

Assessment of the Diagnostic Value of Sentinel Lymph Node Mapping with Blue Dye (Blue Methylene) with Complete Lymph Node Dissection for Endometrial Cancer Staging Surgery (a Multicenter, Prospective, Cross-Sectional Study)

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Article Info

[10.30699/jogcr.8.1.17](#)

Received: 2022/03/16

Accepted: 2022/05/11

Published Online: 2022/12/27

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ABSTRACT

Background: In gynecological cancers, sentinel lymph node (SLN) mapping has been proposed as an alternative to complete lymph node dissection. The goal of this study as to determine sentinel lymph node mapping's sensitivity, specificity, NPV, and PPV versus gold standard surgery (complete lymph node dissection) in patients with metastatic illness.

Method: A total of 26 patients were prospectively enrolled from spring 2021 to winter 2022. Sentinel lymph node mapping with complete pelvic lymph node dissection was done in all of them, and para-aortic lymphadenectomy was detected in 11 (43.33%) patients.

Result: At least one sentinel lymph node was successfully mapped in 22 (84.6%) patients. Three patients (11.5%) had positive lymph nodes, with an 85.7% sensitivity (95 percent CI) to detect node positive illness, a specificity of 74.93%, an NPV of 99.6 (99.5 CI), and a PPV of 5.9% calculated. There were no serious adverse effects due to blue dye.

Conclusion: The accuracy rate of sentinel lymph node mapping by methylene blue was 75.1%.

Keywords: Endometrial carcinoma, sentinel lymph node mapping, methylene blue, IHC

Introduction

Endometrial cancer is the most common gynecological cancer in the United States, with the vast majority of cases (78%) confined to the uterus (**1, 2**). The surgical staging protocols for endometrial cancer patients are total abdominal hysterectomy plus bilateral salpingo-oophorectomy (TAH+BSO) with lymphadenectomy, which would assist gynecologists in making proper adjuvant therapy decisions for patients with lymph node metastases (**3**). Furthermore, lymphadenectomy as a part of endometrial cancer staging surgery reduces the risk of recurrence (**3**). Although complete pelvic and para-aortic lymphadenectomy is a part of endometrial cancer staging surgery, it itself has several disadvantages, including major morbidities

like lymphedema, lymphocyst formation, vascular injuries, and genitofemoral nerve injury (**4, 5**). Also, this is clinically difficult to achieve in patients with morbid obesity (who make up the majority of endometrial cancer patients) (**6, 7**). As a result, in recent years, sentinel lymph node dissection has taken the place of Patients who are node positive benefit from adjuvant treatment in terms of survival (like chemotherapy and radiation). Moreover, lymphadenectomy, as part of staging in endometrial cancer patients, reduces the risk of recurrence (**3**). The mapping of the sentinel lymph node in endometrial cancer staging surgery started in 1996. In addition, 55 studies (including 4905 women) have been completed till 2016 (**1, 6**). A systematic review and meta-analysis of 55

studies found that the patients' mean age was 62 years (95% CI) and their mean BMI was 30 kg/m² (95% CI). 44 percent of studies (95 percent confidence interval) use dye alone to detect sentinel lymph nodes (6). The most frequent material used in the mapping of sentinel lymph nodes was blue dye, for instance, more frequently than indocyanine green (88% versus 12%) (6, 7). The most prevalent blue dye was patent blue dye. The majority of studies (58%) employed cervical dye injection procedures, while 9% (95% CI) used cervical and uterine injections (6,7). In 33% of the studies, only uterine injection was used. (1,6).

In 53 studies (95%CI), the detection rate of sentinel lymph nodes ranged from 23–100% (with a pooled average of 81%), while dye plus radiotracer use had a detection rate of 86%, and the SLN bilateral detection rate was 30%, ranging from 6% to 88%, with a pooled average of 50%. Indocyanine green detected bilateral sentinel lymph nodes 75% of the time, compared to 51% with blue dye. Aortic detection rates ranged from 0% to 84%, with a pooled average of 17%. The average number of SLNs found per subject was 2.9. (6). Non-endometrial histology (e.g., serous, carcinosarcoma, and clear cell) was shown to have no significant difference in SLN detection when compared to endometrial histology ($p = 0.5$) in these studies. The average BMI of the patients was 30 kg/m², and there was no association between SLN detection and BMI. There is no correlation between tumor grade and SLN detection. Previous research found that the number of patients in the trial, their average BMI of 30 kg/m², and their tumor grade had no bearing on the rate of SLN detection. There is no correlation between tumor grade and SLN detection. In previous studies, the use of a cervical injection was linked to a greater likelihood of bilateral sentinel lymph node detection (8-11). Cervical injection was associated with a considerably decreased rate of para-aortic sentinel lymph node identification ($P = 0.003$) when compared to uterine injection. In past studies, there has been no significant difference in the use of radiotracer plus blue dye in comparison with blue dye alone with bilateral or para-aortic sentinel lymph node detection (12, 13), (21). The overall sensitivity of sentinel lymph node identification of metastatic disease was 96 percent in previous investigations (CI 95 percent in 47 studies). In cases with sentinel lymph node metastasis, sentinel lymph nodes were the only positive nodes identified 66 percent of the time (22 studies) (6). The sensitivity of sentinel lymph node identification of metastases was unaffected by study size, injection site, or ultra-staging by IHC cytokeratin staining. When compared to lymph node dissection, the extra operating time for sentinel lymph node mapping ranged from 3 to 40 minutes (in 6 trials) (6). In one investigation,

sentinel lymph node mapping was found to cause less blood loss than lymph node dissection. One patient had an allergic reaction to blue dye in one of the 24 studies reporting adverse effects (1390 females). There were no other significant side effects associated with sentinel lymph node mapping (6). After sentinel lymph node mapping, patients had serious complications associated with lymph node dissection: (5 lymphoceles, 2 vascular injections) There were no long-term outcomes reported in any of these investigations (6) (a comparison of the risk of lymphedema in patients who had either sentinel lymph node mapping or total lymphadenectomy). There was no difference in progression-free survival in 4-5 investigations, with a median follow-up of 17–50 months (PFS) between Patients who had successful and unsuccessful sentinel lymph node mapping (6). In three studies with a median follow-up of 16–32 months (14, 15), there was no difference in progression-free survival for patients who had primary lymph node dissection.

Procedures: Four academic master surgeons participated in the trial. A standard dose of 1.25 mg/ml of methylene blue solution diluted with 4cc of sterile water and a 5 cc needle, blue dye was injected 1 cc superficially and 1 cc deeply into the 3 and 9 o'clock positions of the uterine cervix, one hour before surgery (anesthesia), achieving a total dose of 5 mg of blue dye. This method was used in all patients, and one hour after the injection of dye, the surgery was begun. For effective mapping, a channel lymph node in at least one hemipelvis was required. In all cases, identified sentinel lymph nodes were collected and labeled for location, and all non-sentinel lymph node tissue within the relevant nodal basins was removed. Surgeons also prepared graphic data on sentinel lymph node location. (Ext iliac, int iliac, obturator, para- aortic, and others). Pathologists handled the sentinel and non-sentinel lymph node specimens, creating two 50 mm paraffin-embedded slides from each segment. The H&E staining was done on one slide, while the IHC staining was done on the other. If the first H&E slide did not reveal any metastatic disease, the reserved slide was stained for cytokeratin staining.

Statistical Analysis: The size of the study is at least 196 lymph nodes (at least 25 patients) as a result of this proportion: $n = \frac{z^2 P(1-P)}{d^2}$ Z=1.96 (by CI of: %95). The sensitivity, specificity, npv, and ppv were calculated for the patients who had at least one sentinel lymph node mapped.

Materials and Methods

This is a multicenter, prospective cross-sectional study. Patients with an early clinical

stage, any histologic type, and histologic grade of endometrial cancer undergoing laparotomy were eligible. Four surgeons from three academic centers related to IUMS (Isfahan University of Medical Science) participated in the trial. The patients received cervical injections of blue dye at 3 and 9 o'clock in the uterine cervix, and following that, the procedure included sentinel lymph node mapping, pelvic lymphadenectomy, and para-aortic lymphadenectomy. IHC was used to ultra-stage negative sentinel lymph nodes (as determined by H & E staining on sections) (Immunohistochemistry for cytokeratin staging). Sensitivity, specificity, NPV, and PPV of sentinel lymph node mapping were calculated as the primary endpoints. The current study is a cross-sectional, multicenter investigation. Participants were enrolled in a group from the outpatient clinics of 3 academic hospitals relevant to IUMS (Isfahan University of Medical Science). Patients were eligible if they had endometrial carcinoma of any grade or histologic type, as determined by endometrial sample (16). Patients were included in the trial regardless of their age if they had the performance status and life expectancy to tolerate surgical staging operations. Evidence of extrauterine illness or any previous therapy was an exclusion factor. Also if they had a previous retro peritoneal surgery or lymphadenectomy, or comorbidities like IHD (ischemic heart disease), DM (diabetes mellitus), or methylene-blue allergy or had a previous retro peritoneal surgery or lymphadenectomy were excluded from the trial. Patients were also disqualified for sentinel lymph node mapping if they had severe intrauterine illness at the time of surgery after methylene blue injection.

Results

Patients were enrolled between the spring of 2021 and the winter of 2022 and they were 56.53 years old on average (a range of 32-70y). The patients' average BMI was 33.7 (kg/m²) (a range of 26 to 36.7). The uterine cervix of all 26 patients had been injected with blue dye (at 3 and 9 o'clock). Four out of 26 (15.3%) patients didn't have any stained lymph nodes (as sentinel lymph nodes). None of the 26 patients experienced serious adverse events such as neurologic injuries, syncope, or vertigo, and none had an allergic reaction to the dye. None of 26 patients had serous histology, and three had endometrioid type grade III. In 22 of the 26 patients (84.4 percent), at least one sentinel lymph node was found. The lymph node detection rate in this trial was 84.4% (Table 1). The SLN bilateral detection rate in this trial was 30%. The para-aortic LN detection rate was 6.9% (in comparison to other studies). The mean number of SLN selected per mapped patient was equal to 4.6% (in comparison to the past studies of 2.9%). The obturator chain (23.7% of 101 SLNs) (17), Ext. iliac chain (20.7% of 101 SLNs) (19), and common iliac chain (17.8% of 101 SLNs) (20) were the most common locations for SLNs to be found. The patient had three SLNs, but only one of them was metastatic, and in the same patient, there was one metastatic non-sentinel lymph node. The histologic grade and type of this patient was grade 2 endometrioid endometrial carcinoma. Myometrial invasion accounted for more than half of the myometrial thickness, LVSI was evident, and the tumor was 5 cm in diameter (Table 2).

Table 1. The factor of high risk endometrial cancer in relation to node status

Tumor size	Node negative N=23	Node positive N=3
< 2cm	13(56.23%)	0
≥ 2 cm	10(43.4%)	3(100)
Grade		
1 or 2	21(91.3%)	(33.3%)1
3	1(4.3%)	(66.6%) 2
non-endometrioid	1(4.3%)	(%)0
LVSI		
Absent	19(82.6%)	0
Present	4(17.3%)	3(100%)
Myometrial Invasion		
< 50%	17(73.9%)	1(33.3%)
≥ 50%	6(26.08%)	2(66.6%)
LUSI		
Absent	23(100%)	1(33.3%)
Present	0 (0%)	2(66.6%)
Age		
<50	4(17.3%)	0(0%)
50-59	16(69.5%)	2(66.6%)
≥60	3(13.04%)	1(33.3%)

Table 2. Sensitivity & specificity of SLNM in our trial

	True positive nodes Based on pathology	True negative nodes Based on pathology
Positive SLNS (blue. nodes)	6	95
Negative SLNS (Non- stained nodes)	1	284

In the other two patients with metastatic SLNs, one of them had metastasis in 2 out of the 2 SLNs. The pathologic grade was grade III. The histologic type was endometrioid endometrial carcinoma. Cervico-stromal invasion (CSI) was present, LVS1 was present, and tumor size was 5.5 cm. and less than 10%. In the other patient, 3 of 6 SLNs were metastatic. Myometrial invasion was more than 50%, crevice stromal invasion (CSI) was present, LUSI (lower segment uterine involvement) was present, and tumor size was 7 cm. According to the aforementioned information, all patients with node positive illness had at least one recognized risk factor for lymph node metastases. Based on the trial data, sensitivity was 85.71%, specificity was 74.93%, PPV was 5.9%, and NPV was 99.6%.

Discussion

With a brief look at the studies around the replacement of SLN mapping in endometrial cancer staging surgery, we found that studies began in 1996 and assessed this method. via different procedures and materials (such as injection of radiotracers, injection of dye to the uterus, injection of the above materials to the uterine cervix, etc.). Based on studies done up to now, methylene blue is the most accessible and cheapest material for the detection of SLNs, because of the most notable point of the least serious adverse effects on the patients (21-26). For example, a large systematic review and meta-analysis (including 55 studies) published in 2016 revealed that only one patient had hypersensitivity to methylene blue and none of them had any serious side effects like neurologic injuries, respiratory events, or lymphedema. For the above reasons, we used blue dye as the safest material for the detection of SLNs. Moreover, materials like TC99m (technetium99m) or ICG (indocyanine green) are not in use for all patients to have lymphosynthesized before surgery for detection of SLN that excesses the time of operation and hospitalization of patients. The other advantage of sentinel lymph node detection is the detection of unusual sites of metastatic lymph nodes, for example int iliac nodes. In our trial, 21.7% of SLNs were in the int- Iliac chain location. Moreover, detection of SLNs could be an important guide to making decisions about the next step of treatment (e.g. adjuvant

therapy in patients with metastatic nodes). Based on prior research, cervical injection boosted the rate of bilateral SLN detection, thus we chose to inject blue dye into the cervix uteri at 3 and 9 o'clock to detect more bilateral SLNs. In the past studies, SLN-bilateral selection has ranged from 6–88% (with a pooled average of 50%). In our trial, this was 30%. The para-aortic detection rate in the other studies ranged from 0–84%.

With a pooled average of 7% (in comparison with our trial: 6.9%). The obturator and external iliac nodes have the most SLNs regions. (Past studies have reported extra-ilial nodes). In previous studies, the pooled sensitivity of SLN detection was 96% (in our study, it was 85.7%), and the pooled Npv was 99.7% (in our study, it was 99.6% as in previous studies.) There was no significant change in the sensitivity of SLN detection of metastases by ultra-staging as in earlier trials. IHC staining revealed that all the non-metastatic sentinel lymph nodes were negative in H & E staining. Also, all the 3 patients with node metastases had at least one sentinel lymph node. In all three patients with node metastases, there is at least one risk factor related to high risk endometrial cancer. One of the advantages of this study is the injection of methylene blue at 3 and 9 o'clock of the cervix. So, with fewer points of injection and available sites of injection, with the cheapest material (methylene blue), the safest method and most acceptable method based on patient opinion and less side effects and the same hospital stay, we get the fair sensitivity of 85.7% and the accuracy of 75.1%. So, sentinel lymph node mapping by injecting cost-effective and the most accessible dye (blue methylene) into fewer points of the cervix, can lead to the detection of metastatic lymph nodes with acceptable sensitivity and negative predictive value. To get an accurate sensitivity, more duration and a greater size of study are recommended.

Conclusion

It seems that age, cervical histology and parity at the start of HPV infection are the most important factors for HPV clearance.

In other words, higher age and the histopathology result of CIN1 compared to normal result and multiparity are associated with HPV persistence or even progression.

Acknowledgment

Hereby I would like to thank Professor Allameh and Professor Behnamfar, my research supervisors, for their patiently instruction, passionate support, and constructive criticism of this study effort. also like to thank Dr. Sabet and Dr. Mousavie for their guidance and help in keeping my development on track.

Also Eto thank the employees at the gynecology department of the Medical Science Institute of Isfahan for their assistance in providing me with the resources I needed to run the application.

Conflict of interest

The authors declare no conflict of interest. The ethical code is IR.MUI.MED.REC.1400.679.

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How to Cite This Article:

Allameh T, Mirazimi MS, Sabet F, Mousavi Seresht L, Behnamfar F. Assessment of the Diagnostic Value of Sentinel Lymph Node Mapping with Blue Dye (Blue Methylene) with Complete Lymph Node Dissection for Endometrial Cancer Staging Surgery (a Multicenter, Prospective, Cross-Sectional Study). *J Obstet Gynecol Cancer Res.* 2023; 8(1):17-22.