Methodology and implications of lymphatic mapping and sentinel lymphadenectomy
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CHAPTER TWELVE

Dynamic Sentinel Node Biopsy for Penile Cancer: Reliability of a Staging Technique

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J Urol, in press
After the first publication in 1960 about the use of the sentinel node status to decide upon performing regional lymph node dissection, it took a long time before the concept of sequential dissemination of cancer was explored further. It was Cabañas who described the existence of a sentinel node in the lymphatic drainage of the penis seventeen years later. Since 1965, lymphangiography was carried out via dorsal lymph vessels of the penis to study the lymphatic drainage patterns. The first-echelon lymph node or sentinel node was shown to be frequently located at the anterior or medial aspect of the superficial epigastric vein. Cabañas excised the sentinel node according to its typical anatomical location. In the absence of tumour in the sentinel node, no metastases were found in the other inguinal lymph nodes in 31 patients. Despite these promising results, this approach did not gain widespread acceptance because the described methodology ignores variability in lymphatic drainage.

The development of the technique of lymphatic mapping by Morton and Cochran from the John Wayne Cancer Institute at the end of the 1980s was a break-through in making the sentinel node concept applicable to various types of malignancies. Their original blue dye technique was complemented a few years later with a radioactive tracer in combination with preoperative lymphoscintigraphy and the intraoperative use of a gamma-ray detection probe. The technique of lymphatic mapping and sentinel node biopsy has been validated by numerous studies in which the predictive value of the first draining lymph node for the status of the other lymph nodes in the lymphatic basin was determined.

A few months after the first sentinel node biopsy for melanoma in The Netherlands Cancer Institute in 1993, this new staging procedure was also introduced in the management of penile cancer. Our initial experience with the dual-tracer technique in combination with lymphoscintigraphy and a gamma-ray detection probe has been published earlier. The purpose of the present study was to determine the reliability of sentinel node biopsy in predicting nodal status of theinguinal regions and determine its prognostic value after increasing the patient number and follow-up period.

**Materials and methods**

From January 1994 to April 2001, 82 consecutive patients with bilateral clinically node-negative penile cancer were prospectively enrolled in this study. In addition, eight patients with a unilateral N1 stage based on physical examination and fine needle aspiration cytology were entered. These patients underwent sentinel node biopsy for the clinically unaffected site. The mean age of the patients was 64 years (range 28 to 90 years). The clinical stage of the primary tumour was T2 in 74 patients and T3 in 16 patients. The histological type was squamous cell carcinoma in all patients. The differentiation grade was grade I in 37 patients, grade II in 40, grade II in 12 and unknown in another patient. Patients were not eligible for the study if the primary tumour was staged as T1 or carcinoma in situ, because of the assumed low risk of occult metastasis. Eighteen patients had previously undergone conservative therapy for penile cancer and presented with recurrent tumour.
The day before surgery, lymphoscintigraphy was performed after intradermal injection of nanocolloid (Amersham Cygne, Eindhoven, the Netherlands) labelled with $^{99m}$Tc. The average net dose of the radiolabelled colloid was 68 MBq (range 36-131 MBq). The tracer was administered at three or four sites around the tumour with a total volume of 0.3 ml to 0.4 ml. Anterior dynamic lymphoscintigraphy was performed during 20 minutes immediately after injection. Images were obtained using a dual-head gamma camera (ADAC, Milpitas, CA, USA) with low energy high-resolution collimators. Subsequently, 5-minute anterior and lateral static images were obtained after 30 minutes and 2 hours post-injection. A hot spot in the inguinal region was considered to be a sentinel node if an afferent lymphatic channel was visualised or the hot spot was the first one seen in a sequential pattern. The location of the sentinel node was marked on the skin using real time imaging and a $^{57}$Co-pen.

Shortly before surgery, 1.0 ml patent blue dye (Blue Patenté V, Laboratoire Guerbet, Aulnay-sous-Bois, France) was intradermally injected around the tumour. The sentinel node was identified and harvested after dissection of blue lymphatic vessels and detection of radioactivity with a gamma ray detection probe (Neoprobe®, Johnson & Johnson Medical, Hamburg, Germany). Sentinel node biopsy was followed by local excision of the primary lesion or penile amputation during the same session. Sentinel nodes were bisected, formalin fixed, paraffin embedded and cut at six or more levels. Paraffin sections were stained with hematoxylin and eosin. Immunohistochemical staining using pankeratin and CAM 5.2 (Becton Dickinson, San Jose, CA, USA) was added to the pathological assessment after the tenth procedure.

Routine confirmatory lymph node dissection was not performed. Standard inguinal lymph node dissection with or without iliac node dissection was reserved for patients with a proven lymph node metastasis. The incision for lymphadenectomy was chosen in such a way that the biopsy scar was included in the resection specimen. Sentinel node negative patients were observed. All patients were seen at two-month intervals during the first two postoperative years, at three-month intervals in the third postoperative year and every six months thereafter. Median follow-up was 36 months (range 5-95 months). Two patients were lost to follow-up because of immigration after five and sixteen months.

The Fisher exact test was used to compare sensitivity and negative predictive value between subgroups of patients with a low and high risk of lymph node metastasis. Disease-specific survival curves were constructed with the Kaplan-Meier method. Analyses were performed with Statistical Package for the Social Sciences software (SPSS, Chicago, Illinois, USA).

Results

Lymphoscintigraphy visualised at least one sentinel node in 88 of 90 patients (98%). Lymphatic drainage to both groins was seen in 71 patients (81%) and unilateral drainage in the other 17 patients (19%). Seven of eight unilateral N1 tumours
showed bilateral drainage and drainage to the unaffected site only was seen in the remaining one. Lymphatic drainage was visualised in all 18 patients who had previously undergone conservative therapy for penile cancer (14 patients with bilateral drainage, 4 patients with unilateral drainage). A total number of 217 sentinel nodes were visualised in 159 inguinal regions with a mean number of 1.4 (range 1 to 5). Surgical exploration was performed in 152 of 172 clinically node-negative inguinal regions. Exploration of the other 20 node-negative basins was not performed because of lymphoscintigraphic non-visualisation (19 basins) or because of the absence of a radioactive count rate higher than the background count rate during operation (1 basin). Only one basin without visualisation of a sentinel node on the lymphoscintigram was explored, but no sentinel node was found. The sentinel node could be successfully identified during operation in 149 of the remaining 151 inguinal basins. The overall surgical identification rate was 98% per inguinal region (149/152) and 98% per patient (88/90). The median time interval between incision and identification of the sentinel node was nine minutes (range 2 to 71 minutes). The results are summarised in figure 1.

**Figure 1.** Scintigraphic and intraoperative findings in 90 penile cancer patients undergoing dynamic sentinel node biopsy. Number of patients (number of inguinal regions) are given.
Differences between the number of visualised and the number of excised sentinel nodes were encountered in 34 inguinal regions of 30 patients. Additional sentinel nodes were excised in 18 inguinal regions. Not all hot spots that were defined as sentinel node could be surgically identified in the other 16 groins. A total of 207 sentinel nodes were harvested with a mean number of 1.4 per basin (range 1-3). Fifty-seven sentinel nodes (28%) were only radioactive and 150 (72%) were both blue and radioactive. The following postoperative complications occurred after dynamic sentinel node biopsy: wound infection in five patients, wound abscess in two, skin edge necrosis in one, wound dehiscence in one and seroma in another patient. Lymphedema after sentinel node biopsy alone did not occur.

Pathological examination revealed sentinel node metastasis in 19 inguinal regions of 18 patients. Four of eight patients with unilateral clinically N1 stage had a tumour-positive sentinel node at the contralateral site. Sentinel node metastasis was found in 15 of 77 grade I or II tumours (19%) and in 3 of 12 grade III carcinomas (25%). The tumour-positive sentinel node was only radioactive in 4 of 19 basins (21%) and both blue and radioactive in the remaining cases. All patients with a tumour-positive sentinel node underwent standard regional lymph node dissection one to two weeks postoperatively. The sentinel node was the only tumour-positive lymph node in 14 of these 19 tumour-positive basins (74%).

Regional recurrence necessitating inguinal node dissection after excision of a tumour-negative sentinel node was seen in four patients. A fifth patient developed a unilateral lymph node metastasis after 27 months in a basin that was not initially explored because a sentinel node was not depicted on the images. This results in a false-negative rate of 22% (5 of 23) and a negative predictive value of 93% (67 of 72). The results of sentinel node biopsy stratified to risk of lymph node metastasis are displayed in table 1.

Table 1. Results of sentinel node biopsy stratified by risk of lymph node metastasis based on differentiation grade of the primary tumour and clinical evaluation of the regional lymph nodes.

<table>
<thead>
<tr>
<th></th>
<th>Low risk (grade I / II, bilateral cN0)</th>
<th>High risk (grade III or unilateral cN1)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. patients</td>
<td>70</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Total no. groins explored</td>
<td>122</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>True-positive SN</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>False-negative SN</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>True-negative SN</td>
<td>108</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>86</td>
<td>70</td>
<td>0.62*</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>98</td>
<td>86</td>
<td>0.029*</td>
</tr>
</tbody>
</table>

SN=sentinel node; *=Fisher exact test

Details of the five cases in which the sentinel node procedure failed to identify lymph node metastasis are provided in table 2. Additional sections at 150 μm intervals of the original tumour-negative sentinel node revealed a micrometastasis in one of four patients (table 2).
Table 2. Characteristics of five patients in whom a regional recurrence occurred after excision of a negative sentinel node (no. 1, 2, 4, 5) or after non-visualisation on lymphoscintigraphy (no. 3). All recurrences were treated with regional lymph node dissection.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Stage</th>
<th>Lymph node status</th>
<th>Regional recurrence</th>
<th>Time to recurrence (months)</th>
<th>Survival (months)</th>
<th>Patient status</th>
<th>Reason for failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>T2G1</td>
<td>SN+</td>
<td>+</td>
<td>7</td>
<td>14</td>
<td>DOD</td>
<td>Sampling error by the pathologist</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>T2G3</td>
<td>SN-</td>
<td>-</td>
<td>4</td>
<td>72</td>
<td>NED</td>
<td>?</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>T2G3</td>
<td>SN+</td>
<td>+</td>
<td>27</td>
<td>60</td>
<td>NED</td>
<td>No tracer uptake by tumour blockage?</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>T2G1</td>
<td>SN-</td>
<td>-</td>
<td>6</td>
<td>8</td>
<td>NED</td>
<td>?</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>T2G2</td>
<td>NE</td>
<td>+</td>
<td>7</td>
<td>8</td>
<td>NED</td>
<td>Not all visualised hot spots were identified</td>
</tr>
</tbody>
</table>

L=left groin; R=right groin; SN+ =tumour-positive sentinel node; SN- =tumour-negative sentinel node; NE=not explored; DOD=dead of disease; NED=alive, no evidence of disease.

The estimated three-year disease specific survival rates were 98% and 71% for patients with a tumour-negative or tumour-positive sentinel node respectively (log rank test, P = 0.0018; figure 2). The only patient with a tumour-negative sentinel node who died because of disease had a sarcomatoid squamous cell carcinoma and developed a local recurrence and distant metastases after four and six months respectively without evidence of lymph node metastasis.

![Figure 2. Kaplan-Meier plot of disease specific survival in patients with a tumour-negative or tumour-positive sentinel node (SN). Eight patients with a unilateral clinical N1 stage were excluded from survival analysis. Numbers of patients at risk are provided at each point of time. P = 0.0018 (log rank test)](image-url)
Discussion

Accurate staging in penile cancer is of importance for selection of patients who may benefit from regional therapy in addition to treatment of the primary carcinoma. The incidence of occult lymph node metastases in clinically node-negative patients is about 20%.\(^6\) Elective inguinal node dissection in all patients will identify these patients, but at the cost of substantial morbidity.\(^7\) Overtreatment resulting from this approach is avoided by adopting a ‘wait and see’ policy with therapeutic lymph node dissection at the time of regional recurrence.\(^8\) The argument against a surveillance policy is the assumed negative impact of delayed lymphadenectomy on survival.\(^9\)

Several investigators have tried to solve the problem of selection of patients in whom earlyinguinal lymphadenectomy is indicated. Histopathological characteristics of the primary tumour have been found to be of predictive value for occult lymph node metastases. These factors are local tumour stage, differentiation grade, depth of invasion and the presence of vascular invasion.\(^8\)\(^-{12}\) However, using these predictors to define patient populations at a low or high risk of nodal involvement will result in substantial false-positive and false-negative rates.\(^10\)

Sentinel node biopsy as proposed by Cabañas was a minimally invasive procedure to determine the lymph node status in a direct way.\(^2\) Unfortunately, selective lymph node dissections based on this static anatomical concept failed to reach the desired diagnostic accuracy.\(^13\)\(^-{14}\) Sentinel node biopsy in the present form includes a radioactive tracer and a vital dye to identify the lymph nodes on a direct drainage pathway from the primary tumour. The overall sensitivity of 78% and negative predictive value of 93% in the present study indicates that this technique needs optimization to become a reliable staging procedure for clinically node-negative penile cancer. The sensitivity of the procedure seems to be lower in patients who were traditionally candidates for elective lymphadenectomy at our institution (grade III primary tumour or unilateral cN1), but this difference was not statistically significant (table 1). This observation stresses the importance of regular follow-up at short intervals to identify and treat recurrences at the earliest possible time. Despite the fact that not all occult lymph node metastases were identified, the sentinel node status provided important prognostic information (figure 2).

Although all sentinel lymph nodes were found using the gamma ray detection probe, the vital dye facilitates intraoperative identification and can discriminate between first- and second-echelon nodes by visualising the afferent lymphatic vessel.

When discrepancies between the two detection methods are found, a lymph node that is only radioactive should be considered as sentinel node if the corresponding hot spot meets the criteria of a sentinel node during lymphoscintigraphy. In case of doubt, a radioactive lymph node should also be considered as sentinel node, which is illustrated by one of the false-negative cases (table 2).

Lymphoscintigraphy is essential to determine the lymphatic drainage pattern and enables preoperative marking of the sentinel node on the skin.\(^15\) Based on their initial experience in penile cancer, investigators at the M.D. Anderson Cancer Center concluded that preoperative lymphoscintigraphy is complementary to intra-
operative lymphatic mapping in the detection of microscopic metastases.\textsuperscript{16} The limited discriminating power of the scintigraphy sometimes results in discrepancies with surgical findings. An unresolved question is whether to explore both inguinal regions in case of unilateral drainage on lymphoscintigraphy. Exploration of one of 20 basins with non-visualisation did not reveal a sentinel node, but a recurrence was found in one of the 19 remaining regions. It may be hypothesised that blockage of the afferent lymphatic vessel by tumour deposit prohibited the ingress of tracer in the sentinel node of the latter patient. Based on this finding, we now recommend exploration of a non-visualised basin. This may result in identification of blue sentinel nodes without radioactivity. In addition, intraoperative palpation of the biopsy wound might reveal firm lymph nodes completely replaced by tumour without tracer uptake. This may prevent false-negative results.

What about the indications for sentinel node biopsy? One may wonder if the lymphatic drainage will be altered due to local treatment. Lymphatic mapping in melanoma is mostly performed after excision of the primary tumour with narrow margins. Several studies in breast cancer did not demonstrate an impaired accuracy of the technique after excisional biopsy.\textsuperscript{17} Lymphatic drainage of the penis seems to be less complex than in these two malignancies which suggest that patients who had undergone previous local excision or partial penile amputation can be included as well.\textsuperscript{18} Ultrasound in combination with fine needle aspiration cytology may be added to the preoperative assessment of the groin to define the indication for sentinel node biopsy. The findings of Moskovic et al. who applied this approach in vulvar cancer are therefore noteworthy, because of the similar dissemination pattern.\textsuperscript{19} De Kanter et al. concluded that sentinel node biopsy could be avoided in 17\% of clinically node-negative breast cancer patients by performing preoperative ultrasonography of the axilla in combination with fine needle aspiration cytology.\textsuperscript{20} Patients with unilateral proven N1 stage can be candidates for lymphatic mapping of the opposite side. In four of eight such patients, the sentinel node on the contralateral side was tumour-negative and only unilateral inguinal lymphadenectomy was performed. However, in one of these patients, a regional recurrence occurred during follow-up (table 2).

Implementation of a new technique requires engagement of all involved disciplines. Nuclear medicine physicians have to perform adequate tracer injection and sequential imaging to visualise all lymph nodes on a direct drainage pathway. The pathologist should scrutinise the lymph node to minimise the risk of a sampling error. The finding of a micrometastasis in retrospect in one of the false-negative cases resulted in the addition of immunohistochemical staining to the routine pathological assessment of sentinel nodes. Finally, the urologist has to be familiar with the technique of intraoperative lymphatic mapping and has to pass a learning phase. Exploration of non-visualised basins should be considered to detect lymph nodes completely replaced by tumour without uptake of tracers. Ultrasonography of the groins may help to identify such lymph nodes preoperatively. Further study is needed to determine if such measures result in improvement of the accuracy of the technique.
Conclusions

With a sensitivity of about 80%, dynamic sentinel node biopsy based on the technique of lymphatic mapping is a promising method to detect occult metastases in penile cancer, but attempts to improve the accuracy have to be evaluated in the future. In combination with frequent control during follow-up, we provide the patient a staging procedure with prognostic information and a low rate of morbidity by preventing unnecessary lymphadenectomy.

Acknowledgement

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References