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Near-infrared image guidance in cancer surgery

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Chapter 3

Randomized comparison of near-infrared fluorescence lymphatic tracers for sentinel lymph node mapping of cervical cancer

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ABSTRACT

Objective

Near-infrared fluorescence imaging using indocyanine green (ICG) has recently been introduced as a novel technique for sentinel lymph node (SLN) mapping in early-stage cervical cancer. Although preclinical research has shown that ICG adsorbed to human serum albumin (ICG:HSA) improves its performance, the need for HSA has not yet been confirmed in cervical cancer patients. The current randomized study aims to determine whether ICG:HSA offers advantages over using ICG alone.

Methods

Eighteen consecutive early-stage cervical cancer patients scheduled to undergo pelvic lymphadenectomy were included. Prior to surgery, 1.6 mL of 500 μ M ICG:HSA or 500 μ M ICG alone was injected transvaginally in 4 quadrants around the tumour. The Mini-FLARE imaging system was used for intraoperative NIR fluorescence detection and quantitation.

Results

SLNs were identified intraoperatively in 78% of the patients. Patient and tumour characteristics were equally distributed over both treatment groups. No significant difference in signal-to-background ratio (9.3 vs. 10.1, $P = .72$) or average number of detected SLNs (2.9 vs 2.7, $P = .84$) was found between the ICG:HSA group and the ICG alone group, respectively.

Conclusions

In conclusion, this double-blind, randomized trial showed no advantage of ICG:HSA over ICG alone for the SLN procedure in early-stage cervical cancer. Further optimization is required to improve the intraoperative detection rate.

INTRODUCTION

As part of surgical management in early-stage cervical cancer, bilateral pelvic lymphadenectomy is considered standard of care. However, pelvic lymphadenectomy is associated with complications, such as lymphedema, the risk of nerve injury, and lymphocyst formation.¹ Because lymph node involvement occurs in approximately 12% to 22% of early-stage cervical cancer patients, the majority of patients do not benefit from a pelvic lymphadenectomy, but may suffer from its complications.^{2,3} Over the last decades, the sentinel lymph node (SLN) concept has proven to be feasible and safe in vulvar cancer, melanoma, and breast cancer for selecting patients who would benefit from lymphadenectomy. In addition, the SLN can be used for ultra staging to aid in the identification of micrometastases.⁴ However, due to the bilateral multifarious drainage pattern of the cervix, the SLN procedure is more challenging in cervical cancer patients than in breast cancer patients, for example. SLN biopsy in early-stage cervical cancer patients has been extensively described using blue dye, a radiocolloid, or a combination of both, with various results.⁵

Intraoperative near-infrared (NIR) fluorescence imaging using indocyanine green (ICG) has emerged as a novel technique for SLN detection in cervical cancer patients.⁶⁻¹⁰ NIR fluorescence imaging has several advantages, such as a relatively high tissue penetration and low autofluorescence. ICG is currently the only clinically available NIR lymphatic tracer. However, due to its relatively low fluorescence brightness (quantum yield) and its small hydrodynamic diameter, it is not an optimal lymphatic tracer. Preclinical work has demonstrated that adsorption of ICG to human serum albumin (HSA, complex is ICG:HSA), by simply mixing it, increases the fluorescence intensity (a threefold) and hydrodynamic diameter.¹¹ These advantages could provide improved detection and retention of the tracer in the SLN. On the other hand, the use of albumin adds cost and complexity to the procedure. Moreover, the use of human blood products, such as HSA, poses regulatory hurdles in certain countries, such as the United States.

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

METHODS

Preparation of Indocyanine Green adsorbed to Human Serum Albumin

Indocyanine green (25-mg vials) was purchased from Pulsion Medical Systems (Munich, Germany) and was resuspended in 10 cc of sterile water. To obtain a 500 μM final concentration, 7.8 mL of the 3.2-mM ICG solution was diluted in 42.8

mL of sterile water for injection 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 studies that showed that the optimal dose of ICG:HSA lies between 400 and 800 μM and has the benefit of minimal manipulation of ICG and albumin volumes.^{6,12}

Intraoperative Near-Infrared Imaging System (Mini-FLARE)

SLN mapping was performed using the Mini-Fluorescence-Assisted Resection and Exploration (Mini-FLARE) image-guided surgery system as described previously.¹² Briefly, the system consists of 2 wavelength isolated light sources: a “white” light source, generating 26,600 lx of 400-650 nm light, and a “near-infrared” light source, generating 7.7 mW/cm² of 760 nm light. Color video and NIR fluorescence images are simultaneously acquired and displayed in real time using custom optics and software that separate the color video and NIR fluorescence images. A pseudo-colored (lime green) merged image of the color video and NIR fluorescence images is also displayed. The imaging head is attached to a flexible gooseneck arm, which permits positioning of the imaging head at extreme angles virtually anywhere over the surgical field. For intraoperative use, the imaging head and imaging system pole stand are wrapped in a sterile shield and drape (Medical Technique Inc., Tucson, AZ).

Clinical Trial

The double-blind, randomized, noninferiority 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. All patients planning to undergo a total pelvic lymphadenectomy for early stage cervical cancer were eligible for participation in the study. Exclusion criteria were pregnancy, lactation, or an allergy to iodine, shellfish, or indocyanine green. All patients gave informed consent and were anonymized. Patients were randomized by the Department of Clinical Pharmacy. Treatment allocation was performed by block randomization. Before the start of surgery, 1.6 mL of 500 μM ICG:HSA or ICG alone was transvaginally injected by the surgeon submucosally in 4 quadrants of the cervix. Directly after injection, following surgical scrub and sterile covering of the operation field, a laparotomy was performed.

Prior to the systematic pelvic lymphadenectomy, all standard locations (along the external iliac vessels and the hypogastric artery, the common iliac artery, the obturator fossa and the lateral parametrium) were examined for NIR fluorescence using the Mini-FLARE imaging system. Both the surgeon and the Mini-FLARE operator, who was responsible for analyzing the data, were blinded to the treatment allocation. All

fluorescent hotspots, containing one or more lymph nodes, were denominated as SLNs and were subsequently resected and measured for fluorescence *ex vivo*. Subsequently, the standard systemic pelvic lymphadenectomy was performed and all resected lymph nodes were also measured for fluorescence *ex vivo*. Lymphadenectomy consisted of removal of all lymph node-bearing fatty tissue along the external iliac vessels, the common iliac artery, the hypogastric artery, and from the obturator fossa.¹³ Also, the area lateral to and underneath the superior vesical arteries (lateral parametrium) was cleared from the lymphatic tissue. The radical hysterectomy or abdominal trachelectomy were performed according to the standard procedure. Afterwards, all resected lymph nodes were examined by routine histopathological analysis; lymph nodes were fixed in formalin and embedded in paraffin for routine hematoxylin and eosin staining. SLNs and non-SLNs were examined separately. Additionally, a substantial part of the SLNs and clinical suspected nodes were subsequently examined using a cytokeratin staining.

Power Calculation and Statistical Analysis

The power calculation is based on data from previous studies, in which *in vitro* a threefold difference in fluorescent brightness (quantum yield) was reported between ICG alone and ICG:HSA and *in vivo* signal-to-background ratio of 10.1 ± 1.2 was observed during SLN detection by ICG:HSA.^{6,11} These data revealed that 18 patients are needed to achieve 91% power to detect non-inferiority using a one-sided, 2-sample t test ($\alpha = 0.025$) with a margin of equivalence of 5.0 while assuming no difference between the signal-to-background ratio (SBR) of ICG:HSA and ICG alone. For statistical analysis, SPSS statistical software package (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

Patient characteristics

Eighteen consecutive early-stage cervical cancer patients scheduled to undergo a pelvic lymphadenectomy were randomized to ICG:HSA or ICG alone for NIR fluorescence SLN imaging. All patients were clinically staged as FIGO stage IB1 cervical cancer. Of these 18 patients, the median age was 40 years (range, 28-67), median body mass index was 25 (21-38), and median tumour size was 1.6 cm (range, 0.3-5.0 cm). In one patient in whom preoperative pathology was not conclusive (either cervical cancer or endometrial cancer), postoperative pathological evaluation showed endometrial

cancer. In all patients a complete bilateral pelvic lymphadenectomy was performed, except for one case. In this case the surgeon did not complete the lymphadenectomy, due to pathologically proven metastatic lymphatic disease by intraoperative frozen section and excessive blood loss during surgery. Patient and tumour characteristics and previous treatment were equally distributed over the treatment groups (Table 1).

Table 1. Patient and Tumour Characteristics

Characteristic	ICG:HSA (N = 9)		ICG alone (N = 9)		P
	N	%	N	%	
Age (median, range)	40 (28 - 67)		40 (30 - 50)		.86
Body mass index (median, range)	25 (22 - 38)		23 (21 - 34)		.13
Previous procedures					
No local treatment	3	33	3	33	.77
Local excision	2	23	2	23	
Conization	3	33	4	44	
Local excision + Neoadjuvant chemotherapy	1	11	0	0	
Histological type					
Squamous cell carcinoma	4	44.5	5	56.5	.57
Adenocarcinoma	4	44.5	4	44.5	
Endometroid adenocarcinoma	1	11	0	0	
Type of tumour excision					
Radical hysterectomy	6	67	8	89	.26
Radical trachelectomy	3	33	1	11	
Type of lymphadenectomy					
Complete bilateral lymphadenectomy	8	89	9	100	.30
Incomplete bilateral lymphadenectomy	1	11	0	0	
Tumour size (median, range)	1.4 (0.6 - 5.0)		1.8 (0.3 - 3.6)		.88

NIR Fluorescence Imaging

The results of the NIR fluorescence detection of SLNs are presented in Table 2. During surgery, NIR fluorescence imaging enabled identification of 1 or more fluorescent hotspots in 14 out of 18 (78%) patients (Figure 1). In 11 patients (61%) fluorescent hotspots were identified bilaterally, whereas in 3 patients (17%) hotspots were identified unilaterally. In 4 patients (22%) no fluorescent hotspots were detected intraoperatively. Average time between injection of ICG:HSA or ICG alone and imaging was 43 ± 14 min and did not lengthen the standard of care surgery. On average, 2.8 ± 2.2 hotspots were detected intraoperatively by NIR fluorescence, yielding an average of 4.2 ± 3.7 SLNs per patient at pathological examination. An average of 24.5 (range, 9-46) lymph nodes per patient were harvested when a complete bilateral lymphadenectomy was performed.

In addition, all lymph nodes were examined for fluorescence ex vivo directly after surgical resection in the operating room. In 11 of 18 patients, a total of 29 additional fluorescent hotspots could be identified, which were not identified during

in vivo NIR fluorescence imaging. When the ex vivo detected fluorescent hotspots and the in vivo detected fluorescent hotspots were combined, NIR fluorescence imaging enabled identification of 1 or more fluorescent hotspots in 16 out of 18 (89%) patients, thus in 2 more patients compared to in vivo identification alone. In 13 patients (72%) these were identified bilaterally, in 3 patients (17%) unilaterally, and in 2 patients (11%) there were no fluorescent hotspots detected in vivo or ex vivo.

Histological analysis showed lymph node metastases in 6 out of 18 patients of whom 4 patients had macrometastases (> 2 mm) and 2 patients had micrometastases (≤ 2 mm).¹⁴ In 5 of 6 patients with lymph node metastases, one or more tumour positive nodes were fluorescent. Though, in 2 of these 6 patients, the fluorescent nodes were only detected ex vivo. Furthermore, in 1 patient, the resection specimen contained a nonfluorescent fibrotic nodule with mature fat cells, some lymphoid infiltration, and a micrometastatic lesion. No metastases were found in this patient's fluorescent nodes.

Table 2. SLN Identification Results

Characteristic	Total (18 subjects)		ICG:HSA (9 subjects)		ICG alone (9 subjects)		P
	N	%	N	%	N	%	
Intraoperative detection rate							
Bilateral	11	61	5	56	6	67	.13
Unilateral	3	17	3	33	0	0	
None	4	22	1	11	3	33	
Average number of intraoperative NIR hotspots \pm SD	2.8 \pm 2.2		2.9 \pm 1.7		2.7 \pm 2.6		.84
Signal-to-background ratio	9.7 \pm 3.9		9.3 \pm 3.6		10.1 \pm 4.5		.72
Average lymph nodes in lymphadenectomy \pm SD	24.5 \pm 8.8		23.4 \pm 4.3		25.6 \pm 11.6		.62
Average number of additional hotspots ex vivo \pm SD	1.6 \pm 2.0		1.0 \pm 1.3		2.2 \pm 2.4		.21
Presence of fluorescent nodes							
Bilateral	13	72	6	67	7	78	.82
Unilateral	3	17	2	22	1	11	
None	2	11	1	11	1	11	
Histology							
Negative	12	67	7	78	5	56	.51
ITC/micrometastasis	2	11	1	11	1	11	
Macrometastasis	4	22	1	11	3	33	
False negative rate							
Intraoperatively detected nodes	3/6		1/2		2/4		
All fluorescent nodes	1/6		1/2		0/4		

ICG:HSA, indocyanine green adsorbed to human serum albumin; ITC, isolated tumour cells

Comparison between treatment groups

The primary endpoint of this study was the average brightness of the SLN, expressed in SBR. The average SBR of ICG:HSA (9.3 ± 3.6) and ICG alone (10.1 ± 4.5) were not significantly different ($P = .72$). No significant difference was observed in the average number of in vivo identified fluorescent hotspots between ICG:HSA and ICG alone (2.9 ± 1.7 vs 2.7 ± 2.6 , $P = .84$). Similarly, there was no significant difference in intraoperative detection rate ($P = .13$) or presence of fluorescent nodes after ex vivo inspection ($P = .82$).

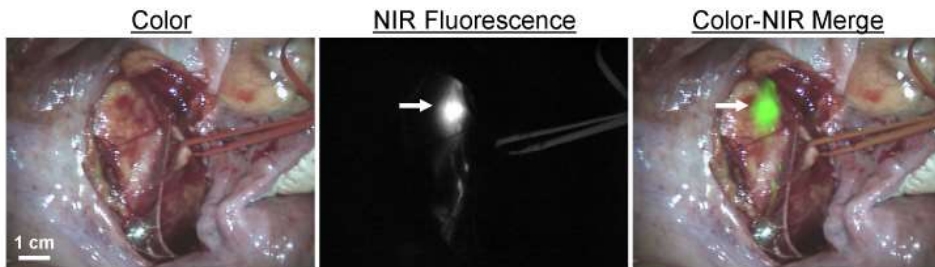


Fig 1. NIR Fluorescence-Based SLN Mapping using ICG:HSA and Mini-FLARE: Identification of a SLN (arrow), located along the right iliac vessels, with NIR fluorescence imaging is demonstrated in a cervical cancer patient after administration of 500 μ M ICG:HSA.

DISCUSSION

The current 18-patient clinical study confirmed the feasibility of the SLN procedure in early-stage cervical cancer using NIR fluorescence imaging with ICG. The overall intraoperative detection rate was 78%, and in 61% of all patients fluorescent nodes could be identified bilaterally. This double-blind randomized trial did not show any advantages of ICG:HSA over ICG alone in SBR, average number of intraoperatively detected fluorescent hotspots, or percentage of patients in whom fluorescent nodes could be detected.

The recent introduction of NIR fluorescence imaging has provided new opportunities in the field of SLN mapping. Preclinical studies indicated that adsorption of ICG to HSA increases the fluorescence intensity and the hydrodynamic diameter, thereby providing improved detection and better retention in the SLN.¹¹ In the current study, ICG:HSA performed equally well as ICG alone. This is in concordance with a previous study performed by our group in which no differences were found between the use of ICG:HSA and ICG alone in SLN mapping in breast cancer patients.¹⁵ In lymph fluids a high protein constitution is found.¹⁶ A potential explanation for the lack of difference between ICG:HSA and ICG alone is that ICG rapidly binds to endogenous proteins when drained in the lymphatic system, eliminating the need for premixing ICG and HSA.

The detection rate in this study is in concordance with previously reported detection rates in studies using NIR fluorescence for SLN mapping in early-stage cervical cancer, which vary from 60% to 100%.⁶⁻⁹ Large studies in early-stage cervical cancer patients using radiocolloid tracers reported higher detection rates varying from 88% to 99%.¹⁷⁻²³ Possible explanations for the lower detection rate observed in this study could be the inclusions of patients with larger tumours (>2 cm) and, especially, the learning curve associated with cervical injection of the tracer and the intraoperatively exposure of tissue to maximize NIR fluorescence detection.^{17,24} Unlike the present study, our initial 9-patient study of NIR fluorescence SLN mapping in cervical cancer utilized two clinicians for all NIR lymphatic tracer injections. Although the learning curve could be of significant effect on the intraoperative identification rate and time needed to identify the SLN, it is unlikely that the learning curve interferes with the SBR, which was used to power the present trial and to test difference between ICG alone and ICG:HSA. Assessment of the learning curve will be essential before NIR fluorescence can be compared to conventional techniques using radiocolloid tracers in a sufficient powered study.

After resection, additional fluorescent lymph nodes, which were missed during intraoperative inspection with the Mini-FLARE, were identified in the resection specimens of 11 out of the 18 patients. Intraoperative detection of these additional fluorescent nodes would have considerably improved the detection rate and the false negative rate. Difficulty in the intraoperative detection of these nodes could be based on the relatively limited tissue penetration of NIR light (several millimeters) compared to radiocolloid tracers, or the learning curve associated with positioning tissue intraoperatively to maximize NIR fluorescence detection. Moreover, radiocolloid tracers permit preoperative localization of the SLN, which assists surgical planning. Still, radiocolloids have the disadvantage that there is no real-time visualization during the operation, and detection is hampered if the SLNs are located near the point of the tracer injection. NIR fluorescence imaging is suitable for real-time identification and detection of SLNs located in the vicinity of the injection site. In addition, NIR fluorescence has as a much better tissue penetration when compared to blue dyes. Therefore, a combination of a radiocolloid and a NIR fluorescence tracer might be preferable. In a recent study, van der Poel et al. reported the successful use of such a multimodal tracer combining radioactivity and NIR fluorescence in sentinel lymph node mapping in prostate cancer patients using a NIR fluorescence laparoscope.²⁵

In conclusion, this double-blind, randomized trial showed no advantage of ICG:HSA in comparison to ICG alone for the SLN procedure in early-stage cervical cancer. Further optimization of NIR fluorescence imaging is required to improve the intraoperative detection rate to permit this technique to compete with radiocolloid tracers.

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