Abstract. The aim of this study was to detect lymphatic spread by serial step-section technique in non-sentinel lymph nodes (NSLNs), which were earlier assessed as negative by histological examination. Patients and Methods: Inguinal dissection specimens of 13 men with penile cancer were investigated. The LNs were sectioned at multiple levels (150 μm-intervals) and then H&E- and immuno-stained for cytokeratin (Lu-5). Results: 196 LNs of 13 men were examined. In 2 out of 13 patients (15%) previously ranked as pN0, minimal lymph node involvement was detected by serial step sections and both immunohistochemistry and H&E staining. Both patients have had an uneventful follow-up of currently 62 and 16 months. Conclusion: Conventional histological examination of NSLNs fails to detect lymphatic spread in penile cancer. Step-section technique at 3 section levels, rather than immunohistochemistry, helps to safely detect minimal metastatic disease. The prognostic relevance is still unclear and has to be investigated in larger cohort studies.

Correspondence to: Carsten Maik Naumann, Department of Urology and Pediatric Urology, University Hospital Schleswig Holstein, Campus Kiel, Arnold Heller Str. 3, 24105 Kiel, Germany. Tel: +49 4315974411, Fax: +49 4315971957, e-mail: c.naumann@urology.uni-kiel.de

Key Words: Penile carcinoma, lymph node metastases, micrometastases, non-sentinel lymph nodes.
Patients and Methods

We investigated inguinal lymph nodes (LNs) of 13 patients from three participating centers, who had been staged previously as pN0 by conventional examination. For this purpose, the lymph nodes were processed according to the Association of Directors of Anatomic and Surgical Pathology, ADASP recommendations for processing and reporting of lymph node specimens submitted for evaluation of metastatic disease (8).

Six serial 3-4 μm sections were cut from 123 lymph nodes of the first nine patients at six consecutive levels (150 μm-intervals) in a pre-study setting. The first two consecutive sections of each level were H&E–stained and immunolabelled (anti-cytokeratin, Lu-5), respectively. 73 lymph nodes of the remaining four patients were investigated at three levels only, but otherwise by identical methodology.

For immunolabelling, the paraffin sections were deparaffinized and rehydrated, then the epitopes were retrieved in a TEC buffer in a stainless-steel pressure cooker for 2.3 min, after the pressure had reached the maximum. At the end of the retrieval phase, the slides were left for 20 min to cool at room temperature. The buffer was then exchanged for tris-buffered saline (TBS) and left for a few minutes. Endogenous peroxidase was quenched with H2O2. The slides were then incubated in PBS with rabbit serum (Dako, Glostrup, Denmark) at a dilution of 1:10 for 20 min. The slides were incubated with anti-pan-cytokeratin (human) clone Lu-5 Mouse IgG (1:100; Vector Laboratories Inc., Burlingame, CA, USA) in the antibody diluent (Dako) for 30 min. After washing with TBS, the sections were incubated with the secondary biotinylated anti-mouse IgG (H. L) antibody (1:100; Vector Laboratories Inc., Burlingame, CA, USA) in the antibody diluent (Dako for 30 min and washed in TBS. Immunoreactivity was detected with an ABC-DAB reaction (Vector Biologo, Kronshagen, Germany), then the sections were weakly counterstained with haematoxylin. Sections of known epithelial lymph node metastases were used as a positive control. Pure antibody diluent was substituted for the primary antibody as a negative control.

Positive findings were documented with respect to the staining method, number, section level and size of the lymph node involvement.

95% Confidence intervals (CI) were calculated by the exact method based on binomial distribution.

Results

196 negatively staged lymph nodes of 13 patients were investigated. The clinical and pathological data of the patients and the tumours are shown in Table I.

Three of the 196 nodes belonging to two different patients harboured metastatic deposits (Figure 1). Thus, conventional histopathological examination had failed to detect metastatic deposits in the inguinal lymph node specimen in two out of thirteen patients, 15% (0.02-0.45 95% CI). The clinicopathological data of these patients are shown in Table II.

All metastatic deposits were detected within the first three sections. Investigation of sections four to six within the pre-study setting did not provide any additional information compared to the first three sections in 123 lymph nodes, therefore the maximum number of investigated levels was reduced from six to three consecutive levels for the remaining 73 lymph nodes.

In this series, anticytokeratin immunostaining (Lu-5) and H&E staining were performed. All metastatic deposits were identified by H&E staining, independent of Lu-5 immunostaining. Therefore, immunostaining did not provide any additional value with regard to the detection of minimal metastatic disease.

Discussion

The presence of lymph node involvement is one of the most reliable prognostic factors in penile cancer (1, 2). Moreover, early removal of occult metastatic disease seems to improve survival when compared to lymph node dissection in view of clinically evident disease (10, 11). Sentinel lymph node biopsy has been shown to be a useful staging tool by balancing staging accuracy and morbidity of this procedure (12). However, several authors have oncological concerns when it comes to accepting sentinel node biopsy as a standard staging procedure (13, 14). Moreover, due to the high costs and the multidisciplinary approach, this technique may be unavailable to many physicians, especially in developing countries where penile carcinoma is a common problem (2, 15). Therefore, conventional lymph node dissection is still considered the gold standard for lymph node staging by most urologists worldwide.

Small patient numbers and variations in histological assessment of the primary tumour may contribute to highly divergent findings of lymph node involvement for the same tumour stages, grades or risk groups (1-6). But a 2.4-fold higher rate of lymph node involvement in the surveillance group compared to those patients who underwent immediate, prophylactic lymph node dissection (7) suggests that nodal understaging is an underestimated methodological problem in lymph node staging of penile cancer.

In a first attempt, Biedrzycki et al. performed immunohistochemical staining of negative NSLNs in 13 patients with penile carcinoma. By using two different
anticytokeratin antibodies, they found 1 out of 217 lymph nodes to contain a single micrometastasis, which had been overlooked by routine staining (16). Since the step section technique was not performed in this study, additional metastatic disease may have been missed in this series. This prompted us to perform an extensive analysis of NSLN, using serial histological sections and IHC. We were able to show that conventional histological lymph node examination fails to detect lymph node metastases of <2 mm (micrometastases) and >2 mm in up to 15%.

Furthermore, our study suggests that the examination of three consecutive section levels at 150 μm intervals is sufficient to prove or exclude lymph node involvement in NSLN. The current results are in line with those of Fréneaux et al., who proved that in 95% of the cases the screening of the first three levels is sufficient for the detection of positive diagnostic events (17). But additional studies need to be carried out to evaluate the clinical validity of this method in penile cancer diagnostics.

In order to further reduce expensive and time-consuming investigations, the step section technique could be restricted to patients with no or minimal nodal involvement in the conventional examination. It is important, however, to ask how patient management will be affected if the result of this technique is positive, while the conventional histopathological examination of the non-sentinel nodes is negative. In this setting, it might make sense to provide either a more extensive surgical approach, or adjuvant treatment or just a more intensive follow-up schedule. The histopathological results of this study in addition to the further clinical course of the affected patients do not provide a final answer to this question. Both patients have proved to be cancer-free, but the metastatic burden and the number of involved lymph nodes – as detected by serial sectioning – was also still very low in both patients. However, this in combination with a favourable lymph node ratio allows for an excellent prognosis.

Meticulous examination of the lymph nodes with exhaustive serial sectioning increases the detection rate of isolated tumour

Table II. Clinicopathological data of the patients with undetected metastatic disease by conventional lymph node processing of inguinal lymph node specimen.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary tumour</th>
<th>pN-status (conventional)</th>
<th>pN-status (Step sections)</th>
<th>Size and level of newly detected metastatic deposit (mm)</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>pT2, G3, L1, V0, R0</td>
<td>pN0 (0/19)</td>
<td>pN1mi (1/19)</td>
<td>1st level: 1.22</td>
<td>Uneventful for 67 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd level: 1.44</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>pT2, G2, L0, V0, R0</td>
<td>pN0 (0/14)</td>
<td>Left: N1mi (1/6) Right: N1 (1/8)</td>
<td>Left: 1st level: 0.95 Right: 1st level: 1.61 3rd level: 2.10</td>
<td>Uneventful for 16 months</td>
</tr>
</tbody>
</table>

Figure 1. A: H&E section of a lymph node micrometastasis (Patient A), B: anti-Cytokeratin, Lu-5 immunohistochemistry showing the same metastasis.
cells and micrometastases; thus, raising further questions on the prognostic role of the microinvolvement of lymph nodes. Based on the findings of Kroon et al., who found additional lymph node involvement only when sentinel nodes showed macroinvolvement (>2 mm), a conservative approach in penile carcinoma might be acceptable (19). Koenig et al. showed that the absence of occult tumour cells in LN, irrespective of the conventional nodal status, showed a significant survival benefit for patients with esophageal carcinoma. Moreover, survival of patients with nodal microinvolvement nearly equaled that with histologically proven overt nodal involvement (pN1) (20). In the SCC of the vulva, Narayansingh et al. found micrometastases in 42% of the cases (n=31) by additional four sections at 250 μm intervals, associated with a 20-fold higher risk of recurrence (21).

In breast cancer, the clinical implication of the extent of lymph node involvement (whether isolated tumour cells or larger) continues to raise substantial uncertainty and controversy. In a very recent study on 3,158 patients, the occurrence of micrometastasis was found to be independently correlated with an increased risk of distant metastases only in patients who underwent axillary lymph node dissection. No significant prognostic effect was ascertained for the presence of microinvolvement in patients submitted to SLN biopsy, which suggests a crucial role of the number of sections and the size of the intervals (22). In this study, NSLNs were scrutinized with 3-6 sections, cut at 250-500 μm intervals, whereas SLNs were examined by complete serial sectioning at 50-100 μm intervals. As a result, some macrometastases in patients of the axillary lymph node dissection (ALND) group may have been misclassified as micrometastases.

A very recent study on melanoma patients established that patients with micrometastases (<0.1 mm) have the same prognosis as sentinel node-negative patients and can be spared a complete lymph node dissection. A <0.2 mm cut-off for micrometastases, as is suggested for breast cancer, does not seem correct for melanoma, as 10% additional nodal positivity was found (23). It remains to be established, which cut-off value defining “biologically false positive” metastatic burden is the most suitable in penile cancer. As in other tumour entities and SCCs in other primary sites, the prognostic impact of microinvolvement of the lymph nodes and its clinical implications in penile carcinoma have to be further investigated.

Conventional histological examination of NSLNs fails to detect micro- and macroinvolvement of lymph nodes in penile cancer. Step section technique at 3 levels (150 μm-intervals), rather than immunohistochemistry, helps to safely detect additional metastatic disease. The prognostic relevance, particularly of microinvolvement, needs to be investigated in larger cohort studies. Furthermore, the results of this study have to be taken into account when investigating prognostic parameters for the development of lymph node metastases. In addition to sampling errors resulting from small patient cohorts, the failure to detect nodal spread by the conventional technique is likely to lead to diverse findings of lymph node involvement in this rare disease.

Acknowledgements

We thank Ms Almut Kalz, Department of Urology and Pediatric Urology, University Hospital Schleswig Holstein, Campus Kiel, Germany, for proof-reading this manuscript.

References

14 Ficarra V and Galfano A: Should the dynamic sentinel node biopsy (DSNB) be considered the gold standard in the evaluation of lymph node status in patients with penile carcinoma? Eur Urol.


Received October 7, 2009
Accepted January 5, 2010