

Sentinel lymph node detection using blue dye in patients with early stage cervical cancer: a pilot study

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ABSTRACT

Aims: This study aimed to determine the feasibility of sentinel lymph node (SLN) detection using lymphatic mapping with intraoperative isosulfan blue dye application in patients with early stage invasive cervical cancer who underwent radical hysterectomy and pelvic lymphadenectomy.

Methods: From June 2009 to December 2010, 16 patients undergoing radical hysterectomy for early-stage cervical cancer (stage IA1, IB1) were offered lymph node mapping for identification of the SLN. The SLN mapping was done after intracervical isosulphane blue injection preoperatively. All patients underwent SLN identification via laparotomy followed by a complete pelvic and paraaortic lymph node dissection and radical hysterectomy.

Results: The median age of the patients was 47 years (range: 36-68 years). The histological types identified included squamous cell carcinoma (12 cases), adenosquamous carcinoma (3 cases), and adenocarcinoma (1 case). The FIGO staging for the patients revealed Ia1 in 5 cases and Ib1 in 11 cases. A total of 417 lymph nodes (minimum 12, maximum 51), including SLNs, were removed from 16 patients. A total of 8 SLNs were identified in 5 patients: 1 in 3 patients, 2 in 1 patient, and 3 in 1 patient. All SLNs were unilateral. The technical failure rate of the procedure, defined as the inability to identify an SLN, was 68.8% (11/16), and the total SLN detection rate was 31.2% (5/16). Immunohistochemical examinations found no metastases in any of the SLNs. Ultrastaging also did not identify micrometastasis in any of the SLNs. Additionally, there were no metastases detected in the non-SLN s of patients who did not exhibit metastasis in the SLNs. Consequently, the false-negative rate could not be determined.

Conclusion: According to our preliminary study, although SLN mapping with blue dye is a low-cost, accessible, and easy-to-perform method, it has a low detection rate of SLN.

Keywords: Cervical cancer, sentinel lymph node, lymphatic metastasis

INTRODUCTION

Cervical cancer is a leading cause of mortality among women. In 2020, approximately 604,000 women were diagnosed with cervical cancer worldwide, resulting in about 342,000 deaths from the disease.¹ In Türkiye, there are 32.8 million women aged 15 years and older who are at risk of developing cervical cancer. Current estimates suggest that each year, 2,532 women in Türkiye receive a diagnosis of cervical cancer, and 1,245 women die from the disease.²

In the case of early-stage cervical cancer, the status of the lymph nodes is the most important prognostic factor and significantly influences adjuvant treatment planning. Patients with negative lymph nodes and clear surgical margins have a very favorable prognosis. The likelihood of recurrence in these patients is low, with a pelvic recurrence rate of about 10%.³ To determine the status of the lymph nodes, lymphadenectomy remains the gold standard. If lymph node metastases are present at the time of primary surgery, the 5-year survival rate decreases from 85% to 50%.⁴⁻⁶

In patients with stage IB1 cervical cancer, the incidence of positive lymph nodes is approximately 15%. This indicates that over 80% of these patients who undergo lymphadenectomy do not benefit from the procedure. However, they still experience the associated increase in operative time, blood loss, and risks of complications such as lymphocyst formation and lymphedema.⁷ Sentinel lymph node (SLN) biopsy in cancer surgery serves to reduce the morbidity associated with complete lymph node dissection while enhancing the detection rate of metastatic lymph nodes. Assessment of SLNs provides an opportunity to stage patients intraoperatively and avoid complete pelvic lymph node dissection, thereby reducing its associated morbidities.

Currently, there is no definitive method to determine lymph node metastasis preoperatively, and systemic pelvic lymphadenectomy remains the gold standard procedure for evaluating lymph node status.⁸ However, in recent years, lymphatic mapping has been used in cervical cancer

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cases to identify the first lymph node station draining the tumor. Different techniques are employed for this purpose, including blue dye, radiocolloid labeling, preoperative lymphoscintigraphy/intraoperative gamma probe, or indocyanine green (ICG).

This study aimed to determine the feasibility of SLN detection using lymphatic mapping with intraoperative blue dye application in patients with early stage cervical cancer.

METHODS

Ethics

This prospective cohort study was derived from a thesis (SB;2011;805516) and received approval from the Zeynep Kamil Women and Children's Diseases Training and Research Hospital Ethics Committee (Date: 20.04.2009, Decision No: 04096443). All experiments were conducted following the ethical guidelines of the Helsinki Declaration. Informed consent was obtained from every patient enrolled in this study.

From June 2009 to December 2010, 16 patients with early stage (Ia1-Ib1) cervical cancer diagnosed in Zeynep Kamil Women and Children's Diseases Training and Research Hospital were enrolled. Patients with a history of preoperative pelvic radiotherapy or chemotherapy, patients diagnosed with clinical stage Ib1 and higher, or patients who declined to participate in the study were excluded. Before surgery, a total of 4 ml of 1% isosulfan blue (Istanbul University Faculty of Pharmacy) was injected peritumorally into the cervix, with 1 ml administered at each of the 3, 6, 9, and 12 o'clock positions using a 25-gauge needle under direct visualization. The depth of injection was between 0.5 and 1 cm (Figure 1). In patients who have undergone a preoperative conization or LEEP, the injections were given into the remnant cervical tissue. The surgical procedure then commenced with an incision made through the median infraumbilical region.

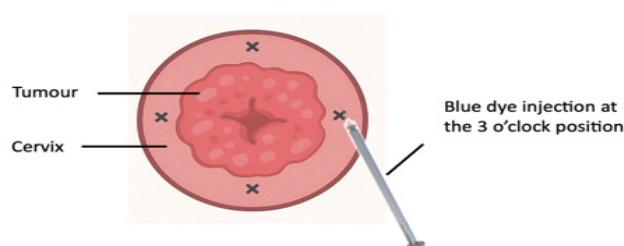


Figure 1. Illustration of the blue dye injection. Blue dye was injected into the area near the normal cervix and tumor interface at four specific quadrants (3, 6, 9, and 12 o'clock)

The interval between the dye injection and the retroperitoneal lymph node dissection was carefully monitored, ensuring it was at least 15 minutes for each patient, with a maximum of 30 minutes. Maximum effort was made for a precise and bloodless dissection to prevent bleeding from vessels and facilitate the localization of lymph nodes. During the retroperitoneal dissection, blue-stained lymph nodes were examined, and all identified blue-stained lymph nodes were classified as sentinel nodes via direct intraoperative inspection. The number and

locations of the SLNs were documented. Then, a standard pelvic lymphadenectomy was performed, followed by a radical hysterectomy.

SLNs were all sent for frozen section. If the lymph node looked normal, a random section was examined to look for the presence of cancer cells. For the final pathological analysis, all SLNs were sent for ultrastaging, which involved cutting two consecutive sections, each 4 µm thick, at 150 µm intervals from each paraffin block until no lymph node tissue remained. The first section was stained with hematoxylin and eosin, while the second section was examined immunohistochemically using anti-cytokeratin antibodies AE1/AE3 (Leica Biosystems; New Castle; UK). Non-sentinel lymph nodes (non-SLNs), only routine H&E staining (one or two sections per node) was performed.

Statistical Analysis

Data were entered in a computerized database and analyzed using SPSS software (Version 11.0 for Windows, SPSS, Inc., Chicago, IL). In this report, we examined the detection rate and diagnostic accuracy of the SLN concept. The procedure was considered "technically inadequate" if no sentinel node could be detected, as previously described in the literature.⁹ This category includes patients who received an insufficient injection of dye, exhibited minimal or no dye uptake, or showed visible dye uptake without an identifiable sentinel node. A "false-negative" study refers to cases where all SLNs identified in a basin are histologically negative, yet tumors are present in other lymph nodes within the same basin. A false-negative case is specifically defined as a situation where metastasis is histologically confirmed in non-SLNs while the SLNs are negative. The following formulas were used;

Detection rate: Patients with successful SLN identification/patients enrolled

False negative rate: False negatives/(true positives+false negatives)

Continuous variables (e.g., age, injection-to-identification time) were expressed as medians with interquartile ranges (IQRs) and compared using the Mann-Whitney U test. Categorical variables (e.g., histological type, FIGO stage, tumor size category, and SLN detection status) were summarized as frequencies and percentages and analyzed using the Chi-square test or Fisher's exact test, as appropriate.

RESULTS

16 cervical cancer patients who applied to our clinic during the study period and were decided to undergo surgical treatment were included in the study. The median age of the patients was 47 years (range: 36-68 years). Histopathological subtypes included squamous cell carcinoma (n=12), adenosquamous carcinoma (n=3), and adenocarcinoma (n=1). The FIGO staging for the patients revealed Ia1 in 5 cases and Ib1 in 11 cases (Table 1).

Tumor size was determined from histological samples. Among the patients with stage Ib1, 3 had tumor diameters smaller than 2 cm, and 8 had tumor diameters larger than 2 cm. 5 patients underwent cervical conization or LEEP before

lymphatic mapping and surgical extirpation. All but one patient underwent type III radical hysterectomy with pelvic and paraaortic lymphadenectomy. In one case, paraaortic dissection was omitted due to comorbidities.

Table 1. Patient, tumor and procedure characteristics

Characteristics	Number (%)
Age (years)	
36-68	16 (100)
Stage	
1a1	5 (31)
1b1	11 (68)
≤20 mm	3 (27)
>20 mm	8 (72)
Histology	
Squamous	12 (75)
Adenosquamous	3 (18)
Adenocarcinoma	1 (6)
The amount of LN's (min 12-max 51)	417
LVSI	
Yes	6 (37)
No	10 (62)
Time between SLN detection and dye application	
18 minutes (min 15-max 30)	16 (100)
Preceding LEEP/conization*	
Yes	5
No	11
Surgery	
Tip3 hysterectomy+PPLND [†]	15(93)
Tip3 hysterectomy+PLND [§]	1(6)

Stage: FIGO (International Federation of Gynecology and Obstetrics) 2009, LN: Lymph node, LVSI: Lymphovascular space invasion, SLN: Sentinel lymph node, LEEP: Loop electrosurgical excision procedure, PPLND: Paraortic and pelvic lymph node dissection, PLND: Pelvic lymph node dissection. Min: Minimum, Max: Maximum

Based on insights from previous studies, we initially used 2 ml of isosulfan blue injected at the 3 o'clock and 9 o'clock positions in the first four patients at the beginning of our study. When we were unable to identify the SLN in these cases, we suspected that the failure to detect the SLN was due to an insufficient amount of blue dye injected. Consequently, we decided to increase the dosage of blue dye and administered 4 ml of isosulfan blue at the 3 o'clock, 6 o'clock, 9 o'clock, and 12 o'clock positions. SLNs were detected at a median of 23 minutes (IQR, 15-30 minutes) after injection.

A total of 417 lymph nodes (range,12-51), including SLNs, were removed from 16 patients. 8 SLNs were identified in 5 patients (31.2%). The nodes were located in the left internal iliac (3 patients), right internal iliac (1 patient), and left obturator (1 patient) regions. All SLNs were unilateral (**Figure 2**). The technical failure rate-defined as no SLN detected-was 68.8% (11/16) and the total SLN detection rate was 31.2% (5/16) (DR: 31.2%).

In two patients, suspicious areas stained blue in the parametrial region were sent to the pathology department in separate containers. Histopathological examination revealed that these areas did not contain lymphoid tissue. In the other two patients, where no SLNs were found, routine H&E revealed carcinoma metastases in three lymph nodes that appeared grossly metastatic during surgery. Immunohistochemical

examinations revealed no metastases in any of the SLNs. Ultrastaging had not detected micrometastasis in any of the SLNs. Furthermore, no metastasis was detected in the non-SLNs of any patient who did not show metastasis in the SLNs. Consequently, the false-negative rate could not be calculated.

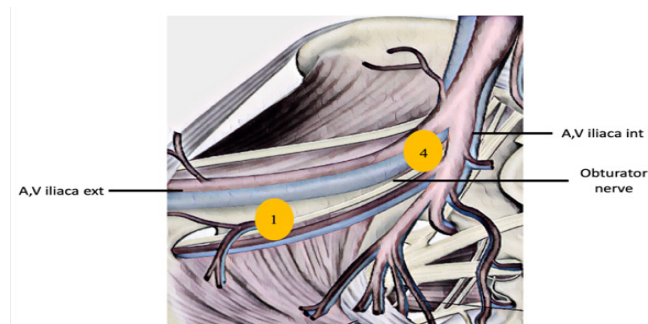


Figure 2. Anatomical localizations of detected sentinel lymph nodes; 1: Obturator region 4: Internal iliac region

No SLNs were detected in patients with prior conization or LEEP. When evaluated chronologically after the beginning of the study, SLNs could not be identified in the first five cases. As the study progressed; the detection rate improved gradually with surgical experience. The order of SLN identification in the 16 cases is presented in **Table 2**.

Table 2. Chronological order of SLN detection

Case	Blu dye (ml)	SLN	LNMet	Case	Blu dye (ml)	SLN	LNmet
1	2 ml	No	No	9	4 ml	No	No
2	2 ml	No	Yes	10*	4 ml	No	No
3*	2 ml	No	No	11	4 ml	No	No
4*	2 ml	No	No	12*	4 ml	No	Yes
5	4 ml	No	No	13	4 ml	Yes	No
6	4 ml	Yes	No	14	4 ml	Yes	No
7*	4 ml	No	No	15	4 ml	Yes	No
8	4 ml	Yes	No	16	4 ml	No	No

*Patient who underwent preoperative conization/LEEP, SLN: Sentinel lymph node, LEEP: Loop electrosurgical excision procedure

No allergic reactions, desaturation events, or intraoperative complications related to blue dye occurred. All patients exhibited transient bluish urine for 24-48 hours postoperatively.

DISCUSSION

Sentinel lymphadenectomy techniques have been effectively utilized in the treatment of breast cancer and melanoma patients, potentially reducing morbidity by allowing omission of complete lymphadenectomy in some patients.¹⁰ Cervical cancer appears to be a suitable cancer for SLN application due to the anatomical accessibility of the cervix and the sequential nature of its lymphatic drainage.

Several methods can be used to determine SLNs in cervical cancer. In Lymphoscintigraphy, after a cervical injection of radiolabeled Technetium 99 m (Tc 99 m) colloid, a preoperative lymphoscintigram is obtained to localize the nodules. The procedure takes approximately 1 hour. The radioisotope injection can be administered the day before or the morning

of surgery. The SLNs are identified using laparoscopic or open surgery gamma probes, or a handheld open-procedure SLN gamma probe, peroperatively. In the blue dye method, blue dye (either isosulfan blue or methylene blue) is injected in the operating room at the time of the surgery while the patient is under anesthesia. Unlike lymphoscintigraphy, blue dye mapping can be performed intraoperatively without the need for nuclear medicine facilities. Additionally, the blue dye is inexpensive and readily available. Recently, ICG has also been introduced as a tracer in this setting. This study aimed to evaluate the effectiveness of the blue dye method for SLN screening in patients with early-stage cervical cancer.^{11,12}

Several studies have attempted to detect SLN in cervical cancer using blue dye, and varying detection rates have been achieved. In a study of 20 cases, O'Boyle et al.⁹ investigated the feasibility of SLN detection by applying blue dye to all four quadrants of the cervix. Although SLN pathology represented the sampled lymphatic basin in all cases, the overall detection rate was 60%. The researchers noted that the SLN detection rate with this technique was lower in patients with tumors >4 cm compared to those with tumors <4 cm. Malur et al.¹³ conducted research involving a series of 50 patients, including 18 with stage II or higher disease. They utilized lymphatic mapping with preoperative lymphoscintigraphy and an intraoperative blue dye technique. Their findings revealed that sentinel nodes were identified in 90% of patients when using a combination of blue dye and radiocolloid, compared to only 55% with blue dye alone. Additionally, another study by Levenback et al.⁷ examined patients with smaller tumors, which are less likely to displace lymph nodes. In this study, the detection rate of SLNs using the blue dye method alone was 26%, but this rate increased to 93-100% when the combined technique was used.¹⁴ Likewise, Echt et al.⁴ identified sentinel nodes in just 2 of 13 (15%) patients with cervical cancer using blue dye only.

In our study, the SLN detection rate with the blue dye method was determined as 31%, which is well below the desired values and aligns with the findings from other single-center studies mentioned earlier. However, multicenter trials have demonstrated significantly higher detection and sensitivity rates when combined tracer methods are used.

After single-institution case series had demonstrated the feasibility of the SLN concept in cervical cancer, the Arbeitsgemeinschaft Gynaekologische Onkologie Group (AGO) conducted a prospective, multicenter cohort study to evaluate the detection rate and diagnostic accuracy of the SLN mapping. 590 patients underwent lymph node detection after labeling with technetium, patent blue, or both. The detection rate of pelvic sentinel nodes was 82% for blue dye and 93.5% for the combination of technetium and patent blue, which was significantly higher than that of blue dye. However, in patients with tumor diameters lower than 20 mm, an inverse correlation was found between detection rate and tumor size. Likewise, the sensitivity of the procedure was higher with bilateral detection (87.2%) and with combined substances applied (80.3%) compared with the total population.¹⁵

The SENTICOL study established the reliability of combined blue dye and radiocolloid SLN mapping in early cervical cancer,

reporting a 92% sensitivity and 98.2% negative predictive value with bilateral detection in the majority of cases.¹⁶ Similarly, the recent SENTIX international trial confirmed that SLN biopsy without systematic lymphadenectomy is safe and accurate, with bilateral detection rates exceeding 90%, particularly when ICG or combined tracers are used.¹⁷ These findings are also consistent with those reported by Zhang et al.¹⁸ in a meta-analysis of over 2,200 patients, which found pooled detection rates of 75.2% for blue dye, 84% for combined techniques, and 85.5% for ICG, confirming that blue dye alone is the least sensitive tracer. Therefore; our study's lower detection rate may be attributed to the exclusive use of blue dye and the inclusion of several patients with tumors larger than 2 cm.

Since the uterus is positioned at the center of the pelvis, it is essential to detect SLNs on both sides to accurately represent the complete drainage area. The rate of bilateral SLN detection varies between 19% and 90% on various studies.¹⁹⁻²² Although unilateral lymph node metastases have been reported in 73% of patients, detection of unilateral SLN is not considered reliable.²¹ Researchers has shown that the likelihood of successfully detecting bilateral SLNs increases when the interval between applying the blue dye and reaching the retroperitoneum, along with the subsequent detection of the SLNs, exceeds 60-90 minutes. An excessively short interval between the injection and the beginning of the dissection was noticed as a source of failure in successfully detecting SLNs.²⁴

In our study, we were unable to identify bilateral SLNs. Our maximum allowed time for retroperitoneal dissection to locate the SLN following the cervical blue dye injection was 30 minutes. If this timeframe was exceeded, we deemed the procedure unsuccessful and proceeded with a radical hysterectomy. We believe that one of the factors contributing to the low detection rate observed in our study may be the limited waiting period after the injection of the blue dye before evaluating the lymph nodes.

The minimum recommended amount of blue dye for SLN detection is 2 ml; however, many studies suggest using 4 ml of dye and injecting it into all four quadrants of the cervix. In our study, we initially applied 2 ml of blue dye to the cervix in the first four patients. Since SLNs were not detected in any of these cases, we increased the amount to 4 ml for the remaining patients and injected the dye into all four quadrants of the cervix. Despite this adjustment, detection rates remained low, suggesting that dye volume alone was not the limiting factor.

It can be hypothesized that previous surgical interventions may significantly alter the cervical lymphatic drainage system. However, in a study involving 39 patients, of which 16 had undergone preoperative conization, researchers found that this procedure did not affect the likelihood of detecting a SLN during exploration.⁷ Similarly, both prior conization and cancer stage are unlikely to influence the failure rate in another study of 35 cases.²⁵ In our study, none of the five patients with prior conization had detectable SLNs, but the small sample size prevents definitive conclusions.

The rate of SLN detection increases with experience in areas such as the depth at which the tracer is applied to the cervical

stroma, modification of the technique in the presence of bulky tumors, accurate determination of injection sites, and timing for retroperitoneal access. It is difficult to determine the optimal number of cases before a surgeon is considered competent in performing the procedure. In a study to determine the optimal experience required to minimize the false-negative rate of SLN biopsy for breast cancer, conducted by McMaster et al.,¹⁰ before abandoning routine axillary dissection in favor of SLN biopsy, surgeons should perform at least 20 SLN cases with acceptable results. The researchers concluded that the learning curve is very shallow and prolonged, and false negative results and failure to find the sentinel nodes continue even after 20-30 cases. In vulvar cancer, the learning curve for sentinel node mapping is achieved after approximately 10 cases.²⁶ In contrast, for cervical cancer, the learning curve is likely to be around 30 cases, as the intracervical injection is technically more challenging and the lymphatic drainage of the cervix is considerably more complex, involving three primary trunks on each side.¹⁴ Our institution had no prior experience with SLN mapping, which likely contributed to our modest detection rate.

In our study, all procedures were conducted through laparotomy by a single surgeon. There is no evidence to suggest that laparoscopy is superior in terms of SLN detection rates or sensitivity.²⁷ Therefore, the low SLN detection rate we observed is not attributable to our surgical approach.

Isosulfan blue can cause adverse effects, such as swelling and itching in the hands, feet, abdomen, and neck, and allergic reactions in less than 1% of patients. Additionally, isosulfan blue may occasionally cause a temporary decrease in oxygen saturation, as indicated by pulse oximetry. Fortunately, no adverse effects were observed in any patient in our patients.¹¹

Recent studies have reported promising outcomes with the use of ICG as a tracer in SLN mapping. A meta-analysis evaluated whether ICG offered any advantages over traditional tracers in SLN mapping for uterine malignancies. The researchers found that ICG SLN mapping improved both overall and bilateral detection rates by 27% compared to blue dyes. However, no significant differences in overall and bilateral detection rates were observed between ICG and Tc. Additionally, when comparing the effectiveness of ICG to that of a combination of blue dyes and Tc, no notable differences in overall detection rates were detected between the two groups.¹²

Despite the relatively low detection rate observed in our study, the clinical significance of blue dye SLN mapping remains considerable, particularly in resource-limited healthcare settings. The blue dye technique offers a practical, low-cost alternative to methods that require advanced nuclear medicine facilities or near-infrared imaging systems. Its simplicity allows intraoperative application without specialized equipment or personnel, making it accessible to a broader range of surgical centers, especially in low- and middle-income countries. While combined techniques using radiocolloid or ICG achieve higher detection and bilateral mapping rates, blue dye mapping remains clinically valuable as a feasible, cost-effective option for centers where these technologies are not available, thereby contributing to the

broader implementation of SLN concepts in cervical cancer management worldwide.

The National Comprehensive Cancer Network (NCCN) Guidelines recommend SLN mapping for selected stage I cervical cancer cases, particularly tumors <2 cm, using combined tracer techniques (blue dye with Tc99m or ICG).²⁸

Limitations

Our study has several limitations. It was designed as a prospective cohort study. Although our hospital serves as a reference facility for gynecologic oncology patients, the number of patients included during the study period was limited. While the surgical team gained experience with the procedure throughout the study, we did not reach the number of patients needed for the learning curve of the SLN procedure, as indicated by the literature. These factors contributed to the low detection rate achieved in our study. Additionally, despite ultrastaging, it was not possible to calculate NPV because we did not detect SLN metastasis in any patient.

CONCLUSION

Our preliminary findings indicate that the blue dye technique has limitations, particularly in its low rate of SLN identification. Nevertheless, in facilities lacking the infrastructure for Tc 99m or ICG applications, the blue dye method remains a viable option for SLN detection. This approach is cost-effective, easily accessible, and associated with a low incidence of side effects.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Zeynep Kamil Women and Children's Diseases Training and Research Hospital Ethics Committee (Date: 20.04.2009, Decision No: 04096443).

Informed Consent

Written informed consent was obtained from all individual participants prior to their inclusion in the study. Participants were fully informed about the study's aims, procedures, potential risks and benefits, and their rights-including the right to withdraw at any time without consequence. All participants voluntarily signed a written informed consent form.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

Design: A.K.; Data collection and processing: S.B.; Analysis: S.B.; Literature review: S.B.; Article writing: S.B.; Critical review: A.K., S.B.

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