ORIGINAL PAPER / GYNECOLOGY

VIA MEDICA

The impact of low volume lymph node metastases and stage migration after pathologic ultrastaging of non-sentinel lymph nodes in early-stage cervical cancer: a study of 54 patients with 4.2 years of follow up

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ABSTRACT

Objectives: To assess the significance of pathologic ultrastaging (PU) of sentinel (SLN) and non-sentinel (nSLN) lymph nodes (LNs) and the influence on cancer staging in patients with International Federation of Gynecology and Obstetrics (FIGO) stage IA2-IB1 cervical cancer.

Material and methods: A retrospective study was conducted with 54 patients divided into two equal-sized groups. In test group (n1), at least one SLN/patient was detected with blue dye. All excised LNs in this group were subjected to PU (4 µm slices/150 µm intervals) with hematoxylin-eosin staining and immunohistochemistry (AE1-AE3 antibodies). In none of the control group (n2) was PU performed, but in 2 patients SLN concept was performed. Patients in both groups underwent radical hysterectomy and lymphadenectomy. The effect of PU was expressed in puTNM and compared with both standard pTNM and FIGO systems. The influence of PU on patients' disease-free survival (DFS) and overall survival (OS) was assessed using Kaplan-Meier curves.

Results: In total, 516 LNs were extracted (66 SLNs, 36% bilaterally). Micrometastases (MIC) or isolated tumor cells (ITC) were detected in 34 of the 482 LNs (7.1%), including 16 MICs and 9 ITC in non-SLNs. False negative rates were: 3.7%/side-specific, and 7.4%/both sides. The use of PU resulted in stage change in 2 cases (N and M status change), FIGO stage did not changed. No PU impact on DFS or OS was observed.

Conclusions: The risk of TNM stage migration in early cervical cancer is low, is more likely in inattentively evaluated patients, and has indeterminate prognostic and predictive value. Selection of cases with cT ≤ 2 cm and cN0 is sufficient to avoid the risk of improper staging.

Key words: cervical cancer; ultrastaging; non-sentinel node; micrometastases

Ginekologia Polska 2019; 90, 1: 20–30

INTRODUCTION

Sentinel lymph node detection (SLND) is a strategy involving closer inspection of the lymph nodes (LNs) which are most likely to be the first draining nodes of the tumor and to harbor malignancy [1]. The clinical use of sentinel lymph node (SLN) biopsy was a significant milestone in surgical oncology [2]. In gynecologic oncol-ogy, SLN biopsy has found its place in the surgical treat-ment of patients with vulvar cancer [3]. In cervical cancer (CC) and endometrial cancer, the role of SLN staining and detection is still being intensely investigated [4–7]. Cervical cancer spreads primarily locally and through the lymphatic system. LN status is one of the most im-portant predictive and prognostic factors in early stage

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CC detection, where other metastases usually occur at more advanced stages. An increase in early CC diagnoses allows for adjusting the extent of uterine resection to the stage of the disease. The same is valid for the scope of lymphadenectomy (LND). When LN metastases is considered essential, SLND seems to be an alternative to complete pelvic LND for some patients. However, the benefits of SLND may be observed in all stages [8–11]. The method primarily allows, among others, the detection of micrometastases (MICs) that have been proven to have the same significance for the patient's prognosis as the detection of macrometastases (MAC) [6].

The US National Comprehensive Cancer Network (NCCN) strongly recommends the application of SLN concept in early-stage CC (as a part of LND - Recommendation 2A) [12], and the same is stated in the European Society of Gynecology and Obstetrics (ESGO) Guidelines [13]. Early-stage CC includes stage IIA1 according to the International Federation of Gynecology and Obstetrics (FIGO) staging system [14] or is defined by sizes up to 2–4 cm [11, 13, 15].

The lymph node sparing approach induces some uncertainties. It is possible that small metastases (MICs) may stay occult unless a non-SLNs (nSLNs) assessment is performed. Recently, FIGO finally added LNs status to their staging system, because the N feature is the most important prognostic factor in many cancers [14]. Unfortunately, imaging methods for smaller metastases (up to 5 mm) still have their limitations [16, 17].

Objectives

The aim of this study was to assess the risk of non-sentinel low volume LN disease (LVLND) coverage of MIC and/or isolated tumor cells (ITC) in cases of both positive and negative SLN, when all LNs are examined by pathological ultrastaging (PU) in early-stage CC. Therefore, it was expected that the histopathological evaluation of all LNs (nSLNs) by PU should validate SLND as a method.

Meanwhile, PU, a non-routine method used for evaluation of LNs, may potentially allow the post-operative detection of stage migration. Clarifying the extent and role of such migration is important in the context of the adequacy of surgical recovery and adjuvant therapy planning.

Comparing the FIGO and TNM staging systems, as well as TNM including PU, together with understanding the significance and possible threats of cancer staging changes are important for enabling us to balance therapy between the desire to minimize unfavorable sequelae of surgery and maximize the oncological outcomes.

MATERIAL AND METHODS

Between the 1st of January 2010 and the 30th of April 2014, 122 patients undergoing surgery for CC FIGO stage IA to IIA2, were entered into the study database, 54 of them were FIGO IA2-IB1. This group of 54 patients was divided into two equal groups: n1 (PU performed) and n2 (control, no PU performed). Group n1 included 1 case of SLND alone, 26 cases of SLND within LND, and the re-evaluation of all extracted LNs. Group n2 included 25 cases of LND without SLN concept, and 2 cases of unsuccessful SLND. All patients underwent surgery suitable to their FIGO stage according to the current NCCN guidelines. The patients either received cisplatin-based chemoradiation, or only underwent surgery where adjuvant treatment was not advisable.

Technique

Before surgery, 1 mL (10 mg) of methylene blue (Sterop Pharmacobel, Belgium) was injected in each of four punctures of the vaginal portion of the cervix (22 G) in the 3, 6, 9 and 12 o'clock locations, approximately 0.5 cm from the tumor border, and perpendicular to the cervix surface. This was administered 10–15 minutes prior to surgical skin incision and 20–25 minutes prior to opening of the retroperitoneal space. Then the radical hysterectomy was performed (Piver class III in most cases) [18]. The LNs were considered as "sentinel" by their blue staining as specified by the surgeon intraoperatively or by the pathologist postoperatively (in cases where the staining was invisible macroscopically). Lymph nodes were removed by separating the labeled (SLNs) from the non-labeled nodes (nSLNs). If there was no labeling on a side, all LNs on that side were regarded as nSLN.

Staging procedure

Lymph node samples were embedded in paraffin blocks and subjected to standard histopathological analysis. After the final diagnosis was provided, the LNs were subjected to multiple sectioning every 150 μm and evaluated by PU. In brief, the method is based on layered cutting of the paraffin-embedded tissues in 4 μm micro cuts at even distances apart (150 μm). The slices were placed on silanized glass slides, two samples from each level, the first stained with hematoxylin and eosin (HE), and the second subjected to immunohistochemistry (IHC) with cytokeratin (CK) monoclonal antibodies AE1/AE3 (Dako, Denmark). LNs were simultaneously examined by two researchers (MS and SW) under an optic microscope and measured manually (the maximal diameter of each lesion was noted). The schematic representation of a LN with MAC, MIC and ITC is shown in Figure 1. The occurrence of MAC or MIC, and of ITC, in each LN that had been
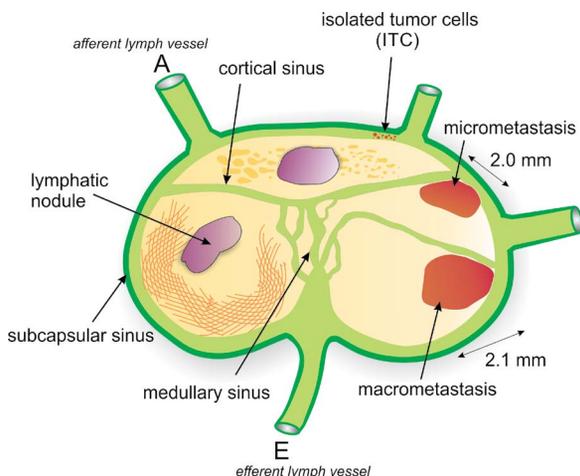
Figure 1. Schematic representation of the definitions of macro-, micrometastases and isolated tumor cells

removed was determined separately. The results have been included in their entirety in the classification of pathological (p) TNM as "puN1", "puN1mi" and "puN0i (+)". In the latter two cases, if the LNs contained only MIC(s) and/or only ITC, the number of LNs involved are given in brackets, for example: "puN1mi,I (+) (3LN, 1LN)". If the LNs contained MAC or MIC/ITC, this information is given at the end of Figure 1 in parentheses. With respect to SLNs, it was assumed that in cases of the successful staining of one or both sides of the pelvis the additional information on the ratio of positive LNs to the number of SLNs should be given in parentheses.

Statistical analysis

The study involved the independence chi-square test to analyze the relationship between dichotomous variables. In cases of quantitative predictors, a normality assumption was evaluated using Kolmogorov's test with the Lilliefors correction. Then the t-Student's test was used to examine the relationship between normally distributed predictors and dichotomous dependent variables, whereas the nonparametric Mann-Whitney U test was used for non-normally distributed predictors. Receiver Operating Characteristic (ROC) curves were determined and the AUC (area under ROC) was calculated to obtain the power of the discriminant variables. The criterion for selection of the optimal cut-off point was the maximization of the Youden index. In all the tests, the significance level was $p = 0.05$, and analysis was performed using Statistica 10 (StatSoft Inc., Poland).

RESULTS

The general characteristics of the patients are summarized in Table 1. LSVI was the only statistically sig-

nificant difference between groups n1 and n2. Detailed information on cancer staging of the n1 group is given in Table 2. In the n1 group, the bilateral detection of SLN was found in 11 of 27 patients (36%), which was a total of 38 hemi-pelvises out of 54 (70%). A total of 516 LNs (5599 slides) were removed from the whole study group (mean 19.4 per patient), and this included 66 SLNs (12.8%, mean 2.5 per patient). Lesions in LNs were found in 11 out of 27 patients, all at stage FIGO IB1 (2009-2017). In total, 56 LNs (10.9%) contained lesions, and the most prevalent lesions were MAC (22 times), MIC (22 times), and ITC (12 times). Micrometastases were found in 6 (9.1%) of the SLNs and in 16 (3.6%) of the 450 nSLNs. Macrometastases were found in 7 SLNs and ITC in 3 SLNs; whereas 15 nSLNs contained MAC and 9 nSLNs contained ITC.

Retrospectively, our intention of using SLN concept and PU allowed the detection of a larger number of LNs compared with a routine approach with surgery and examination (resulting in an average increase of 4 LNs extracted, $p = 0.046$). On average, patients with unilateral staining were about 9.5 years older than those identified with bilateral SLND (p=0.0326). In 10 cases, MIC was found in both examinations (HE and IHC), and in 1 case by IHC only. In 3 patients, metastasis or ITC in LNs were found in the SLN(s) only, in 3 cases exclusively in nSLN(s), and in 5 patients, changes occurred in both SLN(s) and nSLN(s) (Tab. 3). A relationship was found between the occurrence of MAC in SLN and MIC in nSLN ($p = 0.0072$). The tumor metastases data on LN status (SLN and nSLN), including the number of cases and corresponding ITC in LNs, are presented in Table 4. Quantitative and topographic division are shown in Figure 2.

Prognostic factors and nSLN status

In 24 cases of n1 (88.8%) the SLN status predicted the status of nSLN. In the n1 group the sensitivity and specificity of SLN detection per site was 60% and 89.3%, respectively. Lesions in nSLN were significantly more frequent if the primary lesion was > 2 cm than for those of ≤ 2 cm (73% vs 27%, respectively, $p = 0.01068$); and in the cases of a positive LVSI the significance was $p = 0.04285$. The histological type of tumor, grade and depth of infiltration did not show any statistically significant relationship with MAC, MIC and ITC detected in all LNs and in the subgroup of nSLNs as well. In patients with MACs in SLNs, the MACs and MICs in the nSLNs were detected significantly more frequently, without affecting the incidence of ITC ($p = 0.0142$). The cutoff value of invasion depth for the risk of events in LNs was ≥ 8 mm as measured by Youden's index (Fig. 3).

False negatives cases

Results considered as false negative cases, were found in three patients of n1. The actual false negative

Table 1. Patients and tumor characteristics in study (n1) and control (n2) groups

Group	n1 = 27 (%)	n2 = 27 (%)	p-value
Age (median age 54, range 23–65) 20–39 40–59 ≥ 60	8 (30) 12 (44) 7 (26)	2 (7.5) 18 (66.5) 7 (26)	P = 0.354
BMI* (median, range)	25 (19–37)	23 (17.5–39)	P = 0.608
Stage according to FIGO (2009–2017) IA2 IB1	1 (96) 26 (4)	2 (7.5) 25 (92.5)	P = 0.55245
Type of surgery PRS II (pLND) PRS III (pLND) PRS III (p+paLND) PRS III (pLND) PRS III (SLND)* PRS III (pLND)	1 (4) 19 (70) 3 (11) 2 (7) 1 (4) 1 (4)	2 (7.5) 18 (66.5) 5 (18.5) 0 2 (7.5) 0	p^ = 0.7613
Histopathological tumor size in cm (median, range)	2.5; 0.6–5.5	2.5; 0.45–6.0	P = 0.567
Histology Squamous cell carcinoma Adenocarcinoma Adenosquamous carcinoma	24 (89) 1 (7) 2 (4)	25 (92.5) 2 (7.5) 0	P = 0.308
Number of resected and examined lymph nodes	516	382	
Number of patients with positive lymph nodes (MAC+/MIC+)	7 (26)	5 (18.5)	p* = 0.5076
Number of patients with events in lymph nodes (MAC+/-MIC+/-ITC)	11 (41)	5 (18.5)	p* = 0.0706
LVSI	15 (56)	7 (26)	p^ = 0.037
Grade G1 G2 G3	2 (7) 15 (56) 10 (37)	4 (15) 20 (74) 3 (11)	P = 0.07615
Depth of invasion ≤ 10 mm 10.1–19.9 mm ≥ 20 mm	16 (59) 10 (37) 1 (4)	13 (48) 13 (48) 1 (4)	P = 0.555

BS — bilateral salpingectomy; BSO — bilateral salpingo-oophorectomy; BMI — body mass index (calculated by Web application at https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm; CON — cone biopsy; FIGO – International Federation of Gynecology and Obstetrics; G — grade; pLND — pelvic lymphadenectomy; paLND — paraaortic lymphadenectomy; PRS — hysterectomy according to Piver classification; SLND — sentinel lymph nodes dissection; p — probability of U-Mann-Whitney test (corrected for continuity); p^ — probability of the Chi-square test of independence; p* — two-tailed test for two structure indicators

rate was 2/27 (7.4%) per patient and the side-specific false negative rate was 1/38 (2.6%). This is due to the fact that ITC are not metastases; and in the case of the side-specific false negative, the coloration was present on the opposite hemi-pelvis to the false negative result (Tab. 5).

Stage migration

Extending the histopathological examination of all LNs allowed the identification of additional metastases in 34 of 482 LNs (7.1%) — LVLND; comprised of 22 MIC and 12 ITC. After PU was performed, the N feature was adjusted to the actual stage from pN0 to p(u)N1 in 1 case (patient PM, detection of MIC in pelvic nSLNs, 3.7%). In one other case, the M feature was changed from cMx to p(u)M1 (patient MW, PALN nSLN, 3.7%), as shown

in Tables 2 and 6. A detailed summary of changes to both the TNM and FIGO classifications after PU was performed is presented in Table 6. These changes and the differences between the groups in the classifications prior to, and after PU did not reach statistical significance, and did not influence the results.

Survival analysis

During the observation period, with a median of 4.2 years, comparing the two groups (n1 – PU and n2 – no PU), and also comparing between the PU group of patients and those without LN-positivity (including ITC), against the control group, there was no statistically significant difference observed in either the overall survival (OS) rate or the disease-free survival (DFS) rate of the patients (Fig. 4).

No.	Initials	FIGO staging (2009–2017)	Primary pTNM after surgery¶	Restaging after ultrastaging (puTNM)	Depth of invasion (mm)	LVSI	Type of surgery	Lymph nodes
Table 2. Detailed presentation of data on the staging of each patient in the test group (n1 = 27)								
1.	JB	IB1	pT1b1N0Mx	pT1b1 (3.2 cm) uN0 (0/26, 0/5sn) Mx	13	Yes	PRS III	P
2.	LM	IB1	pT1b1N0Mx	pT1b1 (2.7 cm) uN0 (0/24, 0/2sn) Mx	15	No	PRS III	P + PA
3.	TI	IB1	pT1b1N0Mx	pT1b1 (3.3 cm) uN0 (0/36, 0/3sn) Mx	20	No	PRS III	P + PA
4.	OE	IB1	pT2a1N0Mx	pT2a1 (3.7 cm) uN0 (0/29, 0/2sn) Mx	12	Yes	PRS III	P
5.	KA	IA2	pT1a2N0M0	pT1a2 (0.6 cm) uN0 (0/9, 0/2sn) Mx	4	No	PRS II	P
6.	MM	IB1	pT1b1N0Mx	pT1b2 (5.5 cm) uN0 (0/37, 0/4 sn) Mx	12	No	PRS III	P + PA
7.	TM	IB1	pT1b1N1Mx	pT1b1 (2.8 cm) uN1 (6/16, 4/7 sn, mic) Mx	10	Yes	PRS III	P
8.	LD	IB1	pT1b1N0Mx	pT1b1 (0.6cm) uN0 (0/5, 0/3sn) Mx	6	No	PRS III	P
9.	WJ	IB1	pT1b1N0Mx	pT1b1 (2.5 cm) uN0 (i+) (0/8, 0/1 sn, 4LN) Mx	16	Yes	PRS III (LPS)	P
10.	JM	IB1	pT2a1N0Mx	pT1b1 (3.0 cm) uN0 (i+) (0/12, 0/2sn, 2 LN) Mx	9	Yes	PRS III	P
11.	BE	IB1	pT1b1N0Mx	pT1b1 (0.7 cm) uN0 (0/9, 0/2sn) Mx	7	Yes	PRS III	P
12.	KT	IB1	pT1b1N0Mx	pT1b1 (3.0) uN0 (i+) (0/12, 0/2sn, 1snLN) Mx	5	No	PRS III (LPS)	P
13.	PM	IB1	pT1b1N0Mx	pT1b1 (1.0 cm) uN1mi, i (+) (3/5, 1/2sn, 1 LN) Mx	10	Yes	PRS III	P
14.	WW	IB1	pT1b1N0Mx	pT1b1 (0.8 cm) uN0 (0/17, 0/1sn) Mx	5	No	PRS III	P
15.	JL	IB1	pT1b1N0Mx	pT1b1 (2.0 cm) uN0(i+) (0/20, 0/1 sn, 4LN) Mx	15	Yes	PRS III	P
16.	SD	IB1	pT2a1N1Mx	pT2a1 (3.0 cm) uN1 (19/24, 2/2sn, mic)	4	Yes	PRS III	P
17.	PE	IB1	pT1b1N1Mx	pT1b1 (3.5 cm) uN1 (8/19, 4/4sn, mic) Mx	14	Yes	PRS III (RSO, LS)	P
18.	WJ	IB1	pT1b1N0Mx	pT1b1 (0.8 cm) uN0 (0/15, 0/1sn) Mx	8	No	PRS III (BS)	P
19.	OS	IB1	pT1b1N1Mx	pT1b1 (0.8 cm) uN1 (1/12, 0/1sn) Mx	8	Yes	PRS III	P
20.	GB	IB1	pT1b1N0Mx	pT1b1 (2.4 cm) uN0 (0/53, 0/4sn) Mx	5.5	No	PRS III	P
21.	DM	IB1	pT2a1N0Mx	pT2a1 (2.0 cm) uN0 (0/24, 0/3 sn) Mx	6	Yes	PRS III (BS)	P
22.	MW	IB1	pT1b1N0Mx	pT1b1 (2.0 cm) N0 (0/10, 0/1sn) Mx	12.5	Yes	PRS III	P
23.	BM	IB1	pT1b1N1Mx	pT1b1 (2.7 cm) uN1mi (1/20, 1/2 sn) Mx	12	Yes	PRS III	P
24.	LM	IB1	pT1b1N0 (sn) Mx	pT1b1 (0.6 cm) uN0 (sn) (0/3sn) Mx	6	No	PRS III (SLND)	P
25.	SJA	IB1	pT1b1N0Mx	pT1b1 (2.5 cm) uN0 (0/24, 0/3 sn) Mx	10	No	PRS III	P
26.	KT	IB1	pT1b1N0Mx	pT1b1 (0.6 cm) uN0 (0/20, 0/2sn) Mx	6.1	Yes	PRS III	P
27.	MW	IB1	pT1b2N1Mx	pT1b2 (4.2 cm) uN1 (6/27, 0/1 sn, mic) M1	19	No	PRS III	P + Pa

BS — bilateral salpingectomy; BSO — bilateral salpingo-oophorectomy, i (+) — presence of ITC; LND — lymphadenectomy; No. — case number; LPS — laparoscopy; P — pelvic lymphadenectomy; PA — para-aortic lymphadenectomy; PRS II,III — Piver-Rutledge-Smith surgery type II,III; R/LS — right/left salpingectomy; R/LSO — right/left salpingo-oophorectomy; SLND — sentinel lymph node(s) dissection; ¶ based on routine sectioning of specimens; · based on ultrathin sectioning

DISCUSSION

The purpose of our study was to evaluate the reliability of the determination of metastases in nSLNs by examining the presence of any type of tumor deposits (MAC, MIC, ITC) in early-stage CC and by comparing the outcomes with a group of patients without sentinel concept. Using one labeling method (36% with double-sided staining), the false positive rate was 3.7% for hemipelvis, and 7.4% in total. In this study, a change of N stage (from pN0 to puN1) took place in one case. In one out of four cases of para-aortic lymph nodes (PALNs) removal, an unstained LN with MIC (distant metastasis) was confirmed, resulting in the change of that patient's cancer stage (puM1). The metastatic lesion size was 2 mm, which was initially invisible in routine processing. There were no differences in survival between groups, but the groups differed in their LVSIs and the number of LNs removed. The first issue of differing LVSIs can be explained only by contingency, and the latter of the number of LNs removed, by improved "navigation" in the pelvis using SLND. Besides these facts, the ultrastaging patients' material was collected after that of the non-ultrastaging ones. There was no influence of PU on the prognoses, which may be an effect of the small size of our study group (n = 27) which would impact the statistically defined differences, and in such a case, even a 15–20% difference in hazard ratio will be insignificant.

In this study only one marking method was utilized – blue dye. In our previous study, the bilateral detection

Table 3. Cases of events in lymph nodes from the test group (n1) with additional information on tumor size, ultrastaging, SLN identification side, SLN and nSLN condition (according to lesion domination per site and LNs) as well as detected lesions in SLN/nSLN including stained side

Case number (Initials, group number)	SLN detection		LN condition				Positive, indeterminated or other
	Right side	Left side	Right side		Left side		
			SLN	nSLN	SLN	nSLN	
1. (TM) (7)	+	+	MAC (1)	-	MAC (1)	-	MAC (1) and MIC (1) in indeterminated LNs
2. (WJ) (9)	-	+	-	-	-	ITC (4)	
3. (JM) (10)	+	-	ITC (1)	ITC (1)	-	-	
4. (KT) (12)	+	-	ITC (1)	-	-	-	
5. (PM) (13)	+	+	-	-	MIC (1)	MIC (2) ITC (1)	
6. (JL) (15)	-	+	-	-	ITC (1)	ITC (2)	ITC in left parametrial LN (1)
7. (SD) (16)	+	+	MIC (1)	MAC (2) MIC (6)	MAC (1)	MAC (7) MIC (2)	
8. (PE) (17)	+	+	MAC (1)	-	MAC (3)	MAC (3) MIC (1)	
9. (OS) (19)	-	+	-	MAC (1)	-	-	
10. (BM) (23)	+	+	MIC (1)	-	-	-	
11. (MW) (27)	+	-	-	MIC (2)	-	MAC (1) MIC (2)	MIC (1) in paraaortic LN

‡ according to TNM system (2010); LN — lymph node; MAC — macrometastasis; MIC — micrometastasis; ITC — isolated tumor cells; SLN — sentinel lymph node; nSLN — non-sentinel lymph node

Table 4. Lymph nodes' events deciding on staging in 11 patients with division into sentinel lymph nodes (SLNs) and non-sentinel lymph nodes (nSLNs) and isolated tumor cells (ITCs)

SLN status	nSLN status	n [%]	SLN-ITC	nSLN-ITC
-	-	4 (36.36)	3	2
-	+	2 (18.18)	0	0
+	-	1 (9.09)	0	0
+	+	4 (36.36)	0	1
ALL		11 (100)	3	3

Chi-square test, p = 0.12168 - no statistically significant relationship; Left side of the table: positive (+) SLN or nSLN is the diagnosis of macrmetastasis(es) or micrometastasis(es) in the lymph node, ITC - isolated tumor cells; Right side of the table: the number of ITC cases in the sentinel (SLN-ITC) and non-sentinel (nSLN-ITC) lymph nodes, respectively

rate was 66% while using a three-step combined technique [19]. Taking the false negative results per side in our current study (3.7%) and the results per both sides of the previous study (3%), the feasibility of SLND is almost the same. The literature also describes cases of very low detection in cases of double marking [20]. Van de Lande et al. published their systematic review, summarizing 23 studies (842 patients) with early-stage CC, indicating no difference in the cumulative sensitivity analysis of SLN detection of a combined technetium-colloid (^{99m}Tc) with the blue dye method in comparison with blue dye staining alone [21].

A high percentage of unilateral SLN detection may be caused by a significant number of tumors being between 2 and 4 cm (median diameter 2.5 cm) or by the learning curve of performing injections. Both could affect the distribution of blue dye along lymphatic channels. However, due to SLN definition, it is doubtful whether, in the absence of bilateral SLN detection, the LNs removed from the non-marked side can be named as nSLN. Having nSLN on the non-marked side seems to be reasonable given the assumption that, despite the bilateral cervical flow of the lymph, the SLN may exist only on one side and is therefore independent of the method's lymphatic marking ability. Notwithstanding, the aim of our present study was to evaluate the prevalence of LVLND in all the LNs detected with PU performance, irrespective of the quality of administration.

Even though the subject of SLN in CC is not new, there are still only a few studies dedicated to nSLN. According to the authors' knowledge, only 10 studies on PU in early-stage CC, including evaluation of non-SLNs, have been published up to the date of our study (Tab. 7) [22–31].

Marchiole and co-authors [30] in early 2000 evaluated material like ours (574 LNs in 29 patients with CC IA1[LVSI+] - IB1 FIGO stages), in most cases (27/29) using a single staining method. They stated that the number of false negative results was too high (13%) to recognize SLND as safe.

Slama et al. examined 225 patients with CC (FIGO IA2-IIB) in a situation in which at least one SLN was detected with
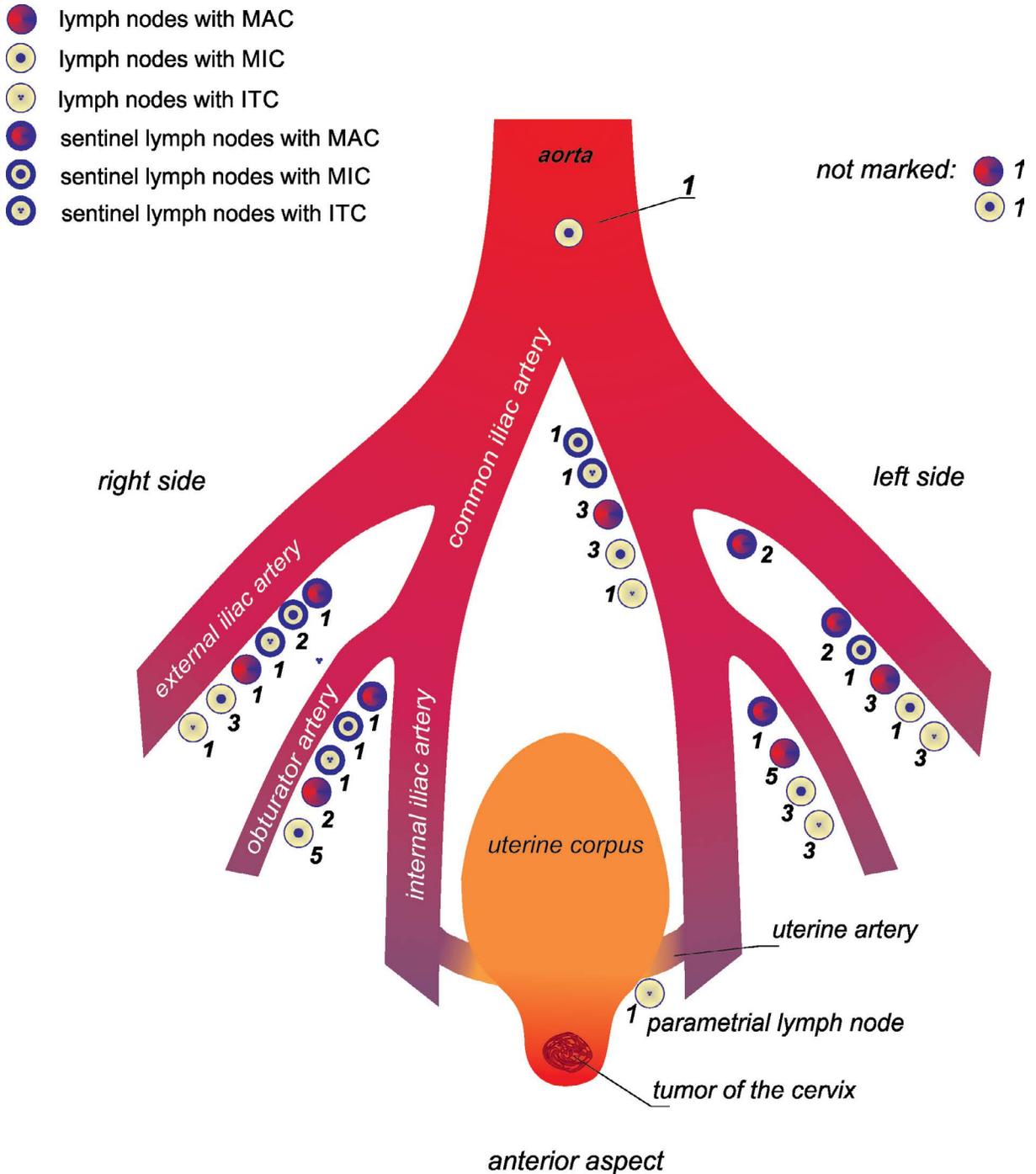
Figure 2. Distribution of lymph nodes events in 27 patients with ultrastaging performed

blue dye and radiotracer and they observed a low sensitivity in establishing the SLN status [32]. The authors emphasized that a tumor size above 20cm^3 and LVSI increase the risk of a false negative result.

In the most recent research, Cibula et al. carefully selected 17 cases with CC FIGO stage IB1 (≥ 3 cm, 8 patients), IB2 (7 patients), and IIB (2 patients), with bilateral SLN identification using an application of blue dye and

radiotracer [31]. Their average of 2.5 SLNs per patient is the same as in our study. Nevertheless, Cibula et al. obtained 100% sensitivity in metastasis determination in SLN tested by PU. Due to the central position of the cervix, the possibility of obtaining false results in the SLN evaluation should be considered as associated with the side of the pelvis. We obtained similar results in our present study; however, 3 false negative results per patient

Youden index = 0.32
Cuf-off point 8.00
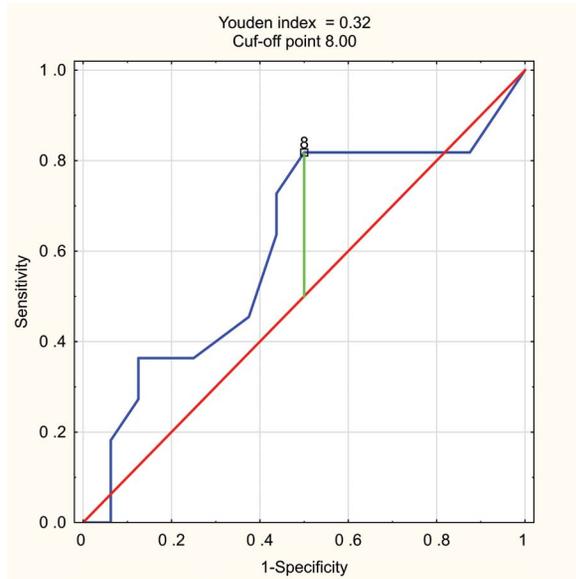
Figure 3. Graph presenting the cut-off value of the depth of tumor invasion for higher risk of invasion to lymph nodes

and DFS, after removing more than 16 nSLN from tumors in IB2–II FIGO stages and LVLND in SLN [33]. Fregnani et al. reported on FIGO IB–IIA cancer stages and found that only a high number of pelvic LNs (> 20) affects the prognosis [28].

Sakuragi et al. performed a study on patients with IB1-IB2 FIGO stages and determined the frequency of metastases in pelvic LNs to be 11.5%, and of metastases in PALNs to be 2.1%, but the prognostic impact was not evaluated [34].

Nodal and distant stage migration in the PU context has not been evaluated to date. Meva et al. performed an analysis of 54 patients with CC and presented computed tomography (CT) findings on the individual FIGO stages. The authors determined there was consistency between FIGO and pTNM in only 59% of their cases [35].

It seems clear that tumors smaller than 2cm in diameter have a lower risk of nSLNs and therefore limiting surgery is safe. Hsu et al. indicated the crucial role of defining the maximal tumor diameter and proposed two scenarios for patients with FIGO stage IB1 cancer < 2cm for better control of the disease, by performing parametrectomy. The system incorporated resource setting, frozen section of SLNs and age. In cases of positive SLNs on frozen section or an age < 50 in lower resource setting, parametrectomy can be omitted [36]. However,

were found (all in cases of unilateral detection, including a case with ITC), a result that can be due to our larger study group or to our adopted definitions.

Zaal et al. examined a group of 645 CC patients (FIGO IA-IIB), and observed a prognostic benefit relating to OS

Table 5. False negative results per patient

Case description	Type of change in the nSLN	Labeling	Commentary
40-years old, BMI 19, SCC, G2, pT = 2.5 cm, invasion 16 mm, LVSI (+)	ITC on the labeled side	Left-sided only	The result is actually not false negative (ITC are not metastases)
62-years old, BMI 29, prior conization 3.5 weeks earlier, SCC, G2, pT = 0.8 cm, LVSI (-), uterine fibroids	MAC on the non-labeled side	Left-sided only	The result is actually not false negative (different sides)
57-years old, BMI 22, SCC, G3, pT = 4.2 cm, invasion 19 mm, LVSI (-)	MIC on the labeled and non-labeled side, one paraaortic	Right-sided only	The result is false-negative

BMI — body mass index; G — grade; ITC — isolated tumor cells; MAC — macrometastasis; LVSI — lymph-vascular space invasion; MIC — micrometastasis; SCC — squamous cell carcinoma

Table 6. Changes in the TNM staging system after histopathological ultrastaging in the test group (n1 = 27)

Feature	Change	Difference
Stage	· clinical (FIGO) vs. pathological ultrastaging, p(u)TNM	5 cases (no. 4, 10, 16, 21, 27)
	· in pathology review: routine (standard processing) vs. ultrastaging 1. T feature · from pT1a2 in p(u)T1b1 · from pT2a1 in p(u)T1b1	1 case (no. 6) 1 case (no. 10)
	2. N feature - pN0 in p(u)N1	1 case (no. 13)
	3. M feature - pMx in p(u)M1	1 case (no. 27)
Potential change in prognosis	As a result of change in N feature	No differences in OS and DFS (no. 13)
	As a result of change in M feature	No difference in OS and DFS (no. 27)

DFS — disease free survival; FIGO — International Federation of Gynecology and Obstetrics; OS — overall survival; TNM — Tumor, Node, Metastasis system
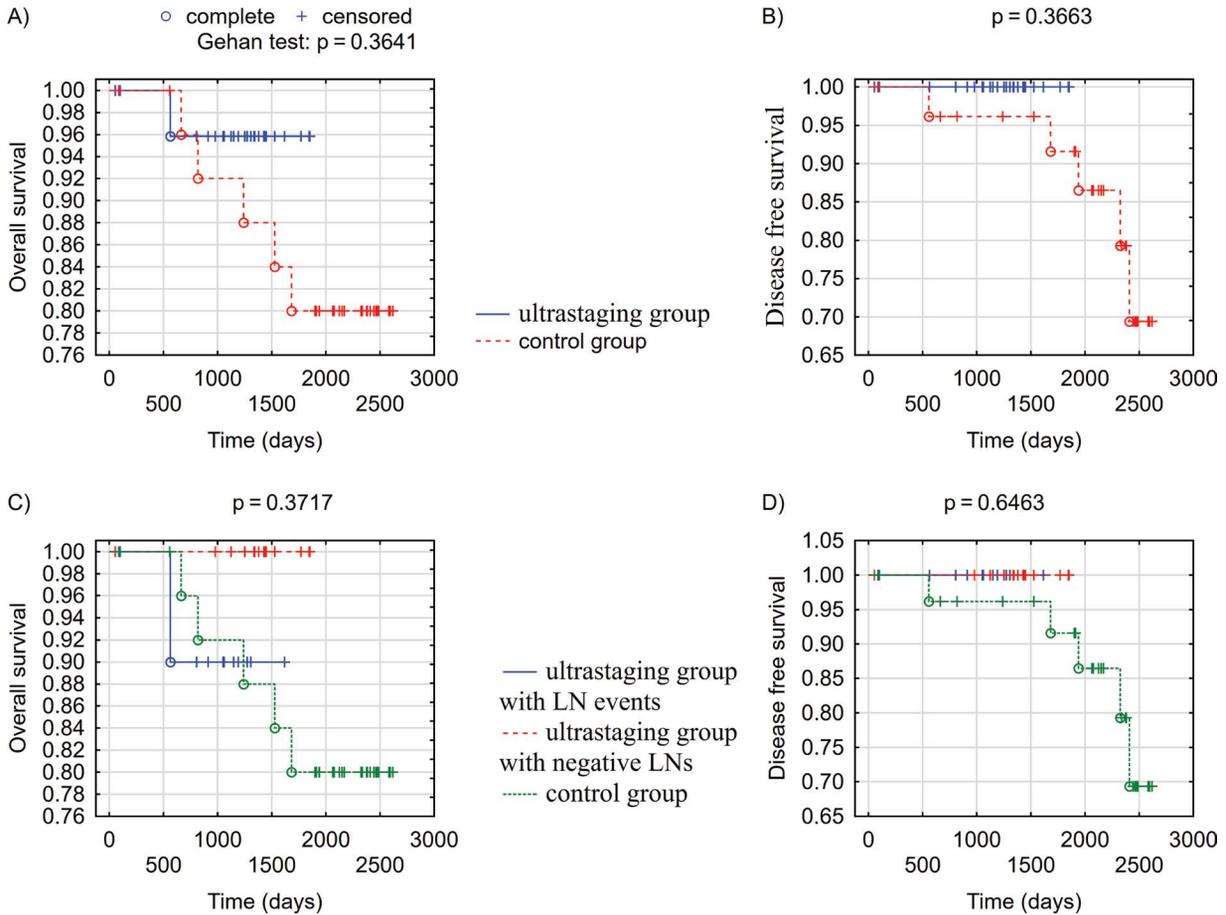
Figure 4. Overall and disease-free survival analysis of patients' study groups: **A)** overall survival and **B)** disease-free survival among ultrastaging group and control groups, **C)** overall survival and **D)** disease-free survival among LN positive and LN negative ultrastaging subgroups, and control group

their study was on "very early", rather than "early" cancer stages [11, 15].

The under-staged patient in our study was the one who had the second largest tumor, pT = 4.2 cm. Two pT1b2 cases (pT = 4.2 cm and pT = 5.6 cm) showed the deficiencies of the clinical classification. Additionally, there were 6 positive LNs detected, with tumor infiltration depth of 19 mm, without LVSI positivity. Application of SLND procedures indicates that possible intra- and postoperative decisions should be categorically restricted to smaller tumors (according to literature: < 4 cm, preferably ≤ 2cm or ≤ 20 cm^3). Although imaging modalities have not been adopted in FIGO staging requirements and TNM, it is only rarely use in CC staging for early stage patients. Our study supports the usefulness of assessing tumor diameter, or volumetric modality (with ultrasound or MRI), which is also what the ESGO guidelines noted [13].

CONCLUSIONS

Micrometastases in early-stage CC (FIGO IA2-IB1) occurs 2.5 times more often in SLNs than in nSLNs. Their detection is possible by extending the histological exami-nation by PU. The risk of finding MICs in nSLNs increases with tumor size (> 2 cm) and with the infiltration of the lymphatic perivascular space (LVSI). Utilization of PU allows information on the actual CC clinical stage to be obtained. The SLN status determines the state of the remaining LNs so long as there is a bilateral identification of SLN. In the absence of SLN detection on one side one cannot reliably conclude on the status of the nodes on the ipsilateral side since there is the risk of obtaining a false diagnostic result and of underestimating the stage. Although selection of cases with cT ≤ 2cm and cN0 does not eliminate the risk of improper staging, stage migration in early CC, due to incorporation of pathologic ultrastaging, is less probable and yet, has an immeasurable prognostic and predictive impact. The only way the prognosis would be different will be where PU identifies metastases that would warrant adjuvant therapy in a setting that would otherwise not receive adjuvant therapy.

Table 7. Previous studies in which patients had ultra-thin cutting of SLN and nSLN and staining in hematoxylin and eosin, immunohistochemistry, or otherwise

Author	Cancer stage FIGO (2009–2017)	N patients	Patients N+ (MAC)	Patients N+ (MIC)	Methods of ultrastaging	Cutting at the distance	Percent of positive nSLN with negative SLN	N lymph nodes	Ref.
Barranger et al., 2003	IA2-IIA	13	0	0	HES & IHC (CK AE1-AE3) in negative LNs in routine examination	3 mm	0	21	[22]
Marchiolé et al., 2004	IA1-IB1	29	3	5	HES & IHC (CK AE1-AE3) in negative LNs in routine examination	200 µm	13	574	[30]
Popa et al., 2006	I-IIA	36	0	0	HE & IHC	40 µm for HE (6 levels), additional level between 3th and 4th was examined with IHC (AE1/3)	0	≥ 926 (The calculation from indirect data)	[29]
Fregnani et al., 2006†	IB-IIA	289	37	11	HE & IHC (CK AE1-AE3)	nd	nd	5599	[28]
Okamoto et al., 2009	IB1	10	1	1	HE & IHC (CK AE1-AE3)	200 µm	0	309	[27]
Ogawa et al., 2010	IA-IIB	82	31	8	HE	nd	0	157	[25]
Martínez et al., 2010	IA2-IB1	41	4	2	IHC (CK AE1-AE3)	200 µm	0	798	[26]
Lou et al., 2013‡	„Early"	30	9	68	PCR (CK19)	nd	18.2	268	[24]
Śniadecki et al., 2014	IA-IIA	12	3	2 (1)*	HE lub/i IHC (CK AE1-AE3)	150 µm	8	280	[23]
Cibula et al., 2016	IB1 ≥ 3 cm, IB2, IIB	17	1	4	HE & IHC (CK AE1-AE3)	150 µm	0	573	[31]

CK — cytokeratin; HES — hematoxylin-eosin-safron; N+ (MAC) — macrometastases to the lymph nodes; N+ (MIC) — micrometastases to the lymph nodes; nd — insufficient data; *one additional micrometastasis to macrometastasis in one of the lymph nodes; † in this paper all the lymph nodes were examined in ultrastaging without performing the labeling of sentinel lymph nodes; ‡ Article in Chinese, only summary is available

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