Promising results using sentinel node biopsy as a substitute for radical lymphadenectomy in endometrial cancer staging

Berit Jul Mosgaard[1], Vibeke Ravn Skovlund[2] & Helle Westergren Hendel[3]

ABSTRACT

INTRODUCTION: The objective of this study was to evaluate the efficacy of the sentinel node (SN) procedure in endometrial cancer patients.

MATERIAL AND METHODS: This was a prospective follow-up study including patients referred to Herlev Hospital, Denmark, to be treated for endometrial cancer in the period from October 2005 to December 2008. Hysteroscopy was performed with a 4.5 mm hysteroscope. Injections of 100-150 MBq ^{99m}Tc-traced colloid were administered subendometrially, and a dynamic scintigram was made. SN(s) identified with a gammaprobe were resected at the operation, and frozen sections were performed, followed by radical pelvic and para-aortic lymphadenectomy.

RESULTS: A total of 32 patients were included. Among patients without clinical macro-metastases (n = 27), the SNs were detected by gamma probe in 23 (85.2%), and in most patients (n = 17, 74.0%) one (n = 12) or two (n = 5) SNs were found. The consistency between the scintigram and per-operative findings increased from 50.0% to 78.9% when the dose of ^{99m}Tc was increased to 150 MBq, mostly because the detection failure rate was lower at the higher dose: 4.8% versus 18.2%. By frozen section all macro-metastases were confirmed, but only one micro-metastasis was diagnosed. All subsequent lymph node metastases found in the final histology were found in sentinel nodes, i.e. no false negative sentinel nodes were found.

CONCLUSION: The SN procedure can be used for endometrial cancer and it has a high detection rate and no false negative SNs were detected. The sensitivity of the SN procedure may be increased by the use of single-photon emission computed tomography (SPECT)/computed tomography (CT) and peroperative cytokeratin (CK) staining of the SN(s).

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TRIAL REGISTRATION: The study was registered with the Danish Data Protection Agency.

The impact of pelvic and para-aortic lymphadenectomy is controversial. The therapeutic effect is regarded as debatable and because the patients who suffer from endometrial cancer are often elderly with co-morbidities they may have an increased perioperative risk of complications and long term side-effects [1-3]. This is reflected in the national Danish recommendations (2005) that include pelvic lymphadenectomy in high risk patients only. Furthermore, para-aortic lymphadenectomy is not recommended except in exceptional cases.

Among 600 endometrial cancer patients, a significantly improved overall survival was demonstrated in patients having pelvic and para-aortic lymphadenectomy compared with patients having pelvic lymphadenectomy only. The difference was shown in both intermediate-risk and high-risk patients. Furthermore, it has been shown that even low-risk patients may have lymph node metastases [3].

The previous studies evaluating the SN biopsy in endometrial cancer are small and not comparable due to differences in procedures used. The detection rate, sensitivity and negative predictive value, therefore, remain unknown [1, 2, 4-7]. The aim of the present study was to evaluate the feasibility and safety of the sentinel node (SN) procedure in endometrial cancer patients in a routine clinical setting before initiating studies to determine if the SN procedure can be used as a substitute for radical pelvic and para-aortic lymphadenectomy in endometrial cancer staging.

MATERIAL AND METHODS

From October 2005 to December 2008, patients referred to the Department of Gynaecology and Obstetrics, Herlev Hospital, Denmark, who were to be treated for an adenocarcinoma of the corpus uteri were invited to participate in the study. Patients with low-risk tumours (grade I and < 50 % myometrial invasion) who were at high risk of perioperative complications were not included.

Hysteroscopic procedure

Hysteroscopy was performed with a 4.5 mm hysteroscope (Compact Miniature Hysteroscope 0°, Storz) in the morning on the day of the operation, without or with local analgesia (Sandonest 20 mg/ml, Septodont, France) by a single investigator trained in hysteroscopy.

In case the tumour was well defined in the uterine

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1) Department of Gynaecology and Obstetrics, Herlev Hospital
2) Department of Pathology, Herlev Hospital
3) Department of Clinical Physiology, Herlev Hospital

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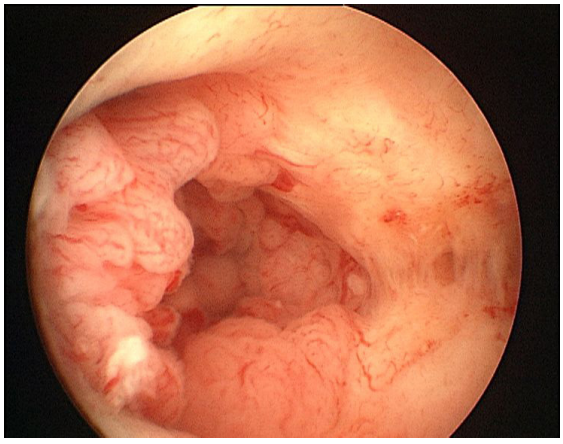

Endometrial cancer seen by hysteroscopy.

cavity, subendometrial injections of 100 or 150 MBq ^{99m}Tc-traced colloid (100-150 MBq ^{99m}Tc-nanocoll in 3 ml NaCl) were given in four sites around the tumour (peritumoural injection). If the tumour was not well defined or diffuse in the whole cavity, the injections were given in the fundus, in the posterior, anterior, and lateral walls at midlevel (quadrant injection).

Scintigraphy

Imaging began no more than 15 min. after completion of the injections and included dynamic imaging (30 sec. per frame in 30 min.) and static imaging (300 sec.) when the SN was identified. Either a Skylight (Phillips, The Netherlands) or a Forte (Phillips, The Netherlands) gamma camera equipped with a Vertex general-purpose (VXGP) collimator was used. Image sizes were 64 × 64 for dynamic images and 256 × 256 for static images. A transmission image for body contour was obtained using a ^{57}Co flood source placed between the patient and the camera for 10-15 sec. during the static image acquisition

Operative procedure and gamma probe detection

The operation was performed via a medial incision. Peritoneal washing for cytology was performed. This procedure was followed by total hysterectomy and bilateral salpingo-oophorectomy before identification of the SN(s) in order to avoid disturbing radiation from the uterus. Clinically evident lymph node metastases, i.e. macrometastases, was evaluated by frozen section and further handled as SN, well aware that substantial tumour infiltration in a lymph node excludes the patient from the SN procedure. SN(s) were identified with a gamma probe (Neoprobe Corporation, Dublin, Ohio 43017-1367). SN(s) were resected and sent to frozen section and cytological investigation. A bilateral radical lymphadenectomy in the pelvic region was performed including lymph nodes from the vasae iliaca communis and externa to the fossa obturaturia, and para-aortic lymphadenectomy was performed from the aorta bifurcation to the renal vein. In case of serous, clear-cell or undiffentiated carcinoma, omentectomy was also performed.

Histology

The SNs removed were investigated by frozen section and cytological investigation. If size permitted, the SNs were divided into two equal parts; one part for frozen section and one for imprints stained by toluidine blue (Toluidine blue, Merck 1.15930). The latter was formalin-fixed and paraffin-embedded together with the lymph node tissue used for frozen section. In case the frozen section, imprint and subsequent routine sections of all SN tissue blocks stained with haematoxylin and eosin (H/E) were negative, additional pairs of sections were subsequently cut with three sections per mm throughout the lymph node tissue. One section of each pair was H/E-stained, the other section was immunostained with cytokeratin (CK) 1% AE1/AE3 using the Envision staining kit (K4000, DAKO, A/S Denmark). All subsequently removed lymph node tissue was formalin-fixed and paraffin-embedded and subsequently studied by H/E-staining on two 4 µm step-sections from each tissue block. In case of metastases, it was noted whether these were single cells (< 10 cells), micro-metastasis (< 2 mm), and if perinodal spread was present.

Statistics

Fisher's exact test was used for testing difference in consistency between scintigram and perioperative findings according to the two different doses of ^{99m}Tc-traced colloid.

Ethics

All patients gave their informed consent. The study was in accordance with the Helsinki II declaration and was approved by the local ethical committee (KA 05007). The radiation dose to the patient was 1 mSv.

Trial registration: The Danish Data Protection Agency.

RESULTS

Study population

A total of 32 patients were included. The characteristics of the patients are given in **Table 1**.

! ABBREVIATIONS

CK = cytokeratin
Co = cobalt
FIGO = International Federation of Gynecology and Obstetrics
MBq = megabecquerel
SN = sentinel node
Tc = technetium

TABLE 1

Patient characteristics (N = 32).

Age, yrs, mean (range)	67.2 (56-83)
Body mass index, kg/m^2, mean (range)	25.09 (15.8-32.9)
Postmenopausal, n (%)	31 (96.9)
ASA score, n (%)	
1	7 (21.9)
2	24 (75.0)
3	1 (3.1)
Co-morbidity, n (%)	
No (except hypertension)	21 (65.6)
Yes	11 (34.4)
Previous abdominal surgery, n (%)	
No	26 (81.3)
Yes, lower abdomen	6 (18.8)
Yes, upper abdomen	0 (0)
Follow-up July 2010, months, mean (range)	31 (2-54)

ASA = American Society of Anesthesiologists.

TABLE 2

Operation and perioperative findings (N = 32).

Perioperative complications, n	0
Operating time, min., mean (range)	135 (80-240)
Blood loss, ml, mean (range)	318 (100-700)
Surgical procedures, n (%)	
Simple hysterectomy, BSO	28 (87.5)
Simple hysterectomy, BSO, omentectomy	3 (9.4)
Radical hysterectomy, BSO	1 (3.1)
Pelvic and para-aortic lymphadenectomy	25 (78.1)
Pelvic lymphadenectomy only	6 (18.8)
No lymphadenectomy[a]	1 (3.1)
SN, n, mean (range)	1,55 (1-4)
Radioactivity in SN, counts, median (range)	
No. 1	425 (13-20,000)
No. 2	200 (30-12,000)
No. 3	43 (15-960)
No. 4	217 (200-235)
Scintigram vs peroperative findings, n, mean (%)	
Consistency	19 (70.4)
Pos. scintigram, SN not found peroperatively	3 (11.1)
Neg. scintigram, SN found peroperatively	3 (11.1)
Other location of SN	1 (3.7)
Neg. scintigram, SN not found peroperatively	1 (3.7)
Macroscopic lymph node metastases, n, mean (%)	
Yes	5[b] (15.6)
No	27 (84.4)

BSO = bilateral salpingooopherectomy; SN = sentinel node.
a) Due to widespread macroscopic lymph node metastases.
b) 1 patient with clinically observed macro metastases had a malignant B-cell lymphoma without metastasis from the endometrial cancer.

Hysteroscopic procedure

The hysteroscopic procedure was uncomplicated and easy to perform in 27 patients. In five patients, however, the access to the uterine cavity was difficult or the image/overview of the cavity was suboptimal due to bleeding. Most of the patients had a localized tumour (n = 23), most often in the fundus (n = 9) or posterior wall (n = 8). A minority had a diffuse tumour (n = 9). In one patient, cervical involvement was visualized by the hysteroscopy.

Injection procedure and dose of ^{99m}Tc-nanocoll

Peritumoral injections were given in 23 patients. Quadrant injections were given in nine patients.

The first 11 patients were injected with 100 MBq ^{99m}Tc, but due to difficulties in detecting the SNs during operation, the dose was increased to 150 MBq in the remaining 21 patients.

Scintigraphic results

In eight patients, the SNs were not visualized. This was independent of the injection procedure (peritumoral or quadrant) and of the injected dose (100 MBq or 150 MBq). In the remaining 24 patients, a SN was visualized in one location in the majority of cases (90.6%). Sixteen SNs were found in the pelvis, four SNs in relation to the aortic bifurcation, and four SNs in the para-aortic region.

Perioperative findings

Five patients had clinically suspicious lymph nodes revealed at operation; four patients turned out to have metastases and one patient to have lymphoma (all with non-visualization of SN on the scintigram). Among the remaining patients (n = 27), the SN(s) were detected by gamma probe in 23 (85.2%) patients, **Table 2**. The consistency between the scintigram and the peroperative findings using a gamma probe rose from 50.0% to 78.9% when the dose of ^{99m}Tc was increased from 100 to 150 MBq (p = 0.18), and the peroperative detection rate rose from 62.5% to 94.7% (p = 0.06) **Table 3**.

Lymphadenectomy

In total, 26 of 32 patients had full pelvine and para-aortic lymphadenectomy. In one patient, lymphadenectomy was not performed due to widespread lymph node metastases revealed at surgery, and in six patients para-aortic lymphadenectomy was not performed due to adhesions from previous surgery or obesity; meanwhile, all these patients had SN(s) in the pelvis.

Histopathology

Among the 23 patients with a sufficient SN procedure preoperatively, eight patients had lymph node metastases. Among all 32 patients, suspicious lymph nodes found perioperatively in four patients and subsequently histopathologically analysed as if they were SN peroperatively showed metastases by frozen section. In two of

TABLE 3

The consistency between scintigram and preoperative findings.

	100 MBq (n = 8)	150 MBq (n = 19)	Total (n = 27)	p-value for difference between 100 MBq and 150 MBq[a]
SN in scintigram, mean, n (% (range))	6 (75 (34.9-96.8))	16 (84.2 (60.4-96.6))	22 (81.5 (61.9-93.7))	0.6
SN peroperatively, mean, n (% (range))	5 (62.5 (24.5-91.5))	18 (94.7 (74.0-99.9))	23 (85.2 (66.3-95.8))	0.06
Concordance, mean, n (% (range))	4 (50 (15.7-84.3))	15 (78.9 (54.4-93.9))	19 (70.4 (49.8-86.2))	0.18
False neg. SN, n	0	0	0	–

SN = sentinel node.
a) Fisher's exact test.

these cases, metastases were > 2mm, but only one micro-metastasis was diagnosed and only one (single cell) was suspected perioperatively by frozen section and imprinting. After step sectioning and CK staining, another four patients turned out to have lymph node metastases.

Among the 27 patients without clinically established lymph node metastases, five had microscopic lymph node metastases; of these, four patients had single-cell metastases, i.e. < 10 tumour cells in the lymph node. In all but one case, which was seen in an H/E-stained step section also, these single cells were only demonstrable in step sections stained with anti-CK staining.

In all eight patients, the lymph node metastases were demonstrated at least in the SN(s), and for some in additional nodes, too.

Among the nine patients in whom SN could not be detected preoperatively, the final histology showed lymph node metastases in one patient (micro-metastasis in two of 25 lymph nodes).

Lymph node status

Six patients had lymph node metastases in the pelvis only (66.7%), two patients had para-aortic lymph node metastases only (22.2%) and one patient had both pelvic and para-aortic lymph node metastases (11.1%). Four of nine patients had metastasis to more than one lymph node (**Table 4**)

Nine patients (28.1%) were up-staged to IIIc because of the results of the radical lymphadenectomy. Among these nine patients, five (15.6 %) were without clinically suspect lymph nodes, Table 4.

DISCUSSION

This pilot study shows that the SN procedure is safely and presumably has a high validity when performed in endometrial cancer patients by means of hysterocopically guided subendometrial injection of radio-labelled (at least 150 MBq is recommended) preoperative scintigraphy and intraoperative gamma-detection probe examination.

Our results are in accordance with the results from other small studies [5, 6, 8-10]. A direct comparison, however, is not possible as different techniques were used. In the present study, the detection rate was markedly improved when using a dose of 150 MBq ^{99m}Tc compared with 100 MBq (95.2% versus 62.5%, p = 0.06). The average detection rate (85.2%) was as high as in other studies using ^{99m}Tc-nanocoll only [10], and as high as in studies also using injections of Patent Blue [5, 6, 8]; 61-100% detection rates [9, 11]. No other studies have compared different doses of ^{99m}Tc-nanocoll.

Positive cytology was not found in early stages in this study, although the hysteroscopic procedure may increase its frequency. The prognosis due to peritoneal tumour cell dissemination in endometrial cancer does not seem to be worse than in ovarian cancer [12]. In our

TABLE 4

Histology of lymph node metastasis and stage distribution (N = 32).

Lymph nodes removed, n (range)	
Pelvic right/left	14/12 (2-35/3-29)
Para-aortic[a]	10 (4-18)
Pelvic and para-aortic total	36 (14-65)
Lymph node metastases from endometrial cancer, n (%)	
0	23 (71.9)
1	5 (15.6)
2	1 (3.1)
≥ 3	3 (9.4)
Severity of lymph node metastases, n (%)	
Macroscopic	4 (12.5)
Micrometastasis (< 2 mm), without perinodal outgrowth	1 (3.1)
Single cells (< 10 cells)	4 (12.5)
Lymph node metastasis localisation, n (%)	
Pelvic right only	2 (22.2)
Pelvic left only	1 (11.1)
Pelvic only, both right and left	3 (33.3)
Para-aortic only	2 (22.2)
Pelvic and para-aortic	1 (11.1)
FIGO stage without/with lymphadenectomy, n	
Ia	2/2
Ib	14[b]/12
Ic	8/5
IIb	3/3
IIIc	4[c]/9[d]

FIGO = International Federation of Gynecology and Obstetrics.
a) Among patients with para-aortic lymphadenectomy.
b) 1 patient with macrometastases clinically had a malignant B-cell lymphoma without metastases from the endometrial cancer.
c) 4 patients with macroscopic lymphnode metastases could be clinically staged as st IIIc.
d) The 5 patients with micrometastases were clinically stage I (2 were stage 2 Ib and 3 were stage Ic).

study, the hysteroscopy was easily performed and well tolerated by the patients, and the detection rate was as high as by subserous injections at laparotomy [6]. Different locations of injection have previously been tested: In the cervix [5, 9, 13], in the subserosal myometrium [11] combined with injections into the cervix and the uterine fundus, and hysteroscopically guided subendometrial injections like in this study [6, 9, 10]. Detection rates have generally been reported to be high (88-100%), except in one study [6].

We found good agreement between the scintigram and the peroperative localization of SN (up to 78.9%). This is superior to Ballester et al [14] which is probably owed to a shorter time interval between the scintigraphy and the surgical SN mapping (same-day versus day-before). In the present study, metastases were demonstrated in nine of 32 patients. In eight patients, the metastases were demonstrated in the SN, in one case by frozen section peroperatively, i.e. no false negative SNs were found. Only the four clinically suspect lymph node metastases were demonstrated by the frozen section procedure. The additional metastases in SN were found by step-sectioning and CK staining, and in most cases they were very small, representing single CK-positive tumour cells. It is presently unclear whether such single cells have any prognostic impact and whether in this way the lymphadenectomy should have any therapeutic consequence. In one patient with micro metastasis, SN could not be detected preoperatively. It is not an accepted approach to include clinically suspect lymph nodes in the SN procedure. In breast or vulva carcinoma, these lymph nodes are preoperatively diagnosed by fine needle aspiration if possible. By routine procedure you investigate 1-2 H/E-stained sections per tissue block to exclude lymph node metastases. By this procedure, no false negative SNs were demonstrated in this study. Using serially cut sections throughout the lymph node does not significantly improve the detection rate of lymph node metastases, but should be performed to state "no false negative SNs". However, according to the SN procedure in vulva cancer and breast cancer, this situation will give rise to radical lymphadenectomy, and staging will therefore be sufficient. Previous studies have found metastases in 5-25% of the patients [5, 8]. In general, the negative predictive value of the SN technique is high.

We found para-aortic SN only in six patients and additional pelvine SN in three patients. Three patients had metastases to para-aortic sentinel lymph nodes. In two of these patients, the metastases were only demonstrated in these nodes. If we still hesitate to perform the para-aortic lymphadenectomy, then 15-20% of the patients in intermediate-risk and high-risk endometrial cancers will be under-staged [15-17]. The method could increase the detection of micrometastasis, which is probably found only rarely by the standard procedure and this could perhaps help identify patients who have early relapse even after extensive staging operation without any initial detection of disseminated disease. Due to the possibly increased perioperative risk and the long-term side effects of full pelvine and para-aortic lymphadenectomy, SN should be further investigated as a possible substitute for extensive surgery in lymph node staging. As metastases are often very small, a valid negative SN status can only be obtained post-operatively by step-sectioning and CK staining throughout the SN. A positive SN may, however, be obtained peroperatively by frozen section in approx. half of the positive cases. The sensitivity of the SN procedure may be increased by the use of SPECT/CT and peroperative CK staining of the SN(s), a new perspective to be included in future studies.

CORRESPONDENCE: *Berit Jul Mosgaard*, Gynækologisk-Obstetrisk Afdeling, Herlev Hospital, 2730 Herlev, Denmark. E-mail: bjmosgaard@dadlnet.dk

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