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Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer

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Summary

Design: prospective experimental study. *Purpose of investigation:* The purpose of this study was to evaluate feasibility and reliability of in vivo sentinel lymph node (SLN) mapping in patients with endometrial cancer and to verify a modified method of application of subserosal blue dye. Detection substance was applied subserosally in the uterine edges vicinity the round ligament of uterus and uterine vessels in the isthmic portion of the uterus. *Materials and Methods:* Eighteen patients with intermediate and high-risk endometrial cancer Stages I- II were subjected to staging laparotomy with intraoperative detection of SLNs and subsequent completion of the pelvic and para-aortic lymphadenectomies. Harvested SLN was routinely examined by classical haematoxylin eosin staining and in case of negativity, immunohistochemistry with anti-keratin antibodies AE1/AE3 was applied. *Results:* Total of 773 lymph nodes were removed in 18 patients: pelvic 420 (54%) and para-aortic 353 (46%). SLNs were detected in 16 of 18 patients totalling 59 nodes (7.6% of all nodes). Forty-eight were identified in the pelvic area (81%) and 11 nodes (19%) in the para-aortic area. Three metastatic SLNs were found in two patients (11%). No false negative nodes were demonstrated. *Conclusion:* Experimental study results indicate that the proposed modified approach to label SLNs is applicable. The presented modified approach brings the highest added value namely in women with a myomatous uterus and scars from previous surgical procedures on the uterus.

Key words: Endometrial cancer; Sentinel node; Blue dye; Lymphadenectomy; Staging; Laparotomy.

Introduction

Sentinel lymph node (SLN) mapping has become a cornerstone of oncologic surgery since it is a proven method of nodal disease identification. The SLN concept is frequently used in carcinoma of the cervix, vulva, and breast. Optimization of sentinel lymph nodes detection in endometrial cancer is currently still subjected to ongoing research.

Endometrial cancer is one of the most common malignancies among women in developed countries [1]. Survival rate generally depends on metastatic lymph nodes. Survival rate of 90% has been reported in case of lymph node metastatic negativity, whereas survival was 75% and 38% in those with the involvement of pelvic and para-aortic lymph nodes [2, 3]. Prognostic factors in patients with endometrial cancer include lymph node positivity, depth of myometrial invasion, and tumor grade. Hysterectomy, bilateral adnexectomy, and pelvic and para-aortic lymphadenectomies were recommended as a well proven standard by FIGO in 1988 [4]. Complete surgical staging has its well-known disadvantages in both intraoperative and early postoperative period.

The SLN is defined as the first lymph node into which the primary tumor is drained. It is assumed that in case of metastatic SLN negativity the other nodes are also negative. SLN mapping should be as good as a systematic lymphadenectomy in the identification of patients with lymph node dissemination, while reducing the morbidity associated with an extensive surgical procedure. SLN biopsy can be considered a compromise between comprehensive surgical staging and the complete omission of lymphadenectomy. The positive impact of lymphadenectomy is the diagnostic value of lymph nodes histological examination, and selection of patients with the benefit from subsequent adjuvant therapy. Staging the procedure according to FIGO cannot be reliably determined without the knowledge of lymph node status [5]. Surgical lymph node staging is more accurate compared to pre-surgical staging based on imaging methods [6]. From a therapeutic point of view, the efficacy of lymphadenectomy is controversial as demonstrated by the ASTEC study [7].

The aim of the present study was a feasibility test of modified application method of detection subserosally injected substance. The authors present the success of this application schema especially in women with uterine body pathologies such as fibroids and scars from previous surgeries.

Materials and Methods

All consecutive patients with intermediate and high-risk endometrial carcinoma Stages I-II who were operated from June, 2012 through February, 2014 and met inclusion and exclusion criteria (Table 1) were recruited in the study. Methodology of the study was approved by local ethical committee. Basic clini-

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Table 1. — *Inclusion and exclusion criteria*

Inclusion criteria	Exclusion criteria
1. Endometrial carcinoma proven by biopsy - hysteroscopy and curettage. 2. Expert oncogynaecological staging ultrasonic and clinical examination with preoperative evaluation of the depth of invasion into the myometrium. 3. Stage Ia - G3, Ib - G2, G3 II- G1 2.3 4. Informed consent	1. FIGO III- IV endometrial carcinomas and other histological subtypes of adenocarcinoma with the exception of endometrial 2. Distant metastases 3. Contraindications to surgery

Table 2. — *Number of removed lymph nodes and sentinel node count.*

Number of patients with complete pelvic and para-aortic lymphadenectomy	n=18
Total number of harvested lymph nodes	773
Number of pelvic nodes	420 (54 %)
Number of para-aortic nodes	353 (46 %)
Total number of sentinel nodes	59 (7.6 %)
Number of sentinel nodes right side of pelvis	28
Number of sentinel nodes left side of pelvis	20
Number of para-aortic sentinel nodes	11
Number of metastatic sentinel nodes	3 (11% patients)
Number of falsely negative sentinel nodes	0

cal data were obtained in all patients and all patients underwent experimental modelling in the detection of Slants. The patients were operated on by two surgeons. Patients underwent low midline incision with extension above navel under general anesthesia. After exploration of the abdominal cavity, lavage was performed by default. The uterus was fixed neither by fundus nor by the uterine edges and it was only supported by two fingers during the application of the detection substance (Figure 1). Four ml of Patent Blau (2.5%) was used as a detection agent. It was split into two syringes- one per each half of the uterus. Subcutaneous needle was used for substernal application. Application location was subserosally in the uterine edges from dorsal side of uterus body in the level of ligamentum ovarii proprium and uterine vascular bundles in the isthmic part of the uterus. Injection depth was approximately one mm and the substance was inserted gradually. The location was spot coagulated immediately after the injection to prevent the leakage of detection substance and subsequent contamination of the surgical field. Same application scheme was used for the second half of the uterus. Only four punctures to the uterus were applied. A ten-minute delay aimed to allow for sufficient uptake of lymphotropic agents followed. The procedure then continued by the dissection of pelvic peritoneum to inspect retroperitoneal spaces like pararectal, paravesical, and obturator fossa. Subsequently the retroperitoneum in the radix of mesentery was digested to visualize para-aortic space. Blue node was removed and identified as sentinel describing the anatomical area of location and laterality. Standard extrafascial hysterectomy with bilateral adnexectomy and systematic pelvic and para-aortal lymphadenectomies to the level of the renal veins was completed.

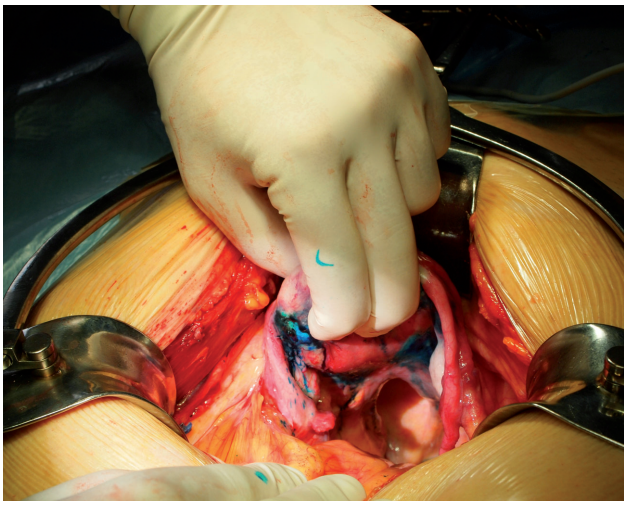

Figure 1. — Subserosal application of blue dye in the uterine edges from dorsal side of uterus body.

Histological processing

A pathologist evaluated SLNs at first with haematoxilin eosin staining and then all negative sentinel nodes were processed by sentinel node ultra-section technique. Six sections in one node at 200 μm intervals were performed. An additional cut was ammended between the third and fourth section upon which immunohistochemistry examinations were applied; it was namely a mouse monoclonal antibody anti AE 1/AE 3 cytokeratine. Non SLNs were stained only by haematoxilin eosin.

Results

Altogether 18 patients (mean age 66 ± 7.68 years (range 52 - 77) with intermediate and high-grade endometrial cancer Stage I-II underwent surgical intervention ranging hysterectomy, bilateral adnexectomy, detection of SLNs and subsequent complete pelvic and para-aortic lymphadnectomy during the period from June 2012 through February 2014.

Average BMI 32.5 ± 5.4 (min 23 - max 44). Total of 773 lymph nodes were removed, out of which 420 were pelvic lymph nodes (54%) and 353 (46%) were para-aortic lymph nodes. The average harvest of the pelvic lymph nodes was 28.4 ± (min 15 -max 34) and para-aortic lymph nodes was 24.5 (min 11 - max 28) (Table 2).

SLNs were detected in 16 of 18 patients (detection rate 88%). In total there were 48 pelvic SLNs. Eleven para-aortic SLNs were removed in nine patients (detection rate 50%), two para-aortic nodes were detected in one patient. Para-aortic SLNs were always detected in combination with pelvic SLNs; no isolated para-aortic SLNs were found. Three metastatic involvements of lymph nodes, two pelvic, and one para-aortic, were found in two patients (11.1%). Metastases to one pelvic and one para-aortic SLNs were found in one patient. Metastatic lymph nodes were confirmed by classical staining haematoxilin eosin and all negative sentinel nodes also proven by immunohistochem-

istry. None of the patients had false-negative SLNs. Detection of SLN failed in two patients. Bowel adhesion to the uterine fundus was present in one patient. After adhesiolysis subsequent application of detecting substance had contaminated the surgical field. In the second patient, the application was successful, but no blue node was detected, and the reason for this failure was not determined.

Localization of pelvic SLNs were: external iliac vessels n=12 (25%), internal iliac vessels n=18 (37%), obturator fossa n=15 (31%), and presacral space n=3 (6%). Para-aortic SLNs were located as in the following: two nodes in paracaval space, two nodes intera interaortocaval space, four nodes in supramesenteric para-aortal space, and three nodes in inframesenteric para-aortal space.

Discussion

Despite the fact that SLN mapping in endometrial cancer recently underwent intensive development, each of the currently used methods has some limitations moreover patient sample set was small and therefore a question of SLN detection optimisation approach still remains. SLN labelling approach techniques in endometrial cancer according to recent publications are: hysteroscopic application [8-13], subserosal application to the uterine body [14-18], cervical application [19-25], and combination of subserosal and cervical applications.

The authors present their data on a modified method of subserosal application of blue dye in 18 women who underwent surgery for intermediate and high-risk patients. SLNs were detected in 16 of 18 patients (detection rate 88%). Para-aortic SLNs were detected always together with pelvic nodes and detection rate for sentinel para-aortic lymph nodes was 50%. After SLN detection, complete pelvic and para-aortic lymphadenectomies were performed. In two patients positive lymph nodes were found (11.1%).

SLN identification with subserosal injection into the uterine body had been researched in six studies. The detection rate ranged from 45-92%.

Burke *et al.* in 1996 first presented SLN detection protocol with subserosal blue dye application in endometrial cancer [14]. In 15 patients blue dye was applicated subserosally in three sites in the sagittal line of the uterine body in total amount of three millilitres. Fallopian tubes were occluded. After ten minutes of dye uptake, SLNs were detected and complete pelvic and para-aortic lymphadenectomies were performed. Detection rates of SLNs were 67% and 27% lymph nodes were positive.

Lopes *et al.* in 2007 applied three ml of blue dye in 40 women [15]. Detection rate of SLNs was 78%. Complete surgical staging, including pelvic and para-aortic lymphadnenectomy were performed.

Altgassen *et al.* in 25 patients used four ml of blue dye subserosally by applying eight injections into the uterine body, four from the ventral and four from the dorsal site, with minimal manipulation with uterine body and any occlusion of the fallopian tubes were performed [16]. Detection rate was 92% which is the highest achieved and 12% of positive nodes were diagnosed. Only in selected patients para-aortic lymphadenectomy was performed.

Li *et al.* in 20 patients administered four ml of blue dye subserosally in five sites [17]. SLNs detection rate was 75% and 10% of nodes were involved. Para-aortic lymphadenectomy was also only performed in selected patients.

Frumovitz *et al.* in 18 patients described combination of subserosally applicated blue dye and ^{99m}Tc [18]. Injections were applied in three sites. Detection rate of SLNs was 45%. This is the lowest detection rate in the subserosal technique and the reason in unclear. Positive lymphatic nodes were not diagnosed.

Robova *et al.* in 67 patients also used a combination of blue dye and ^{99m}Tc [12]. Higher detection rate of 73% was achieved by using same technique as Frumovitz *et al.* Positive lymphatic nodes were diagnosed in 5.5%.

In the present study, the authors achieved a detection rate of 88% which is comparable with other studies. They consider a benefit that this study included only patients with intermediate and high-risk endometrial cancer and that negative sentinel nodes were examined by immunohistochemistry. After detection of sentinel nodes, pelvic and para-aortic lymphadenectomies were completed.

Four injections were administered from the dorsal side of uterus body close to the uterine edges. This technique of application eliminates the question of how deeply and where to subserosally inject detection substance at various thicknesses of the myometrium, especially in myomatous uterus, adenomyosis, previous surgical interventions on the uterus, and the adhesive process in the pelvis. In the present authors' application scheme, the detection substance is injected from the dorsal side of the uterus, and they see an advantage in that it eliminates handling and application through vesicouterine fold in the isthmic portion of the uterus and subsequently diffuse blue stain, which reduces the clarity of the surgery field.

From the experience of the previous application of blue dye, short term coagulation at the place after application it appears to be practical, which leads to minimize backflow of detection substance and prevents contamination of the surgical field. The advantages include the simplicity of the method without the need for other associated methods like lymphoscintigraphy, hysteroscopy, and is a technique with the shortest learning curve.

The limitation of this study is that the presented method is based only on blue dye detection and small number of patients. Application with usage of another dye for example indocyanine-green or combination with ^{99m}Tc nanocolloid should increases the detection rate [22, 24, 25]. Based on the multicenters prospective studies SENTI_ENDO [26] and the study Khoury Collado *et al.* [27] show that immunohistochemistry evaluation of the SLN and ultra-staging of the SLN may be even more sensitive than a full lymphadenectomy,

with lymph nodes evaluated by conventional pathology. However, the clinical importance of isolated tumor cells discovered in a lymph node that is negative by traditional histological analysis is still not known.

Conclusion

The present experimental study offers an alternative to the already published application schemas. It appears particularly advantageous for patients with myomatous uterus with scars after surgical procedures and during the adhesive process in the pelvis. Recent literature survey indicates that pathologist and immunohistochemical processing play a crucial role in SLNs examination.

Acknowledgments

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