The sentinel node concept in early cervical cancer performs well in tumors smaller than 2 cm.

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Abstract

Objective. The aim of the study was to evaluate the sentinel node (SLN) concept for lymphatic mapping in early stage cervical cancer.

Methods. 105 women with early stage (1a1-2a) cervical cancer were scheduled for the sentinel node procedure in conjunction with a complete pelvic lymphadenectomy. The day before surgery, 1-1,5 mL 120MBq Tc^{99} albumin nanocolloid was injected submucosally at four points around the tumor followed by a lymphoscintigram (LSG) to achieve an overview of the radiotracer uptake.

Results. During surgery, the overall detection rate (gamma probe) of at least one SLN was 90% (94/105 women) whereas at least one SLN was identified in 94% (61/65 women) with a tumor $\leq$ 2 cm. Bilateral SLNs were identified in 62/105 (59%) of the women.

Among 18 women with any metastatic lymph node 17 had a metastatic SLN (sensitivity 94%, 95% CI 73-100%). Among 61 women with a tumor $\leq$ 2 cm, all 5 women with any metastatic lymph node also had a metastatic SLN (sensitivity 100%). One woman with a 1.5 cm squamous epithelial carcinoma had metastatic positive SLNs on each side but also one metastatic bulky (>2 cm) node without radiotracer uptake. The negative predictive value for patients with cervical cancers $\leq$ 2 cm was 100%.

Conclusions. The SLN-technique seems to be an accurate method for identifying lymph node metastases in cervical cancer patients with tumors 2 cm or smaller. In case of a unilateral SLN only, a complete lymphadenectomy should be performed on the radionegative side. All bulky nodes must be removed.

Introduction

In early cervical cancer, identification of tumor spread to regional lymph nodes is mandatory to schedule patients for adequate treatment and to provide prognostic information. So far, a complete pelvic lymphadenectomy is usually performed.

However, a complete pelvic lymphadenectomy is associated with short and long term morbidity such as lymphedema, lymphocele and pelvic nerve impairment [1].

The sentinel node (SLN) concept has been proven safe in early carcinoma of the breast [2, 3, 4], avoiding total axillary dissection. For the same reasons, the SLN technique is now commonly used in the evaluation of certain malignant melanomas [5, 6]. Moreover, several reports confirm that the SLN concept is safe for lymphatic mapping also in squamous cell carcinoma of the vulva [7] .

In cervical cancer, studies have indicated that the pelvic SLN status may accurately predict the state of the regional lymph nodes [8]. Thus, the SLN concept in early cervical cancer possibly could reduce morbidity, caused by a complete lymphadenectomy and be beneficial for these patients[9].

The aim of the study was to evaluate feasibility, accuracy, and technical failure rate of the SLN concept in early stage cervical cancer with the perioperative use of a gamma probe aided by a preoperative lymphoscintigram (LSG). We also wanted to evaluate the feasibility of the SLN concept in conjunction with a robot assisted laparoscopic approach.

Materials and methods

From March 2005 to April 2009 a total of 105 women presenting with early stage (1a1-2a) cervical cancer were scheduled for the sentinel node procedure in conjunction with a complete pelvic lymphadenectomy, at the department of Obstetrics and Gynecology at Lund University Hospital, Lund, Sweden. In 90 of 105 patients (86 %) a robot assisted laparoscopic approach (da Vinci Surgical system, Intuitive surgical Inc, Sunnyvale, CA) was used, whereas the remainder had either open surgery or a traditional laparoscopic approach (Table 1).

As radiotracer, we used 1.5 mL (120MBq) Tc^{99} human-albumin nanocolloid (GIPHARMA, Saluggia, Italy). Under direct visualization a four quadrant submucosal peritumoral injection of the radiotracer was performed approximately 18 hours before onset of surgery by the surgeon or the assisting surgeon. Immediately after the injection, a 15 minute dynamic (anterior) LSG with a final picture after 45-60 minutes was performed. For logistic reasons, seven patients were injected with Tc-99 the morning of the surgery, but had no LSG.

During surgery we used a gamma probe (Neo2000® laparoscopic probe, Neoprobe Corporation, Dublin OHIO*)* to detect the SLN having the LSG chart exposed for additional guidance. With the probe, we systematically scanned the pelvic side walls, the presacral area and the paraaortic area up to the level of the inferior mesenteric artery. Any lymph node with a radioactivity of at least five times the background count was considered a SLN and was sent separately for patho-histological evaluation (frozen section as well as full final evaluation). We also separately removed enlarged but radionegative nodes. Then, a complete bilateral pelvic lymphadenectomy was performed starting with the common iliac nodes (boundary five centimeters cranial of the bifurcation of the iliac artery), followed by the external iliac nodes (distal boundary the Cloquet's node, lateral boundary the genito-femoral nerve), and the obturator nodes (distal boundary the pubic bone, dorsal boundary the obturator nerve).

If case of metastatic nodes the radical hysterectomy was abandoned in favor of radiation treatment with concomitant weekly cisplatinum. For the histological examination, the SLNs were divided in at least two pieces for frozen section, and at least one section was stained from each piece with haematoxylin and eosin (H&E), and evaluated microscopically peroperatively. Thereafter, the tissue was fixed in 4% phosphate buffered formaldehyde and further processed for permanent sections. If no metastases were found at least two additional sections were obtained from paraffin-embedded tissue, at distances of 0.2 mm and stained with H&E. Beginning in December 2007 negative SLN slides were additionally stained by a pan cytokeratin cocktail MNF116 (Dako Canada, ON) immunoperoxidase stain. The remaining non-SLNs were fixed in 4% phosphate buffered formaldehyde. After fixation, each lymph node was cut in 3 mm thick slices and at least 1 slice per lymph node was histo-pathologically analyzed after staining with H&E.

The performances of the diagnostic tests are summarized by sensitivity, specificity and negative predictive values with exact confidence intervals (CI) based on the binomial distribution.

The use of the radioactive tracer Technetium^{99} (Tc^{99}) was approved by the local authorities, and the study was approved by the Regional Ethical Board, University of Lund.

Results

The median age of the patients was 40 years (range 24-76). The clinico-pathologic characteristics of the patients are summarized in Table 1. The tumor was less or equal to 2 centimeters in 62 % (*n*=65), and larger than 2 centimeters in 38% (*n*=40) as measured preoperatively by visualization, CT-scan or MRI (mean 1.8 centimeters and median 1.5 centimeters). Of the women, 60 (57%) had a squamous cell carcinoma, 44 (42%) had an adenocarcinoma and one woman had a tumor with a predominant neuro-endocrine histopathology. The most frequent stage was 1b1 (66%). The vast majority of surgical procedures (86 % n=90) were performed by robot assisted laparoscopy. One patient was converted from robot assisted laparoscopy to laparotomy due to robot arm failure. Radical trachelectomy was performed in 9 patients (of which 4 with the robot), 83 patients had radical hysterectomy and in 13 patients only pelvic lymphadenectomy was performed, since lymph node metastases were diagnosed during surgery.

The overall detection rate of at least one SLN was 90% (94/105 patients) and 94% (61/65 patients) in patients with tumor equal or smaller than 2 cm (Table 2). Bilateral SLNs were identified in 59% (62/105) of the patients. In patients with tumor equal or smaller than 2 cm, bilateral SLNs were detected in 65 % (42/65), whereas in patients with tumor larger than 2 cm, bilateral SLNs were found in only 50% (20/40). No difference in detection rate between squamous cell carcinomas and adenocarcinomas was observed. The LSG showed “hot” SLNs in 85 out of 97 patients (88%), which was slightly less compared with the detection rate with the gamma probe, (Table 2). The median number of SLN/side was 1 (range 0-4) on both the right and left side. The mean number of SLN/side was 1.4 (SD 1.1) on the right side and 1.2 (SD 1.1) on the left side. The mean number of removed and analyzed pelvic lymph nodes per side was 12.2 (SD 6.4) on the right side and 11.6 (SD 5.6) on the left side. Two women both with tumors larger than 3 centimeters had no identified SLNs either with the probe or with the

LSG but one had a bulky metastatic node and the other woman had metastases in 14 out of 27 analyzed lymph nodes.

Among 18 women with at least one metastatic lymph node, 17 also had a metastatic SLN. One woman with a stage 2a squamous cell carcinoma of 3.5 centimeters had one metastatic non SLN on the left side but the bilateral (one on each side) SLNs were without tumor. Five out of the 61 women (8%) with a tumor size of 2 centimeters or less had lymph node metastases, all identified in SLNs (sensitivity 100%) (Table 2). Another woman with a stage 1b1 squamous carcinoma of 1.5 centimeters had metastatic SLNs on both sides but also one radionegative metastatic bulky node. The negative predictive value for patients with cervical cancers diameter equal to 2 centimeters or less was 100%.

Laparoscopic robot assisted pelvic SLN -procedure was performed in 90 patients. In the separate analyses including only the laparoscopic robot assisted procedures, the detection rate, sensitivity and negative predictive value did not differ from the total material.

The intraoperative frozen section of SLNs identified metastatic disease in 14 out of 18 patients, with metastatic SLNs in the final histology. The remaining four “frozen section negative” SLNs contained micrometastases between 0.1 and 0.5 mm. Metastases were found exclusively in the SLNs in 14 out of 18 patients. Two of these patients had metastases in two of the SLNs

Frozen section confirmed metastases in the five patients with bulky (>2 cm) suspicious metastatic nodes identified during surgery. In two women the bulky nodes were gamma-positive with the probe. Another patient had bilateral metastatic SLN in addition to one radio-negative bulky metastatic node (this patient is mentioned above). In two patients with bulky metastatic nodes no SLN were identified.

Discussion

The detection rate of a SLN in this study was 90% which is similar to other published series [10, 11]. In tumors equal or smaller than 2 cm the detection rate was higher (94 %) as also shown by Altgassen et al [11]. The combined use of radiotracer and blue dye may increase the detection rate a few percent. However, it may be confusing if several options for definitions of SLNs are possible. Moreover the timing of the blue dye injection is crucial, and any delay in the protocol could influence the accuracy of the tracing. In addition, there is a small risk of allergic reactions using the blue dye [12], why we refrained from the blue dye technique. In other tumors types such as malignant melanoma, breast cancer, vulvar cancer or penis cancer the SLN concept is reliable when the tumor is not too large [13].

Since the lymphovascular drainage from cervix divides from the midline to both pelvic side walls the SLN has to be detected per hemi-pelvis rather than per patient[14]. In the literature, the bilateral detection rate is reported between 24-88% compared with 59% in our series [3, 10, 11]. Several of the false negative SLNs in the literature have been in patients with unilateral SLNs, and the “false” negative metastatic node was found on the contralateral side [10, 15]. Thus, if no SLN at one pelvic side wall is identified a complete lymphadenectomy on this side must be performed.

From studies in breast and vulvar cancer it is known that bulky metastatic nodes may cease to receive lymphatic flow due to blockage of the lymphatic channels [16]. In this study five patients had bulky suspiciously metastatic nodes at surgery and metastases were found in all these nodes. One patient had a radionegative bulky metastatic node on one side in addition to bilateral metastatic SLN. In a study of Altgassen et al. there was no data on or any discussion about enlarged or suspicious-lymph nodes [11]. When the disease is metastatic, the lymphatic flow may bypass-a bulky metastatic node and the radiotracer can take another route and identify any possible lymph node as SLN. In our study this may have been the

explanation for one false negative SLN in a stage 2 A 3.5 cm large tumor. Pre-operative imaging by MRI and/or CT scan increases the possibility to identify enlarged bulky nodes. Furthermore, we believe the enhanced visualization with the robotic laparoscopic 3D vision and magnification facilitates the identification of lymph nodes in general and non-SLN tumor suspect nodes in particular adding extra accuracy to the SLN concept.

In patients with tumors size 2 centimeters or less the sensitivity for the SLN concept was 100 % as all five women with lymph node metastases were identified. Four SLNs were negative in the frozen section but micro metastases less than 0.5 mm were found at serial sectioning and staining with H&E. The false negative SLNs in frozen section indicate the importance of further formalin fixation and serial sectioning of the SLNs. In our study, intraoperative assessment of SLNs allowed immediate detection of metastases to determine whether radical hysterectomy or chemoradiation should be performed. Serial sectioning to evaluate the SLNs has demonstrated an increased detection rate of metastases in up to 10–15 %. It has been shown in breast cancer patients that 10 % had occult lymph node metastases, 16 % in the SLNs and 4% in other lymph [17]. However, the clinical significance of a micro-metastasis (0.2-2.0 mm) or even smaller tumor cell conglomerates (<0.2 mm) is not yet determined but those patients may have increased risk of loco-regional recurrences. In cervical cancer a local regional recurrence worsens the prognosis significantly and leads to major surgery, often combined with chemoradiation in cases where the recurrent tumor may be curable. The search for micro-metastases by serial sectioning of all lymph nodes is time consuming whereas serial sectioning on targeted SLNs only is less labor intense and may result in high metastatic yield.

The SLN concept with a gamma probe and a pre-operative LSG may improve the chance to find metastasis in unusual locations such as the presacral area, the higher common iliac region and the lower para-aortic areas, where up to 10% of the metastatic

nodes are found [3]. On the other hand, radioactive lymph nodes may be difficult to find close to the cervix, due to background radioactivity from the injection in the cervix. However, if the parametria contain metastatic lymph nodes the nodes are removed and analyzed en blocque with the cervical specimen at radical hysterectomy or radical trachelectomy.

The negative predictive value for a SLN free of disease in this study was 99%, which indicating a low probability of failure. In a recent case control study the SLN concept has detected an increased number of lymph node metastases (17%) compared to a complete lymphadenectomy (7%) (Ref Gortzak-Uzan Gynecologic Oncology 116 (2010) 28–32). If the patients are divided by tumor size preoperatively, the negative predictive value for tumors equal to 2 centimeters or smaller was in this study 100%. Thus, the concept for tumors equal to 2 centimeters or less is safe. In cervical tumors 2 centimeters or less, a similar high negative predictive value 99.1 % has been shown in a large multicenter study by Altgassen et al [11]. On the other hand, in our study the negative predictive value for patients with tumors larger than 2 centimeter was 95 %.

Pre-operative LSG may enhance the possibility to detect SLNs in the presacral and the common iliac artery or lower para-aortic region. However, in our material the detection rate in the pre-operative LSG was lower, compared with the per-operative gamma probe corresponding with other reports [18]. Though, SPECT-CT with three dimensional images may improve pre-operative imaging and make the detection easier and more precise [19,20] .

Studies with other new techniques such as CT-PET have shown high specificity in predicting metastatic lymph nodes but limited sensitivity. CT-PET may be used as a part of the preoperative investigation of cervical cancer patients but cannot replace the lymphatic surgery, as CT-PET presently is unable to identify metastases less than 4 millimeters [21].

The results from the present and other studies indicate a role for SLN concept in patients with cervical tumors 2 centimeters or less and show a low false negative rate. If there is no identifiable SLN on either of the pelvic walls of the patient, a complete lymphadenectomy should be performed at this side. For reasons discussed earlier it is important that bulky nodes are removed. For the early cancer of 2 centimeters or less without bulky nodes and a detectable SLN on each pelvic side there is reason to recommend a sharp SLN protocol instead of a complete pelvic lymph node extraction. The recommendation would include a follow up protocol with an observational prospective multi-center study, including QualityofLife analyses to find negative side effect of the complete pelvic gland extraction compared to the sharp SLN concept, but also be initiated to further establish the safety of omitting complete lymphadenectomy in patients with no metastases in sentinel nodes.

Conflict of Interest Statement

None of the authors has any conflict of interest related to this work.

References

[1] Musch M, Klevecka V, Roggenbuck U, Kroepfl D. Complications of pelvic lymphadenectomy in 1,380 patients undergoing radical retropubic prostatectomy between 1993 and 2006. J Urol 2008;179:923-8; discussion 928-9.

[2] Veronesi P, Rodriguez-Fernandez J, Intra M. Controversies in the use of sentinel nodes: microinvasion, post surgery and after preoperative systemic treatment. Breast 2007;16 Suppl 2:S67-70.

[3] Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D *et al*. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol 2005;98:281-8.

[4] Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994; 220:391-8; discussion 398-401.

[5] Callejo Peixoto I, Meneses e Sousa J. Clinical and biological aspects of sentinel node biopsy in malignant melanoma--an update. Clin Transl Oncol 2005;7:145-9.

[6] Reintgen D, Pendas S, Jakub J, Swor G, Giuliano R, Bauer J *et al*. National trials involving lymphatic mapping for melanoma: the Multicenter Selective Lymphadenectomy Trial, the Sunbelt Melanoma Trial, and the Florida Melanoma Trial. Semin Oncol 2004;31:363-73.

[7] de Hullu JA, Hollema H, Piers DA, Verheijen RH, van Diest PJ, Mourits MJ *et al.* Sentinel lymph node procedure is highly accurate in squamous cell carcinoma of the vulva. J Clin Oncol 2000;18:2811-6.

[8] Popa I, Plante M, Renaud MC, Roy M, Tetu B. Negative sentinel lymph node accurately predicts negative status of pelvic lymph nodes in uterine cervix carcinoma. Gynecol Oncol 2006;103:649-53.

[9] Levenback CF, van der Zee AGJ, Rob L, Plante M, Covens A, Schneider A *et al.* Sentinel lymph node biopsy in patients with gynecologic cancers. Expert panel statement from the International Sentinel Node Society Meeting February 21, 2008. Gynecol Oncol 2009;114:151-156.

[10] Hauspy J, Beiner M, Harley I, Ehrlich L, Rasty G, Covens A. Sentinel lymph nodes in early stage cervical cancer. Gynecol Oncol 2007;105:285-90.

[11] Altgassen C, Hertel H, Brandstadt A, Kohler C, Durst M, Schneider A. Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO Study Group. J Clin Oncol 2008;26:2943-51.

[12] King TA, Fey JV, Van Zee KJ, Heerdt AS, Gemignani ML, Port ER *et al.* A prospective analysis of the effect of blue-dye volume on sentinel lymph node mapping success and incidence of allergic reaction in patients with breast cancer. Ann Surg Oncol 2004;11:535-41.

[13] Hauspy J, Beiner M, Harley I, Ehrlich L, Rasty G, Covens A. Sentinel lymph node in vulvar cancer. Cancer 2007;110:1015-23.

[14] Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol 2003;91:494-503.

[15] Levenback C, Coleman RL, Burke TW, Lin WM, Erdman W, Deavers M et al. Lymphatic mapping and sentinel node identification in patients with cervix cancer undergoing radical hysterectomy and pelvic lymphadenectomy. J Clin Oncol 2002;20: 688-93.

[16] Fons G, ter Rahe B, Sloof G, de Hullu J, van der Velden J. Failure in the detection of the sentinel lymph node with a combined technique of radioactive tracer and blue dye in a patient with cancer of the vulva and a single positive lymph node. Gynecol Oncol 2004;92:981-4.

[17] Weaver DL: Assessing the significance of occult micrometastases in axillary lymph nodes from breast cancer patients. Breast J 2006;12:291-3.

[18] Vieira SC, Sousa RB, Tavares MB, Silva JB, Abreu BA, Santos LG *et al*. Preoperative pelvic lymphoscintigraphy is of limited usefulness for sentinel lymph node detection in cervical cancer. Eur J Obstet Gynecol Reprod Biol 2009;145:96-9.

[19] Ibusuki M, Yamamoto Y, Kawasoe T, Shiraishi S, Tomiguchi S, Yamashita Y, *et al.* Potential advantage of preoperative three-dimensional mapping of sentinel nodes in breast cancer by a hybrid single photon emission CT (SPECT)/CT system. Surg Oncol 2009 May 11 (Epub ahead of print).

[20] Vermeeren L, Valdes Olmos RA, Meinhardt W, Bex A, van der Poel HG, Vogel WV *et al.* Value of SPECT/CT for detection and anatomic localization of sentinel lymph nodes before laparoscopic sentinel node lymphadenectomy in prostate carcinoma. J Nucl Med 2009;50:865-70.

[21] Chung HH, Park NH, Kim JW, Song YS, Chung JK, Kang SB. Role of integrated PET-CT in pelvic lymph node staging of cervical cancer before radical hysterectomy. Gynecol Obstet Invest 2009;67:61-6.