Reliability of Sentinel Lymph Node Biopsy for Regional Staging of Head and Neck Merkel Cell Carcinoma

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Objective: To determine (1) the reliability of sentinel lymph node (SLN) biopsy and (2) the need for cytokeratin 20 (CK-20) immunostaining in the staging of head and neck Merkel cell carcinoma (MCC).

Design: Retrospective cohort study (median follow-up of 34.5 months).

Setting: Tertiary care center.

Patients: Ten patients with head and neck MCC who underwent regional staging with SLN biopsy (SLNB) and CK-20 immunostaining.

Interventions: Sentinel lymph nodes were identified using preoperative lymphoscintigraphy, intraoperative gamma probe, and isosulfan blue dye. The SLNs were evaluated with hematoxylin-eosin and CK-20 immunostaining. Patients with negative SLNB results were followed up clinically.

Main Outcome Measures: Percentage of positive SLNs, regional recurrence in the setting of a negative finding from SLNB, and percentage of positive SLNs requiring CK-20 immunostaining for diagnosis of micrometastatic MCC.

Results: At least 1 SLN was identified in every patient. Of 24 nodes, 19 (79%) were from the neck region and 5 (21%) were from the parotid basin. Two of the 24 SLNs, in 2 (20%) of 10 patients, were positive for metastatic disease. Both positive SLNs appeared negative on hematoxylin-eosin–stained sections, but small foci of micrometastatic MCC were identified with CK-20 immunostaining. No cranial nerve complications occurred. Regional failure in the setting of a negative finding on SLNB was observed in 1 (12%) of 8 patients.

Conclusions: Biopsy of SLNs represents a safe and reliable technique for regional staging of MCC of the head and neck. It provides pathologists with a limited number of SLNs for focused analysis, which is imperative because hematoxylin-eosin immunostaining is often insufficient for identifying micrometastatic MCC. The use of anti–CK-20 antibody allows accurate identification of micrometastatic MCC.

sive, yet highly accurate, means to evaluate nodal basins. In doing so, the technique identifies patients harboring occult nodal disease who warrant therapeutic lymphadenectomy (TLND) and adjuvant therapy while sparing the remaining patients without regional disease the morbidity associated with complete lymphadenectomy and irradiation.17 Surgeons have been hesitant to apply this staging technique within the H&N region because of concern for discordant cervical lymphatic vessels,18 potential damage to vital structures such as the facial nerve,19 technical difficulties, and the necessity for nuclear medicine staff as well as pathologists who specialize in the SLNB.20 The objective of this retrospective cohort study was to determine the reliability of SLNB for regional staging of H&N MCC. Our study also investigated the necessity of cyto-keratin 20 (CK-20) immunohistochemical staining for accurate identification of micrometastatic MCC within sentinel lymph nodes (SLNs). We chose CK-20 because it is currently the most sensitive and specific marker for MCC.

METHODS

Approval for this study was granted by the University of Michigan Medical School Institutional Review Board for Human Subject Research. This retrospective cohort study included 10 consecutive patients treated for H&N MCC who were staged by means of SLNB by 2 senior surgeons (C.R.B. and T.N.T.). Patients were identified through a query of the prospective University of Michigan Pathology Database from January 1, 1995, through May 31, 2003. All patients who had histologically proven MCC in the clinical absence of regional or distant metastasis were counseled for SLNB. Patients whose disease was staged using SLNB were included in the analysis. Patients who underwent prior MCC excision with wide margins or prior neck surgery were excluded because of the decreased accuracy in identifying the true SLNs. A minimum follow-up of 1 year was required.

All patients underwent preoperative lymphoscintigraphy to determine the number, location, and laterality of nodal basins at risk for metastatic disease. The lymphoscintigraphy was performed 2 to 4 hours before surgery by means of techniques previously described.20,21 Technetium Tc 99m sulfur colloid (CIS-US Inc, Bedford, Mass), 2 to 4 µCi (0.074-0.148 MBq), was injected intradermally into the 4 quadrants surrounding the primary MCC lesion. Planar imaging (E.CAM; Siemens, Hoffman Estates, Ill) was performed 15 to 30 minutes after injection.

Intraoperative lymphatic mapping with isosulfan blue dye (1% Lymphazurin; Hirsch Industries Inc, Richmond, Va) was performed by means of previously described techniques.20 Approximately 1 mL of dye was injected into the intradermal layer surrounding the MCC lesion. After wide local excision of the primary lesion with 1- to 2-cm margins, nodal basins were evaluated for increased radioactivity with a handheld gamma probe (Navigator GP5; RMD Instruments, Watertown, Mass). A 1- to 3-cm incision was made overlying the areas of increased radioactivity. A preauricular incision was used for SLNB in the parotid region.

Sentinel lymph nodes were identified with a combination of gamma probe and blue dye. Each SLN was individually dissected from surrounding tissue. Facial nerve monitoring (Viking; Nicolet Instrument Corp, Madison, Wis) was used for SLNB within the parotid nodal basin. The staging procedure was considered complete when all nodal basins had minimal background radioactivity relative to the primary lesion and SLNs. All SLNs were sent for histologic evaluation with formalin-fixed permanent sections. In brief, histologic evaluation included serial sectioning of the SLNs at 2- to 3-mm intervals and H&E staining. Immunohistochemical staining with anti-CK-20 (1:25 dilution; Dako Corp, Carpinteria, Calif) was performed for all SLNs that were negative on initial H&E examination. Patients with a positive MCC SLN were counseled on the role of TLND and adjuvant radiation therapy. The remaining patients with a negative SLN biopsy were followed up clinically.

The University of Michigan Pathology Database was used to define the population demographics. Main outcome measures included the percentage of positive SLNs, regional recurrence in the setting of a negative result on SLNB (false-negative rate), facial nerve injury, H&E staining, and CK-20 immunostaining.

RESULTS

Ten patients treated between January 1995 and May 2003 met the inclusion criteria for this study. Six were female and 4 were male. The median patient age was 74 years (age range, 55-85 years). The distribution of the primary lesions and associated demographics are listed in Table 1.

Through the combined techniques of lymphoscintigraphy, intraoperative gamma probe, and isosulfan blue dye, at least 1 SLN was found in 100% of the cases; a total

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Primary Site</th>
<th>Tumor Size, cm</th>
<th>No. of SLNs</th>
<th>No. of Positive SLNs</th>
<th>Follow-up, mo</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/74</td>
<td>Cheek</td>
<td>1.6 × 1.3</td>
<td>2</td>
<td>1</td>
<td>38</td>
<td>NED</td>
</tr>
<tr>
<td>2/F/73</td>
<td>Brow</td>
<td>3 × 1.7</td>
<td>2</td>
<td>1</td>
<td>45</td>
<td>NED</td>
</tr>
<tr>
<td>3/M/84</td>
<td>Cheek</td>
<td>NA*</td>
<td>1</td>
<td>0</td>
<td>33</td>
<td>NED</td>
</tr>
<tr>
<td>4/F/55</td>
<td>Eyelid/cheek</td>
<td>NA*</td>
<td>2</td>
<td>0</td>
<td>31</td>
<td>NED</td>
</tr>
<tr>
<td>5/M/74</td>
<td>Nasal dorsum</td>
<td>NA*</td>
<td>3</td>
<td>0</td>
<td>30</td>
<td>Regional recurrence†</td>
</tr>
<tr>
<td>6/F/84F</td>
<td>Nasal ala</td>
<td>NA*</td>
<td>4</td>
<td>0</td>
<td>28</td>
<td>NED</td>
</tr>
<tr>
<td>7/M/83</td>
<td>Lower eyelid</td>
<td>NA*</td>
<td>3</td>
<td>0</td>
<td>27</td>
<td>NED</td>
</tr>
<tr>
<td>8/M/85</td>
<td>Infralobular</td>
<td>NA*</td>
<td>2</td>
<td>0</td>
<td>58</td>
<td>NED</td>
</tr>
<tr>
<td>9/F/71</td>
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<td>2.2 × 1.6</td>
<td>4</td>
<td>0</td>
<td>57</td>
<td>NED</td>
</tr>
<tr>
<td>10/F/68</td>
<td>Nasal ala</td>
<td>NA*</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>NED</td>
</tr>
</tbody>
</table>

Table 1: Demographics of Head and Neck Merkel Cell Carcinoma

Abbreviations: NA, not available; NED, no evidence of disease; SLN, sentinel lymph node.

†Case contributing to false-negative rate on SLN biopsy findings.
of 24 SLNs were identified. The average number of SLNs identified per patient was 2.4 (range, 1-4 nodes). Of the 24 SLNs, 19 (79%) were identified in neck nodal basins. The remaining 5 SLNs (21%) were harvested from the parotid bed. All 5 patients whose nodes drained to the parotid basin underwent successful SLNB. Two (20%) of 10 patients demonstrated bilateral nodal drainage. Both individuals presented with midline nasal MCC.

There was minimal morbidity related to this procedure. No anaphylactic reactions occurred after injection of isosulfan blue dye. There were no cases of cranial nerve damage, and all patients demonstrated normal postoperative facial nerve function. Damage to vital neck vascular structures did not occur.

Two (20%) of 10 patients had occult nodal MCC identified by SLNB and were classified as SLN positive. In each case, H&E staining of the serially sectioned SLN failed to demonstrate MCC. Micrometastatic disease was identified only after CK-20 immunostaining (Figure). Both patients were counseled about TLND, but because of significant comorbidities they refused surgery and elected regional radiation treatment. Both patients were disease free at a median follow-up of 41.5 months (38 and 45 months).

The remaining 8 patients (80%), who lacked evidence of micrometastatic MCC with both H&E and CK-20 immunostaining, were classified as SLN negative. This group was followed up clinically for a median of 34 months (range, 12-58 months). Nine months after SLNB, 1 patient (12%) developed recurrent disease within a previously mapped nodal basin in which all SLNs were originally negative for micrometastatic disease. Therefore, we report our regional failure rate in the setting of a negative SLNB finding, also referred to as the false-negative rate, to be 12% at a median follow-up interval of 34 months. The patient who failed regionally required salvage bilateral TLNDs because the original location of the primary tumor was midline on the nasal dorsum. The remaining harvested 38 lymph nodes were negative for metastatic disease. Adjuvant radiation therapy to the nodal basins was administered after surgery, and the patient remained disease free 30 months after initial diagnosis.

In the current study, we demonstrated the utility of SLNB for the regional staging of H&N MCC. We successfully identified an SLN in 100% of patients without damage to vital H&N structures, including the facial nerve. Our reported rate of occult nodal metastasis in 20% of patients is comparable with that reported in other MCC studies that used staging elective lymph node dissection (ELND). At first glance, our retrospective study involving 10 patients may seem small. Unfortunately, the rarity of MCC has limited reports in the literature to case studies and anecdotal findings. Other authors have successfully used SLNB for the staging of H&N MCC (Table 2). Currently, our SLNB study represents the largest H&N series to date. Although our mean follow-up of 40 months might be considered modest, MCC is known for aggressive regional recurrence, usually within the first 12 months after initial diagnosis.

Both patients in our study with micrometastatic nodal disease declined TLND because of medical problems and elected instead to receive regional radiation therapy. At a mean follow-up of 41.5 months, both remained free of disease. This disease-free interval is promising, given the 16% to 37% regional failure rate reported in the literature for patients who underwent TLND for treatment of palpable regional disease at the time of initial diagnosis. Our disease-free interval further supports that early diagnosis of occult nodal disease may increase disease-free survival compared with watchful waiting in which regional nodal basins are treated only after clinically palpable disease develops.

Only 1 (12%) of 8 patients with a negative SLNB finding developed regional recurrence in a previously mapped nodal basin (false negative). This failure rate is acceptable given that previous reports cite high failure rates in the untreated neck ranging from 63% to 75%. Our failure occurred in a patient referred to our institution after undergoing an excisional biopsy. We reexcised the primary site to achieve adequate 2-cm margins, and SLNB was performed. Although the patient did not undergo pre-
vicious wide local excision, final pathologic review of the reexcised specimen demonstrated scar without residual MCC. Inability to inject radiocolloid and dye in the exact primary lesion site and the disruption of the draining lymphatics may have contributed to the SLNB failure. This failure highlights the importance of performing a small excisional biopsy for diagnostic purposes, followed by treatment with simultaneous wide local excision and SLNB as a definitive, second-staged procedure.

The high rate and associated poor prognosis of MCC regional metastasis has caused many surgeons to advocate prophylactic treatment of the neck with ELND for local disease. Although improved regional control and disease-free intervals have been reported in the setting of ELND vs TLND, to our knowledge no study to date has demonstrated a survival benefit. This lack of survival benefit has been cited as the most compelling argument against ELND. Elective lymph node dissection is not without morbidity, which must be kept in mind because MCC is a disease of elderly patients, with a mean age older than 65 years. We agree with proponents of ELND who contend that the information on nodal status is helpful in selecting patients who may benefit from further adjuvant therapy. However, we view SLNB as a less invasive means of gaining this prognostic information.

The current National Comprehensive Cancer Network guidelines recommend that all patients with a positive SLNB result undergo TLND, as demonstrated by adjuvant irradiation to the primary site as well as the in-transit lymphatics and draining lymph nodes. Previous regional failures within the neck after radiation therapy have been attributed to inaccurate identification of draining nodal basins. O’Brien et al demonstrated a 34% discordance between the clinical prediction of lymphatic drainage and lymphoscintigraphy findings in 97 cases of H&N cutaneous melanoma. Identification of appropriate draining lymph nodes can be especially challenging for midline lesions of the face. The use of SLNB provides an opportunity to accurately identify draining nodal basins on an individualized basis. In addition, National Comprehensive Cancer Network guidelines recommend adjuvant radiation treatment only to the primary site for SLN-negative patients. In doing so, individuals can be spared the side effects associated with neck irradiation.

The most important benefit in using SLNB, as opposed to ELND, for regional staging is the ability to provide the pathologist with a limited number of lymph nodes for thorough evaluation. Identification of occult MCC metastasis warrants rigorous pathological analysis, which should include serial sectioning of the lymph nodes at highest risk for occult disease and H&E staining, followed by immunohistochemical staining with CK-20 for nodes that fail to demonstrate MCC on initial H&E staining. It is well recognized that CK-20 immunostaining is the most sensitive and specific means to corroborate the diagnosis of MCC. Our study supports the necessity of MCC-specific immunochemical staining. Both patients with a positive SLN lacked histologic evidence of micrometastasis on H&E staining. A small focus of tumor was identified only after the use of CK-20 immunostaining. It would be impractical and extremely expensive to thoroughly evaluate the many nodes obtained via ELND with the serial sectioning and CK-20 staining required to diagnostically micrometastatic MCC.

### CONCLUSIONS

Merkel cell carcinoma remains both a diagnostic and therapeutic challenge. This retrospective study demonstrates that SLNB can be performed reliably and safely in the H&N region to identify occult regional disease. In addition, we demonstrated that standard H&E staining alone is often inadequate in identifying micrometastatic MCC in the SLN because diagnosis was achieved only after immunostaining for CK-20.

Merkel cell carcinoma is recognized as an aggressive cutaneous malignancy that requires accurate staging at initial presentation. The rarity of MCC necessitates multi-institutional clinical trials to identify treatment options that truly impact patient survival. Clinical trials will be successful only if a homogeneous population of patients with MCC is identified through accurate regional staging. Without accurate pathologic staging, stratification is impossible and results of clinical trials will re-
main inconsistent and difficult to interpret. Fortunately, SLNB provides a minimally invasive and focused means to stage MCC.

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REFERENCES