The Accuracy of Intraoperative Frozen Section of the Inguinal Sentinel Lymph Node in Vulvar Cancer

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Abstract. Background: Limited data are available for the accuracy of intraoperative frozen section analysis of inguinal sentinel lymph node in patients with vulvar cancer. Patients and Methods: Forty-four patients with vulvar cancer treated with separate incisions for inguinal sentinel lymph node dissection between 2001 and 2007 were evaluated in the present study. Results: Out of 44 patients, 3 had a false-negative intraoperative frozen section result due to micrometastasis. No false-positive result of the intraoperative frozen section analysis was obtained. We identified two studies, which exclusively examined the false-negative rate of frozen section analysis of the sentinel lymph node. Data of these 3 studies were pooled, yielding an overall underdiagnosis of frozen section analysis in 6/128 (4.7%) patients, resulting in sensitivity, specificity and positive and negative predictive values of 88.5%, 100%, 100% and 93.2%, respectively. Conclusion: Intraoperative frozen section analysis of the inguinal sentinel lymph nodes exhibits good sensitivity/specificity characteristics for the assessment of inguinal lymph node involvement in patients with vulvar cancer.

Vulvar cancer is most commonly observed in elderly women (1), but the number of younger woman with this disease appears to be increasing (2, 3). The sentinel lymph node is defined as the first draining lymph node of a specific anatomical region (4, 5), and is usually identified either using a blue dye or a radioactive tracer (6, 7). Sentinel lymph node dissection has replaced complete lymph node dissection providing important predictive and prognostic information in patients with melanoma and breast cancer (8-10). The advantages of sentinel lymph node dissection include less surgical and postoperative morbidity such as a reduced rate of wound breakdown, as well as long-term sequelae such as chronic lymph edema (11, 12).

In gynaecological malignancies, the sentinel lymph node procedure has also been extensively evaluated (13, 14). In vulvar cancer, various reports have shown good sensitivity/specificity characteristics of the inguinal sentinel lymph node procedure. Frozen section analysis is an integral part of any sentinel lymph node procedure. After the inguinal sentinel lymph node is identified and surgically removed, frozen section analysis is performed. A histologically negative, i.e. tumor-free, sentinel lymph node is thought to be predictive for all other inguinal lymph nodes of the respective groin. This allows inguinal lymph node dissection to be aborted, sparing the patient unnecessary surgical and postoperative morbidity. In contrast to other malignancies, limited data are available on the diagnostic accuracy of frozen section analysis in patients with vulvar cancer.

The aim of the present study was to provide data on the diagnostic accuracy of the inguinal sentinel lymph node procedure in patients with vulvar cancer.

Patients and Methods

Medical records of 44 consecutive patients with vulvar cancer who had undergone the sentinel lymph node technique without it being followed by a complete inguinal lymph node dissection between January 2001 and August 2007 were reviewed.

All patients were treated in the Department of Obstetrics and Gynecology at the Medical University of Vienna. During the first three years following implementation of the sentinel lymph node dissection in cases of vulvar cancer in 1998, the new technique was always followed by a complete inguinal lymph node dissection at our department. From 2001, all patients were offered the sentinel lymph node technique using intraoperative frozen section analysis so that in tumor-free cases, i.e. negative lymph node assessment, complete inguinal lymph node dissection could be avoided.

Patients with vulvar cancer with an invasion depth of no more than 1 mm, vulvar melanoma, adenocarcinoma, basal cell cancer, verrucous carcinoma and patients with prior chemotherapy, pelvic or inguinal radiotherapy or prior vulvar surgery were excluded.

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All patients with T1 and T2 tumors without clinically suspicious inguinal nodes were enrolled. The mean age of the patients was 70.0 (±15.1) years. Thirty-four patients had a tumor at least 2 cm from the midline, and no considerable palpable tumor on the contralateral side, therefore only the unilateral groin was dissected. In 10 patients, a bilateral groin procedure was essential, so in total 54 groin tumors were eligible for intraoperative frozen section analysis.

Two to three hours before surgery technetium$^{99m}$ microcolloidal albumin containing 15 MBq tracer in 0.4 ml saline (Alburex, Pharmaceutical Nycomed Amersham, Braunschweig, Germany) was intradermally injected in each quadrant around the tumor in all patients. Static images were taken 2 h after dynamic scintigraphy. The final site of the sentinel node was marked with a cobalt penmarker and before surgery the skin was marked with indelible ink to guide the surgeon. In 4 out of 44 cases (9%), the isosulfan blue method was additionally used, by injection of 1.0 ml peritumorally, to identify the afferent lymphatic channels and more easily trace the sentinel node.

All frozen section specimens were analysed by a board-certified pathologist, specialized in gynecological pathology. Intraoperative pathological examination was performed according to a defined protocol. The sentinel node was cut perpendicular to the longest axis at intervals of 2 to 3 mm. When the lymph node was too small (2-3 mm) it was submitted either intact or divided in half for frozen section. Briefly, the unfixed lymph node tissue was frozen rapidly in a cryostat to approximately −20˚C. Subsequently, the frozen tissue block was cut with a microtome parallel to the long axis of the node at 4 μm slices. Four to six serial frozen sections (4 μm-thick) were cut in one level. The sections were placed on a glass slide and stained with hematoxylin and eosin (H&E) for histological analysis during operation time. Thereafter, the remaining lymph node tissue was fixed in buffered 4% formaldehyde (pH 7.2) and embedded in paraffin. In brief, nodes were first cut into 2- to 3-mm blocks and then each block was cut into 400-μm slices. One 4-μm-thick section was taken from each slice and routinely stained with H&E. In cases of negative sentinel node assessment, additional immunohistochemical (IHC) cytookeratin examination was performed. Non-sentinel lymph nodes removed during inguinal dissection were processed by routine histological techniques by cutting into serial sections at 4 μm and then stained using the standard H&E staining protocol. Non-sentinel nodes were not investigated by frozen section analysis. Prior to the staining procedure the sections of the non-frozen section specimens were deparaffinized in xylene and rehydrated via graded ethanol. Findings at surgery were correlated with the final pathology report.

Medline was used (12.02.2008; search terms: vulvar cancer, sentinel node, frozen section analysis, intraoperative diagnosis) to identify cohort studies, systematic reviews and meta-analyses assessing the accuracy of intraoperative frozen section analysis in woman with vulvar cancer. Studies were included if they were published as complete reports in English. Studies were eligible if they exclusively investigated vulvar cancer and if they reported absolute numbers. All p-values are two-tailed and a p-value <0.05 was considered statistically significant. We performed univariate and multivariate linear regression models with underdiagnosis (frozen section analysis: negative/definitive histology: positive) and overdiagnosis (frozen section analysis: positive/definitive histology:negative), dichotomized as categorical variables, as the dependent variable and patient age, tumor size, tumor grade, tumor stage as the independent variables. The relationship between these variables is described by the standardized β coefficient and respective p-values. Statistical software SPSS 11.0 for Windows (SPSS Inc., Chigo, IL, USA) was used for statistical analysis.

<table>
<thead>
<tr>
<th>Sentinel lymph node dissection and frozen section analysis</th>
<th>Number of patients</th>
<th>Number of groins</th>
<th>Patients age at surgery (years)</th>
<th>Tumor stage</th>
<th>Lymph node status</th>
<th>Histological grade</th>
<th>Removed lymph nodes for frozen section</th>
<th>Operation time (min)</th>
<th>Length of hospital stay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44</td>
<td>54</td>
<td>70.0 (±15.1)</td>
<td>pT1</td>
<td>pN0</td>
<td>G1</td>
<td>120</td>
<td>92 (±37.2)</td>
<td>15.9 (±11.9)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pT2</td>
<td>pN1</td>
<td>G2</td>
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<td>G3</td>
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</table>

Values are given either as numbers or as means (±standard deviation).

Results

In our series of 44 patients, 10 women underwent bilateral sentinel lymphonodectomy of the groin, therefore 54 groin samples were investigated and analysed by frozen section. A total of 120 lymph nodes were evaluated by frozen section technology: 26 were tumor positive, 94 tumor negative while 3/94 were falsely negative (3.2%).

Samples from 3/44 patients were classified as false-negatives in frozen section analysis. In one case, the result of frozen section was indecisive; this result was finally classified as negative. No false-positive result of the intraoperative frozen section analysis was obtained. The number of non-sentinel nodes which were stained by the H&E procedure was 289, 63 tumor-positive and 226 tumor-negative. pT1 and pT2 tumors were found in 30 and 14 cases, respectively. pN0, pN1, pN2 tumours were found in 27, 10 and 7 patients respectively. The mean operation time was 92 (±37.2) minutes; the length of hospital stay was 15.9 (±11.4) days (Table I). The three patients with false-negative results from frozen section analysis underwent secondary complete lymph node dissection, with negative results for the remaining nodes.

In a univariate analysis, the investigated tumor characteristics, namely tumor stage ($p=0.51$), tumor grade ($p=0.08$) and tumor size ($p=0.33$), as well as patients’ age ($p=0.41$) did not influence the accuracy of intraoperative frozen section analysis of the sentinel inguinal lymph node. Of note, the results of the statistical analysis are limited due to the small cohort of patients enrolled.
Sixteen studies were found to have investigated the role of the inguinal sentinel lymph node in patients with vulvar cancer. Two studies exclusively examined the false-negative rate in frozen sections during the operation (15, 16): 1/41 and 2/43 false-negative cases were observed, respectively (Table II). Data of these two studies were pooled with ours, yielding an overall underdiagnosis in frozen section analysis in 6/128 (4.7%) patients resulting in a sensitivity, specificity and positive and negative predictive values of 88.5%, 100%, 100% and 93.2%, respectively.

**Discussion**

Sentinel node biopsy is under investigation as a method to avoid complete groin dissection in women with vulvar cancer in order to reduce the number of acute and long-term complications such as wound breakdown or lymphedema of the legs (17).

In our present analysis of 44 women, we found 3 false-negative sentinel lymph nodes assessments during intraoperative frozen section analysis, with an overall sensitivity of 85.0%. In the pooled analysis of the published studies including our data, we found that intraoperative frozen section analysis had an overall sensitivity of 88.5% and an overall positive predictive value of 100%. Overdiagnosis and underdiagnosis occurred in 0% and 4.7%, respectively.

The sentinel node technique allows the submission of one or a few nodes to more enhanced pathological analysis, termed ultrastaging (18, 19). This generally involves taking serial sections through the node and application of specific IHC staining for tumor antigens in order to identify micrometastases. The value of IHC staining of sentinel lymph nodes is controversial. Application of IHC staining and serial sectioning for the evaluation of sentinel nodes from axillary dissections in breast cancer patients increased the detection of metastatic disease by 10.0 to 15.0% (20, 21). On the other hand a retrospective analysis of 707 axillary nodes from 34 node-negative breast cancer patients compared step sectioning to IHC staining and found no added benefit with the addition of IHC staining (22).

The use of IHC staining to detect micrometastasis in sentinel lymph node in patients with vulvar cancer is equally controversial. The majority of the studies recommend serial sectioning and IHC staining in the evaluation of all sentinel nodes obtained from patients with vulvar malignancies (18).

In recent studies (19, 23-30), the detection rate with serial sectioning and IHC staining was 100% with no false-negative nodes, but there are also studies which report no metastasis by detection without the addition of cytokeratin IHC staining (27, 28).

Our reported data are in accordance with previous reports describing the difficulty of establishing a diagnosis of metastatic lymph nodes in vulvar cancer in general, and during frozen section analysis in particular (15, 16). Patients intraoperatively found to have negative sentinel nodes may benefit from a less invasive procedure. The clinical relevance of missing a micrometastasis due to frozen section diagnosis is discussed controversially (31-33).

The limitation of our own study is the relatively small cohort and the retrospective design. However, we are the first to summarise the published data with respect to the accuracy of intraoperative frozen section analysis of the inguinal sentinel lymph node in patients with vulvar cancer. We conclude that intraoperative frozen section diagnosis of the groin lymph nodes in patients with vulvar cancer is reliable.

**References**


