

Clinical feasibility and diagnostic accuracy of detecting micrometastatic lymph node disease in sentinel and non-sentinel lymph nodes in cervical cancer: outcomes and implications

Możliwości kliniczne i dokładność diagnostyczna wykrywania mikroprzerzutowej choroby węzłowej w węzłach chłonnych wartowniczych i pozawartowniczych w raku szyjki macicy: wyniki i implikacje

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Abstract

Background: Lymph node (LN) micrometastatic disease has come to prominence since ultrastaging was shown to improve the quality of LN procedures in epithelial cancers. The aim of the study was to evaluate the feasibility and diagnostic usefulness of detecting micrometastases in sentinel (SLN) and non-sentinel LNs (nSLN) in cervical cancer.

Material and methods: Twelve consecutive patients with cervical cancer stages IA to IIA, classified according to the Union for International Cancer Control (UICC) and divided into two groups: A (7) and B (5), with and without SLN procedure with methylene blue dye, who underwent radical hysterectomy and lymph nodes removal, were recruited for the study. All LNs were evaluated in hematoxylin-eosin (HE) staining and immunohistochemically (IHC) in ultra-staging with anti-cytokeratin AE1/AE3 antibodies. A detailed analysis was performed with regard to the technical and histopathological aspects of the procedure.

Results: More LNs could be extracted and studied in group A as compared to group B (210 vs. 70, mean 30 vs. 14, respectively, $p < 0.0005$). A total of 13 SLNs were extracted, and the identification rate was 71% (5/7 in group A). One micrometastatic LN was found in each of the groups (16% cases), but the preliminary classification of the advancement stage was changed only in 1 case from the labeled nodes group (group A - from pN0 with HE to pN1 with IHC).

Conclusions: Presence or absence of metastases in SLN(s) should not be sufficient amount of information for a surgeon or an oncologist, who ought to have data about all of the removed lymph nodes (sent to ultrastaging). In order for the surgery to be performed properly, it is vital to ensure that SLNs were removed. Assessment of the N status ought to be taken into consideration in the classification according to the International Federation of Gynecology and Obstetrics (FIGO).

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Streszczenie

Cel pracy: Mikroprzerzutowa choroba węzłowa zyskała na znaczeniu odkąd wykazano, że ultrastaging poprawia jakość procedur operacyjnych z usuwaniem węzłów chłonnych w nowotworach nabłonkowych. Celem pracy była ocena możliwości jak i przydatności diagnostycznej wykrywania mikroprzerzutów zarówno w węzłach wartowniczych (SLN) jak i pozawartowniczych (nSLN) w raku szyjki macicy.

Materiał i metody: 12 kolejnych pacjentek z rakiem szyjki macicy sklasyfikowanych wg Unii dla Międzynarodowej Kontroli nad Rakiem (UICC) w stopniach od IA do IIA w dwóch grupach A (7) i B (5) odpowiednio z i bez procedury SLN z użyciem błękitu metylenowego poddano radykalnej histerektomii i limfadenktomii. Wszystkie węzły chłonne oceniono w barwieniu hematoksylina – eozyna (HE) i immunohistochemiczne (IHC) w ultrastagingu z użyciem przeciwciał przeciwko cytokeratynom AE1/AE3. Szczegółową analizę wykonano w odniesieniu do aspektów technicznych i histopatologicznych procedury.

Wyniki: Więcej węzłów chłonnych można było usunąć i zbadać w grupie A niż w grupie B (210 vs 70, średnio 30 vs 14, $p < 0,0005$). Wykryto 13 SLN, a poziom identyfikacji wyniósł 71% (5/7 w grupie A). Po jednym węźle chłonnym z mikroprzerzutami znaleziono w obu grupach (16% przypadków), ale wstępna klasyfikacja nowotworu została zmieniona tylko w jednym przypadku w grupie z oznakowywanymi węzłami (grupa A – z pN0 z HE do pN1 w IHC).

Wnioski: Obecność lub brak przerzutów do SLN nie powinna być wystarczająca dla chirurga i onkologa, którzy powinni mieć informację o wszystkich usuniętych węzłach chłonnych (przesłanych do ultrastagingu). Istotnym warunkiem przeprowadzenia prawidłowej operacji węzłowej jest upewnienie się, że materiał pooperacyjny zawiera węzły wartownicze. Ocenianie stanu węzłów chłonnych powinno być brane pod uwagę do oceny wg klasyfikacji FIGO.

Słowa kluczowe: **mikroprzerzuty / ultrastaging / pozawartownicze węzły chłonne / rak szyjki macicy / stopniowanie zaawansowania raka /**

Introduction

Micrometastases (micNs) are tumor deposits ranging from at least 200-cell clusters up to 2-mm in diameter nodules in a lymph node [1]. Micrometastases have been found to have the same prognostic significance as macrometastases in cervical cancer [2]. To the best of our knowledge, no study so far has focused on the feasibility and clinical impact of detecting micNs in all lymph nodes. In the largest study of Cibula et al., the prognostic role of non-sentinel lymph nodes (nSLNs) was not specified [2]. Introduction of ultrastaging into pathological staging has brought broader spectrum of lymph node assessment in some cancers of the epithelial origin [3-6].

However, it does not seem to be time-effective in the intra-surgical context [7]. This technique has an increased sensitivity but requires a special method of SLN labeling with the use of radioisotope, and/or by providing a dye absorbed by lymph vessels and relating nodes. The time itself is a strong limitation of assessing LNs in ultrastaging setting because this method requires one or two working days of a single pathologist (according to our own experience), thus being relatively time-consuming.

In recent years, SLN has achieved a growing amount of focus and attention. However, in the context of skip or occult metastases which might occur in up to 6% of cervical cancer cases (stage Ib2-IIb according to the International Federation of Gynecology and Obstetrics, FIGO) [8-9], unknown proportion of positive micNs per stage, unclear significance of micNs in the nSLNs, and unexpected lymph vessel basins in each particular patient, might constitute an indication to remove all lymph nodes. This procedure may be performed in order to further extend the

field of radiotherapy for better cancer control [10]. The question whether ultrastaging is to be used in the assessment of all lymph nodes remains a source of much debate [10-12]. Our study was designed to enhance our understanding about the spreading of cancer cells from the primary operable cervical cancer. However, SLN detection and nSLN ultrastaging might also maintain their own importance in advanced stages of the disease [12].

Material and methods

Twelve consecutive patients with primary cervical cancer, confirmed by histologic examination and clinically eligible for radical hysterectomy, were evaluated in the study. All patients underwent a radical hysterectomy (Piver-Rutledge II-III) with different extent of lymphadenectomy and all but two had bilateral oophorectomy.

In group A, 4 ml of methylene blue was injected into the cervix submucosa to 6, 3, 9 and 12 hours of the vaginal portion outside the tumor before each surgical procedure. All but one case were squamous cell type histology. The specimens were routinely assessed with the exception of the lymph nodes that were subjected to ultrastaging. Briefly, the lymph nodes were cut into 2-mm slices. Each of these slices was divided into two blocks and finally cut at 150 μ m in order to evaluate them histologically. Sections were stained with hematoxylin-eosin (HE) and ultrastaged with anti-cytokeratin AE1/AE3 antibodies (DAKO North America, Inc. 6392 Via Real, Carpinteria, CA 93013, USA).

The details of the procedure had been previously described in another study [6]. Statistical analysis was performed with Statistica v. 10 package (Statsoft Inc., Poland).

Table 1. Basic characteristics of the study group.

Group	Case	Age	Cancer stage (TNM)	pT (cm)	LVSI	Grade	Surgery	SLN localization
A (sn+)	1	39	pT2b2N1mic (1/30, 5sn)Mx	4,7	X	2	TR, W-M + LDp+pa+ps	LO(mic), RO, LCI
	2	55	pT2b2*N0(0/21, 5sn)Mx	4,5	+	2	W-M + LDp (ie)	RO, LO
	3	65	pT1b1N0(0/10, 1sn)Mx	2	-	2	W-M + LDp	LO
	4	43	pT1b1N0(0/22, 1sn)Mx	2,7	-	2	W-M + LDp (ie)+pa	LO
	5	23	pT1b1N0(0/35, 1sn)Mx	3,3	-	3	W-M + LDp+pa+ps	RO
	6	70	pT1a1(2mm)N0(0/50, 0sn)Mx	0,25	-	1	W-M + LDp+pa	-
	7	63	cT1b1*N0(0/42, 0sn)Mx	0	X	1	W-M + LDp	-
B (sn-)	8	52	cT2apN1(3/23,+1mic)Mx	X	X	2	BA + LDp+pa (ie)	-
	9	67	pT1b1N0(0/6)Mx	2	-	X***	W-M + LDp	-
	10	65	pT1b2N0(0/13)Mx	5	+	3	W-M + LDp (ie)	-
	11	59	pT0**N0(0/14)Mx	0	X	X	W-M + LD (ie)	-
	12	34	pT1b1N0(0/14)Mx	3	+	2	W-M + LDp+pa	-

Abbreviations: (sn+) – labeling of the sentinel lymph nodes; (sn-) – without labeling of the sentinel lymph nodes; TNM – staging according to UICC Tumor-Node-Metastasis System; LVSI – lymphovascular space invasion; ma – macrometastasis; mic – micrometastasis; X – this feature could not be assessed; TR – tumor resection; WM – Wertheim-Meigs surgery; LO – left obturator lymph node(s); RO – right obturator lymph node(s); LCI – left common iliac lymph node(s); BA – bilateral adnexectomy; LD – lymphadenectomy; LDp – pelvic lymphadenectomy; LDpa – paraaortic lymphadenectomy; LDps – presacral lymphadenectomy; ie – intraoperative examination *after previous conisation; **result based on excisional biopsy (cancer in the cervical polyp); ***adenosquamous carcinoma, grade was not given

The study was approved by the local Independent Bioethical Commission of Medical University of Gdansk. A written informed consent was obtained from all study participants.

Results

Out of 12 patients, 7 (group A) underwent the SLN procedure, and 5 (group B) were not subjected to this procedure (Table 1). Mean age was 51 and 55 years in groups A and B, respectively. In case of 2 patients from group A – 1 with stage pT1a1(2mm) N0(0/50)Mx and 1 with cT1b1pN0(0/42)Mx – we failed to identify SLNs. The total number of lymph nodes was 280 (group A: 210, mean: 30 nodes/patient, range 10-50; group B: 70, mean: 14 nodes/patient, range 6–23, $p < 0.0005$, two-sided t-Student test). In group A, 2 (28%) out of 7 patients had no labeled lymph nodes found through dye injection. Both patients were among the oldest in that group. Out of the 7 patients, optimal (two-sided) labeling of the lymph nodes basin was successful in only 2 subjects, who had the largest cervical change in the study population (pT2b). Thirteen SLNs were identified in group A (13/210, 6.2%). Metastases were found in 2 patients out of 12 (16%), 1 in each group (Table 1). In the first patient (case 1, group A) the exophytic vaginal cervical tumor was removed using monopolar coagulation; 4 ml of blue dye was injected into the macroscopically unchanged cervix outside the previous tumor, then radical hysterectomy was performed. Five SLNs could be identified, including only one metastatic node of the left obturator group (Nmic diameter of 1.5 mm *via* ultrastaging). Sentinel nodes were present in the following groups: left obturator (1/2), left common iliac (0/2) and right obturator (0/1). In the second patient (case 8, group B), with cT2apN1(3/23,+1mic)Mx, 3 involved lymph nodes were found, including three with macroscopic metastases (in the right obturator node, in the left obturator node and in the left interiliac node).

Next to one of them (in the left obturator node) the one more Nmic became apparent only in ultrastaging. No blue dye was injected. No micrometastases were found in any of the lymph nodes removed and checked intraoperatively.

Discussion

The usefulness of SLNs labeling remains to be fully elucidated. In our recent publication regarding lymph nodes in general, we have discussed many problems emerging from the studies on lymph nodes in cervical cancer, and referred to some of them in the current study (i.e. tumor stage, number of extracted lymph nodes and additional diagnostic power of labeling SLNs in comparison to no labeling) [13]. The basic criterion for the correctness of the study should be bilateral detection of SLNs. Although the optimal mapping of both sides was achieved only in 2 out of 7 patients in the current study, all subjects underwent a thorough examination of the lymph nodes in ultrastaging, what allowed to detect all possible metastases. Marchiolè et al., were among the first to raise the question of validity of lymph node concept in cervical cancer [14]. They analyzed 26 patients with negative lymph node metastases on routine histological examination and have found 2 micNs in SLNs and 3 additional micNs in nSLNs (19% when considering SLNs and 11.5% when considering only non-sentinel lymph nodes). The frequency of detection of micNs in our group was also similar (16%) – 1 case for each group with and without the SLN procedure. Certainly, a comprehensive examination of the lymph nodes allows to avoid the problem of patient selection for the SLN procedure and to omit the question of tumor size. Indeed, the border of 2cm indicating a tumor-related factor hampers the detection of SLN, but at the same time it increases the risk of finding non-SLN micNs [15-16]. On the other hand, some studies were not able to demonstrate

micNs and draw different conclusions, despite a small number of cases but a relatively large number of removed lymph nodes [17]. Determination of the lymphatic vessels space invasion (LVSI) constitutes another factor negatively influencing the occurrence of lymph node metastases. Juretzka et al., detected the presence of LVSI in 12 out of 49 retrospectively reviewed patients with FIGO IA2-IB2 lymph node negative cervical cancer and found 3 LVSI cases among 4 patients with micNs. There were 6.7% and 50% recurrences, respectively in both groups, the node-negative and node-positive [18]. In a study by Marchiolè et al., reporting on recurrent cervical cancer with median follow-up time of 36.8 months, LVSI was found in 77% of cases and micNs were found in 35% of cases as compared to 42% for LVSI and 4% for micN in the group without recurrence [19]. However, it seems that there may be an independence between LVSI and positive lymph nodes or tumor size as well, as was demonstrated by our investigation, but also by a much larger study of Cibula et al. [6]. In our study, the participants, at various stages of early cervical cancer in a variety of clinical situations, were subjected to the radical tumor and lymph node surgery and comprehensive lymph nodes assessment. It represents a fairly wide range of analyses as the new patients will be introduced to the study.

Conclusions

Small sample size did not allow us to draw any definite conclusions. Regardless, it can be clearly seen that without the use of the blue dye a statistically lower number of lymph nodes can be identified and excised, as compared to the use of the blue dye marker (mean 30 vs. 14). In addition, tumor micrometastases were observed in both groups, which stresses the importance of lymph nodes ultrastaging, rather than focusing only on labeling and checking the SLNs. Sentinel lymph nodes are most commonly detected in the group of pelvic lymph nodes corresponding to the group of obturator lymph nodes. In addition to the criteria of bilateral mapping of the SLNs, it should be aimed to get this group of lymph nodes among others SLSs and nSLNs. Ultrastaging allowed the detection of additional micNs in 2 patients, but it did not change the pN status in 1 case (additional micN to 3 macrometastases in case no. 8). In the case no. 1, the status of the pN has changed into a higher stage (from pN0 with HE to pN1mic with IHC).

Lymph nodes remain an important issue, as it concerns an introduction of node stage differentiation across FIGO stages into N1-N2-N3 or at least N1, which is not yet recognized like in the Tumor-Node-Metastasis system of UICC. On the contrary, the N feature may be ignored and FIGO staging may not include lymph nodes metastases as a separate prognostic and predictive factor. It seems that the potential applicability of lymph node biopsy should be taken into account before each surgery for better staging. It seems also apparent that, based on a series of experiments, it is impossible to determine the usefulness of ultrastaging. It is therefore reasonable to propose an international trial to analyze cost-, time- and long-term prognostic effectiveness of removal and ultrastaging of all the lymph nodes at all stages of cervical cancer.

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