Histopathological validation of the sentinel node concept in cervical cancer

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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 sentinel and non-sentinel nodes (non-SNs).

Materials and methods: From July 2001 to March 2003, 18 patients (median age, 48 years) with cervical cancer (stage IA2, one patient; stage IB1, nine patients; stage IB2, three patients; stage IIA, three patients; and stage IIB, two patients) 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: A mean of 2.4 SNs (range 1–5) and 8 non-SNs (range 4–14) were excised per patient. Eight SNs (18.2%) from five patients (27.8%) were found to be metastatic at the final histological assessment, including two macrometastatic SNs, three micrometastatic SNs and isolated tumor cells in three SNs. In 13 patients, no metastatic SN involvement was detected by H&E and IHC staining. In these 13 patients, 106 non-SNs were examined by serial sectioning and IHC, and none was found to be metastatic.

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

Key words: cervical cancer, immunohistochemistry, laparoscopy, lymph node metastasis, sentinel node biopsy

Introduction

Lymph node status is both a major prognostic factor and a decision criterion for adjuvant therapy in patients with cervical cancer. Pelvic lymph node metastases are only detected in 0–17% and 12–27% of women with stage IB and stage IIA cervical cancer, respectively [1–4]. Thus, as many as four out of five patients derive no benefit from pelvic lymphadenectomy; on the contrary, this procedure may increase morbidity, especially when postoperative radiotherapy is required [5]. Alternative modes of pelvic node status assessment, including imaging techniques, have not yet equalled the ‘gold standard’ method, namely histological examination of nodes recovered from the pelvic dissection specimen [6].

Recently, efforts have been made to reduce the morbidity of surgical treatment, without compromising survival, by developing laparoscopic or vaginal approaches. The sentinel node (SN) procedure has emerged as an alternative to systematic lymphadenectomy, and also to reduce the morbidity of this procedure. The theory of SN procedure was first formulated for penile cancer [7]. It implies that lymph nodes draining any one site have a hierarchical organisation through which lymph flows in a systematic order. Metastasis from a tumor drained by these lymph nodes will be first arrested by the most proximal node or nodes in this orderly arrangement. 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. Substantial evidence regarding the validity of the SN hypothesis has now been obtained by multiple studies in the literature, especially in breast cancer [8]. These studies have demonstrated that the SN is the most likely node to harbor metastatic disease in breast cancer, and when the SN is uninvolved with metastatic disease, it is highly unlikely that disease will be found in any of the non-SNs. But 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. Using serial sectioning and IHC to examine the SNs and non-SNs, Turner et al. provided histological support for the SN concept in breast cancer [9].

The validity of the SN procedure has subsequently been established in melanoma [10], vulvar cancer [11] and breast cancer [12]. In contrast, the SN procedure is still under evaluation in cervical cancer [13–16].
In this study, we used serial sections and IHC staining to examine both SNs and non-SNs. The aim was to determine whether the SN is truly the lymph node most likely to harbor metastatic tumors and to assess the true histological false-negative rate of SN in patients with cervical cancer.

Patients and methods

Eighteen consecutive women referred to our institution with cervical cancer from July 2001 to March 2003 were included in the study. The inclusion criteria were an age of between 18 and 75 years and biopsy-confirmed cervical cancer. All the patients underwent preoperative blood sampling, chest X-ray examination and pelvic magnetic resonance imaging (MRI). The disease stage was classified as recommended by the International Federation of Gynecology and Obstetrics (FIGO) [17].

Patients with stage IA2 or IB1 disease who did not qualify for neo-adjuvant chemo-radiotherapy underwent a laparoscopic SN procedure with combined detection together with systematic pelvic lymphadenectomy, and laparoscopic radical hysterectomy, the Schauta–Amreich operation or trachelectomy, depending on tumor size and the patient’s desire to preserve her childbearing potential.

Patients with stage IB2, IIA or IIB disease who qualified for neo-adjuvant chemo-radiotherapy underwent combined external irradiation and brachytherapy, with concomitant chemotherapy. Neo-adjuvant therapy was followed by the laparoscopic SN procedure, as described above, plus systematic pelvic lymphadenectomy and radical hysterectomy.

In keeping with our institutional guidelines, para-aortic lymphadenectomy was performed in both groups when metastatic pelvic lymph nodes (identified by intraoperative pathological examination) or a para-aortic sentinel node was detected.

The protocol was approved by our Institutional Review Board. All the patients gave their written consent after receiving all the relevant information, including the potential adverse effects of Patent Blue, radiocolloid, general anesthesia and the laparoscopic procedure, and the possible need to convert to open surgery.

SN procedure

All operations were performed by the same two surgeons (E.B. and E.D.), as previously described [14]. Briefly, four pericervical injections of 0.2 ml (20 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, using a triple-head γ camera (Irix; Marconi, Cleveland, OH, USA).

Under general anesthesia, the patient was placed in a low lithotomy position. A speculum was placed in the vagina, and Patent Blue (Bleu Patenté; Guerbet, Évry, France) was injected pericervically with a 25-gauge spinal needle at 3 and 9 o’clock (1 ml per injection). After Patent Blue injection, the pelvic and lower para-aortic regions were carefully inspected by laparoscopy for lymph ducts and specific dye uptake by lymph vessels. ‘Hot’ pelvic and para-aortic nodes were located using an endoscopic γ probe (Eurorad, Strasbourg, France) inserted through the 12-mm suprapubic trocar. Hot nodes were sought before opening the peritoneum.

When the SN had been located, 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, Elancourt, France).

Systematic laparoscopic bilateral pelvic lymphadenectomy was performed after the SN procedure. All nodal tissue along the obturator fossa and the external vessels up to the iliac bifurcation was dissected free and extracted in an endoscopic bag. The absence of residual pelvic or para-aortic radioactivity was verified before laparoscopic radical hysterectomy, the Schauta–Amreich operation or trachelectomy.

Histopathology

SN and other nodes were inspected by the same pathologist (A.C.). Grossly metastatic nodes were sectioned. SNs that appeared normal were cut along the long axis. All SNs were submitted to intraoperative imprint cytology on each half node. Air-dried cytological smears were obtained by scraping the cut surfaces and stained with a rapid May–Grünwald–Giemsa method. Each half-SN was sectioned at 3-mm intervals. Each 3-mm section was analyzed by four additional levels of 150 μm and four parallel sections; one was used for hematoxylin and eosin (H&E) staining, and H&E-negative sections were examined by IHC with an anti-cytokeratin antibody cocktail (Cytokeratin AE1-AE3; Dako, 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 had metastatic tumor by H&E or IHC staining, then cytokeratin IHC and multiple sectioning were not undertaken on corresponding non-SNs. 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 nodal metastases was estimated with an eyepiece micrometer. Micrometastasis was defined as a single focus of metastatic disease per node, measuring ≤ 2 mm. The presence of single non-cohesive tumor cells was recorded. SNs were recorded as positive when they contained macrometastases, micrometastases or isolated tumor cells.

SN analysis

SNs were recorded as blue-stained and/or hot (in vivo radioactivity exceeding three times the background). The false-negative rate was calculated 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.

Results

Characteristics of the patients

The median age of the 18 patients was 48 years (range 31–66 years). Demographic data and tumor characteristics are shown in Table 1. Median tumor size was 30 mm (range 12–60 mm). The median body mass index was 22.6 kg/m² (range 18–34.2 kg/m²). Four patients were nulliparous. Ten patients were post-menopausal, of whom two were on hormone replacement therapy.

The SN procedure used both Patent Blue and radioactive colloid in every case. None of the patients had MRI evidence of lymph node involvement.

Histopathology

The 18 SN procedures yielded a mean of 2.4 SNs per patient (range 1–5). A total of 44 SNs were removed. Of these, 31 were both blue and hot (70.5%), 10 were hot alone (22.7%) and three were blue alone (6.8%). The mean number of removed pelvic lymph nodes, including SNs, was 10.5 per patient (range 4–17).

None of the 44 SNs showed signs of malignancy on intraoperative imprint cytology. Therefore, para-aortic lymphadenectomy was never performed during initial surgery.
Eight SNs (18.2%) from five patients (27.8%) were found to be metastatic at the final histological assessment. Of these eight positive SNs, three were diagnosed by H&E staining and five by IHC.

H&E staining revealed two macrometastatic SNs and one micrometastatic SN (1 mm) in two patients with stage IA2 and IB2 disease. These three positive SNs (solely hot), located in the medial external iliac area, were the only histopathologically positive lymph nodes in these two patients. The first patient, with two positive SNs, who underwent trachelectomy for stage IA2 disease, subsequently underwent laparoscopic hysterectomy, bilateral ovarian transposition and para-aortic lymphadenectomy; none of 20 excised para-aortic lymph nodes were metastatic, and no tumor residue was found in the uterus.

Immunohistochemical analysis revealed micrometastases in two SNs and isolated tumor cells in three SNs from a total of three patients. The two micrometastatic SNs (one hot and blue, and one solely hot), found in the same patient, were located bilaterally in the obturator fossae. The second patient had single malignant cells in two SNs (both hot and blue) located bilaterally, in the medial external iliac region. The third patient had a single malignant cell in a single SN (solely blue) located in the medial external iliac region. The SNs were the only histopathologically positive lymph nodes in these three patients.

The SNs of 13 patients were negative by H&E and IHC staining, and their non-SNs were therefore extensively examined. A total of 106 non-SNs from these 13 patients were examined by serial sectioning and IHC, and none was positive. Thus, the true histological false-negative rate of SN using multiple sections and IHC examination of all non-SNs for metastasis was 0%.

No further external radiotherapy was prescribed to women with nodal micrometastases or isolated cancer cells detected by IHC staining.

Two patients relapsed after a median overall follow-up of 12 months (range 3–23 months). The first patient had a centropelvic recurrence 19 months after primary surgery. She initially had stage IB1, well-differentiated squamous cell carcinoma of the cervix (30 mm diameter) with no lymphovascular space, parametrial or vaginal wall involvement. Combined H&E and IHC analysis of the SN and the 11 pelvic non-SNs was negative. The clear surgical margin was 10 mm. The second patient, who had stage IB1, moderately differentiated squamous cell carcinoma of the cervix, relapsed 23 months after primary surgery with centropelvic disease. The initial cervical tumor measured 35 mm, and involved the lymphovascular space and right parametrium. Combined H&E and IHC staining of the SN and the 10 pelvic non-SNs was negative.

**Discussion**

The most important index of the relevance of 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 physiological significance of that node or the more extensive histopathological technique of lymph node examination. To our knowledge, only one study performed by Turner et al. has validated the concept of the SN procedure in breast cancer [9]. 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. We used the same approach in 18 patients with cervical cancer, and found that the SN procedure was perfectly reliable. This is the first validation of the SN procedure based on combined IHC and H&E analysis in this setting.

Pelvic lymph node status is the main prognostic factor in cervical cancer. Patients with histologically normal pelvic lymph nodes and clear surgical margins around the primary tumor have a good prognosis and a low risk of recurrence [18, 19]. Nonetheless, 10% of these patients still relapse in the pelvic region [20], probably from histologically occult nodal micrometastases [21]. As in
breast cancer [22], the prognostic significance of occult metastasis is controversial in cervical cancer. Five (28%) of 18 patients had nodal metastases. IHC identified SN metastasis in three patients, which would have been missed by simple H&E staining. Combined IHC and H&E examination thus increased the detection rate to 28%, from 11% with H&E staining alone. In our institution their presence detected by IHC does not influence the treatment regimen. Using reverse-transcriptase PCR analysis, van Trappen et al. found that 50% of early cervical tumors shed tumor cells to pelvic lymph nodes, and that this was associated with poor patient outcome [23]. Likewise, in patients with endometrial cancer, Yabushita et al. showed that positive cytokeratin staining of lymph nodes that were H&E-negative was a risk factor for recurrence [24]. A major advantage of the SN approach is that it avoids multiple sectioning and IHC staining of all excised nodes, saving both time and money.

The major problem arising during intraoperative analysis in patients with cervical cancer is the choice of appropriate pelvic lymph node stations. Currently, this choice is based on node consistency, size and aspect. Frozen section or imprint cytology analysis of SN potentially increases the diagnostic accuracy for lymph node metastasis relative to intraoperative histology based on macroscopic features. Intraoperative SN analysis will guide the surgical management of patients with cervical cancer. In particular, the uterus may be preserved in young patients with early cervical cancer when no SN metastasis was found during intraoperative analysis. In contrast, the detection of SN metastasis during surgery calls for pelvic lymphadenectomy to be extended to the para-aortic area and avoids unnecessary radical hysterectomy. Our results suggest that intraoperative imprint cytology of SNs is unreliable in patients with cervical cancer. Frozen section analysis is probably the most accurate technique for intraoperative analysis of SN histological status in patients with cervical cancer.

Two of our patients had locoregional recurrences, even though extensive histological analysis of both SNs and non-SNs had been negative. The first patient underwent post-operative brachytherapy. This patient was initially treated by Schauta–Amreich operation with a length of 20 mm removed from the vaginal cuff. After the diagnosis of recurrence, chemotherapy was performed followed by laparotomic pelvectomy. This patient is alive 7 months after treatment. The possible risk factor for recurrence in this patient was the large size of the primary tumor (30 mm). Other putative prognostic factors, such as anemia and biological findings, remain to be validated [25–27]. This locoregional recurrence could also be explained by tumor emboli in lymphatic vessels which did not reach the nodes. The second patient had lymphovascular space invasion by the primary tumor, and also right parametrial involvement, both of which are risk factors for recurrence in patients with cervical cancer [28, 29]. This patient was initially treated by laparoscopic radical hysterectomy with lengths of 15 and 20 cm removed from the vaginal cuff and parametria, respectively. This patient underwent post-operative brachytherapy. After the diagnosis of recurrence, a laparotomic pelvectomy was performed followed by chemotherapy. This patient is also alive.

Our patients underwent only an obturator and external iliac lymphadenectomy, which explains why relatively few lymph nodes were removed. During subsequent systemic intraoperative examination, lymphadenectomy was extended to the common iliac and para-aortic regions only in patients with positive SNs. The mean number of excised lymph nodes per pelvic site was in keeping with data from a French multicenter study based on the same surgical protocol [30]. This protocol is justified by studies which demonstrate the following: (i) that lymphatic channels draining laterally to the obturator and external iliac lymph nodes are the most important cervical drainage route; (ii) a low incidence of skip metastasis; and (iii) similar overall survival and disease-free survival rates compared with extensive lymphadenectomy [1, 2].

Although we reported SN detection and false-negative rates of 100% and 0%, respectively, an SN can not be detected in all cases, and a false-negative rate of 5% is acceptable.

In conclusion, our results suggest that the SN procedure reliably predicts the metastatic status of the regional lymphatic basin in patients with cervical cancer. However, larger studies are required before recommending routine use of the SN approach.

### References


