Ultrastaging Improves Detection of Metastases in Sentinel Lymph Nodes of Uterine Cervix Squamous Cell Carcinoma

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Abstract: The technique of sentinel lymph node (SLN) detection is increasingly being applied in patients with uterine cervix carcinoma. This study presents the pathologic findings of SLNs in 48 such patients. The institutional pathology files were searched for all patients with a diagnosis of cervical squamous cell carcinoma who had SLNs reported. Patient age, follow-up, tumor size, presence/absence of lymphatic invasion, number and status of SLNs and non-SLNs, location of SLNs, and size of metastases in SLNs were recorded. All SLNs were sectioned in 2-mm slices perpendicular to the long axis and submitted entirely for microscopic examination. For all SLNs negative on the initial hematoxylin and eosin (H&E) stained slides, an ultrastaging protocol was performed consisting of 5 sets of slides at 40-μm intervals (1 H&E slide + 2 unstained slides), representing an additional 5 intervals. Lymph nodes negative by the additional H&E intervals had immunohistochemistry for cytokeratin performed on 1 unstained slide. Forty-eight patients ranging from 25 to 62 years of age had a total of 208 SLNs removed. Fifteen (31%) patients had positive SLNs with 1 to 5 positive SLNs per case. The metastasis size ranged from a single cell to 27 mm. Twelve patients had metastasis detected by routine processing in 23 SLNs, whereas ultrastaging detected metastases in 3 SLNs of 3 additional patients. In 2 patients with metastasis detected by ultrastaging, the metastasis was detected by wide H&E intervals (level 2 for 1 patient; level 3 for 1 patient); in 1 patient, the metastasis was detected only by immunohistochemistry and consisted of a single cell. Of the 15 patients with positive SLNs, 3 patients had a total of 6 positive non-SLNs. All of the patients with a positive SLN are currently living. Thirty-three (69%) patients had negative SLNs. Of these, 1 patient had a single positive non-SLN for a false negative rate of 6.25%. Negative SLN predicts negative non-SLN. For most patients with a positive SLN, the SLN will be the only metastasis detected; a minority of patients with a positive SLN may have a positive non-SLN.

Key Words: sentinel lymph node, ultrastaging, uterine cervix, squamous cell carcinoma, metastasis

Uterine cervix carcinoma is the third most common gynecologic malignancy. It is estimated that in the year 2007, 11,150 women will be diagnosed with this type of tumor whereas 3670 women will die from this disease. The most important prognostic factor in early stage cervical carcinoma is the presence or absence of metastatic carcinoma in the pelvic lymph nodes. To that end, the majority of patients with cervical carcinoma undergo pelvic lymph node dissection. However, the reported incidence of nodal metastases is not high, ranging from 0% to 16% in patients with tumors ≤2.0 cm to 15% to 31% in patients with IB carcinoma overall. Therefore, at least two-thirds of patients with cervical carcinoma theoretically could be spared a complete lymph node dissection with the attendant potential morbidities of nerve/vessel injury, lymphocyst formation, and lymphedema.

Recent efforts to identify those women who may not require a full pelvic lymph node dissection have concentrated on sentinel lymph node (SLN) identification. The SLN is defined as the first lymph node draining an anatomic site involved by tumor. If the SLN is histologically negative for metastatic disease, then the remaining lymph nodes of that chain also should be negative. Women with histologically negative SLNs could therefore be spared a more extensive lymph node dissection. An initial SLN biopsy in lieu of a complete lymph node dissection has become the standard of care for breast carcinoma and melanoma. The feasibility of SLN identification in cervical carcinoma has been well documented. The majority of these studies have focused on the clinical process of SLN identification and the predictive value of the SLN result. Many of these series have been relatively small with a tendency to include all types of cervical carcinoma. It is unknown whether the tumor type would behave differently in a series with a predominant histologic type.
influences findings such as the typical size of lymph node metastasis and the risk of metastasis to a SLN. Therefore, this study has been restricted to the evaluation of SLNs in patients with pure squamous cell carcinoma of the cervix. Most of the literature on the subject of SLN mapping in patients with cervical carcinoma has been clinically driven. There has been little attention paid to either the pathologic findings in the SLN or how the SLN should be processed for histologic examination. In this series, the clinical and pathologic features of 48 patients with squamous cell carcinoma of the cervix who underwent SLN mapping are presented, with an emphasis on the need to examine SLNs in histologic detail. A proposed method for such an examination is introduced on the basis of the findings in this case series.

MATERIALS AND METHODS

The surgical pathology files at The University of Texas MD Anderson Cancer Center Department of Pathology, were searched for all patients with squamous cell carcinoma of the cervix who had SLNs reported during a period of 9 years (year 1998 to 2006). Clinical features recorded included patient age, adjuvant treatment, and follow-up. Histopathologic features recorded included tumor size, presence or absence of lymphovascular space invasion, the number of SLNs and non-SLNs per case, the presence of metastases in SLNs and non-SLNs, and the size and location of the metastases in the SLNs. All SLN slides were reviewed at MD Anderson Cancer Center. All available SLN slides were re-reviewed for the purpose of this study.

All lymph nodes that were designated “sentinel” intraoperatively were serially sectioned at 2-mm intervals perpendicular to the long axis and entirely submitted for routine processing and hematoxylin and eosin (H&E) staining. Any SLN negative by routine examination was further examined by an ultrastaging protocol consisting of 3 consecutive sections (5-μm thick), each obtained at 5 levels (40-μm interval). The first section of each level was stained with a keratin cocktail to confirm the negative histologic impression.

The keratin cocktail used in the immunohistochemistry laboratory at MD Anderson Cancer Center is composed of 4 antibodies: AE1/AE3 (DAKO, 1:50, Carpinteria, CA), CAM5.2 (Becton Dickinson, 1:50, San Jose, CA), Cytokeratin MNF116 (DAKO, 1:50, Carpinteria, CA), and Keratin 8 and 18 (Zymed, 1:25, South San Francisco, CA). The un-stained slides were dried at 37°C for 30 minutes before being placed into a BondMax automated immunostainer (Vision BioSystems, San Francisco, CA). Epitope retrieval was performed with enzyme pretreatment for 5 minutes, and a polymer detection system was used. Diaminobenzidine was used as the chromagen.

To calculate the probability that a metastasis of a certain size would be detected using our institutional ultrastaging protocol, the formula \((0.275 + d)/2\) was used in which \(d\) is the diameter of the metastasis. The other component of the numerator, 0.275, is derived from the portion of each serial section of lymph node examined in detail according to the ultrastaging protocol (5 segments of 55 μm each composed of a 40-μm wide interval plus three 5-μm thick sections). This model is only an approximation and assumes that metastases are spherical and is applicable for a single metastasis. Statistical comparisons were carried out using Wilcoxon rank-sum tests (R package software) and the Fisher exact test (Stat software).

RESULTS

SLNs were identified in 48 patients with International Federation of Gynecology and Obstetrics stage IA-IB2 squamous cell carcinoma of the cervix. A total of 208 SLNs were identified with a median of 3.5 SLN/patient (range: 1 to 15). Thirty-three patients had negative SLNs and 15 patients had at least 1 SLN positive for metastasis. The clinicopathologic features of cases with and without metastasis to a SLN are compared in Table 1. Features of the 15 cases with positive SLNs are summarized in Table 2.

### TABLE 1. Clinicopathologic Features of Cases With and Without Positive Sentinel Lymph Nodes

<table>
<thead>
<tr>
<th></th>
<th>Positive SLN</th>
<th>Negative SLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>37.5 ± 6</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>Median follow-up (mo)</td>
<td>48, all patients alive NED</td>
<td>52, one patient DOD</td>
</tr>
<tr>
<td>Mean tumor size (cm)</td>
<td>2.3 ± 1.3</td>
<td>2.3 ± 1.3</td>
</tr>
<tr>
<td>Vascular invasion present</td>
<td>12/15 patients</td>
<td>13/33 patients</td>
</tr>
<tr>
<td>Total SLN identified</td>
<td>76</td>
<td>127</td>
</tr>
<tr>
<td>Total non-SLN identified</td>
<td>156</td>
<td>797</td>
</tr>
<tr>
<td>Number, positive non-SLN</td>
<td>10 SLN (3 patients)</td>
<td>1 (1 patient)*</td>
</tr>
<tr>
<td>Most common SLN site</td>
<td>External iliac</td>
<td>External iliac</td>
</tr>
</tbody>
</table>

DOD indicates dead of disease; NED, no evidence of disease; SLN, sentinel lymph node.

*Represents the 1 patient who died of disease.
In the SLN negative group (127 SLNs total; approximately 4 SLNs/patient), the patient aged from 25 to 62 years (mean: 41 ± 9 y; median: 38 y). The follow-up in this group ranged from 3 to 85 months (mean: 50.2 ± 24.6 mo). Six patients received adjuvant therapy, which, in 2 cases, consisted of preoperative radiation therapy. One of the two patients receiving preoperative radiation therapy had an intracavitary system. The other patient received a single small dose of pelvic radiation before opting for surgery as definitive therapy. Twenty-six patients received no additional therapy beyond radical hysterectomy and pelvic lymph node dissection. In 1 patient, treatment after pelvic lymph node dissection was unknown. Tumor size in the SLN negative group ranged from 0.5 to 6.0 cm (mean 2.3 ± 1.3 cm) with 11 poorly differentiated tumors, 14 moderately differentiated tumors, and 1 well differentiated tumor. In 2 patients, there was no residual invasive carcinoma. Thirteen patients had lymphovascular invasion and 17 patients had no lymphovascular invasion. The presence or absence of lymphovascular invasion could not be confirmed in 3 patients. Nineteen patients had bilateral SLNs identified, whereas 14 had unilateral SLNs identified. The most frequent sites for SLNs included external iliac (40 lymph nodes), obturator (40 lymph nodes), and common iliac (18 lymph nodes). Thirty-two patients with negative SLNs also had negative non-SLNs. Only 1 patient had a negative SLN with positive non-SLNs. This patient, an early patient in the series, had a 5.0-cm cervical tumor with 1 SLN in the left common iliac basin identified. Nineteen non-SLNs were identified of which 4 had metastases. The positive non-SLNs were bilateral, and in 3, the lymph node was grossly replaced by tumor. At least one of the positive non-SLNs, based on the specimen designation, was located in a lymphatic basin closer to the tumor than the identified SLN. The tissue block from this negative SLN was subsequently exhausted at 40-μm intervals and no metastatic carcinoma was identified. This patient accounted for the 1 documented death in the entire series of 48 patients. Twenty-eight patients with negative SLNs are alive with no evidence of disease; 4 patients have been lost to follow-up.

Fifteen patients had 26 positive SLNs (out of 76 SLNs total; approximately 5 SLNs/patient). Patient age ranged from 31 to 51 years (mean: 37.5 ± 6 y, median: 38 y), and follow-up ranged from 6 to 78 months (mean 42.2 ± 24.5 mo). Twelve patients received adjuvant therapy (radiation or chemoradiation) after surgery, and 2 patients elected to receive no further therapy. One patient was lost to follow-up shortly after surgery; therefore, it is unknown whether that patient received adjuvant therapy. The other patients in this group are currently alive and without evidence of disease. The tumor size in this group ranged from 1.0 to 3.5 cm (mean 2.3 ± 1.3 cm) with 11 poorly differentiated tumors, 3 moderately differentiated tumors, and 1 well differentiated tumor. No significant relation between tumor grade and the risk of a positive SLN could be demonstrated (\( P = 0.239 \)) if moderately and poorly differentiated tumors were considered as separate groups, \( P = 1.0 \) if moderately and poorly differentiated tumors were considered as a single high-grade group). Lymphovascular invasion was present in 12 cases and absent in 2 cases. The presence of lymphovascular invasion correlated strongly with having a positive SLN when compared with the group with negative SLN (\( P < 0.005 \)). The presence or absence of lymphovascular invasion could not be confirmed in 1 case. In 13 cases, bilateral SLNs were identified. In 2 cases, the SLN was unilateral. The most common sites identified for SLNs included external iliac (20 lymph nodes), obturator (19 lymph nodes), and common iliac (16 lymph nodes). The positive SLNs were noted in the following locations:

### Table 2. Features of Cases With Positive Sentinel Lymph Nodes

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Tumor Size (cm)</th>
<th>Lymphatic/Vascular Invasion</th>
<th>Positive SLN/Total SLN</th>
<th>Metastasis Size (mm)</th>
<th>Ultrastaging Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>IB1</td>
<td>2.7</td>
<td>Extensive</td>
<td>5/11</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>Case 2</td>
<td>IB1</td>
<td>2.0</td>
<td>Not stated</td>
<td>5/6</td>
<td>Unknown</td>
</tr>
<tr>
<td>Case 3</td>
<td>IB1</td>
<td>2.2</td>
<td>Extensive</td>
<td>1/5</td>
<td>3.0</td>
</tr>
<tr>
<td>Case 4</td>
<td>IB1</td>
<td>1.0</td>
<td>Present</td>
<td>1/2</td>
<td>Single cell</td>
</tr>
<tr>
<td>Case 5</td>
<td>IB2</td>
<td>1.0</td>
<td>Extensive</td>
<td>1/5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Case 6</td>
<td>IB1</td>
<td>1.7</td>
<td>Present</td>
<td>2/2</td>
<td>0.5, 27</td>
</tr>
<tr>
<td>Case 7</td>
<td>IB1</td>
<td>2.3</td>
<td>Present</td>
<td>1/8</td>
<td>1.0</td>
</tr>
<tr>
<td>Case 8</td>
<td>IB1</td>
<td>1.0</td>
<td>Present</td>
<td>1/5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Case 9</td>
<td>IB1</td>
<td>2.5</td>
<td>Present</td>
<td>1/2</td>
<td>Unknown</td>
</tr>
<tr>
<td>Case 10</td>
<td>IB1</td>
<td>3.5</td>
<td>No</td>
<td>1/3</td>
<td>Unknown</td>
</tr>
<tr>
<td>Case 11</td>
<td>IB1</td>
<td>1.5</td>
<td>No</td>
<td>1/3</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Case 12</td>
<td>IB1</td>
<td>2.5</td>
<td>Present</td>
<td>1/5</td>
<td>4.5</td>
</tr>
<tr>
<td>Case 13</td>
<td>IB1</td>
<td>3.5</td>
<td>Present</td>
<td>2/7</td>
<td>1.5, 1.6</td>
</tr>
<tr>
<td>Case 14</td>
<td>IB1</td>
<td>1.0</td>
<td>Present</td>
<td>1/4</td>
<td>11.0</td>
</tr>
<tr>
<td>Case 15</td>
<td>IB2</td>
<td>4.7</td>
<td>Extensive</td>
<td>2/5</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

H&E indicates hematoxylin and eosin; IHC, immunohistochemistry; SLN, sentinel lymph node.
FIGURE 1. Metastatic carcinoma in 2 sentinel lymph nodes detected by ultrastaging. A, Invasive, poorly differentiated squamous cell carcinoma with vascular invasion (arrow), case 5 (100×). B, Initial H&E level of a right external iliac sentinel lymph node (200×), case 5. C, H&E level 3 in same area as image (B) (200×) with metastatic squamous cell carcinoma in a subcapsular sinus (arrow). D, Invasive, poorly differentiated squamous cell carcinoma, case 7 (200×). E, Metastatic carcinoma in a left external iliac sentinel lymph node (200×), case 7. F, Carcinoma in image (E) is highlighted by cytokeratin immunohistochemistry (200×).
external iliac (11 SLNs), obturator (7 SLNs), parametrical (5 SLNs), junctional (1 SLN), common iliac (1 SLN), and para-aortic (1 SLN). None of the positive SLNs had extracapsular extension. Metastasis size ranged from isolated tumor cells identified only by immunohistochemistry to grossly visible tumor (up to 27 mm) with a median metastasis size of 1.5 mm. Ten of twenty-six SLNs had metastases < 2.0 mm in greatest dimension (single cell to 1.6 mm; median size: 0.9 mm). The smallest 3 metastases, all ≤0.1 mm (single cell and 2 cell clusters), were observed in the subcapsular sinus of the involved lymph node. The larger metastases were located within the subcapsular cortex. Positive SLNs were identified on initial section in 12 cases. The ultrastaging protocol identified 3 additional SLNs with metastases in 3 additional patients. In 2 patients, the positive SLN was identified on the additional H&E wide intervals (levels 2 to 5 in 1 case, level 3 in 1 case) (Fig. 1). The third patient had a keratin positive cell in the subcapsular sinus of a lymph node. This cell was not identified on any subsequent H&E levels and its nature could not be further investigated. The prognostic significance of such single cells is currently unknown. However, for the purpose of this study, the patient is included in the positive SLN group to illustrate the findings of the ultrastaging protocol.

Three of the fifteen patients with positive SLNs also had positive non-SLNs. In 1 patient, a right parametrical non-SLN with metastasis on the same side as a positive external iliac SLN was detected (case 12). For the second patient, positive left parametrical and left external iliac SLNs were identified along with 3 additional positive left parametrical non-SLNs. This patient also had a right pelvic non-SLN with metastatic carcinoma. A right external iliac SLN had also been identified but was negative after H&E levels and immunoperoxidase staining for cytokeratin (case 13). Additional levels at 40-μm intervals until exhaustion of the tissue block from the right external iliac SLN were performed and showed no evidence of metastatic carcinoma. Although the negative right external iliac SLN was technically a false negative for the right side, positive SLNs were identified on the opposite side, and the patient was appropriately triaged to adjuvant therapy. The third patient had a nonsentinel left obturator lymph node with metastasis in addition to a positive left obturator SLN (case 14). Of the variables, age, number of SLNs, number of positive SLNs, number of non-SLNs, method of SLN metastasis detection (ie, whether the metastasis was found on ultrastaging or initial processing), size of SLN metastasis, presence of vascular invasion, and SLN location, only the number of non-SLNs sampled was significantly (P ≤0.02) associated with the presence of positive non-SLNs. That is, patients with SLN metastases and higher numbers of non-SLNs sampled had an increased likelihood of finding metastasis in a non-SLN.

On the basis of the current ultrastaging protocol used at our institution, the approximate probability of detecting a metastasis of 0.9 mm (the median size for metastases < 2.0 mm in this study) is 59%. The probability decreases to 19% for a metastasis diameter of 0.1 mm.

DISCUSSION

SLN mapping, in lieu of a full pelvic and para-aortic lymph node dissection, holds the promise of less surgical-related morbidity for patients with operable cervical carcinoma. In theory, patients with negative SLNs and no other adverse prognostic features would require no further therapy. Therefore, before SLN mapping can become the standard of care in cervical carcinoma, it is necessary to gain a better understanding of the pathology of these lymph nodes. Insights gained from such studies can guide future direction for how these lymph nodes should be processed to ensure that a clinically significant metastasis is not missed.

In this series of 48 patients with early stage (IA-IB2) squamous cell carcinoma of the cervix, 15 patients had metastases to a SLN (31.25%). Only 1 patient had a false negative SLN for a false negative rate of 6.25%. It should be noted that in the setting of patients with grossly positive lymph nodes, as in this case, that tumor-replaced SLNs may not have reliable dye/tracer uptake. Of the patients with positive SLNs, 3 (20%) had additional positive non-SLNs. Our findings are consistent with previously published series of SLNs in cervical carcinoma.1–7,9–15,16–18,20–32,34–37,39,40,42–44

The published series to date of SLN in the setting of uterine cervix carcinoma have established the feasibility of SLN identification. From at least 37 such series, the reported range of patients with positive SLNs is 0% to 36%. The majority of the reported patients with a positive SLN did not have metastases to non-SLNs, with only 6 series reporting >40% of patients with a positive SLN having metastasis to a non-SLN.5,9,28,34,39,40 Twenty-one of the thirty-seven SLN studies reviewed during the preparation of this series reported a 0% false negative rate.1–4,6,7,11,12,17,18,21,22,26,27,29,31,32,36,40,42,44 In the remaining 12 studies, in which the issue of the false negative rate was addressed, the reported false negative rate ranged from 2.8% to 37%.5,8,16,20,23,24,28,30,34,35,37,39 The widely ranging false negative rates in these studies (0% to 37% overall) are most likely reflections of the variation in SLN identification technique and the variation in the histopathologic examination of the SLNs. It should be emphasized that none of these studies establish a consensus as to how SLNs should be processed for histopathologic examination or whether ultrastaging should be performed.

In our series, 3 patients with initially negative SLNs were found to have a positive SLN as a result of ultrastaging (2 patients on wide H&E intervals; 1 patient by immunohistochemistry) improving our detection rate by 25%. A majority of the published SLN series used some combination of additional H&E sections and/or cytokeratin staining on SLN that were histologically negative on initial tissue section.1,5,7,9,17,18,20,22–24,26,28–32,34–36,39,40,42,44 In 11 of these studies, the more extensive histopathologic investigation yielded an improved detection rate for metastasis in an SLN.4,5,7,9,17,24,30,31,39,44 However, the method varied between studies. Most performed at least some type of
cytokeratin stain, but not all of the series used H&E levels. Among investigators, the number of levels ranged from 1 additional level to up to 5 additional levels and the interval between levels ranged from 40 to 250 μm. This is significant because each method has a different potential for detecting metastases < 1.0 mm.

In our institution, we section all lymph nodes designated as sentinel perpendicular to the long axis in 2.0-mm thick slices. Although more tissue blocks are created, this method allows for a larger surface area of the lymphoid tissue, along with the subcapsular space, to be examined. If the initial H&E stained slide from each block is negative for metastasis, 5 additional H&E levels spaced at 40-μm intervals are obtained along with 2 unstained slides at each level. Because a significant number of patients (up to 25% in this study) may have a positive lymph node, the ultrastaging protocol is performed subsequent to the initial H&E section so that potentially unnecessary levels are avoided. Although it is an unstained slide from the first level that is usually selected for keratin staining to confirm the negative results by H&E, 2 unstained slides accompany each level. This insures that there will be material available for further investigation should the deeper levels have findings suspicious for, but not diagnostic of, metastasis. Between the initial processing and wide intervals, nearly two-thirds of the tissue block is used. Nearly 1.0 mm of tissue is removed when obtaining the initial H&E section for the purposes of facing the block and achieving a representative section of the tissue and is, therefore, not examined microscopically. This is common practice in obtaining routine sections for histologic examination. In the SLN further processed for ultrastaging, an additional 275 μm of the tissue are examined in further detail. At each of 5 levels spaced at 40-μm intervals, 1 H&E stained slide (5-μm thick section) plus 2 unstained slides (5-μm thick sections) for further study (total of 55-μm per level × 5) are examined. Approximately 725 μm of the tissue remains in the tissue block unexamined.

In theory, all metastases > 2.0 mm should be detected as the lymph node tissue is cut into 2.0-mm thick slices. Therefore, the ultrastaging protocol aims to detect those metastases that could be potentially missed by this grossing protocol. Ten lymph nodes had metastases < 2.0 mm. On the basis of the median size of 0.9 mm for this group, the approximate probability of detecting metastases of this size on the basis of our current ultrastaging protocol is 59%. The probability goes down to 19% for metastases approaching 0.1 mm (100 μm). The reason for a relatively low probability of detection despite a rigorous examination rests in the approximately 725 μm of tissue remaining in the block after the processing for ultrastaging. To examine the likelihood of the presence of metastases in the remaining lymphoid tissue, the tissue blocks of the false negative SLNs (1 patient with a false negative SLN and 1 patient with a false negative SLN on 1 side but positive SLNs on the contralateral side) were sectioned in their entirety at 40-μm intervals and no metastatic carcinoma was seen. This required the examination of 64 additional slides from 4 tissue blocks. Had all of the negative SLNs been processed in this manner, the workload would have been too burdensome. However, by increasing the wide interval size but maintaining the same number of slides, more of the tissue block is examined and the approximate probability of detecting a submillimeter metastasis will increase without increasing the workload. For example, the approximate probability of detecting a metastasis of 0.9 mm increases to > 95% using a protocol of 5 intervals at 250 μm, assuming < 650 μm of tissue are spent in obtaining the initial H&E section. The chance of detecting a 100-μm metastasis by this method increases to approximately 70%. The tradeoff would be the potential for missing isolated tumor cell clusters. It is understood, however, that by any method, there is only a small chance of detecting single cells and isolated cell clusters unless one examines the entire block at 5-μm intervals, a time and cost-prohibitive option. In this series, the metastases detected by additional H&E levels were detected on levels 2 and 3. Although it could be argued that only 3 levels are necessary, we advocate for 5 levels because of the statistical model suggesting an increased rate of detection owing to the histologic examination of more of the tissue block. Likewise, because narrower intervals resulted in more unexamined tissue, we would advocate that the intervals between levels be of such a distance that the block is sectioned through. Ultimately, an ultrastaging protocol should be optimized to detect the smallest clinically significant metastasis.

However, there is no published study to date unequivocally establishing a minimum size criterion for clinical significance. Up to 15% of patients with IB cervical carcinoma and negative lymph nodes may recur, and it is possible that some of these recurrences could be owing to metastases not detected by standard processing of lymph nodes. Three retrospective studies of histologically negative lymph nodes from patients with stage I cervical carcinoma have indeed demonstrated the presence of metastatic carcinoma upon further examination. In 2 studies, immunoperoxidase staining for cytokeratin demonstrated metastatic carcinoma in 8% and 15% of patients who initially had histologically negative lymph nodes. One of these studies specifically examined metastases < 2.0 mm detected by an immunostain for keratin and reported that half of these patients with metastases developed a recurrence. A third study used reverse transcription-polymerase chain reaction for cytokeratin and found that up to 44% of histologically negative lymph nodes harbored evidence for occult metastasis. Follow-up in this study was limited, and only 2 patients with molecular evidence of metastasis ultimately had recurrent disease. The clinical course of IB cervical carcinoma and the results of these studies would suggest that microscopic (and even submicroscopic) metastases may be significant, yet these studies do not go further to suggest ultimately how much disease burden is clinically significant. Interestingly, in our study, 2 patients elected not to receive adjuvant...
therapy. One patient had a 0.1-mm focus of tumor in 1 SLN and the other had a single keratin positive cell in a SLN. Both are alive without evidence of disease 49 and 58 months after diagnosis, respectively. It should also be noted that in this study, there were no positive non-SLNs with SLN metastases ≤ 1.0 mm. As there are conflicting results for what may constitute a clinically significant SLN metastasis, further studies with long follow-up will be required. To that end, standardization of ultrastaging protocols between institutions may be helpful to increase the uniformity of results.

Our series and others have demonstrated that routine processing is insufficient in the evaluation of SLN. In our experience, H&E intervals were more helpful in detecting microscopic metastases than immunohistochemistry. However, some of the previously published series document the use of only cytokeratin staining as helpful in identifying metastases in SLNs.2,4,30 Cytokeratin staining is particularly useful in identifying single cell metastases, as in one of our cases. Therefore, it should remain as part of an ultrastaging protocol.

In conclusion, the protocol for the processing of SLN should be aimed at optimizing the detection of most metastases. Such a protocol should include thin sectioning of the lymph node during gross examination followed by an ultrastaging protocol for all SLNs negative after routine histologic processing. The ultrastaging protocol should include a combination of H&E levels and immunohistochemistry. Because metastases may be seen on deeper levels, more than 1 additional H&E stained slide should be examined. Although the current protocol in place at our institution seems satisfactory, the theoretical increase in the detection of submillimeter metastases by increasing the interval between the H&E levels and thereby decreasing the amount of unexamined tissue in the block has led to a change from the existing protocol. Optimization and creation of a standard ultrastaging protocol may yield true positive and false negative rates for SLNs that more accurately reflect lymphatic metastases in patients with cervical squamous cell carcinoma. The information obtained from a large number of identically processed lymph nodes from multiple institutions may determine whether a critical threshold for metastasis size exists, so that the ultrastaging protocol can be further optimized toward the detection of clinically significant metastases.

REFERENCES


