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Original Scientific Paper

SENTINEL LYMPH NODE BIOPSY IN LOCALLY ADVANCED CERVICAL CANCER: A PROSPECTIVE, SINGLE-CENTER STUDY

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SUMMARY – Cervical cancer is one of the most common malignant tumors of the female reproductive system in women aged 15-35 years. Sentinel lymph node (SLN) biopsy is a diagnostic procedure used to determine status of local or regional lymph nodes of the tumor. In this article, we report the main results of our prospective study designed to assess diagnostic accuracy of SLN biopsy in women with early-stage cervical cancer using our modified tracer application technique. A total of 49 women with an early stage cervical carcinoma (FIGO stage IA2-IIA1) underwent a SLN procedure at the Zagreb University Hospital Center, Zagreb, Croatia. Radical abdominal hysterectomy with bilateral pelvic lymph node dissection was performed in all study women. We performed SLN biopsy with diluted methylene blue injected submucosally prior to surgery at 4 quadrants of the cervix and at 4 quadrants at the level of the cervix transition to vaginal vaults. The application was successfully performed in all women. In total, 46 women with FIGO staging IA2 (19.57%), IB1 (71.73%), IB2 (6.53%), IIA1 (2.17%) were enrolled in the study. A total of 1338 lymph nodes were removed, and 254 of these were SLNs with the mean value of 2.76 *per* pelvic side. Of these 254 SLNs, there were 13 positive SLNs in 7 women, whereas 241 non-SLNs were observed. Detection rate according to the number of participants and number of pelvic sides with at least one SLN detected on each side was 100%. In total, 92 pelvis sides were analyzed and at least one SLN was found on each side. Thus, bilateral SLN detection rate was 100%. Lymph node metastases were detected on 9 (9.8%) pelvic sides and false-negative rate was 1.09%. The sensitivity and negative predictive values were 88.9% and 98.9%, respectively. There were no differences in detection rate according to patient age, tumor size and FIGO stage. Interiliac and obturator fossa was the most common localization of SLN in 84.4% of cases. To the best of our knowledge, this is the first prospective study presenting a new modified tracer application technique in the SLN biopsy procedure. We present a novel, simple and feasible technique with well established points of application and volumes of tracer, demonstrating high overall and bilateral detection rates even in tumors >2 cm. Special emphasis should be on using this technique with indocyanine green application.

Keywords: *Cervical cancer; Sentinel lymph node; Radical abdominal hysterectomy*

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Introduction

Cervical cancer is one of the most common malignant tumors of the female reproductive system in women aged 15-35 years[1]. Radical hysterectomy or trachelectomy with bilateral pelvic and/or paraaortic lymphadenectomy is the standard treatment of early stages of cervical carcinoma with associated complications of urinary retention, infection, sexual dysfunction, and loss of fertility. The risk of lymph node metastasis in early cervical cancer is around 15%[2], which makes lymphadenectomy unnecessary in 80% of cases with increasing lymphedema, lymphocele, neurovascular and ureteral injury, or blood loss secondary to the dissection.

Sentinel lymph node (SLN) biopsy is a diagnostic procedure used to determine status of local or regional lymph nodes of the tumor with an intent to take targeted lymph node and reduce radicality of the procedure. However, in a systematic review of more than 50 studies involved, a wide range of detection rates between 50% to 100% have been reported[3]. Using a variety of mapping protocols (different types, volumes of tracers, and different application sites of tracers) and reporting different detection rate analyzed *per* patient (and not *per* pelvic side), makes diagnostic accuracy of SLN in cervical cancer unclear.

Complex lymph pathway and midline localization of the cervix is why drainage is bilateral in most cases of cervical cancer[4]. Therefore, bilateral detection rate of SLN in cervical cancer needs to be high, otherwise too many women will have to undergo at least ipsilateral complete lymphadenectomy on the pelvic side where the SLN is not identified[5].

In this article, we report the main results of our prospective study designed to assess diagnostic accuracy of SLN biopsy in women with early stage cervical cancer using our modified tracer application technique. The primary objective was to find feasible and not time-consuming technique of tracer application that might have high SLN detection rate not depending on the size and *Federation Internationale de Gynecologie et d'Obstetrique* (FIGO) stage of tumor. The secondary objective was to assess bilateral detection rate, sensitivity and negative predictive value of SLN biopsy while performing bilateral lymphadenectomy as a routine procedure.

Material and Methods

Patient selection

The study included 49 women with early stage cervical carcinoma (FIGO stage IA2-IIA1) that underwent a SLN procedure at the Zagreb University Hospital Center, Zagreb, Croatia. Radical abdominal hysterectomy with bilateral pelvic lymph node dissection was performed in all women. This prospective study was approved by the institutional Review Board and written informed consent was obtained from every woman. The inclusion criteria were FIGO (2009) stage IA2-IIA1 cervical carcinoma regardless of histologic type (squamous cell carcinoma, endocervical adenocarcinoma, adenosquamous carcinoma, and clear cell carcinoma). Magnetic resonance imaging (MRI), pelvic computed tomography (CT) and pelvic ultrasound were performed systematically in enrolled women before the procedure. Enlarged lymph node detected by the above diagnostic tools, pregnancy, and bulky disease making injection in healthy tissue impossible were excluded from the study.

Sentinel lymph node tracer application technique

Solution with 2 mL of methylene blue and 6 mL of physiological fluid, mixed together in sterile fashion, was made before starting a procedure. The application of 8 mL diluted tracer was carried out immediately before starting the surgery at eight different points while the woman was under general anesthesia. Diluted methylene blue was injected submucosally prior to surgery at 4 quadrants of the cervix and at 4 quadrants at the level of cervix transition to vaginal vaults. Tracer was injected in four quadrants at 3, 6, 9 and 12 o'clock position. In each quadrant, 1 mL of diluted blue dye was applied not deeper than 5 mm and the procedure was always performed by the same surgeon. The syringe was kept in place for a few seconds after the application to prevent retrograde leakage of the tracer. The procedure continued as planned with opening of the retroperitoneal spaces. Every blue node detected (Fig. 1) was considered to be SLN and was removed separately from each side of pelvis. Bilateral pelvic lymphadenectomy and radical hysterectomy were performed in all women. No side effects of the blue dye application were reported during the study. SLNs were visible in all cases without the need of dose repeat

intraoperatively. The application was successfully performed in all women.

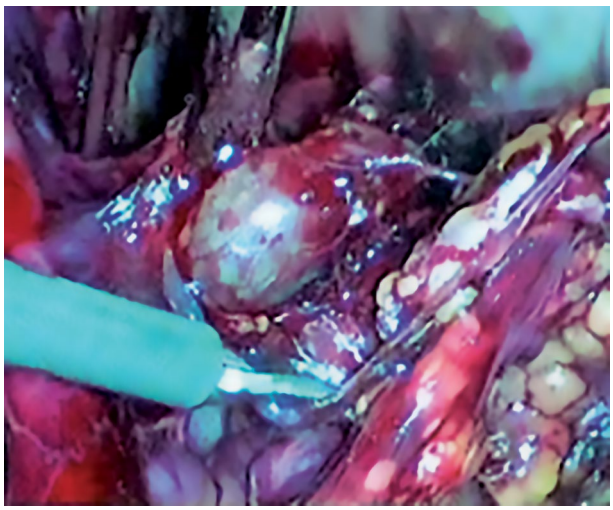

Fig. 1. Sentinel lymph node intraoperatively.

Histology

Sentinel lymph nodes were fixed 24 hours in 4% buffered formalin and sliced in 2 mm sections before embedding in paraffin. Non-sentinel lymph nodes and other specimens were fixed in 10% buffered formalin for a permanent section procedure. After fixation of lymph nodes, 4 μm thick sections were stained with hematoxylin and eosin, and analyzed by the most experienced pathologist at the department.

Statistical analysis

Data were analyzed according to the number of women and number of pelvic sides. On the first analysis, the SLN detection rate was defined as the ratio of the number of women with at least one SLN detected to all study women. The sensitivity was defined as the ratio of the number of sides with at least one involved SLN to the number of sides with at least one involved node among the sides with at least one detected SLN. On the second analysis, detection rate by pelvic sides was defined as the ratio of the number of sides with at least one detected SLN to all pelvic sides. A false-negative finding was considered when lymphatic mapping showed drainage to one or more SLNs in a pelvic side, biopsy of the SLNs revealed no metastases, and the patient had at least one metastatic non-SLN. The SPSS version 21.0 statistical software was used on data analyses.

Results

We excluded three women from the study because of the loss of data or withdrawal from the study. In total, 46 women with FIGO staging IA2 (19.57%), IB1 (71.73%), IB2 (6.53%) and IIA1 (2.17%) were enrolled in the study. A total of 1338 lymph nodes were removed, 254 were SLNs, mean 2.76 *per* pelvic side. Of 254 SLNs, there were 13 positive SLNs in 7 women, while 241 non-SLNs were observed in total. We performed detection 30 minutes after local injection of the tracer. Detection rate according to the number of participants and number of pelvic sides with at least one SLN detected on each side was 100%. In total, 92 sides of pelvis were analyzed and at least one SLN was found on each side. Thus, bilateral SLN detection rate was 100%. Lymph node metastases were detected on 9 (9.8%) pelvic sides and false-negative rate was 1.09%. The sensitivity and negative predictive values were 88.9% and 98.9%, respectively.

In our cohort, there were 52% of women with tumor size ≤2 cm and 48% with tumor size >2 cm. Tumor size >4 cm was identified in 6.5% of the study population. There were no differences in detection rate according to patient age, tumor size and FIGO stage. Interiliac and obturator fossa was the most common localization of SLN in 84.4% of cases. Although we had three women with tumor size >4 cm, we decided to perform a surgical procedure because of egzophytic tumor growth and without parametrial involvement on clinical and MRI findings. A positive non-SLN lymph node with metastasis less than 1 mm was found in only one woman.

Discussion

To the best of our knowledge, this is the first prospective study presenting a new modified tracer application technique in the SLN biopsy procedure. Our findings confirmed diagnostic usefulness and feasibility of SLN detection in early-stage cervical cancer. Overall, SLN detection rates using our technique were 100% with bilateral detection in 100% of women.

First question in SLN isolation is which marker to use. In the first published article on the issue, SLN detection rate with lymphazurine dye was 15.4%[6]. Combining different markers, blue dye (methylene blue, isosulfan blue, patent blue) and isotope (^{99m}Tc), detection rate rises without conclusion which marker is better if used alone[7,8].

In the SENTICOL study, detection rate with at least one detected SLN did not show a statistically significant difference between the groups with radionuclide and blue dye marker and was 93.9% and 89.9%, respectively[9]. A systematic review reports higher SLN detection rate in the combined group (97%) than in the group with blue dye or isotope alone (88% and 92%, respectively)[10]. It is necessary to emphasize that in these studies, detection rate was defined as the ratio of the number of patients with at least one SLN detected to all women. Bilateral detection of SLN in the SENTICOL study was 68.8%, 66.7% and 76.5% for blue dye, isotope marker and combination of these two markers, respectively[5]. This implies that in 30% of cases, we should perform lymphadenectomy on pelvic side where SLN was not detected, so the SLN biopsy procedure in the reported study is not beneficial in reducing the procedure radicality.

Recently published studies used ^{99m}Tc-labeled phytate with bilateral detection rate of 98% and 84%[11,12]. Although using ^{99m}Tc-labeled phytate demonstrates good overall detection rate, the procedure is time-consuming and technically demanding. Other limitations are differences in volumes and concentrations of blue dye and isotopes among published studies. Detection rate of SLN was higher when using a higher dose of blue dye (50% in 1.5 mL group *vs*. 83% in 2 mL and 90% in 4 mL group)[13]. Some studies report even higher detection rate (100%) using 3.5-4 mL blue dye, although they do not report tumor size[14]. Techniques of applying tracers were almost the same in all studies at 3 and 9 or 3, 6, 9 and 12 o'clock position with differences in depth, which was shown to be effective if tracer was applied in cervical tissue without tumor[15]. Up to recent findings reported in the literature, most studies did not mention whether injection was administered intra- or subepithelially[10], as we did in our report, and this could theoretically make a difference in SLN detection and other preoperative features.

With respect to the above mentioned limitations, we designed our tracer application technique to improve bilateral SLN detection in early-stage cervical cancer.

A recent systematic review of 67 studies showed significant differences in bilateral SLN detection rate, with greater bilateral detection rate in the group with tumors <2 cm[3]. Our study demonstrated better bilateral detection rate, even in the group of tumors >2 cm.

To increase feasibility and to overcome technical demands, we decided to use methylene blue as a single tracer for our SLN marking technique. The idea of using methylene blue mixed with physiological fluid was to increase the volume given in each point of cervix to improve absorption. We avoided a possible limitation of tracer uptake because of obstructed stromal lymph channels, with application in eight predefined points of cervix. A complex cervical lymphatic drainage was another rationale for increasing the number of application points. Using eight predefined points of application, we encompassed a wider area, increasing the likelihood of applying tracer to healthy tissue of the cervix, thus increasing absorption into a larger number of lymph channels. As cervical lymphatics drain bilaterally, SLN must be detected and interpreted *per* hemipelvis in order to be considered acceptable isolated procedure[4].

With establishement of application points, we reduced the operating time and use of time-consuming diagnostic methods preoperatively for detection of healthy tissue, reported in previous studies[10]. In our study, we had 17.4% (8/46) of women with positive metastatic lymph nodes. Bilateral detection rate of SLN was 100%. The false-negative rate was 1.09% and negative predictive value was 98.8%. There were no differences in bilateral SLN detection rate according to tumor size (≤2 cm and >2 cm), FIGO stage, and patient age.

Studies report on low overall and bilateral SLN detection rates in tumors >2 cm[3,15]. Careful marker application technique is essential for successful SLN detection. Our study confirmed feasibility of SLN biopsy, even in tumors >2 cm.

To conclude, we present a novel, simple and feasible technique with well established points of application and volumes of tracer, demonstrating high overall and bilateral detection rates even in tumors >2 cm. Special

emphasis should be on using this tehnique with indocyanine green application.

The potential lack of enrolled patients could be a possible limitation of our study. To validate our modified SLN tracer application technique, further studies should be performed.

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Sažetak

BIOPSIJA *SENTINEL* LIMFNOG ČVORA KOD LOKALNO UZNAPREDOVALOG KARCINOMA CERVIKSA: PROSPEKTIVNA, JEDNOCENTRIČNA STUDIJA

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Rak vrata maternice jedan je od najčešćih malignih tumora ženskog reproduktivnog sustava u žena u dobi od 15 do 35 godina. Biopsija *sentinel* limfnih čvorova (SLN) dijagnostički je postupak koji se rabi za određivanje statusa lokalnih ili regionalnih limfnih čvorova tumora. U ovom radu prikazujemo glavne rezultate naše prospektivne studije osmišljene s ciljem procjene dijagnostičke točnosti SLN biopsije u žena s ranim stadijem karcinoma cerviksa primjenom naše modificirane tehnike aplikacije modrila. Ukupno je 49 žena s karcinomom cerviksa u ranom stadiju (FIGO stadij IA2–IIA1) podvrgnuto postupku SLN biopsije u Kliničkom bolničkom centru Zagreb, Zagreb, Hrvatska. Kod svih ispitanica učinjena je radikalna abdominalna histerektomija s obostranom disekcijom zdjeličnih limfnih čvorova. SLN biopsija izvedena je injiciranjem razrijeđenog metilenskog modrila submukozno, prije operacije, u četiri kvadranta vrata maternice i u četiri kvadranta prijelaza vrata maternice u vaginalne svodove. Aplikacija je uspješno provedena kod svih bolesnica. U studiju je uključeno 46 žena sa stadijima FIGO IA2 (19,57%), IB1 (71,73%), IB2 (6,53%) i IIA1 (2,17%). Ukupno je odstranjeno 1338 limfnih čvorova, od čega 254 SLN, s prosjekom od 2,76 čvorova po zdjeličnoj strani. Od 254 identificiranih SLN pozitivno je bilo 13 čvorova u 7 bolesnica, dok je 241 čvor bio negativan. Stopa detekcije prema broju ispitanica i broju zdjeličnih strana s barem jednim detektiranim SLN-om bila je 100%. Ukupno su analizirane 92 zdjelične strane, a barem jedan SLN pronađen je na svakoj strani. Tako je stopa bilateralne detekcije iznosila 100%. Metastaze u limfnim čvorovima otkrivene su na 9 (9,8%) zdjeličnih strana, a stopa lažno negativnih nalaza bila je 1,09%. Osjetljivost i negativna prediktivna vrijednost iznosile su 88,9% odnosno 98,9%. Nije bilo razlika u stopi detekcije prema dobi bolesnica, veličini tumora i FIGO stadiju. Interilijačna regija i obturatorna jama bile su najčešće lokalizacije SLN u 84,4% slučajeva. Prema našim saznanjima, ovo je prva prospektivna studija koja prikazuje novu modificiranu tehniku aplikacije modrila u postupku SLN biopsije. Predstavljamo novu, jednostavnu i izvedivu tehniku s jasno definiranim točkama aplikacije i volumenima modrila, koja pokazuje visoke ukupne i bilateralne stope detekcije čak i kod tumora >2 cm. Poseban naglasak treba staviti na primjenu ove tehnike u kombinaciji s indocijaninskim kontrastom.

Ključne riječi: *Rak vrata maternice; Sentinel limfni čvor; Radikalna abdominalna histerektomija*