The sentinel node concept in endometrial cancer: histopathologic validation by serial section and immunohistochemistry

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Received 30 January 2007; revised 21 May 2007; accepted 22 May 2007

Background: The sentinel node (SN) is defined as the first node in the lymphatic system that drains a tumor site. If the SN is not metastatic, then all other nodes should also be disease-free. We used serial sections and immunohistochemical (IHC) staining to examine both SN and non-sentinel nodes (non-SNs).

Patients and methods: Twenty-three patients (median age 69 years) with early endometrial cancer underwent a laparoscopic SN procedure based on a combined detection method, followed by complete laparoscopic pelvic lymphadenectomy. If the SN was free of metastasis by both hematoxylin and eosin (H&E) and IHC staining, all non-SNs were also examined by the combined staining method.

Results: SNs were identified in 19 patients (82.6%). A total of 47 SNs were removed (mean 2.5). Ten SNs (21.3%) from five patients (26.3%) were found to be metastatic at the final histologic assessment. In 14 patients, no metastatic SN involvement was detected by H&E and IHC staining. In these 14 patients, 120 non-SNs were examined by serial sectioning and IHC, and none were found to be metastatic.

Conclusion: The SN procedure appears to reliably predict the metastatic status of the regional lymphatic basin in patients with early endometrial cancer.

Key words: endometrial cancer, immunohistochemistry, micrometastasis, pathologic staging, patent blue, radiocolloid, sentinel node

Introduction

Endometrial carcinoma is the most frequent gynecologic malignancy in industrialized countries. In the United States, 41,200 new cases of endometrial cancer and 7,350 deaths were reported in 2006 [1]. In Europe, 4,600 new cases of endometrial cancer were diagnosed in 1995, corresponding to a standardized incidence rate of 13.6 cases per 100,000 women.

Surgical management of early-stage endometrial cancer includes peritoneal cytology, total hysterectomy with bilateral salpingo-oophorectomy and lymph node sampling. Histological grade, the depth of myometrial involvement and lymph node status are the main prognostic factors in endometrial cancer [2]. The International Federation of Obstetricians and Gynecologists [3] recommends pelvic lymphadenectomy to assess lymph node status in this setting, whereas the Gynaecological Oncology Group recommends systematic pelvic and para-aortic lymphadenectomy [4, 5]. Alternative modes for assessing the status of pelvic lymph nodes, including imaging techniques, have not yet equaled the ‘gold standard’ method, namely histological examination of nodes from the pelvic dissection specimen [6].

Lymphatic mapping with sentinel lymph node biopsy has emerged as an alternative to systematic lymphadenectomy, and also reduces the morbidity of this procedure. The sentinel node (SN) has been explored in many solid tumor sites and has become the standard of care in patients with cutaneous melanoma and breast cancer [7–9]. Few studies have examined the use of the SN procedure in endometrial cancer [10–23]. In endometrial cancer, the SN procedure is increasingly used as an alternative to systematic lymphadenectomy, with the aims of avoiding excess morbidity and improving lymph node staging, while at the same time preserving the control of regional disease. Endometrial cancer is ideal for lymphatic mapping because the lymphatic drainage is ambiguous and complex. Potential at-risk lymph node basins are found along the obturator, external iliac and aortic vessels. Identification of a reliable SN would preclude the need for complete lymphadenectomy, which is particularly valuable for morbidly obese patients in whom lymphadenectomy can often be difficult to perform. Finally, with the application of minimally invasive approaches
to endometrial cancer, the combination of laparoscopic hysterectomy and SN biopsy constitutes an appealing alternative to current practice.

The concept of SN biopsy is based on two basic principles: the existence of an orderly and predictable pattern of lymphatic drainage to a regional lymph node basin, and the functioning of a first lymph node as an effective filter for tumor cells. These nodes (or node) are the SNs and are predictive of the local nodal network. Therefore, in theory, the identification of SNs and their histological status can be used to determine the extent of nodal dissection required.

An important parameter in studies concerning this question is the false-negative rate, which corresponds to the number of false-negative procedures divided by the sum of the true-positive and false-negative procedures (1 – sensitivity), although some investigators calculate it in their own way. These authors did not perform the same histopathological analysis for SNs and non-SNs; thus, it has been argued that the enhanced detection of ‘occult’ metastases in the SNs may reflect the more intensive histological technique using serial sectioning and immunohistochemistry (IHC) rather than the physiological significance of the SN.

In the current study, we applied cytokeratin IHC staining to non-SNs if no metastatic SN involvement was detected by hematoxylin and eosin (H&E) and IHC staining. Our purpose was to determine whether the SN is truly the pelvic or aortic lymph node most likely to harbor metastatic tumor and to assess the true histologic false-negative rate of SN procedure at our institution in patient with endometrial cancer.

**patients and methods**

From July 2002 to December 2004, 23 consecutive patients with endometrial cancer were included in this prospective study. The inclusion criteria were biopsy-confirmed endometrial cancer of clinical stage I or II according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) [3]. All the patients underwent pre-operative blood sampling, chest radiography, hysteroscopy and pelvic magnetic resonance imaging (MRI).

Patients with stage I disease underwent laparoscopic treatment, including peritoneal washing, bilateral salpingo-oophorectomy, the SN procedure, systematic pelvic lymphadenectomy and laparoscopically assisted vaginal hysterectomy (LAVH). Patients with stage II disease underwent a peritoneal washing, bilateral salpingo-oophorectomy, the SN procedure followed by systematic pelvic lymphadenectomy and laparoscopic radical hysterectomy. In accordance with the French National Federation of Cancer [24] and the FIGO [3], no systematic para-aortic lymphadenectomy was performed. However, in accordance with our institutional protocol, para-aortic lymphadenectomy was performed when a para-aortic SN was detected or positive SNs were found by intraoperative histologic examination.

All the patients gave their written consent after receiving relevant information, including the potential adverse effects of patent blue, radiocolloid, general anesthesia and laparoscopy, and the possible need to convert to open surgery.

**sn procedure**

Four pericervical injections (1.5 cm depth) of 0.2 ml (10 MBq each) of unfiltered technetium sulfur colloid (Nanocis, CIS Bio International, Saclay, France) were administered with a 25-gauge spinal needle on the day before surgery. Scintigraphic images were obtained 2 h after the injections and then every 30 min until the SN was visualized.

Under general anesthesia, the patients were placed in a low lithotomy position. A speculum was placed in the vagina and patent blue (Bleu Paténeté V, Guerbet Laboratory, Issy les Moulineaux, France) was injected pericervically (1 ml per injection, 1.5 cm deep) with a 25-gauge spinal needle, at 3 and 9 o’clock.

After patent blue injection, the pelvic and lower para-aortic regions were carefully inspected for lymph ducts and specific dye uptake by lymph nodes. ‘Hot’ pelvic and para-aortic nodes were located by using an endoscopic gamma probe (Eurorad, Strasbourg, France) inserted through the 12-mm suprapubic trocar. Hot nodes were detected before opening the peritoneum. The gamma probe was angled laterally to avoid detection of residual radioactivity at the injection site.

After location of the SN, the peritoneum was opened above the external iliac vessels to the round ligament. Each blue and/or ‘hot’ node was removed separately in an endoscopic bag (Endocatch, Auto Suture Company, Elancourt, France).

Laparoscopic bilateral pelvic lymphadenectomy was performed systematically after the SN procedure. All node tissue along the obturator fossa and the external vessels, up to the iliac bifurcation, was extracted in an endoscopic bag. The absence of residual pelvic or para-aortic radioactivity was verified before LAVH or laparoscopic radical hysterectomy.

In accordance with the French and FIGO guidelines, para-aortic lymphadenectomy was not performed.

**histopathologic evaluation**

A pathologist inspected SNs and other pelvic nodes. Grossly metastatic nodes were sectioned. Normal-appearing SNs were cut perpendicular to the long axis. All SNs were examined intra-operatively by imprint cytology. Air-dried cytologic smears were prepared by scraping the cut surfaces and were stained using a rapid May–Grnwald–Giems method. Each half SN was sectioned at 3-mm intervals and each 3-mm section of SN was analyzed by four additional levels of 150 μm and four parallel sections; the four first sections were used for H&E staining and the four following ones were then examined by IHC with an anti-cytokeratin antibody cocktail (Cytokeratin AE1-AE3, Dako Corporation, Glostrup, Denmark).

Other nodes (non-SNs) were totally submitted and blocked individually following 3-mm distances and H&E staining. Further examination of non-SN specimens depended on SN histology. If the SN was metastatic-free by H&E and IHC, all corresponding non-SNs were examined as for the SNs, using serial sectioning and IHC staining.

The size of node metastases was estimated with an eyepiece micrometer. Micrometastasis was defined as a single focus of metastatic disease between 0.2 mm and no more 2 mm in a given node. The presence of single non-cohesive tumor cells (submicrometastasis or isolated single cells) was reported and defined as metastatic disease no more than 0.2 mm. SNs were considered positive for tumor involvement when they contained micrometastases, micrometastases or isolated tumor cells.

**analysis**

SNs were recorded as blue-stained and/or ‘hot’ (i.e. ex vivo count exceeding three times background radioactivity). The false-negative rate was defined as the number of procedures with a negative SN and one or more positive non-SNs divided by the number of procedures with any positive pelvic lymph node(s).

**results**

**patient characteristics**

The demographic data and tumor characteristics for the 23 patients are shown in Table 1. Median age was 69 years.
Among the 10 positive SNs, H&E staining revealed macrometastases in three SNs from two patients. These three SNs (both blue and hot) were all located in the medial external iliac region. One of these two patients with stage I endometrial cancer had a macrometastatic SN and a positive ipsilateral pelvic node.

Immunohistochemical analysis revealed micrometastases in six SNs and isolated tumor cells in one SN (three patients). Of these three patients, two had grade 3 and one had grade 2 endometrial cancer. Four of the six micrometastatic SNs were located in the interiliac area, one in the obturator fossa and one in the medial external iliac region. All non-SNs were negative.

The SNs of 14 patients (37 SNs) were negative by H&E and IHC staining, and their non-SNs were therefore extensively examined. A total of 120 non-SNs from these 14 patients were examined by serial section and IHC, and none were positive (no macrometastasis, micrometastasis or isolated single cells). Thus, the true histologic false-negative rate of SNs using multiple sections and IHC examination of all non-SNs for metastasis was 0%.

**Discussion**

The main problem with the SN procedure is the false-negative rate, i.e. the number of procedures in which the SN is negative but one or more pelvic non-SNs are positive, divided by the number of procedures in which any pelvic lymph node is positive. A false-negative finding understages the patient, and may result in an incorrect decision regarding the need for adjuvant therapy. Previous studies have examined the non-SNs only with standard H&E to evaluate the false-negative rate of the SN procedure. Therefore, it was not possible to determine whether detection of a metastasis in the SN was due to the physiologic significance of that node or the more extensive histopathologic technique of lymph node examination.

To validate the accuracy of lymphatic mapping and SN biopsy for patients with endometrial carcinoma, we examined both SNs and non-SNs using the same histopathological techniques. We performed multilevel sectioning and IHC analysis of tumor-free SNs as well as tumor-free non-SNs. Among the 14 patients who had negative pelvic SNs on multilevel sectioning and IHC analysis, all non-SNs, using the same histopathological analysis (serial sectioning and IHC analysis), were metastasis-free. The true negative predictive value of SN biopsy was 100%. To our knowledge, this is the first validation of the SN procedure in endometrial cancer based on combined IHC and H&E analysis of SNs and non-SNs in this setting.

The extensive histopathological analysis could be performed before the potential diffusion of the SN procedure in solid cancer. However, few studies have validated the concept of the SN procedure in other solid cancers.

In cervical cancer, only three studies have evaluated the accuracy of the SN concept [25–27]. Among 21 patients, Marchiò et al. [25], using serial sections and IHC staining to examine both SNs and non-SNs, determined whether the SN is truly the lymph node most likely to harbor metastatic tumor and assessed the 'true' histologic false-negative rate of SN in
patients with cervical cancer. Using a colorimetric method alone, the authors found a high false-negative rate of the SN biopsy, raising questions about the validity of this concept in cervical cancer. Indeed, the extended analysis of non-SNs by serial sectioning and IHC allowed the detection of only one micrometastasis in one non-SN, increasing the false-negative rate to 12.5%. In contrast to Marchiole et al. [25], Barranger et al. [26] reported a study of 18 patients with cervical cancer who underwent a laparoscopic SN procedure using a combined detection method. If the SN was free of metastasis by both H&E and IHC staining, all non-SNs were also examined by the combined staining method. In 13 patients, no metastatic SN involvement was detected by H&E and IHC staining. In these 13 patients, non-SNs were examined by serial sectioning and IHC, and none were found to be metastatic. This confirms that the SN procedure appears to reliably predict the metastatic status of the regional lymphatic basin in patients with cervical cancer. A largest study by Popa et al. [27] correlated the SN status in cervical cancer (stages I and IIA) with non-SN among 36 patients with negative SN on final histology. Using a combined detection method, no metastasis was found in any SN or non-SN by step sections and IHC. These results confirm that the SN procedure by combined detection reliably predicts the metastatic status of the regional lymphatic basin in patients with cervical cancer. However, larger studies are required before recommending the routine use of the SN approach.

Turner et al. [28] validated the concept of the SN procedure in breast cancer. They used IHC and serial sectioning to examine the SNs and non-SNs in 103 patients with breast cancer. In 60 patients whose SNs were metastasis-free, only one among 1087 non-SNs was found to be metastatic. The false-negative rate was 0.97%, equating to one misdiagnosed patient among 103. Intraoperative lymphatic mapping and sentinel lymphadenectomy using blue dye and 99m-technetium-labeled sulfur colloid were performed. Stitzenberg et al. [29] confirmed these results, validating the SN hypothesis in breast cancer. All axillary lymph nodes that were tumor free by H&E underwent additional sectioning and staining with H&E and an IHC stain. Occult SN metastases were identified in 12.7% of cases. None of the 724 non-SNs examined contained occult metastases. The SN false-negative rate was zero. This study confirms histopathologically that the SN has biologic significance as the axillary node most likely to harbor metastatic tumor. Standardization of the handling, sectioning and staining of the SN is necessary as lymphatic mapping and sentinel lymphadenectomy become integrated into the care of patients with breast cancer.

Using the SN procedure with blue dye alone in 124 patients with colorectal cancer, Wong et al. [30] showed that metastases were identified in 13 of 278 SNs and in only 5 of 1829 non-SNs (P < 0.001). The authors concluded that if the SN is negative by both H&E and IHC analysis, the probability of finding metastases in non-SN is low. In the same way, Bembenek et al. [31] in a group of 33 patients with colon cancer classified as SN negative, analyzed 1011 nodes by step sections and IHC: 14 of the 70 SNs (20%) but only 37 of 941 non-SNs had micrometastases or nanometastases (size less than 0.2 mm). In both these studies the number of patients with negative SNs and positive non-SNs by serial section and IHC, which would allow us to calculate the true false-negative rate, was not reported.

In 22 patients with oral and oropharyngeal squamous cell carcinoma, Nieuwenhuis et al. [32] validated the SN hypothesis using radioactive detection alone. In the patients with 13 radioactive SNs, none of the non-radioactive nodes (non-SNs) at final histopathologic analysis, including serial sections and IHC, contained metastasis.

In our study, occult metastases were identified in 7 of the 10 metastatic SNs. In the English literature, only three studies performed extensive histopathological analysis by serial sections and IHC [15, 19]. The prognostic significance of micrometastases is controversial in endometrial cancer. The SN biopsy based on a small number of nodes removed by patient allows focused analysis, such as multiple sectioning and IHC staining. This pathological analysis of the SNs allows the identification of significant occult metastases, which may potentially explain pelvic recurrence. Yabushita et al. [33] reported that IHC expression of cytokeratin in lymph nodes with undetected metastases by H&E staining is a risk factor for recurrence in stage I endometrial cancer.

One the limitations of this study is that the tracers were injected into the cervix, even though they were pursuing the spread of an endometrial carcinoma, not reflecting the corpus uterine drainage but the cervical pathway.
Another limitation of this study is the absence of the para-aortic lymphadenectomy to really evaluate the true false-negative rate.

In conclusion, our results suggest that the SN procedure reliably predicts the metastatic status of the regional lymphatic basin in patients with early-endometrial cancer. However, larger studies are required to confirm our initial findings.

**conflict of interest statement**

None declared.

**references**