Risk factors for failure of sentinel lymph node mapping using indocyanine green/near-infrared fluorescent imaging in endometrial cancer

Salih Taşkın[1] · Mustafa Erkan Sarı[2] · Duygu Altın[1] · Cevriye Cansız Ersöz[3] · Ali Gökçe[1] · Seher Yüksel[3] · Duygu Kankaya[3] · Fırat Ortaç[1]

Abstract

Purpose Side-specific systematic lymphadenectomy is suggested if sentinel lymph node (SLN) mapping failed in early stages endometrial cancer. This study aimed to evaluate the risk factors associated with failed mapping which may lead to modify SLN mapping technique, increase the success of SLN mapping and reduce the necessity of systematic lymphadenectomy.
Methods Patients with early stage endometrial cancer were included in this study. All patients underwent SLN mapping with indocyanine green/near-infrared compatible surgical platforms. Indocyanine green was injected intracervical. "Bilateral mapping" and "failed bilateral SLN mapping (unilateral or bilateral failed mapping)" groups were compared for demographic, clinical, surgical, and pathological features.
Results 101 cases were analyzed. The overall, unilateral, and bilateral SLN detection rates were 94.1%, 19.8%, and 74.3%, respectively. The failed (unilateral or no mapping) bilateral detection rate was 25.7%. Failed bilateral mapping rates were higher in patients with longer cervical and uterine longitudinal lengths, deep myometrial invasion and larger tumor size without statistical significance. Body mass index and operation type were not related with failed mapping. Increasing number of operations or injection of larger volume of indocyanine green (4 mL vs. 2 mL) did not improve mapping rate significantly.
Conclusion Cervical indocyanine green injection may overcome the negative effect of obesity on bilateral mapping. Although there was a negative correlation trend between the longitudinal cervical and uterine lengths and bilateral mapping, this possible relation needs to be confirmed in further studies.

Keywords Sentinel · Mapping · Endometrial cancer · Indocyanine green · Failure

Introduction

Based on the growing literature on sentinel lymph node (SLN) biopsy in uterine cancer, it is well-known that SLN mapping can be a favorable option to assess lymph node status, and the use of SLN algorithm is suggested as an option to evaluate early stage endometrial cancer instead of systematic lymphadenectomy [1–5]. This approach can prevent surgical morbidities of systematic lymphadenectomy and give accurate information about lymph node status, which is critical in deciding the type and extent of adjuvant treatment required.

The blue dye, technetium-99, or indocyanine green have been used as tracers for SLN mapping in endometrial cancer, and on achieving bilateral mapping, the SLN(s) are resected, and no further systematic lymph node dissection is needed—according to the National Comprehensive Cancer Network SLN algorithm. However, if SLN mapping is not achieved in one hemipelvis, a side-specific lymphadenectomy should be performed. Despite the improvements in bilateral mapping rates with the use of indocyanine green [6, 7], the mapping fails in at least 20–25% of cases [8, 9] with a subsequent need for systematic lymphadenectomy. An analysis of these cases can be beneficial to understand the factors affecting

✉ Salih Taşkın
  salihtaskin@yahoo.com

1 Department of Obstetrics and Gynecology, Ankara University School of Medicine, 06100 Ankara, Turkey

2 Department of Obstetrics and Gynecology, Ankara Training and Research Hospital, Ankara, Turkey

3 Department of Pathology, Ankara University School of Medicine, Ankara, Turkey

mapping failure, improve the SLN algorithm, and reduce the subsequent need for side-specific lymphadenectomy.

Therefore, the primary objective of our study was to report the detection rate of SLN using indocyanine green/near-infrared fluorescent technology in endometrial cancer and determine the risk factors associated with mapping failure.

Materials and methods

This study included patients with clinical early stage uterine cancer who underwent SLN mapping algorithm using indocyanine green/near-infrared fluorescent technology between April 2017 and October 2018. The institutional review board approved the study protocol, and informed consent was obtained from all the patients. Patients with extrauterine disease proven with preoperative clinical and imaging studies and any synchronous malignancy were excluded. Clinical early stage cancer was defined as a disease confined to the uterus.

A 25-mg vial of indocyanine green powder (Pulsion Medical Systems, Feldkirchen, Germany) was diluted in 20 mL of saline and injected by the same author (ST) into the uterine cervix at 3 and 9 o'clock locations [half deep (1 cm) and half superficial (3–4 mm)]. A total of 2- or 4-mL injection (1.25 mg/mL) was used in the 1:2 ratio consecutively to enable comparison of the different volumes of mapping failure. The dye was injected at a rate of approximately 10 s in each site. The endoscopic systems compatible with indocyanine green/near-infrared fluorescent technology were used in all surgeries (Spies Full HD D-Light P System, Karl Storz, Tuttlingen, Germany, for laparoscopic and open surgeries and the da Vinci Xi robotic platform, Intuitive Surgical, California, for robotic surgeries).

Surgical steps have been previously defined [10]. Briefly, the mapped SLNs were resected, and any suspicious lymph node was removed regardless of mapping. Subsequently, bilateral pelvic lymphadenectomies were performed if high-risk conditions for anesthesia did not exist. The indications for para-aortic lymphadenectomy were preoperative non-endometrioid histology, grade 3 endometrioid cancer, positive lymph nodes on intraoperative frozen section, or enlarged para-aortic nodes suspicious for malignancy.

Ultra-staging protocol for SLNs was implemented if the SLN was negative on initial hematoxylin–eosin staining as described in our previous reports [10]. Tumor foci larger than 2 mm in a lymph node were considered as macrometastasis, whereas tumor foci of 0.2–2 mm were defined as micrometastasis. The tumor deposits of less than 0.2 mm were defined as isolated tumor cells.

The patients were allocated into two groups for the analysis. The first group (bilateral group) included patients with successful bilateral SLN mapping with at least one SLN obtained from each pelvic side according to the final pathologic evaluation of the tissue labeled as SLN. The second group (failure group) included patients with failed bilateral SLN mapping (unilateral or bilateral failed mapping). The two groups were compared regarding their clinicopathological features.

The medical data of patients were prospectively recorded during the study period. The data included parameters, such as age, menopausal status, body mass index (BMI), longitudinal lengths of the cervix and uterus measured by the pathologist, type of surgery (laparoscopy, robotic, or open), amount of indocyanine green injected (2 or 4 mL), histologic type and grade of the tumor, presence and depth of myometrial invasion, presence of lymphovascular space invasion, tumor size, number of pelvic and para-aortic lymph node(s) removed, number of metastatic pelvic and para-aortic lymph nodes, status of lymphatic metastasis (isolated tumor cells, micrometastasis, or macrometastasis), stage of disease, and SLN detection status (bilateral, unilateral, or none). Additionally, the patients were allocated into four groups based on the timing of surgery as the sequential quarters of the cohort (first, second, third, and fourth quarters) to analyze the effect of experience on the mapping results.

Statistical analyzes were performed using Statistical Package for the Social Sciences software (version 23, SPSS Inc., Chicago, IL, USA). Descriptive data were presented as median (min–max) for continuous variables and as frequency (percentage) for categorical variables. Chi-square test was used to analyze the intergroup differences in categorical variables, and the intergroup comparisons of continuous variables were performed using the Mann–Whitney U test based on the results of their normality tests. A p value of < 0.05 was considered statistically significant. Sensitivity, false negative rate, and negative predictive value were calculated to assess the performance of the SLN algorithm. As all metastatic SLNs must be positive for lymph node metastasis, specificity, positive predictive value, and the false positive rate could not be reported. True positivity was defined as a positive SLN or algorithm in a patient with lymph node metastasis.

Results

A total of 101 patients were analyzed. The median age of the patients was 58 years (range 38–80 years), and median BMI was 33 kg/m^2 (range 21–49 kg/m^2). Most of the patients had minimally invasive surgery (79.2%) either through the robotic (11.9%) or laparoscopic (67.3%) approach. The operation details are summarized in Table 1. The rate of achieving systematic pelvic lymph node dissection was 91.1% (pelvic only 48.5%, pelvic and para-aortic

Table 1 Baseline characteristics of all patients

Characteristics	Values
Age (years), median, range	58 (38–80)
BMI, median, range	33 (21–49)
Menopausal status	
Premenopausal	12 (11.9%)
Postmenopausal	89 (88.1%)
Operation type	
Laparoscopy	68 (67.3%)
Laparotomy	21 (20.8%)
Robotic	12 (11.9%)
Site of lymphadenectomy	
Pelvic only	49 (48.5%)
Pelvic and para-aortic	43 (42.6%)
SLN dissection only	9 (8.9%)
Number of LNs removed, mean	
Number of pelvic LNs removed	20 (1–74)
Number of para-aortic LNs removed	0 (0–53)
Lymphatic metastasis, n	
Macrometastasis	9
Micrometastasis	3
Isolated tumor cells	2
Stage	
IA	64 (63.4%)
IB	11 (10.9%)
II	3 (3%)
IIIA	4 (4%)
IIIC1	10 (9.9%)
IIIC2	4 (4%)
IVB	1 (1%)
SLN detection	
Bilateral mapping	75 (74.3%)
Failure group	26 (25.7%)
Unilateral	20 (19.8%)
None	6 (5.9%)

BMI body mass index, *LN* lymph node, *SLN* sentinel lymph node

42.6%). SLN dissection without systematic lymphadenectomy was performed in 9 patients (8.9%). Atypical endometrial hyperplasia was reported on final histopathologic evaluation in four patients who had endometrial cancer diagnosis prior to the surgery. Clinicopathological features of all patients are summarized in Table 1.

Tissues resected and labeled as SLN from five hemipelvises of different patients did not contain lymph node(s), and these hemipelvises were considered non-mapped. According to the final pathology, the overall SLN detection rate was 94.1% (*n*: 95), bilateral mapping rate was 74.3% (*n*: 75), unilateral mapping rate was 19.8% (*n*: 20), and bilateral non-mapping rate was 5.9% (*n*: 6). Eventually, the "bilateral group" included 75 patients (74.3%) and "failure group" included 26 patients (25.7%).

There was no statistically significant difference between the experience periods (first 25, 50, or 75 cases and the subsequent cases) in terms of overall, unilateral, and bilateral SLN detection rates ($p > 0.05$). No significant difference between the 2 groups (bilateral vs. failure) was noted regarding clinical and pathological features. Median values of uterine and cervical lengths of the whole population were used for comparing failure rates. The failure rates were higher in patients with a cervical longitudinal length ≥ 35 mm compared with < 35 mm (22.4% vs. 32.2%), uterine longitudinal length > 6 cm compared with ≤ 6 cm (19.6% vs. 30.7%), and in patients given 2 mL indocyanine green compared with the patients given 4 mL (30.6% vs. 22.6%). However, these differences were not statistically significant. Age, BMI, stage of disease, menopausal status, and type of surgery were similar in 2 mL (*n*: 36) or 4 mL (*n*: 65) injected patients ($p > 0.05$ for each). Comparison of baseline characteristics of patients in terms of SLN detection status is shown in Table 2. Clinicopathological features of patients with bilateral non-mapping are explained in Table 3.

Lymph node metastasis was identified in 14 patients (13.8%), including 2 isolated tumor cells, 3 micrometastases, and 9 macrometastases. Para-aortic lymph node metastasis was detected in four patients. Three of them had pelvic lymph node metastasis and one had an isolated para-aortic lymph node metastasis.

The SLN algorithm could detect 12 of the 14 lymphatic metastases. There were two false negative results. One of them was an isolated para-aortic lymph node metastasis, and the other was a non-SLN metastasis with tumor-free SLN obtained from the same hemipelvis. Eight of the 14 metastases were seen in the SLNs, and only 1 of these 8 cases also had non-SLN metastasis, 4 of the 14 metastases were found in the non-mapped side, and 3 of them had suspicious nodes. Calculations were made among the patients who underwent systematic lymphadenectomy after SLN mapping algorithm steps (*n*: 92) and the false negative rate was 2.5%, negative predictive value was 97.5%, and sensitivity was 85.7%.

Discussion

In the current study, the overall, unilateral, and bilateral SLN detection rates were 94.1%, 19.8%, and 74.3%, respectively, and these rates are concordant with the previous reports [3–5, 11–13]. The failed (unilateral or no mapping) bilateral detection rate was noted in 25.7% of patients, and these cases were compared with bilateral mapped cases to identify factors that may influence mapping failure.

There are differences between the studies in terms of the injected volume and dosage of indocyanine green. In various

Table 2 Comparison of baseline characteristics of patients in terms of SLN detection status

Characteristics	Bilateral (n = 75)	Failure (unilateral or none) (n = 26)	p
Age (years), mean	59.5 ± 10.21	60.5 ± 7.46	0.66
BMI	33.25 ± 5.92	35.15 ± 7.68	0.31
BMI < 33, n	32 (78%)	9 (22%)	
BMI ≥ 33, n	36 (76.5%)	11 (23.5%)	0.87
Menopausal status			
Premenopausal	11 (91.6%)	1 (8.4%)	
Postmenopausal	64 (71.9%)	25 (28.1%)	0.18
Experience period			
1st quarter (n = 25)	17 (68%)	8 (32%)	
2nd quarter (n = 25)	21 (84%)	4 (16%)	0.6
3th quarter (n = 25)	18 (72%)	7 (28%)	
4th quarter (n = 26)	19 (73%)	7 (27%)	
Cervical longitudinal size, mean	32.5 ± 7.1	34.9 ± 7.1	0.15
≤ 35 mm	52 (77.6%)	15 (22.4%)	0.43
> 35 mm	19 (67.8%)	9 (32.2%)	
Uterine longitudinal size, mean	5.9 ± 1.46	6.4 ± 1.25	0.1
< 6 cm	37 (80.4%)	9 (19.6%)	0.21
≥ 6 cm	34 (69.3%)	15 (30.7%)	
The amount of ICG given			
2 mL	25 (69.4%)	11 (30.6%)	0.38
4 mL	50 (76.9%)	15 (23.1%)	
Operation type			
Laparoscopy	52 (76.4%)	16 (23.6%)	
Laparotomy	16 (76.1%)	5 (23.9%)	0.4
Robotic	7 (58.3%)	5 (41.7%)	
Histologic type			
Atypical hyperplasia	4 (100%)	0	
Endometrioid	62 (72.9%)	23 (27.1%)	0.48
Non-endometrioid	9 (75%)	3 (25%)	
Histologic grade			
Grade 1	37 (75.5%)	12 (24.5%)	
Grade 2	18 (66.7%)	9 (33.3%)	0.66
Grade 3	16 (76.1%)	5 (23.9%)	
LVSI			
Absent	56 (72.7%)	21 (27.3%)	0.83
Present	15 (75%)	5 (25%)	
Myometrial invasion			
Absent	9 (69.2%)	4 (30.8%)	
< 1/2	48 (76.2%)	15 (23.8%)	0.65
> 1/2	14 (66.7%)	7 (33.3%)	
Tumor size (cm)	27.6 ± 16.59	34.7 ± 16.86	0.08
Stage			
Early (I–II)	59 (75.6%)	19 (24.4%)	
Advanced (III–IV)	12 (63.1%)	7 (36.9%)	0.27

BMI body mass index, *ICG* indocyanine Green, *LVSI* lymphovascular space invasion

tumors, injection of large volume and low concentration of indocyanine green was demonstrated to be more successful in SLN mapping [14]. In this study, the failed mapping rates were 30% and 22% for 2- and 4-mL injected volumes, respectively, albeit with the same concentration; however, no statistical significance was noted. A recent study also failed to demonstrate improvement in bilateral mapping rate in endometrial cancer by injecting different volumes and concentrations of indocyanine green (8 mL of 5 mg/mL vs. 4 mL of 1.25 mg/mL) [15].

The tracer injection technique has already been defined, and it is almost standardized and similar for all cases. However, there may be several differences in the cervical structure of each patient. In this study, there was a negative correlation trend between the longitudinal cervical and uterine lengths and bilateral mapping with no statistical significance. Although this relationship was not assessed previously, it was reported that there was no correlation with the previous conization or uterine/cervical surgical intervention and bilateral mapping rates [8, 16]. Nonetheless, it is imperative to evaluate the effect of the cervical volume and structure (atrophic, big, etc.); injected tracer volume and dosage; injection speed and depth on bilateral mapping success. These evaluations can lead to the individualization of the injection procedure based on preoperative cervical measurements.

Obesity can hinder the migration of the tracer due to the fatty lymphatic channel basins and lead to mapping failure. Moreover, the retroperitoneum is too fatty and bloody in obese patients, and it can be difficult for the surgeon to detect an SLN. In a study conducted on 188 patients with early cervical cancer, higher BMI was associated significantly with failed bilateral detection [16]. However, no accurate data exists that can indeed demonstrate that obesity has a detrimental effect on bilateral mapping, typically when indocyanine green is used. Tanner et al. [8] found that the failed mapping rate was higher in obese patients in their entire cohort using blue dye or indocyanine green. However, in the subgroup of patients on whom indocyanine green was used, the adverse effect of obesity on mapping was lost, and they suggested using indocyanine green in patients with BMI > 30 kg/m^2. Additionally, we found that bilateral mapping rates became similar in obese and non-obese patients on using indocyanine green. Therefore, indocyanine green may overcome the negative effect of obesity on bilateral mapping. In our study, there was no negative effect on bilateral mapping even in patients with BMI > 33 kg/m^2. Use of indocyanine green in all our patients can explain the higher rates of bilateral mapping than Tanner et al.'s cohort (75% vs. 62%) [8].

A recent study found stage III or IV disease to be the only factor related to mapping failure [17]. Lymphatic metastasis was suggested as a possible cause for failed

Table 3 Clinicopathological features of patients with no sentinel lymph node detected ($n=6$)

	BMI	Experience period	Operation type	Cx Size	mL	Histopathology	Lymphatic metastasis	Grade	LVSI	Stage
Case 1	39	4. Quarter	L/S	35	4	Endometrioid	Absent	1	No	IA
Case 2	27	4. Quarter	L/T	20	2	Mixed	Present (macro)	3	Yes	IIIC2
Case 3	49	2. Quarter	L/S	30	2	Endometrioid	Absent	2	No	IB
Case 4	33	4. Quarter	L/S	35	2	Endometrioid	Present (macro)	1	Yes	IIIC1
Case 5	30	3. Quarter	L/S	35	2	Endometrioid	Absent	2	No	IA

BMI body mass index, *Cx* Cervix, *mL* amount of ICG given in mL, *L/S* laparoscopy, *L/T* laparotomy, *LVSI* lymphovascular space invasion

mapping owing to the tumor cells that may obstruct the lymphatic channels and avoid the passage of the tracer to the lymph node. Although the rate of mapping failure was higher in advanced stage patients compared to the early stages, our study did not detect a statistically significant difference. Furthermore, 8 of the 14 metastases were detected in SLNs that were not suspicious for metastasis, and it may suggest that SLN mapping still works in the presence of low-volume metastasis. However, lymph node metastases were detected in three patients who had strongly suspicious lymph nodes for metastasis and enlarged lymph nodes, albeit with no SLN mapping.

There was no significant difference between the 2 groups (bilateral vs. failure) in terms of clinical and pathological features. These results are concordant with the previous reports [8, 17] and indicate that mapping failure may be related to injection, surgery technique, or other patient features rather than histopathologic characteristics. By increasing the number of operations during the study period, there was no change or improvement in bilateral mapping rate in our study. Our extensive experience of SLN mapping with blue dye may be an explanation for this result.

In our series, five SLN-labeled specimens were reported to be without a lymph node on final pathology. These cases constituted 20% of the failed mapping group (5/26). This type of mapping failure can hinder the appropriate application of the SLN algorithm. In such instances, an intraoperative frozen section examination was suggested to confirm the existence of a lymph node in the resected specimen [18].

This study is one of the few studies that investigated the factors related to mapping failure. The study population was homogeneous because it was a single center study and the same surgical team performed all the operations. Therefore, the patient-dependent factors could be analyzed independently through surgical practices, including the cervical injection technique and intraoperative steps. Additionally, some new features that have a potential effect on failed mapping were evaluated in this study. However, the relatively small sample size and some missing data were probably the main reasons for the several statistically insignificant differences noted regarding the clinical, histopathologic, and surgical features.

We have the following recommendations for future research to improve the success of the SLN algorithm: (1) assess the uterine and cervical structure preoperatively to individualize the injection technique; (2) use suitable tracer, optimal tracer volume and dose, precise depth and speed of the injection; (3) repeat the injection if SLN is not detected after the first injection [19] or perform a fundal injection if SLN is not detected after the cervical injection; and (4) evaluate the effect of using cervical and fundal injections alone or in combination on the mapping rates.

In conclusion, it seems that using cervical indocyanine green injection can overcome the negative effect of high BMI on bilateral mapping. A trend of inverse correlation was noted between increasing uterine and cervical longitudinal lengths and bilateral mapping with no statistical significance. Similarly, injection of 4-mL indocyanine green provided a higher bilateral mapping rate compared with 2-mL injection with statistical insignificance. Further studies with larger sample sizes are required to confirm these results adequately.

Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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