CHAPTER 2

PREDICTION OF LYMPH NODE METASTASES
IN VULVAR CANCER: A REVIEW

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ABSTRACT

The aim of this study was to review the literature on currently available non- and minimally-invasive diagnostic methods and analysis of primary tumor characteristics for prediction of inguinofemoral lymph node metastases in patients with primary squamous cell carcinoma of the vulva. We used the English language literature in Pubmed and reference lists from selected articles. Search terms included vulvar carcinoma, prognosis, lymph node metastases, ultrasound, computer tomography, magnetic resonance imaging, positron emission tomography, and sentinel lymph node. No study type restrictions were imposed. Currently no noninvasive imaging techniques exist that are able to predict lymph node metastases with a high enough negative predictive value. A depth of invasion ≤ 1mm is the only histopathologic parameter that can exclude patients for inguinofemoral lymphadenectomy. No other clinicopathologic parameter allows exclusion of lymph node metastases with a high enough negative predictive value. The minimally invasive sentinel node procedure is a promising technique for selecting patients for inguinofemoral lymphadenectomy, but its safety has not been proven yet.
INTRODUCTION

Vulvar carcinoma is a rare disease, mainly affecting elderly women. The majority of vulvar carcinomas are squamous cell carcinomas (SCC), which account for 90% of all vulvar carcinomas. The pattern of dissemination of SCC is predominantly lymphogenic to the inguinofemoral lymph nodes. Pelvic and distant metastases are rare and mostly fatal.

Radical vulvectomy with bilateral inguinofemoral lymphadenectomy ‘en bloc’ has been the standard treatment for years in patients with SCC of the vulva with a depth of invasion > 1mm. The morbidity of this surgical treatment was significant: frequent wound breakdown, wound infections, lymphocysts, lymphedema and impressive psychosexual consequences, especially in younger patients. These complications often prolong hospitalization. The most common complication is chronic lymphedema, which has been reported in up to 69% of patients. In a study by de Hullu et al., it was shown that after a median follow-up of 118 months, 47% of patients treated for vulvar carcinoma still experienced either severe pain and/or severe lymphedema in the legs. In the past decades, changes toward less radical treatment have been made in the standard treatment of vulvar carcinoma with depth of invasion > 1mm: wide local excision and ipsi- or bilateral inguinofemoral lymphadenectomy via separate incisions. However, also in early-stage disease (T1 or T2 tumors with clinically unsuspicious inguinofemoral lymph nodes), all patients undergo inguinofemoral lymphadenectomy based on the finding that 20 – 30% will have lymph node metastases. The rationale for this approach is the assumption that prognosis is superior after elective inguinofemoral lymphadenectomy compared with surveillance of the groins. This assumption is primarily based on clinical observations that, by the time groin recurrences manifest themselves, curative therapy frequently is no longer possible, resulting in poor regional control with dismal sequels for the patient. The prognosis for groin recurrences of vulvar carcinoma is very poor, most patients die of disease. No randomized trials have addressed the issue of elective versus delayed inguinofemoral lymphadenectomy in patients with vulvar carcinoma and clinically unsuspicious lymph nodes. Doubt remains, however, about the therapeutic role of inguinofemoral lymphadenectomy in node-negative patients. Of all patients with clinically unsuspicious inguinofemoral lymph nodes, only 20 – 30% have inguinofemoral lymph node metastases. The other 70 – 80% will probably not benefit from the lymphadenectomy but are at risk for its significant morbidity. The morbidity of inguinofemoral lymphadenectomy and the absence of inguinofemoral lymph node metastases in the majority of patients with early-stage disease are compelling arguments to develop methods to define a ‘low-risk’ group. In this ‘low-risk’ group of selected vulvar carcinoma patients, inguinofemoral lymphadenectomy might be omitted.

This review focuses on currently available non- and minimally invasive diagnostic methods and clinicopathologic analysis of primary tumor characteristics for prediction of inguinofemoral lymph node metastases in patients with primary SCC of the vulva.
DATA SOURCES

Relevant studies were identified by a computer search of English language abstracts in the Pubmed database until May 2004. We searched the medical literature using combinations of the following heading terms: vulvar carcinoma, SCC, prognosis, lymph node metastases, ultrasound, computer tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and sentinel lymph node. Outcomes of interest were articles on identification / prediction of lymph node metastases. With the button ‘related articles’ in Pubmed, papers were identified that were not found in the primary search. Reference lists from selected articles were used in order to identify missing relevant publications.

NONINVASIVE METHODS

PALPATION

Palpation of the inguinofemoral lymph nodes is a cheap and simple approach for determining the inguinofemoral lymph node status but is impaired by several conditions: obesity of the patients, small size of the metastases, metastases located deep in the subcutaneous fat tissue, and scar tissue due to former surgery and / or radiation. Initially, the staging system of vulvar carcinoma was based on pretreatment evaluation according to the tumor node metastasis (TNM) classification (Table 1). The information of the inguinofemoral lymph node status was obtained by palpation of the groins (N0: no lymph nodes, N1: non-suspicious lymph nodes and N2: suspicious lymph nodes). In all three groups of the clinical groin classification, errors were made compared to the final pathological review of the lymph nodes after surgery. The overall error rate was nearly 25%. These disappointing results were confirmed by the observation that intraoperative lymph node palpation had positive and negative predictive values of only 56% and 89%, respectively, even when done by experienced gynecologic oncologists. Comparable results were recently reported by Katz et al. From these observations, it appears that the accuracy of palpation is not sufficient to select patients in whom an inguinofemoral lymphadenectomy can be omitted.

ULTRASOUND

Ultrasound is safe, noninvasive, and highly acceptable to patients. Ultrasound has been used to assess nodal status in head and neck tumors, breast carcinoma, upper aerodigestive tract malignancy, and cervical carcinoma. The accuracy of ultrasound ranges between 67 and 95%, depending on the different parameters examined (short axis diameter, long axis diameter, long axis / short axis ratio, shape, nodal vascular pattern, echogenic pattern, and regularity of nodal outline).
TABLE 1. TUMOR NODE METASTASIS (TNM) CLASSIFICATION OF VULVAR CANCER

<table>
<thead>
<tr>
<th>TNM</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Tumor confined to the vulva, 2cm in largest diameter</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor confined to the vulva, &gt; 2cm in diameter</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor of any size with adjacent spread to the urethra and/or vagina and/or perineum and/or anus</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor of any size infiltrating the bladder mucosa and/or the rectal mucosa or including the upper part of the urethral mucosa and/or fixed to the bone</td>
</tr>
<tr>
<td>N0</td>
<td>No nodes palpable</td>
</tr>
<tr>
<td>N1</td>
<td>Nodes palpable in either groin, not enlarged, mobile (not clinically suspect for metastases)</td>
</tr>
<tr>
<td>N2</td>
<td>Nodes palpable in either or both groins, enlarged, firm and mobile (clinically suspect for metastases)</td>
</tr>
<tr>
<td>N3</td>
<td>Fixed or ulcerated nodes</td>
</tr>
<tr>
<td>M0</td>
<td>No clinical metastases</td>
</tr>
<tr>
<td>M1a</td>
<td>Palpable deep pelvic lymph nodes</td>
</tr>
<tr>
<td>M1b</td>
<td>Other distant metastases</td>
</tr>
</tbody>
</table>

Mäkela et al. compared palpation and ultrasound for the detection of metastatic inguinofemoral lymph nodes in 25 patients with vulvar carcinoma. The results of ultrasound were significantly better than those of palpation, with a negative predictive value of 94% for ultrasound. Comparable results were obtained for different lymph node basins in patients with cutaneous melanoma in whom ultrasound also appeared superior to palpation. However, especially due to the low number of vulvar carcinoma patients included in studies, there is currently too little evidence for excluding lymph node metastases with ultrasound alone. Ultrasound has also been combined with ultrasound-guided fine-needle aspiration cytology (FNAC) to improve accuracy by Moskovic et al. This combined technique could accurately predict nodal status in the majority of cases. Falsely negative cytology occurred when the metastatic focus was ≤ 3mm (two false-negative results out of 40 groins). Hall et al., who extended the study of Moskovic et al. to 44 patients, concluded that the combination of ultrasound and FNAC provides a sensitive and specific tool for preoperative assessment (sensitivity 93%, specificity 100%). Another possibility is to combine two sonography criteria as the definition for suspicious lymph nodes (short axis diameter > 8mm and long axis / short axis ratio [L / S ratio] ≤ 2) and to sample suspicious lymph nodes by ultrasound-guided FNAC. Even in the hands of experienced investigators, however, micrometastases will inevitably be missed with this method. Another drawback is that the adequacy of this technique is highly dependent on the skills of the radiologist and that radiologists with these skills are rare. For an overview of above-mentioned studies, see Table 2.

In conclusion, so far, the negative predictive value of ultrasound in patients with vulvar carcinoma (to exclude lymph node metastases) may be not high enough to allow its use as a criterion to omit inguinofemoral lymphadenectomy in selected patients. In combination with FNAC, ultrasound may
become of more importance in the near future, possibly in selecting patients for sentinel node detection or in the follow-up of vulvar carcinoma patients after a negative sentinel node.

**Table 2. Overview of Studies on Determining Inguinal Nodal Status with Ultrasound in Patients with Vulvar Cancer**

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makela 17</td>
<td>25</td>
<td>Ultrasound</td>
<td>82%</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preoperative palpation</td>
<td>9%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intraoperative palpation</td>
<td>55%</td>
<td>90%</td>
</tr>
<tr>
<td>Abang Mohammed 16</td>
<td>20</td>
<td>Ultrasound</td>
<td>83%</td>
<td>88%</td>
</tr>
<tr>
<td>Moskovic 20</td>
<td>24</td>
<td>Ultrasound</td>
<td>85%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytology</td>
<td>58%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Combined</td>
<td>83%</td>
<td>82%</td>
</tr>
<tr>
<td>Hall 21</td>
<td>44</td>
<td>Ultrasound</td>
<td>86%</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytology</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Combined</td>
<td>93%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**CT and MRI**

The only experience with CT in patients with vulvar carcinoma was the measurement of the distance in centimeters between the skin and the underlying inguinofermal lymph nodes for planning of groin radiation. 22,23 No other literature is available on the diagnostic value of CT for detection of inguinofermal lymph node metastases in patients with vulvar carcinoma.

MRI is being used more often in the treatment of gynecological malignancies because MRI offers an opportunity to stage both the primary tumor and the regional lymph nodes. Recently, two studies on identification of inguinal lymph node metastases in vulvar carcinoma by MRI were published. MRI was highly specific for the detection of nodal involvement in 22 patients with vulvar carcinoma (for superficial nodes a specificity of 97% and for deep inguinal nodes 100%). However, the sensitivity for both groups of inguinal nodes was low (respectively, 40% and 50%), which means a high false-negative rate. 24 Another pilot study showed a specificity of 91% and a sensitivity of 89% for detection of inguinal lymph node metastases by MRI in ten patients with vulvar carcinoma. 25 An explanation for the difference in sensitivity between both studies might be that the parameters used to define a lymph node as abnormal were more extensive in the second study. These preliminary data indicate that MRI may have some value for selecting women in whom the risk of nodal metastases is very
low from those in whom the risk is relatively high, but further experience is needed before firm conclusions can be drawn.

**POSITRON EMISSION TOMOGRAPHY**

The clinical use of PET for detection and staging of malignant tumors is rapidly increasing in different malignancies. In the future, PET may obtain a role in diagnostic imaging as well as in monitoring of therapeutic interventions in specific malignancies. In general, limitations exist with regard to false-positive results. Acute or chronic inflammation as well as aspecific reactions following radiotherapy may mimic tumor tissue.

In cutaneous melanoma, the accuracy of detecting lymph node metastases was studied in 56 lymph node basins: PET detected 100% of metastases > 10mm; 83% of metastases 6 – 10mm; and 23% of metastases ≤ 5mm. In stage I and II melanoma, PET was positive in only one of eight patients with a positive sentinel node, yielding a sensitivity of 13%. Comparable results (sensitivity 15%) were obtained in melanoma patients with Breslow thickness > 1.0mm and no palpable lymph nodes. Apparently, PET cannot detect subclinical microscopic disease with acceptable sensitivity.

In SCC of the vulva, sensitivity and specificity of detection of inguinofemoral lymph node metastases by PET using L-[11C]-tyrosine as a tracer were 62% and 89% per groin. Another study with fluorodeoxyglucose showed similar results (sensitivity 67%, specificity 95%). Both studies concluded that PET is not able to adequately predict or exclude presence of inguinofemoral lymph node metastases in patients with SCC of the vulva.

**MINIMALLY INVASIVE METHODS**

**SENTINEL LYMPH NODE PROCEDURE**

The lack of accurate noninvasive techniques to detect inguinofemoral lymph node metastases encouraged the use of the minimally invasive sentinel lymph node procedure. The sentinel lymph node is defined as the first draining lymph node, and the pathology of the sentinel lymph node is considered to be representative for the non-sentinel lymph nodes. It implies that a negative sentinel lymph node predicts the absence of tumor metastases in the other non-sentinel lymph nodes. The sentinel node procedure is already generally accepted as an accurate method of staging in patients with cutaneous melanoma and breast carcinoma, but large randomized trials are still under way to prove its safety and clinical utility.

In 1994, the sentinel node procedure with only blue dye was first described in patients with vulvar carcinoma. In subsequent pilot studies, the sentinel node procedure with the combined technique (preoperative lymphoscintigram with technetium-labeled nanocolloid and blue dye) proved to be feasible in patients with primary vulvar carcinoma.
In 1999, Ansink et al. published a report on the first large series of patients studied to investigate the negative predictive value of the sentinel lymph node procedure in vulvar carcinoma. In this multicenter study, only blue dye was used. Shortly after this publication, DeCicco et al. reported no false-negative sentinel lymph node in 37 patients with preoperative lymphoscintigram and gamma probe-guided surgery. De Hullu et al. used the combined technique and reported no false-negative sentinel lymph node in 59 patients. See Table 3 for an overview of the studies investigating the negative predictive value of a negative sentinel lymph node. Only studies with more than 20 patients are included. In all these studies, the sentinel node procedure was followed by inguinofemoral lymphadenectomy. The results of these studies show an identification rate of nearly 100% when preoperative lymphoscintigram and gamma probe-guided surgery have been performed.

**Table 3. Studies on the Accuracy of the Sentinel Lymph Node Procedure in Vulvar Cancer**

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Blue dye</th>
<th>Tracer</th>
<th>Lympho-Scintigram</th>
<th>Identification rate</th>
<th>False negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ansink</td>
<td>51</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>56%</td>
<td>2</td>
</tr>
<tr>
<td>DeCicco</td>
<td>37</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>De Hullu</td>
<td>59</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Levenback</td>
<td>52</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>88%</td>
<td>0</td>
</tr>
<tr>
<td>Silutz</td>
<td>26</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Moore</td>
<td>21</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Puig-Tintore</td>
<td>26</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>96%</td>
<td>0</td>
</tr>
</tbody>
</table>

* in only 8 of the 26 patients, blue dye was used

An extended study is ongoing in the United States (GOG-173) to further determine the accuracy of the sentinel node procedure in vulvar carcinoma, by performing the sentinel node procedure with isosulfan blue, followed by standard complete lymphadenectomy. Pre- or intraoperative lymphoscintigraphy is optional.

In conclusion, based on 169 patients in five studies, the sentinel node procedure with at least the preoperative use of a radioactive tracer, eventually combined with blue dye, is highly accurate in predicting lymph node metastases, with a negative predictive value of a negative sentinel node of nearly 100%. However, safety and clinical utility need still to be proven in large clinical trials, in which full lymphadenectomy is no longer performed after a negative sentinel lymph node.
Primary Tumor Characteristics

Histopathologic Parameters

In the past, many studies on histopathologic parameters of the primary tumor have been performed in vulvar carcinoma with the aim to define a group with 'low risk on lymph node metastases'. Initially, several authors considered, by analogy with cervical carcinoma, depth of invasion ≤ 5mm as 'microinvasive', with an extremely low risk on inguinofemoral lymph node metastases, but several more recent studies showed that the percentage of patients with inguinofemoral lymph node metastases rises steadily with each millimeter of invasion.49-57 See Table 4 for an overview of five studies, which registered the percentage of inguinofemoral lymph node metastases related to depth of invasion per millimeter in patients with T1 (≤ 2cm) tumors.50,52,55-57

Table 4. Lymph Node Status in Patients with T1 Tumors in Relation to Depth of Invasion

<table>
<thead>
<tr>
<th>Depth of Invasion</th>
<th>Number of Patients</th>
<th>Number of lymph node metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1mm</td>
<td>108</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1.1 – 2mm</td>
<td>92</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td>2.1 – 3mm</td>
<td>79</td>
<td>7 (8.9%)</td>
</tr>
<tr>
<td>3.1 – 4mm</td>
<td>42</td>
<td>13 (25%)</td>
</tr>
<tr>
<td>4.1 – 5mm</td>
<td>25</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>&gt; 5mm</td>
<td>31</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
<td>42 (11.1%)</td>
</tr>
</tbody>
</table>

Only in patients with SCC of the vulva with ≤ 1mm of invasion (stage IA)54 it is now generally accepted to omit inguinofemoral lymphadenectomy based on the low risk of inguinofemoral lymph node metastases, as shown in Table 4. Only occasional reports have been published of patients with stage IA vulvar carcinoma with inguinofemoral lymph node metastases.58-60 Moreover, the long-term results of these patients who underwent only local treatment without inguinofemoral lymphadenectomy are excellent.61

Univariate analyses of other histopathologic variables, such as tumor diameter, presence of lymphovascular invasion, grade of the tumor, growth pattern (pushing versus spray-like), concurrent vulvar intraepithelial neoplasia, site of the lesion, and uni- or multifocality of the tumor, have been performed in different studies, with conflicting results regarding their ability to predict inguinofemoral lymph node metastases. Several authors performed multivariate analyses to define which clinicopathologic factors, apart from depth of invasion, are independently related to inguinofemoral
lymph node metastases. See Table 5 for an overview of these studies. However, it appears to be impossible to define a real ‘low-risk group’ based on these factors. Apart from this inability, it remains to be decided what exactly ‘low risk’ means. When debating a possible acceptable false-negative rate for sentinel node detection in vulvar carcinoma, the Gynecologic Cancer Group of the European Organization for Research and Treatment of Cancer (EORTC) decided that in light of the significant decrease in morbidity by omitting elective lymphadenectomy, a maximum increase of groin recurrences (i.e., a false negative rate) of 6% might be acceptable. In analogy to this discussion, this would imply that primary tumor characteristics should be able to define a group of patients with a risk of metastases < 6%. Within this point of view, inguinofemoral lymphadenectomy could also be omitted in patients with vulvar carcinoma with a depth of invasion of 1.1 – 2.0mm (a risk on lymph node metastases of 5.4%; see Table 4).

In conclusion, based on conventional histopathologic primary tumor characteristics, there is no possibility of defining ‘low-risk groups’ of patients with invasive SCC for whom it is safe to omit inguinofemoral lymphadenectomy, other than patients with tumors with a depth of invasion ≤ 1mm.

**Table 5. Overview of Studies with Multivariate Analyses Concerning Relation Between Clinicopathologic Factors and Inguinofemoral Lymph Node Metastases**

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Variables independently related to lymph node metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homesley</td>
<td>588</td>
<td>Clinical node status, lymphovascular invasion, tumor differentiation (GOG), age, depth of invasion</td>
</tr>
<tr>
<td>Andreasson</td>
<td>122</td>
<td>Tumor site, depth of invasion, tumor size</td>
</tr>
<tr>
<td>Boyce</td>
<td>84</td>
<td>Clinical node status, depth of invasion, growth pattern</td>
</tr>
<tr>
<td>Smyczek-Gargya</td>
<td>168</td>
<td>Clinical node status, tumor size, depth of invasion, tumor grading</td>
</tr>
</tbody>
</table>

**Cell Biologic Parameters**

Apart from the more conventional clinical and histopathologic parameters described above, more recently discovered cell biologic parameters have also been evaluated for their possible value in predicting lymph node metastases in vulvar carcinoma. In the following paragraphs, we will summarize those cell biologic parameters that were analyzed for their usefulness in predicting lymph node metastases.

DNA ploidy status is a prognostic factor for survival in vulvar melanoma but appears to have no prognostic value in SCC of the vulva. Tumors with positive inguinal lymph nodes appeared to be associated with a higher rate of aneuploidy compared to tumors with negative inguinal nodes, but the difference was not significant. However, a significantly higher S-phase fraction (proliferation...
index) was found in tumors from patients with lymph node metastases when compared to tumors from patients with negative lymph nodes (median value in patients with lymph node metastases [n=13] 18.2, in patients with negative lymph nodes [n=26] 8.9). In 1976 the role of human papillomavirus (HPV) was first proposed in the development of carcinoma of the female genital tract. The virally (HPV) encoded oncoproteins E6 and E7 can form a complex with protein products of tumor suppressor genes, E6 binding to the p53 protein and E7 binding to the retinoblastoma gene product. This may lead to loss of the ‘normal’ negative growth control. The relation between HPV infection and vulvar carcinoma is not as clear as it is in cervical carcinoma. About one-third of SCC of the vulva is associated with high-risk HPV infection, particularly HPV types 16 and 18. Data on the relation between the HPV status and the presence of lymph node metastases are conflicting. One study with a small number of patients reported that the rate of metastases in HPV-positive vulvar carcinomas (3 / 7, 43%) was higher than for the HPV-negative carcinomas (4 / 16, 25%). Another larger study, however, found no relation between the presence of HPV and lymph node metastases.

Epidermal growth factor receptor (EGFR) activation plays a key role in cell adhesion, cell locomotion, cell survival, invasion, and angiogenesis, which results in modulation of tumor progression. EGFR expression was analyzed in benign vulvar epithelium, primary vulvar carcinoma, and in groin node metastases. There was a progressive increase in EGFR expression from benign vulvar epithelium to primary malignant vulvar tissue to metastatic lesions within the same patient. Increased expression of EGFR in the primary vulvar malignancy was significantly associated with lymph node metastases and decreased patient survival in a study of 61 patients (an increased EGFR level in tumor tissue identified 71% of lymph node metastases). Twenty-nine percent of the lymph node metastases could, however, not be identified by an increased EGFR level, so EGFR level analysis is not sensitive enough to exclude patients for complete lymphadenectomy.

Overexpression of the tumor suppressor gene p53 in squamous cell vulvar carcinomas varies from 39% to 61% in different studies. No relation was found between overexpression of p53 in the primary tumor and lymph node metastases in patients with vulvar carcinoma.

The tumor suppressor gene retinoblastoma (Rb) has also been investigated in vulvar carcinoma. Loss of Rb immunostaining was observed in about 20% of vulvar SCC but did not seem to play any role for prognosis. The loss of Rb expression increased from FIGO stage I to IV, suggesting that Rb may play a role in tumor progression. Another study showed progressive decrease of Rb2 / p130 (a retinoblastoma-related protein) expression from non-neoplastic epithelial alterations through intraepithelial neoplasia to invasive vulvar carcinoma, suggesting a role for this tumor suppressor gene in vulvar carcinogenesis. There was no correlation between Rb2 / p130 expression and the presence of lymph node metastases.

The presence of the HER-2 / neu immunopositivity was found to be associated with lymph node metastases in early SCC of the vulva as 69% of the node-positive tumors was HER-2 / neu positive.
against 36% of the node-negative tumors (see Table 6).\textsuperscript{69} Assessment of the proliferation-associated markers Ki-67 and Ag-NOR did not contribute to the prediction of lymph node metastases.\textsuperscript{83}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
 & Sensitivity (%) & Specificity (%) & PPV (%) & NPV (%) \\
\hline
HER-2 / neu (N=39) & 69 & 61 & 47 & 80 \\
\hline
HPV (N=23) & 43 & 75 & 43 & 75 \\
\hline
EGF-R (N=61) & 100 & 15 & 26 & 100 \\
\hline
\end{tabular}
\caption{Overview of Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of Cell Biologic Parameters that Showed a Significant Correlation with Lymph Node Metastases}
\end{table}

Studies on pro- and anti-apoptotic parameters in vulvar carcinoma are limited. While Bcl-2 overexpression leads to blocking of apoptosis, overexpression of Bax promotes apoptosis. In a study of 72 vulvar carcinoma patients, immunohistological staining for Bcl-2 showed that Bcl-2 positivity is associated with lymph node metastases (p=0.03).\textsuperscript{84} In contrast, in a preliminary study of 28 patients by Hefler et al., no correlation was found between Bcl-2 expression and lymph node involvement.\textsuperscript{85}

Cyclooxygenase-2 (COX-2) is one of the two isoforms of the key enzyme in the biosynthesis of prostaglandins and (in critical steps of tumor progression) overexpression has been associated with increased metastatic potential. The use of the ratio between COX-2 in the tumor cells and COX-2 in the stroma seems to correlate more effectively with vulvar tumor extension and metastatic lymph node involvement than the levels of COX-2 in tumor cells. In a study of 36 patients with invasive vulvar carcinoma, a higher tumor / stroma COX-2 ratio was observed in cases with lymph node metastases compared to cases without lymph node metastases (p=0.037). It remains to be verified whether assessment of COX-2 status can be useful in the prognostic characterization of vulvar carcinoma.\textsuperscript{86}

Comparative genomic hybridization (CGH) is a powerful tool in the study of carcinoma that has quickly gained recognition for its ability to scan the entire genome for DNA copy number changes that are mapped to chromosome regions. Chromosome regions gained or lost may harbor oncogenes or tumor suppressor genes, respectively. Loss of 4p13-pter, and concomitant loss of 3p and gain in 3q, is the most frequent genetic alteration in SCC of the vulva.\textsuperscript{87} Another study found that gain of 3q was more common in HPV-positive carcinomas compared to HPV-negative carcinomas, while chromosome 8q was more frequently gained in HPV-negative carcinomas. Chromosome arms 3p and 11q were frequently lost in both categories of vulvar carcinoma.\textsuperscript{88} The different genetic changes indicate that there are at least two different types of vulvar carcinoma. However, all studies on CGH included only a limited number of patients. To our knowledge, no articles are available that study the relationship between lymph node metastases and chromosome regions gained or lost.
DISCUSSION

In SCC of the vulva, in particular, a very high negative predictive value for a diagnostic method is necessary to safely omit inguinofemoral lymphadenectomy. With respect to the groin, uni- or bilateral inguinofemoral lymphadenectomy is currently the standard treatment. Because of the high morbidity and complication rate of this surgical procedure, there is a trend towards less radical treatment. In 50 – 60% of the patients, the inguinofemoral lymphadenectomy may only be a diagnostic method to exclude lymph node metastases. It has to be kept in mind that missing lymph node metastases has significant consequences because of the high mortality. This points to the clinical relevance of a reliable non- or minimally invasive technique for detection of lymph node metastases.

No noninvasive procedure (palpation of the groins, ultrasound, MRI, CT, PET) is available that can predict lymph node metastases with a negative predictive value that is high enough to safely omit inguinofemoral lymphadenectomy. Ultrasound combined with fine-needle aspiration shows promising results, but small metastases will eventually be missed by this procedure. However, this method may be helpful preoperatively to exclude patients from the sentinel lymph node procedure by detecting lymph node metastases, leading to immediate inguinofemoral lymphadenectomy.

Studies on different histopathologic parameters showed that inguinofemoral lymphadenectomy can be omitted in tumors with a depth of invasion less than 1mm. No other histopathologic parameters are able to define low-risk groups of patients with invasive SCC of the vulva.

Data on cell biologic parameters are inconclusive, conflicting and the number of patients analyzed is often too small. Some cell biologic parameters showed significant correlation with lymph node metastases: S-phase fraction, HER-2 / neu, HPV positivity, EGFR overexpression, Bcl-2, and COX-2. We calculated the sensitivity, specificity, negative predictive value, and positive predictive value of the parameters of which the necessary data were available in literature (HER-2 / neu, HPV and EGFR; see Table 6). The only parameter with a high enough negative predictive value to exclude lymph node metastases in patients with vulvar carcinoma was EGFR overexpression. However, this parameter had a very low specificity, so very few patients without lymph node metastases will be identified by this technique. Also, the number of patients in these three studies was small (respectively 39, 23, and 61 patients). Further research is necessary to define the consequences of these positive results. Until now, the cell biologic parameters investigated have not been sensitive enough to exclude patients for inguinofemoral lymphadenectomy.

At present, the minimally invasive sentinel lymph node procedure is the most promising diagnostic tool for assessment of lymph node status, but its safety is still to be proven. Extended studies are ongoing to further determine the accuracy and safety of the sentinel node procedure in vulvar carcinoma. For now, wide local excision with uni- or bilateral inguinofemoral lymphadenectomy is still the standard treatment for patients with SCC of the vulva with a depth of invasion > 1mm.
References


