Staging of Regional Lymph Nodes in Melanoma
A Case for Including Nonsentinel Lymph Node Positivity in the American Joint Committee on Cancer Staging System

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IMPORTANCE Survival varies widely in patients with stage III melanoma. The existence of clinical significance for positive nonsentinel lymph node (NSLN) status would warrant consideration for incorporation into the American Joint Committee on Cancer staging system and better prediction of survival.

OBJECTIVE To evaluate whether disease limited to sentinel lymph nodes (SLNs) represents different clinical significance than disease spread into NSLNs.

DESIGN, SETTING, AND PARTICIPANTS The database of the John Wayne Cancer Institute at Saint John’s Health Center, Santa Monica, California, was queried for all patients with SLNs positive for cutaneous melanoma who subsequently underwent completion lymph node dissection.

MAIN OUTCOMES AND MEASURES Disease-free survival, melanoma-specific survival (MSS), and overall survival.

RESULTS A total of 4223 patients underwent SLN biopsy from 1986 to 2012. Of these patients, 329 had a tumor-positive SLN. Of the 329, 250 patients (76.0%) had no additional positive nodes and 79 (24.0%) had a tumor-positive NSLN. Factors predictive of NSLN positivity included older age (P = .04), greater Breslow thickness (P < .001), and ulceration (P < .02). Median overall survival was 178 months for the SLN-only positive group and 42.2 months for the NSLN positive group (5-year overall survival, 72.3% and 46.4%, respectively). Median MSS was not reached for the SLN-only positive group and was 60 months for the NSLN positive group (5-year MSS, 77.8% and 49.5%, respectively). On multivariate analysis, NSLN positivity had a strong association with recurrence (hazard ratio [HR], 1.75; 95% CI, 1.23-2.50; P = .002), shorter overall survival (HR, 2.24; 95% CI, 1.48-3.40; P < .001), and shorter MSS (HR, 2.23; 95% CI, 1.46-3.07; P < .001). To further control for the effects of total positive lymph nodes, comparison was done for patients with only N2 disease (2-3 total positive lymph nodes); the results of this comparison confirmed the independent effect of NSLN status (MSS; P = .04).

CONCLUSIONS AND RELEVANCE Nonsentinel lymph node positivity is one of the most significant prognostic factors in patients with stage III melanoma. Subclassification of melanoma by NSLN tumor status should be considered for the American Joint Committee on Cancer staging system.

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Regional lymph node metastasis in patients with primary cutaneous melanoma is the most important prognostic factor for tumor recurrence and survival. Sentinel lymph node (SLN) biopsy has become one of the most important clinical tools in the staging of melanoma since its introduction by Morton and colleagues. Its ability to detect the 20% of patients with occult lymph node metastases has been validated in the Multicenter Selective Lymphadenectomy Trial (MSLT I). The premise of the SLN importance is that melanoma follows an orderly progression of locoregional spread from the primary site to the draining lymph node basin.

Current guidelines state that all patients with a positive SLN should undergo completion lymph node dissection (CLND) because there is no other reliable means of detecting nonsentinel lymph node (NSLN) metastasis; however, CLND entails the risk of morbidities, including seroma, infection, nerve injury, and lymphedema. In addition, 80% to 90% of the patients who undergo CLND have no additional positive nodes. Given this low positivity rate, many practitioners have begun to question whether CLND is necessary. Whether NSLNs represent a different echelon of nodes has not been validated, and the significance of NSLN metastasis in patients with SLN metastasis is unclear. Several recent studies have suggested that a positive NSLN in the remainder of the lymph basin indicates a poor prognosis. Thus, even if it may not necessarily improve survival, CLND could provide valuable additional information for prognostic staging.

Five-year survival in patients with stage III melanoma ranges from 24% to 72%. As therapies become more promising, patients with worse prognostic factors may become more likely candidates for additional therapies. The official guidelines for staging melanoma were updated in 2009 by the American Joint Committee on Cancer (AJCC). Current AJCC staging for stage III melanoma takes into account primary tumor ulceration and mitoses, nodal tumor burden, and the presence of in-transit metastases. It does not take into account whether the positive nodes are sentinel or nonsentinel.

Refining the AJCC staging system to provide a more accurate prognostic assessment could facilitate selection of patients for adjuvant therapy. In this study, we aimed to determine (1) whether there are clinical factors that can predict NSLN positivity, (2) whether NSLN positivity portends a worse survival than SLN positivity alone, and (3) whether this worse survival rate continues even after adjustment for other factors associated with decreased survival, such as age, sex, Breslow thickness, ulceration, and number of positive nodes.

Methods

Patients were selected from a prospectively maintained database at the John Wayne Cancer Institute at Saint John’s Health Center, and this study of de-identified data was approved for institutional review board exemption. A query was performed to identify 4223 patients who underwent an SLN biopsy from 1986 to 2012. Although selection criteria for SLN biopsy have varied over the course of the study period, in the earlier days of SLN staging the pathology protocol was less extensive and thus less sensitive; however, we have since broadened our indications for SLN examination. These 2 factors may negate each other. Exclusion criteria included a primary tumor other than cutaneous (ie, mucosal or ocular) and no CLND. Demographic and tumor information was collected, including age, sex, primary tumor characteristics (anatomic site, Clark level, Breslow thickness, and ulceration), SLN positivity, and NSLN positivity.

An SLN biopsy was offered to patients with primary melanoma more than 1 mm in Breslow thickness and to selected patients with thickness less than 1 mm who had other predictive features (ulceration, high mitotic rate, young age). Lymphatic mapping was performed with intradermal injection of technetium 99m-filtered sulfur colloid and isosulfan blue at the primary site. Lymphoscintigraphy was used to identify the draining lymph node basin and the SLNs were marked. In the operating room, 1 to 2 mL of isosulfan blue was injected intradermally prior to skin incision. A handheld gamma probe and blue dye visualization were used to identify the SLN. All blue nodes, hot nodes (10% of the hottest count), and palpably suspicious nodes were then sent to the pathology laboratory.

All SLNs were placed in formalin for permanent sectioning. The nodes were paraffin embedded and stained with hematoxylin-eosin and with immunohistochemical stains for S-100 protein, HMB-45, and Melan-A. All patients with positive SLNs then were recommended for CLND. The NSLNs were evaluated by pathologists using hematoxylin-eosin staining of bivalved lymph nodes.

Statistical analysis was performed with commercial software (SAS, version 9.12; SAS Institute, Inc). Clinicopathologic descriptive features were compared in the SLN-only positive group and the NSLN positive group. Survival was determined using the Kaplan-Meier method, and comparisons were made using the log-rank test. Univariate and multivariate Cox regression analyses were performed to determine the importance of NSLN positivity on survival relative to other variables. The Fisher exact test was used to determine the correlation between patient characteristics and NSLN positivity. Primary outcome measures were disease-free survival (DFS), defined as the period from initial primary diagnosis until the first melanoma recurrence; melanoma-specific survival (MSS), defined as the period from the initial primary diagnosis until occurrence of melanoma-specific death; and overall survival, defined as the period from the initial primary diagnosis until the occurrence of death from any reason. Differences at $P < .05$ were considered significant.

Results

We identified 3989 patients who underwent SLN biopsy between 1986 and 2012. Of those patients, 329 had a positive SLN; 250 patients had a positive SLN only and a negative CLND result, and 79 patients had positive NSLNs in addition to a positive SLN. Of those with only SLN positivity, 190 patients (76.0%) had 1 positive node (N1), 52 patients (20.8%) had 2 positive nodes (N2), 6 patients (2.4%) had 3 positive nodes (N2), and 2...
patients (0.8%) had 4 or more positive nodes (N3). Of those with positive NSLNs in addition to their positive SLN, 27 patients (34.2%) had a total of 2 positive nodes (N2), 21 patients (26.6%) had 3 positive nodes (N2), and 31 patients (39.2%) had 4 or more positive nodes (N3).

There was no significant difference in sex distribution, primary tumor location, or histologic characteristics between the SLN-only positive group and the NSLN positive group. The mean age of the SLN-only positive group was 51 years, which was significantly younger than the NSLN positive group (mean age, 56 years; \(P = .04\)). The NSLN positive group tended to have deeper T3 or T4 lesions and higher Clark levels vs the SLN-only positive group (\(P < .001\)). The NSLN positive group also tended to have ulceration of their lesions (\(P < .02\)). Demographics and tumor-specific variables of both the SLN-only positive group and NSLN positive patients are reported in Table 1.

The 5-year DFS was significantly longer for the SLN-only positive group than for the NSLN positive group (\(P < .001\)) (Figure 1A). Median overall survival was 178 months for the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SLN-Only Positivity</th>
<th>NSLN Positivity</th>
<th>(P) Value*</th>
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<tr>
<td>Sex</td>
<td>Male</td>
<td>156 (62.4)</td>
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<td>56</td>
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<td>Trunk</td>
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<td>4 (5.1)</td>
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<td>T2 category: 1.01-2.00</td>
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<td>14 (17.7)</td>
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</table>

Abbreviations: NSLN, nonsentinel lymph node; SLN, sentinel lymph node.

* Statistical analysis performed using \(\chi^2\) and analysis of variance.

Figure 1. Disease-Free Survival, Overall Survival, and Melanoma-Specific Survival

A. Five-year disease-free survival for sentinel lymph node (SLN)–only positive patients was significantly shorter than that of the nonsentinel lymph node (NSLN) positive group (\(P < .001\)). B. Median overall survival was 178 months for SLN-only positive patients and 42.2 months for NSLN positive patients. Five-year overall survival was 72.3% for SLN-only positive patients vs 46.4% for NSLN positive patients (\(P < .001\)). C. Five-year disease-free survival (melanoma-specific survival) was 77.8% for the SLN-only positive group and 46.4% for the NSLN positive group (\(P < .001\)).

Table 1. The 5-year DFS was significantly longer for the SLN-only positive group than for the NSLN positive group (\(P < .001\)) (Figure 1A). Median overall survival was 178 months for the
SLN-only positive group and 42.2 months for the NSLN positive group. The 5-year overall survival was 72.3% for the SLN-only positive group and 46.4% for the NSLN positive group (P < .001) (Figure 1B). Median MSS was not reached for the SLN-only positive group and was 60 months for the NSLN positive group. The 5-year MSS was 77.8% for the SLN-only positive group and 49.5% for the NSLN positive group (P < .001) (Figure 1C).

To determine whether this worse prognosis in the NSLN positive group was attributable to the spread of disease beyond the SLN or simply the result of an increase in the number of involved nodes, analysis was done with adjustment for the higher number of lymph nodes in the NSLN positive group. This group had a hazard ratio (HR) of 1.91 (95% CI, 1.35-2.72; P < .001) for DFS, 2.242; 95% CI, 1.476-3.404; P < .001) for overall survival, and 2.11 (95% CI, 1.46-3.07; P < .05) for MSS.

To determine whether spread of disease beyond the SLN was truly an independent prognostic indicator of decreased survival, we performed a multivariate Cox regression analysis on patient-related, tumor-related, and SLN and NSLN factors. For DFS, multivariate analysis found older age (HR, 1.03; 95% CI, 1.02-1.04; P < .001), male sex (HR, 1.44; 95% CI, 1.02-2.03; P = .04), increasing number of positive SLNs (HR, 1.45; 95% CI, 1.19-1.77; P < .001), and NSLN positivity (HR, 1.75; 95% CI, 1.23-2.50; P = .002) to be predictive of a higher rate of recurrence. Nonsentinel lymph node positivity increased the risk of recurrence, with a 1.7-fold greater likelihood of recurrence compared with SLN-only positivity (Table 2).

For overall survival, multivariate analysis found older age (HR, 1.03; 95% CI, 1.02-1.04; P < .001), male sex (HR, 1.53; 95% CI, 1.03-2.26; P = .03), greater Breslow thickness (HR, 1.03; 95% CI, 1.00-1.06; P = .04), increasing number of positive SLNs (HR, 1.63; 95% CI, 1.30-2.06; P < .001), and NSLN positivity (HR, 1.82; 95% CI, 1.24-2.69; P < .001) to be predictive of shorter overall survival. Nonsentinel lymph node positivity decreased overall survival, with a 1.8-fold risk of death compared with SLN-only positivity (Table 2).

For MSS, multivariate analysis found older age (HR, 1.02; 95% CI, 1.01-1.04; P < .001), greater Breslow thickness (HR, 1.04; 95% CI, 1.01-1.08; P = .007), increasing number of positive SLNs (HR, 1.49; 95% CI, 1.14-1.94; P = .003), and NSLN positivity (HR, 2.242; 95% CI, 1.476-3.404; P < .001) to be predictive of shorter MSS. Nonsentinel lymph node positivity decreased MSS, with a 2.2-fold greater likelihood of death compared with SLN-only positivity (Table 2).

To further control for the total number of positive nodes, comparison was done for patients who had N2 disease only (2-3 positive lymph nodes). This confirmed the independent effect of NSLN status on MSS when controlled for the number of positive nodes (MSS; P = .04) (Figure 2).

Discussion

Our data support a prognostic difference between SLN positivity and NSLN positivity. There is no dispute that SLN biopsy is the most reliable method for nodal staging. Its advent has revolutionized surgery for metastatic melanoma. The prognostic value of SLN positivity has been validated by the first MSLT trial.2 The prognostic information that can be obtained from NSLN positivity is less evident. Several studies have examined the significance of NSLN positivity.

Cascinelli et al10 performed a retrospective analysis on 176 patients: 143 with SLN-only positivity and 33 with NSLN positivity. Results from their analysis showed a 5-year survival of 92.6% for the SLN-only positive group and 60% for the NSLN positive group. Their conclusion was that NSLN analysis allows identification of patients with nodal disease who are at a greater risk of death compared with patients with SLN-only positivity.
different risk level for death. Flaws of this study include the lack of adjustment for the higher number of positive nodes in the NSLN positive group.

Roka et al.10 examined their 85 SLN-positive patients; 67 of these patients had SLN-only positivity and 18 had NSLN positivity. On univariate analysis, recurrence rates were significantly lower in the SLN-only positive group vs the NSLN positive group (87% vs 78%; P = .02), and death from disease was also significantly lower in the SLN-only positive group vs NSLN positive group. This study’s main purpose was to assess factors most strongly associated with positive NSLN status. No multivariate analysis was done, and the investigators did not account for the worse prognosis associated with the higher number of nodes in the NSLN positive group.

Brown et al.12 identified 296 patients with SLN-only positivity and 51 patients with NSLN positivity. The 5-year DFS was 64.8% and 42.6% (P < .001) and overall survival was 64.9% and 49.4% (P < .001), respectively. Their analysis held true when the total number of positive lymph nodes and the NSLN status were evaluated using multivariate analysis (P < .01).

Ghaferi and colleagues13 compared a group of 90 patients with SLN-only positivity with 41 patients with NSLN positivity. Their study showed that a tumor-involved NSLN was a statistically significant predictor of outcome on multivariate analysis. The HR for NSLN positivity was 1.92 (1.27-2.89) for overall survival and 1.79 (95% CI, 1.01-3.19) for distant DFS. Their analysis was limited to patients with 2 to 3 positive nodes to adjust for the worse survival that would be seen with a greater number of positive nodes.

Ariyan et al.11 examined 222 patients who underwent SLN biopsy using their retrospectively maintained database. One hundred eighty-five of these patients were SLN-only positive and 37 patients were NSLN positive. Median survival between these 2 groups was 104 months vs 36 months (P < .001). On adjustment for the number of positive nodes by analyzing patients with an equal number of positive nodes, the presence of NSLN positivity was still associated with worse MSS (66 months vs 34 months; P < .04). On multivariate analysis, positive NSLN was an independent predictor of disease-specific survival (HR, 2.5).

Our data corroborate the findings of these previous studies showing that NSLN positivity appears to be prognostically different than SLN positivity. Currently, all patients with SLN positivity are recommended to undergo a CLND. However, only 20% of these patients have additional metastasis in the remainder of their nodal basin. The MSLT II was designed to determine whether nodal observation is an acceptable alternative to CLND for patients with positive SLNs. Until completion of this trial, CLND should remain standard management for patients with SLN metastasis.

It is clear that the degree of lymph node involvement with metastatic melanoma carries prognostic significance. The number of involved nodes determines nodal stage in the current AJCC system and is directly related to the risk of melanoma-associated death. This significance has been validated in numerous data sets. In addition, the tumor burden of the SLN is associated with NSLN involvement and overall prognosis.

Our study suggests that there is also a qualitative difference in the prognostic significance of tumor-involved SLNs vs tumor-involved SLNs plus NSLNs. Adjusting for the number of involved nodes, the distinction between metastasis only to lymph nodes receiving direct drainage from the primary site (ie, SLNs) and other, higher-echelon nodes remains significant. The source of this difference is unknown, but one can speculate that it is related to either an increased potential for dissemination of the tumor cells or a decreased ability in some SLNs to contain or respond to tumor cells.

Data from this study demonstrate that NSLN involvement is more common with aggressive tumor characteristics (eg, thickness and ulceration) and with host characteristics (eg, age). Prior studies have made it clear that the risk of NSLN metastasis can be quantified, not only based on primary tumor characteristics but also on SLN tumor location and burden. Investigators from our melanoma center have previously shown that NSLN involvement increases significantly if more than 5% of the SLN area is replaced by tumor, although this quantification was not available for the patients in the present analysis. The same biological abilities that allow tumor cells to penetrate initial immune stations may also allow hematogenous dissemination. An additional analysis from our center has demonstrated a decline in lymphatic function in elderly patients with decreased retention or concentration of radiotracers used in SLN mapping. This inability to retain colloid particles may parallel an inability to retain tumor cells, increasing the risk of NSLN metastasis, and perhaps distant-site metastasis. These tumor biological and immunologic questions could be investigated and may provide useful information regarding the process of melanoma metastasis.

In the present study, we did not have access to information regarding potentially important variables, including the tumor burden within the SLNs and the presence of microscopic metastases in NSLNs that were not evident by hematoxylin-eosin staining. Although these data might affect the results of our multivariable analysis, they may not be practical to include in the staging system at this time. Exhaustive sectioning and immunohistochemical staining of NSLNs is likely to be impractical for the pathologist, and no consensus exists regarding the most appropriate measurement system to
quantify SLN tumor burden. At present, NSLN involvement, as identified by current standard pathological processing, carries powerful, independent prognostic information and is simple to assess.

The AJCC guidelines were updated in 2009 and were based on the analysis of 17,600 patients in the AJCC Melanoma Staging Database, including 3307 patients with stage III melanoma. Statistical analyses of survival data determined the factors important for staging and prognosis. We propose that, for the next iteration of the staging system, the committee performs an analysis of the independent prognostic impact of NSLN status. Should that analysis confirm the findings of our series and others, this simple, readily available data point should be included in the next staging system.

**References**