorescent and blue SLN (arrow). Camera exposure times were 250 msec (upper panel), 60 msec (middle panel), and 10 msec (lower panel). Scale bars = 1 cm.

Sentinel lymph node detection

Preoperative lymphoscintigraphy identified at least one SLN in each woman. The SLN was located unilaterally in 14 women and bilateral SLNs were identified in 10 women. In eight women (33%) lymphatic vessels were percutaneously visible using NIR fluorescence, which could assist in the location of the incision (Fig. 2). Average time between skin incision and detection of the first SLN was 10 ± 8 min. Intraoperatively, on average 1.5 ± 1.2 SLNs per participant were identified (Supplementary Video). Of the 35 SLNs identified 35 (100%) were radioactive, 35 (100%) were fluorescent and 27 (77%) were blue. In all women, the NIR fluorescence signal in the SLN was detected before patent blue. In 19 out of the 24 women at least one SLN was detected during surgery. In 25 out of the 34 groins in which a SLN was detected by lymphoscintigraphy at least one SLN was detected. In two women, in whom no SLN could be located intraoperatively, a fluorescent node could be detected in the resection specimen of the inguinofemoral lymphadenectomy following the SLN procedure. Histological analysis showed lymph node metastases in seven out of 24 women of whom four women had macrometastases (> 2 mm) and three had micrometastases (≤ 2 mm). In all women with lymph node metastases at least one of the tumour positive lymph nodes was appointed SLN. No adverse reactions associated with the use of ICG or ICG:HSA occurred.

Comparison between treatment groups

The average SBR of ICG:HSA (10.3 ± 2.5) and ICG alone (11.2 ± 6.0) were not significantly different (P = 0.65) (Table 2). No significant difference was observed in the average number of in vivo identified fluorescent SLNs per groin between ICG:HSA and ICG alone (1.9 ± 1.4 vs. 1.0 ± 0.7, P = 0.06). Similarly, there was no significant difference in intraoperative detection rate (P = 0.27).

COMMENT

The present study confirmed feasibility of NIR fluorescence for optical guidance for intraoperative SLN biopsy using ICG in women with vulvar cancer. All SLNs that were detected by radio guidance could also be detected by NIR fluorescence, however not all SLNs were detected by blue dye staining. This double-blind randomized trial did not show any advantages of ICG:HSA over ICG alone in SBR and average number of intraoperative detected fluorescent SLNs.

NIR fluorescence was of added value during SLN detection, as all SLNs detected could be identified by the direct optical guidance of NIR fluorescence, but only 77% by blue dye staining. Moreover, the NIR fluorescence signal in the SLN could be detected

Table 2. SLN Identification Results

Characteristic	Total (24 subjects)		ICG:HSA (12 subjects)		ICG alone (12 subjects)		P
	N	%	N	%	N	%	
SLNs detected by lymphoscintigraphy							.41
Unilateral	14	42	6	50	8	67	
Bilateral	10	58	6	50	4	33	
Intraoperative detection rate*							
Per patient	19	79	10	83	9	75	.61
Per groin† (34 groins)	25	74	15	83	10	63	.17
Average number of intraoperative identified SLNs per patient ± SD	1.5 ± 1.2		1.9 ± 1.4		1.0 ± 0.7		.06
Number of SLNs identified	35		23		12		
Method of SLN detection							
Radioactive	35	100	23	100	12	100	
Fluorescence	35	100	23	100	12	100	
Blue dye	27	77	16	70	11	92	.09
Signal-to-background ratio	10.7 ± 4.4		10.3 ± 2.5		11.2 ± 6.0		.65
Average time between injection and skin incision ± SD (min)	17 ± 6		18 ± 6		16 ± 6		.39
Average time between skin incision and first SLN detection ± SD (min)	10 ± 9		10 ± 9		11 ± 8		.75
Histology							.50
negative	17	71	8	67	9	75	
ITC/micrometastasis	3	13	1	8	2	17	
macrometastasis	4	16	3	25	1	8	

ICG:HSA, indocyanine green (ICG) adsorbed to human serum albumin (HSA); SLN, sentinel lymph node; ITC, isolated tumour cells
*Detection rate combining NIR fluorescence imaging, the gamma probe, and blue dye staining
†Groins with SLN localization by preoperative lymphoscintigraphy (N=18 ICG:HSA, N=16 ICG alone)

before patent blue in all cases. Blue dye staining and NIR fluorescence both provide real-time optical guidance. Tissue penetration of NIR fluorescent light is significantly higher than penetration of visible light, which enables deeper and earlier visualization of signal by NIR fluorescence. This can assist to determine the location of the incision and provide improved optical guidance during SLN localization. Furthermore, since ICG is diluted to levels invisible to the human eye after injection, no discoloration of the surgical field occurs. It can therefore be questioned whether blue dye can be

omitted when NIR fluorescence is used.[22] Moreover, compared to radioactive lymphatic tracers, NIR fluorescence is not hampered by high background signals of the injection site by the gamma probe, which can interfere SLN detection using the gamma probe. However, due to the limited penetration depth of NIR fluorescence imaging (several mm), radioactive SLN tracers remain necessary for preoperative surgical planning and to detect deeper located SLNs. Therefore, a combination of a radiocolloid tracer for preoperative planning and guidance of deeper located nodes and a NIR fluorescence tracer for real-time optical guidance is advocated.[23,24]

This study is one out of three simultaneously initiated clinical studies to directly compare the lymphatic tracers ICG:HSA and ICG alone.[15,25] In concordance with these studies, no significant difference in SBR and average number of identified SLNs between the lymphatic tracers ICG:HSA and ICG alone was found in vulvar cancer. Since lymph fluids consist of a high protein levels,[26] a potential explanation for the lack of difference between ICG:HSA and ICG alone is that ICG rapidly binds to these endogenous proteins when drained in the lymphatic system, eliminating the need for premixing ICG and HSA. Together, the three clinical studies demonstrate no difference between ICG:HSA and ICG alone for the SLN identification in a large heterogeneous group of women, with different anatomical locations (breast cancer, cervical cancer, and vulvar cancer) and different times from injection to imaging.[15,25]

All SLNs identified intraoperatively could be detected by both radio guidance and NIR fluorescence. Although a high concordance between detection by NIR fluorescence and radio guidance was found, the intraoperative detection rate of the SLN (19 out of 24 women) in this study is relatively low compared to other studies.10 A possible explanation could be that two women underwent previous groin surgery for varicose veins and one underwent a SLN procedure previously. Moreover, in two women fluorescent SLNs could be detected in the lymphadenectomy specimen ex vivo, which if detected intraoperatively would have increased the intraoperative detection rate considerably. Nevertheless, in all women with lymph node metastases, the SLN containing tumour cells could be identified intraoperatively.

In conclusion, this double-blind, randomized trial showed no advantage of using ICG:HSA in comparison to ICG alone and shows the added value of NIR fluorescence for SLN biopsy in vulvar cancer.

ACKNOWLEDGMENTS

The authors thank Margriet J.G. Löwik and Dorien M.A. Berends-van der Meer for their assistance during the participant inclusion process and Lindsey Gendall for editing. This work was supported in part by NIH grants R01-CA-115296 and R21-CA-130297 and the Dutch Cancer Society grant UL2010-4732. This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine, project MUSIS (grant 03O-202). Joost van der Vorst is an MD-medical research trainee funded by The Netherlands Organisation for Health Research and Development (grant 92003593).

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