Clinical Significance of Microscopic Melanoma Metastases in the Nonhottest Sentinel Lymph Nodes

Su Luo, MD; Alice Z. C. Lobo, MD; Kenneth K. Tanabe, MD; Alona Muzikansky, MA; Tyler Durazzo, MD; Arthur Sober, MD; Hensin Tsao, MD, PhD; A. Benedict Cosimi, MD; Donald P. Lawrence, MD; Lyn M. Duncan, MD

IMPORTANCE A practice gap exists in the surgical removal of sentinel lymph nodes, from removal of only the most radioactive (hottest) lymph node to removal of all lymph nodes with radioactivity greater than 10% of the hottest lymph node.

OBJECTIVE To determine the clinical significance of melanoma in sentinel lymph nodes that are not the hottest sentinel node and to determine the risk for disease progression based on sentinel lymph node status and primary tumor characteristics.

DESIGN, SETTING, AND PARTICIPANTS Consecutive patients with cutaneous melanoma with sentinel lymph nodes resected from January 5, 2004, to June 30, 2008, with a mean follow-up of 59 months, at Massachusetts General Hospital were included in this retrospective review. The last year of follow-up was 2012. The operative protocol led to resection of all sentinel lymph nodes with radioactivity greater than 10% of the hottest lymph node. The number of lymph nodes removed, technetium-99m counts for each sentinel lymph node, presence or absence of sentinel lymph node metastases, primary tumor characteristics, disease progression, and melanoma-specific survival were recorded.

MAIN OUTCOMES AND MEASURES Microscopic melanoma metastases in the hottest and nonhottest sentinel lymph nodes and factors that correlate with disease progression and mortality.

RESULTS A total of 1575 sentinel lymph nodes were analyzed in 475 patients. Ninety-one patients (19%) had positive sentinel lymph nodes. Of these, 72 (79%) had metastases in the hottest sentinel lymph node. Of 19 cases with tumor present, but not in the hottest sentinel lymph node, counts ranged from 26% to 97% of the hottest node. Progression occurred in 43% of patients with sentinel node metastasis, regardless of whether the hottest lymph node was positive. In patients with negative sentinel lymph nodes, 11% developed metastases beyond the sentinel lymph node basin and 3.4% recurred in the basin. Mitogenicity of the primary tumor was associated with mortality (odds ratio, 2.435; 95% CI, 1.351-4.391; P < .001). Removing only the hottest sentinel lymph node would have led to false-negative results in 19 of 475 (4%) of all patients and 19 of 91 patients (21%) with positive sentinel lymph nodes. The 8-year survival in patients with at least 1 positive sentinel lymph node was less than 55%. The presence of more than 1 mitosis per square millimeter in the primary cutaneous melanoma was associated with decreased survival.

CONCLUSIONS AND RELEVANCE Microscopic melanoma metastases was associated with disease progression and mortality, whether present in the hottest sentinel lymph node or not. These observations emphasize the importance of removing the less hot nodes, addressing a practice gap in the surgical approach to patients with melanoma.
taging cutaneous melanoma with sentinel lymph node (SLN) mapping is the most important surgical advance in melanoma management in the past quarter century. Unlike carcinoma of the breast, isolated melanoma cells in the SLNs have prognostic significance. Patients with negative SLNs have an estimated 3-year disease-free survival of 90% compared with 60% for patients with positive SLNs. This procedure is offered to patients with clinically localized American Joint Committee on Cancer stage I and II cutaneous melanoma with primary tumors more than 1 mm thick, tumors less than 1 mm with mitotic activity and/or ulceration, or other risk factors such as lymphovascular invasion. When metastatic melanoma is identified in a SLN, including even a solitary tumor cell, patients are upstaged to American Joint Committee on Cancer stage III. The identification of melanoma in a SLN has significant clinical effect. More than 90% of patients with positive SLNs undergo completion lymphadenectomy (CLND) and most also receive adjuvant therapy or targeted therapy. On the other hand, patients with negative SLNs undergo no further surgery or adjuvant therapy.

At Massachusetts General Hospital, SLN biopsy is performed using preoperative lymphoscintigraphy, with technetium-99m-labeled sulfur colloid and sometimes iodosulfan blue dye injected at the site of the primary cutaneous melanoma. A scintillation camera documents static and dynamic images demonstrating pathways of lymphatic drainage to the sentinel nodes. The blue dye enables visualization of the sentinel node(s), while a handheld gamma probe identifies areas of tracer uptake in situ. After removal of the first SLN, the ex vivo radioactive counts accumulated during a 10-second interval are recorded. The remaining lymph nodes are excised if they are estimated to have more than 10% of the counts of the hottest lymph node in situ and then, based on ex vivo counts, the SLNs are determined to be SLNs (>10%) or non-SLNs (≤10% counts of the hottest SLN as measured ex vivo). Laboratory protocols for histological SLN analysis vary widely. In this study, a 9-slide protocol that included 3 levels, 3 hematoxylin and eosin stains, and 6 immunohistochemical stains was used. The clinical significance of microscopic melanoma metastases in patients who did not have tumor in the hottest SLNs was examined in relationship to disease progression and primary tumor characteristics. The number and location of mapped basins in relationship to the rate of node positivity and regional recurrence were also evaluated.

Results

The clinical and pathological features of 475 patients are shown in Table 1. Microscopic SLN metastases were identified in 91 patients (19%) and benign capsular nevi were observed in 38 (8%) (Table 2). There were no significant differences in sex or age between patients without melanoma in the SLNs and patients with SLNs positive for microscopic melanoma metastasis. Primary tumors located in special sites (n = 15, including 11 acral, 3 vulvar, and 1 anal melanoma) were more frequent in the SLN-positive group (P = .01). On the other hand, lentigo maligna melanomas were more frequent in the SLN-negative group (P < .02). The mean primary tumor thickness was greater for patients with...
positive SLNs ($P < .01$), and mitotic activity and vascular invasion were more commonly observed in this group ($P < .001$ and $P < .05$, respectively).

In a multivariate Cox proportional hazards model with an outcome of progression-free survival, using the covariates of SLN status, mitoses per square millimeter, ulcer-
Invasion, neurotropism, microsatellites, regression, vascular invasion, and Clark level, significant association with disease progression was observed for SLN status \((P < .001)\), mitoses per square millimeter \((P < .001)\), vascular invasion \((P = .02)\), and Clark level \((P = .02)\). Mitoses and tumor thickness were entered into the model as continuous variables; the others were entered as class variables. Ulceration, tumor-infiltrating lymphocytes, neural invasion, regression, and microscopic satellites were not significantly different between the 2 groups. Mean and median follow-up from time of SLN mapping to last encounter or date of death were 60 and 64 months, respectively.

While SLNs are usually mapped to a single lymph node basin, occasionally lymphoscintigraphy showed more than 1 basin draining the cutaneous tumor. A single SLN basin was mapped in 417 patients and 2 basins in 58 patients (Figure 1). Of 384 patients with negative SLNs, 340 had 1 negative basin and 44 patients had 2 negative basins. Of the 91 patients with positive SLNs, 77 had SLNs removed from 1 basin and 14 patients had SLNs removed from 2 basins (8 patients with positive SLNs in 1 of 2 basins and 6 with positive SLNs in both mapped basins).

A total of 533 lymph node basins were mapped including 267 axillary, 138 groin (superficial inguinal and external iliac/
pelvic), 113 neck (supraclavicular/jugular/parotid/periauricular/submental), 10 occipital, 2 popliteal, and 3 epitrochlear (Table 3). Metastases were identified in 18% of axillary basins (47 of 267), 25% of groin basins (34 of 138), and 11% of neck basins (12 of 113); there were no statistically significant differences in the rate of node positivity between sites of mapped basins.

More than 3 SLNs were removed on average. In the 91 patients with positive SLNs, 327 nodes were removed, and in the 384 patients with negative SLNs, 1238 nodes were removed (mean, 3.6 and 3.2 SLNs, respectively). On average, 4 or more SLNs were removed in patients without the hottest positive SLNs (mean, 4.9; 19 patients) compared with fewer than 4 in those with the hottest positive SLNs (mean, 3.3; 72 patients) ($P = .02$; Table 2). There was no correlation between the number of SLNs taken and disease progression or survival. In patients with positive SLNs, most commonly only 1 lymph node contained tumor (64 of 91 patients; range, 1-6; median, 1). A survival advantage was not associated with the number of positive SLNs, although our cohort contained only 27 patients with more than 1 positive SLN.

The hottest SLNs contained metastatic melanoma in 72 of 91 cases (79%). Of the 19 patients without tumor in the hottest lymph node, 13 (14%) had melanoma in the second hottest lymph node. Four patients (4%) had no tumor detected in the first or second hottest SLNs but had metastases in the third hottest node. Two patients (2%) had no tumor identified until the fourth hottest lymph node. The hottest lymph node with melanoma metastasis ranged from 26% to 100% of the hottest lymph node removed. There was no significant difference in tumor burden between the hottest and nonhottest positive SLNs (Table 2). While the presence of a SLN metastasis was associated with a reduced recurrence-free and overall melanoma-specific survival ($P < .001$), prognosis was no different if the metastasis was present in the hottest node or not (Figure 2).

Of the 91 patients with positive SLNs, 84 (92%) underwent CLND. Six of 84 patients (7%) had additional positive lymph nodes in the CLND. Two of these patients had tumors in the hottest SLNs; 4 did not but they had tumors in other SLNs. All 6 patients with positive CLND had a melanoma-associated death. Of 78 patients with negative CLND, 29 had a melanoma-specific death. Two of seven patients who did not have CLND died of melanoma-associated causes. Overall, of 91 patients with positive SLNs, 39 (43%) progressed and 37 of these died of melanoma (ie, 41% disease-specific death rate in patients with positive SLNs).

Of the 384 patients with negative SLNs, 43 (11%) progressed to develop metastases; of these, 24 died (mean follow-up, 61 months). Recurrence in the mapped basin occurred in 13 (3.4%). Recurrences occurred a mean of 36 months after the SLN procedure (range, 8-69 months). When patients with negative SLNs were evaluated, tumor thickness did not show an association with tumor progression or survival. On the other hand, mitogenicity showed a powerful correlation with disease progression and survival ($P = .002$) (Figure 2).

In this cohort, 80 patients had stage IB tumors. No SLN metastases were observed in the 59 patients with primary tumor less than 1 mm (mean, 0.79 mm; range, 0.45-0.98 mm). The mean number of SLNs taken in patients with tumors less than 1 mm was 2.8. Four patients with tumor thickness less than 1 mm progressed: 2 developed metastases beyond the SLNs (0.7 mm and 3 mitoses per square millimeter; and 0.82 mm and 4 mitoses per square millimeter) and 2 patients died of metastatic melanoma (0.9 mm and 10 mitoses per square millimeter; and 0.96 mm and 0 mitoses per square millimeter). Sentinel lymph node mapping was performed in 21 patients with 1-mm-thick primary tumors and metastatic melanoma was detected in 4 (19%). Of these 4 patients, 1 died of melanoma, 1 died of other causes, and 2 have remained without recurrence or metastasis at 78 and 74 months after SLN mapping. Additionally, relapse was observed in 2 patients with 1-mm-thick primary tumors and negative SLNs; 1 developed local recurrence and 1 died of metastatic melanoma (one had a mitogenic primary tumor and the other did not). Sentinel lymph node mapping in patients with very thin melanoma remains controversial; nevertheless, in our cohort, 9% of patients with primary tumors 1 mm or less progressed to develop metastases beyond the SLN basin.

### Discussion

Lymphatic mapping and SLN biopsy, first described in 1992, is a well-established technique for identifying micrometastatic disease in regional nodal basins, with relatively minimal morbidity. The tumor status of regional lymph nodes is known to be the most important prognostic factor for patients with early-stage melanoma as evidenced by the 90% vs 60% 3-year disease-free survival of patients without and with positive SLNs, respectively. The 2009 American Joint Committee on Cancer Melanoma Staging Committee recommended SLN biopsy as a staging procedure in patients with primary melanomas thicker than 1 mm or with tumors 1 mm or less in thickness but with ulceration or 1 mitosis per square millimeter and clinically or radiographically uninvolved regional lymph nodes. The prognostic significance of SLN mapping for thin melanomas was reaffirmed by the development
of a joint clinical practice guideline after expert panel review by the American Society of Clinical Oncology and the Society of Surgical Oncology.26

In this study of 475 patients, 19% had microscopic metastases in SLNs and 8% had capsular nodal nevi.16,27 Primary tumor site and sex did not influence the likelihood of SLN metastases.28 Patients with primary tumors in special sites were more frequent in the positive-SLN group (P = .01); however, these tumors were thicker (median, 3.0 mm) than the tumors from other sites (median, 1.5 mm), perhaps explaining the increased rate of metastasis. Sentinel lymph node metastasis correlated with tumor thickness, mitoses per square millimeter, and vascular invasion. Mitotic rate (odds ratio, 2.435; 95% CI, 1.351-4.391; P < .001) and vascular invasion (P = .02) also showed association with progression beyond the SLN in multivariate analysis. While tumor type is usually not prognostically significant when tumor thickness is accounted for, no patient with lentigo maligna melanoma had a positive SLN. This could not be explained by tumor thickness alone given that the patients with lentigo maligna melanoma did not have significantly thinner tumors than patients with other types of melanoma (median, 1.4 vs 1.6 mm).

Similar to prior reports, the most frequently mapped SLN basins were the axilla, groin, and neck, in that order.29 The rate of SLN metastases in cutaneous melanoma is 7% or less.30 In this study, 39% of SLNs were mapped. 

Similartoprioreports, themostfrequentlymappedSLNbasin(progression, A and B) and death from melanoma-associated cause (overall survival, C and D). Sentinel lymph node status and survival in 475 patients with melanoma (A and C). Patients with melanoma (A and C). Patients with microscop melanoma in the hottest SLNs (orange, n = 72), microscopic melanoma not in the hottest SLN (blue, n = 19), and without SLN metastases (black, n = 384) (P < .001). Primary tumor mitogenicity and survival in 323 patients with melanoma and negative SLNs (B and D). Patients with primary tumor with more than 1 mitosis per square millimeter (blue, n = 200), 1 mitosis per square millimeter (orange, n = 19), and no mitosis (black, n = 50) (P < .001).
of SLN positivity (11%-25%) and rate of basin recurrence (1%-5%) were not significantly different between mapped sites. Some basins are more difficult to map accurately, particularly for head and neck primaries; multidirectional drainage, with smaller and more numerous lymph nodes, may serve as confounding factors. While some studies reported higher rates of recurrence after negative SLN mapping in these basins, others did not. Overall, 1565 SLNs were removed from 523 lymph node basins in 475 patients. The mean number of SLNs removed per basin was 3 and did not differ between patients with positive and negative SLNs. The range of 1 to 3 nodes is similar to that reported in other cohorts. In 72% of patients with positive SLNs, the hottest SLNs contained metastatic melanoma. In 4% of patients, or 28% with positive SLNs, metastases were present but not found in the hottest SLNs. The radioactive counts of the SLNs with metastatic melanoma ranged from 26% to 100% of the hottest SLNs. The progression-free survival and overall survival were no different for patients without the hottest positive SLNs and those with the hottest positive SLNs. This finding underscores the importance of removing more than 1 SLN.

While the SLN has been described as the first encountered node in a lymphatic chain that drains a specific primary skin site, the intricacies of lymphatic organization are occasionally complicated. Confirmation of SLN status was historically based on the presence of blue dye but now also relies on nodal radioactivity. While the node that is the most radioactive, or hottest, may be considered the sentinel one, current practice considers SLNs to be those with more than 10% of the counts of the hottest node. Overall, some would champion the concept that SLN status is best determined by the concomitant presence of blue dye and high radioactivity. We demonstrate that the current practice of removing nodes with more than 10% radioactivity of the hottest SLNs is not too exhaustive. Our study demonstrates that, at times, it is not even the second or third, but sometimes it is the fourth, hottest node that contains metastasis. Furthermore, the distribution of tumor size in these nonhottest nodes was comparable with the distribution in the hottest, demonstrating that these nodes were equally significant in tumor burden.

Sentinel lymph node mapping may be offered to patients with primary melanoma 1 mm or less if they have additional risk factors including mitoses, ulceration, and lymphovascular invasion. In this cohort, 9% of patients with primary tumors 1 mm or less progressed with melanoma metastases beyond the SLN basin. In patients with primary melanoma 1 mm or less, the melanoma-specific death rate was 25% (1 of 4) for those with positive SLNs and 4% (3 of 76) for those with negative SLNs. This observation is congruent with previous reports that SLN status has prognostic value for patients with high-risk thin melanomas.

**Conclusions**

Overall, SLN serves as a robust staging parameter for patients with localized cutaneous melanoma. Removing more than 1 SLN provides increased detection of clinically significant metastases. More than 4% of patients had metastases present in SLNs that was not the most radioactive; disease progression was identical to patients with tumors in the hottest SLNs. Closing the practice gap and removing more than just the hottest SLN allows the identification of patients at risk for developing melanoma progression that may benefit from additional surgery and adjuvant therapy.

**ARTICLE INFORMATION**

Accepted for Publication: September 26, 2014. Published Online: April 1, 2015. doi:10.1001/jamasurg.2014.3843.

Author Affiliations: Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston (Luo, Durazzo, Sober, Tsao); Pathology Service and Dermatopathology Unit, Massachusetts General Hospital, Harvard Medical School, Boston (Muzikansky); Division of Transplant Surgery, Massachusetts General Hospital, Harvard Medical School, Boston (Cosimi); Center for Melanoma, Massachusetts General Hospital, Harvard Medical School, Boston (Lawrence).

Author Contributions: Dr Duncan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Copyright 2015 American Medical Association. All rights reserved.
Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported by departmental research funds from the Dermatopathology Unit of the Pathology Service, Massachusetts General Hospital, Boston.

Role of the Funder/Sponsor: The Dermatopathology Unit of the Pathology Service of Massachusetts General Hospital had a role in the design and conduct of the study and collection, management, analysis, and interpretation of the data but not in the preparation, review, or approval of the manuscript and decision to submit the manuscript for publication.

REFERENCES


