Lymphatic Mapping for Melanoma: Long-term Results of Regional Nodal Sampling With Radioguided Surgery

Erik M. Ramnath, MS; Deepa Kamath; Andrea Brobeil; Alec Stall; Vidya Kamath; C. Wayne Cruse, MD; Frank Glass, MD; Jane Messina, MD; Neil Fenske, MD; Claudia Berman, MD; Merrick I. Ross, MD; Alan Cantor, PhD; David Cuthbertson, MS; and Douglas S. Reintgen, MD

The use of lymphatic mapping and sentinel lymph node biopsy is effective in staging patients with primary cutaneous melanoma.

Background: Lymphatic mapping and sentinel lymph node (SLN) biopsy are new techniques used in the surgical treatment of patients with malignant melanoma. These procedures have the potential to change the surgical treatment of the disease to provide a more rational approach to adjuvant therapy. Methods: A prospective database of melanoma patients undergoing lymphatic mapping and SLN biopsy was reviewed to identify prognostic factors for overall and disease-free survival in this patient population. Results: Five-year overall and disease-free survival was 92.3% and 79.0%, with a median follow-up of 17 months. The number of histologically positive SLNs was the most powerful predictor of overall and disease-free survival. Patients with no histologically positive SLNs had a five-year overall and disease-free survival of 97.9% and 93.3%, respectively. Tumor ulceration and Clark level greater than or equal to III were the significant prognostic factors for survival. Conclusions: The use of lymphatic mapping and SLN biopsy effectively stages patients with primary cutaneous melanoma. Additionally, the presence of histologically positive SLNs is the most powerful indicator of overall and disease-free survival for these patients.

Introduction

An estimated 40,300 new cases of invasive melanoma will have been diagnosed in 1997 in the United States alone. This represents a 48% increase in the number of cases diagnosed per year compared with 1989. Although most patients present with clinically negative nodal basins, a subset of these patients will harbor occult metastases in regional lymph nodes. Therefore, the proper surgical management remains a controversial issue. Several retrospective reports have demonstrated a survival benefit for melanoma patients who underwent an elective lymph node dissection (ELND) as part of their primary therapy. However, in one prospective randomized trial, a survival benefit from ELND was demonstrated only in patients 60 years of age or younger, in patients with non-ulcerated melanomas, or in those with lesions between 1 to 2 mm.

In order to better identify those patients at risk for occult metastases in regional lymph nodes, melanoma treatment centers across the country have used lymphatic mapping and sentinel lymph node biopsy to determine which patients require lymphadenectomy. With the use of a vital blue dye mapping agent, SLN identification rates approached 80%. Currently, with improvements in preoperative lymphoscintigraphy and combination intraoperative mapping techniques using both a radiocolloid and vital blue dye mapping agents, the rate of SLN identification is approaching 100%. The histologic status of the regional nodal basin is thought to be the most important prognostic factor in patients with stage I or II melanoma. Additionally, the survival benefit imparted by high-dose systemic adjuvant interferon alfa-2b therapy provides an important rationale for accurate assessment of the nodal basins in patients at risk for metastases.

SLN biopsy is an effective method of detecting disease in the nodal basin without the morbidity and expense associated with formal lymphadenectomy. However, sampling errors during routine histologic evaluation of lymph nodes may underestimate the presence of metastatic disease. Sampling errors can be overcome using a combination of serial sectioning and immunohistochemical staining to improve the detection of microscopic metastases in examined nodes. Recently, reverse transcriptase polymerase chain reaction (RT-PCR) for tyrosinase mRNA has been reported to increase the detection of occult nodal disease.

The use of lymphatic mapping and SLN biopsy can be used to accurately stage nodal basins at risk for occult metastases. The false-negative rate, defined as the percentage of nodal basins that harbor metastases in nodes other than the SLN when the SLN is negative, has been reported to be less than 4% in several studies. This approach may revolutionize the treatment of patients with primary cutaneous melanoma.

The purpose of this study was to identify prognostic factors predictive of disease-free survival and overall survival in patients presenting with primary cutaneous melanomas. Additionally, disease recurrence was evaluated in patients with a negative SLN biopsy.
Materials and Methods

From November 1991 to December 1996, a total of 480 patients with primary cutaneous melanoma underwent lymphatic mapping and SLN biopsy at the H. Lee Moffitt Cancer Center & Research Institute (MCC). These procedures are currently part of the standard of practice at MCC. Patients diagnosed with primary cutaneous melanoma underwent lymphatic mapping if the tumor thickness was at least 1.0 mm or, if less than 1.0 mm in thickness, with a Clark level of at least IV. Additionally, the patient must have no evidence of metastatic disease in the regional lymph nodes or distant sites as determined by physical examination and staging evaluation (chest radiograph and liver function tests).

Patients underwent preoperative lymphoscintigraphy utilizing intradermally injected technetium 99mTc sulfur colloid (450 µCi, CIS-US, Inc, Bedford, Mass) to determine general lymphatic drainage patterns and to identify those basins at risk for metastatic melanoma. Intraoperative lymphatic mapping and SLN biopsy were performed following the intradermal administration of 1 to 3 mL of isosulfan blue dye (Lymphazurin 1%, Hirsch Industries, Inc, Richmond, Va) around the intact tumor or biopsy site immediately preceding the procedure. Preoperative lymphoscintigraphy was performed two to six hours prior to the surgery so the sulfur colloid had time to migrate and concentrate in the SLN. Lymphatic mapping was subsequently performed with the aid of a hand-held gamma counter (Neoprobe, Neoprobe Corp, Dublin, Ohio). In patients undergoing mapping of more than one basin, the basin with the predominant drainage by preoperative lymphoscintigraphy was explored first. An SLN was defined as a lymph node in a regional basin with blue dye localization and/or radiotracer concentrated at a ratio of 10:1 (ex vivo SLN activity/non-SLN activity) or 3:1 ratio of SLN/background activity in vivo. All patients underwent wide local excision of the primary melanoma with margins appropriate for tumor thickness.4,25

Generally, excised SLNs were analyzed by routine histologic examination with a hematoxylin and eosin stain. Detailed immunohistochemical staining using antiserum to the melanoma associated antigen S-100 was performed on all SLN specimens that were negative by hematoxylin and eosin staining.

During our early experiences with lymphatic mapping, patients underwent planned synchronous ELND following a negative SLN biopsy (n = 56). These patients were not included in the evaluation of the natural history following a negative SLN biopsy. Subsequent patients underwent no further surgical therapy if the pathology of the SLN was negative. Patients who demonstrated evidence of metastatic melanoma in their SLN underwent complete lymph node dissection (CLND) of the affected basin.

Postoperative follow-up consisted of physical examination, chest radiograph, and determinations of serum alkaline phosphatase and lactate dehydrogenase levels. Further investigations, including computed tomography, magnetic resonance imaging, and/or nuclear scan, were performed selectively to confirm abnormal findings or symptoms suggestive of metastatic melanoma. Routine surveillance was recommended every three to four months for years 1 and 2, every six months for years 3 to 5, and annually thereafter.

A prospective melanoma database was reviewed to determine relevant clinical information and to identify sites of recurrence. Overall survival, disease-free survival, and time to most recent follow-up (or death) were calculated from the date of primary melanoma diagnosis.

Standard statistical techniques were used. Categorical variables were analyzed by either chi-square or Student’s t test as appropriate. Disease recurrence curves were constructed using the Kaplan-Meier product limit method and were analyzed by the log-rank procedure. Multivariate analyses used to associate covariates to time-dependent endpoints were performed using the Cox proportional hazards regression model.

Patient Characteristics and Prognostic Factors

In this study, 480 patients were evaluated. Of these, successful lymphatic mapping and SLN biopsy were performed in 465 patients. Further analysis of prognostic factors, overall survival, and disease-free survival was limited to a group of 461 patients in whom tumor thickness was evaluable. Table 1 provides a summary of clinical and pathologic characteristics of these 461 patients. The median and mean tumor thicknesses were 2.33 mm and 1.77 mm, respectively. Additionally, 147 (30.8%) of these patients demonstrated ulcerated tumors. The median age was 56.9 years (range = 14 to 93 years), and 59.3% of the patients were men.

Results

Distribution of Mapped Basins

A total of 577 lymph node basins were mapped in 480 patients (1.2 basins per patient). Mapped basins were distributed evenly between the right and left sides. Of the total number of basins mapped, 302 (52.3%) were axillary, 139 (24.1%) were inguinal, 130 (22.5%) were cervical, 5 (0.9%) were in-transit nodes, and the site of the basin was not recorded in one patient (0.2%). Of 389 patients (81.0%) who underwent lymphatic mapping of a single regional basin, 83 (17.3%) underwent lymphatic mapping of two basins, and 8 (1.7%) underwent mapping of three nodal basins.

SLN Identification Rate and Prognostic Factor Distribution

At least one SLN was identified in 465 (96.9%) of the 480 patients evaluated in this study. The most difficult primary site for determining lymphatic drainage was the head and neck region, with a success rate of 95.4%. At least one SLN was identified in 561 of the 577 basins mapped, indicating an overall success rate of 97.2%. A total of 950 SLNs were harvested from these patients, but only 82 SLNs (8.6%) were positive for metastatic disease. However, only 53 (3.3%) of 1,624 non-SLNs harvested were positive for metastatic disease. SLNs positive for metastatic disease were identified in 64 patients, but only 9 patients (14.1%) with histologically positive SLNs demonstrated evidence of nodal metastases in non-SLNs excised with a CLND.

The effect of Breslow thickness on the number of histologically positive SLNs is outlined in Table 2. Increasing Breslow tumor thickness correlated (P<0.001) with an increase in the rate of SLN involvement with metastatic disease, suggesting that patients with increased tumor invasion are at greater risk for developing nodal metastases.

<table>
<thead>
<tr>
<th>Breslow Thickness (mm)</th>
<th>Number of Patients</th>
<th>Number of Positive SLNs</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.76</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.76 to 1.00</td>
<td>75</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>1.00 to 1.50</td>
<td>164</td>
<td>12</td>
<td>7.3</td>
</tr>
<tr>
<td>1.51 to 2.00</td>
<td>251</td>
<td>33</td>
<td>13.1</td>
</tr>
<tr>
<td>2.01 to 4.00</td>
<td>52</td>
<td>15</td>
<td>28.8</td>
</tr>
<tr>
<td>&gt;4.00</td>
<td>461</td>
<td>64</td>
<td>13.9</td>
</tr>
</tbody>
</table>

* Patient population consisted of those having successful SLN biopsy and determinable Breslow thickness. Data were analyzed by chi-square method and a significant difference between all groups was identified (P<0.05).
The distribution of prognostic factors grouped by histologic status of the SLN is presented in Table 3. Patients with a positive SLN were statistically more likely to have thick, axially located, or ulcerated primary tumors. Additionally, a significant difference between SLN groups by patient age and sex was not evident.

Survival Analysis of the Entire Patient Population

The median follow-up time for the entire patient population was 17 months. The calculated five-year overall and disease-free survival rates were 92.3% and 79.0%, respectively (Fig 1). Table 4 provides a summary of prognostic factors for overall survival analyzed by univariate and multivariate methods. Breslow thickness, Clark level greater than or equal to III, number of histologically positive SLNs, presence of any histologically positive SLNs, and percentage of histologically positive SLNs harvested were all statistically significant indicators by univariate analysis (P<0.05). However, only Clark level greater than or equal to III and number of histologically positive SLNs harvested from the patient remained significant indicators of overall survival by multivariate analysis (P<0.05). The results of univariate and multivariate analysis of prognostic factors on disease-free survival are given in Table 5.

Survival Analysis of Patients Without ELND

During our early experience with this technique, 56 patients with stage 1 or stage 2 primary cutaneous melanoma were treated with lymphoscintigraphy, lymphatic mapping, and SLN biopsy. These patients then received a CLND to document our incidence of skip metastases. In this group, 13 patients (23.2%) had at least one SLN positive for metastatic disease, while the SLNs of the remaining 43 (76.8%) were histologically negative. Patients with histologically negative SLNs showed no evidence of nodal disease in any non-SLN removed during ELND. Additionally, none of these patients developed recurrent disease in the regional lymph node basin during the follow-up period. Because the false-negative rate for this procedure at MCC was 0% and had been reported to be less than 4% at other institutions,10,11,13,17 we changed the standard of care at our institute to include a CLND only if patients demonstrated a histologically positive SLN.

In this study, 404 patients underwent SLN biopsy under the new care guidelines. The characteristics of this subset of melanoma patients are given in Table 6. Forty-seven (11.6%) of these patients had histologically positive SLNs. CLND was performed on 41 (87.2%) of the SLN-positive patients. Five patients refused CLND despite recommendations to undergo this procedure. One patient was considered high-risk secondary to other medical problems, and the surgeon decided to follow the patient without further surgery. Three patients (0.8%) of the 357 patients with negative SLNs later developed recurrent disease in the regional lymph node basin. Retrospective analysis of the SLNs of these patients was performed for two of these patients. A specimen was not available for analysis for the third patient. RT-PCR for tyrosinase messenger RNA23 revealed two patients evaluated were positive for micrometastatic disease.

Survival Analysis of Patients With Histologically Negative SLNs

Survival analysis of the 357 patients with histologically negative SLNs was performed to gain information about overall and disease-free survival in this subset of patients. The median follow-up time for this group of patients was 17 months. The calculated five-year overall and disease-free survival rates were 92.3% and 79.0%, respectively (Fig 1). Table 4 provides a summary of prognostic factors for overall survival analyzed by univariate and multivariate methods. Breslow thickness, Clark level greater than or equal to III, ulceration, number of basins mapped, number of histologically positive SLNs, presence of any histologically positive SLNs, and percentage of histologically positive SLNs harvested were all statistically significant indicators by univariate analysis (P<0.05). However, only Clark level greater than or equal to III and number of histologically positive SLNs harvested from the patient remained significant indicators of overall survival by multivariate analysis (P<0.05). The results of univariate and multivariate analysis of prognostic factors on disease-free survival are given in Table 5.

Table 3 - Distribution of Prognostic Factors Grouped by Histologic Status of Sentinel Lymph Nodes Harvested by SLN Biopsy (n=461)*

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Negative SLN</th>
<th>Positive SLN</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.7</td>
<td>54.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>Men (%)</td>
<td>56.1</td>
<td>69.7</td>
</tr>
<tr>
<td>Median Breslow Thickness (mm)</td>
<td>3.2</td>
<td>3.1</td>
<td>0.018**</td>
</tr>
<tr>
<td>Clark level (%)</td>
<td>30.7</td>
<td>24.8</td>
<td>0.919</td>
</tr>
<tr>
<td>Axial location (%)</td>
<td>41.0</td>
<td>56.8</td>
<td>0.011**</td>
</tr>
<tr>
<td>Ulceration (%)</td>
<td>35.5</td>
<td>47.4</td>
<td>0.070</td>
</tr>
</tbody>
</table>

* Patient population consisted of those having had a successful SLN biopsy and a determinable Breslow thickness.
** Indicates a statistically significant comparison between groups as determined by chi-square analysis.

Table 5 - Univariate and Multivariate Analysis of Prognostic Factors Used to Evaluate Disease-Free Survival (n=461)*

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Univariate P-Value</th>
<th>Multivariate P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.048</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Breslow Thickness</td>
<td>0.001**</td>
<td>0.001**</td>
</tr>
<tr>
<td>Clark level &gt;= III</td>
<td>0.001**</td>
<td>0.001**</td>
</tr>
<tr>
<td>Ulceration</td>
<td>0.032</td>
<td>NS</td>
</tr>
<tr>
<td># of basins mapped</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td># of positive SLNs</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Any positive SLNs</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Percent positive SLNs</td>
<td>0.001**</td>
<td>NS</td>
</tr>
</tbody>
</table>

* The patient population consisted of those having undergone a successful SLN biopsy and having a measurable tumor thickness.
** Statistical significance was determined as outlined in Materials and Methods section.

Fig 1 - Overall and disease-free survival curves constructed using the Kaplan-Meier product limit method. The patient population included those having a successful SLN biopsy and having a measurable tumor thickness (n=461).
was 20.2 months, and the five-year overall survival was 97.9% (Fig 2). Table 7 summarizes the results of univariate and multivariate analysis of known prognostic factors on overall survival. Only the presence of tumor ulceration was statistically significant for overall survival by univariate ($P=0.009$) and multivariate ($P=0.008$) analysis. The median follow-up for patients with histologically negative SLNs followed to any recurrence or death was 16.2 months, and the five-year disease-free survival was 93.3% (Fig 2). Table 8 summarizes univariate and multivariate analysis of prognostic factors of disease-free survival for patients with histologically negative SLNs. Clark level greater than or equal to III was predictive of recurrence by univariate and multivariate analysis ($P=0.03$).

Discussion

The combination of lymphatic mapping and SLN biopsy was initially described by Morton et al.\textsuperscript{10} as a method of identifying patients with primary melanoma who may be harboring clinically occult nodal metastases. The technique assumes that lymphatic drainage from the site of the primary tumor follows an orderly progression through afferent lymphatics into SLNs before spreading into non-SLNs found in the regional lymphatic basin.\textsuperscript{11} In this study, we observed that none of the initial 56 patients treated with SLN biopsy followed by CLND demonstrated non-SLN metastases if the SLNs were free from metastases. Additionally, of the entire patient population evaluated, only 9 (14.1%) of 64 patients with histologically positive SLNs demonstrated histologically positive non-SLNs on CLND. These data are consistent with the orderly progression hypothesis.

An SLN positive for metastatic melanoma was identified in 13% of the patients evaluated (Table 2). However, because the median tumor thickness for the patient population was 1.77 mm, the frequency of histologically positive SLNs reported may not represent the frequency of positive SLNs in patients with greater tumor thickness. Therefore, the frequency of positive SLNs was stratified based on tumor thickness (Table 3). From this analysis, we observed that as tumor thickness increases, the number of patients with positive SLNs increases. Additionally, patients with relatively thin melanomas, between 0.76 mm and 1.50 mm, demonstrate a reasonable rate of occult nodal disease on detailed examination of the SLN with serial sectioning and immunohistochemical staining. Therefore, we conclude that offering nodal staging surgery to patients with thinner melanomas is a reasonable course of action because the histologic status of the regional lymph nodes makes such a priori difference in treatment recommendations (ie, CLND or administration of adjuvant interferon alfa-2b). Finally, even patients with thick primary melanomas can benefit from lymphatic mapping and SLN biopsy since data from the M.D. Anderson Cancer Center show that T4 N0 melanoma patients do significantly better than the T4 N1 patients,\textsuperscript{14} and adjuvant interferon alfa-2b did not seem to produce a survival benefit for the T4 N0 subgroup in the ECOG 1684 trial.\textsuperscript{17} Univariate and multivariate analysis was performed on the entire patient population to determine prognostic factors predictive of overall and disease-free survival. Results of this analysis indicate that the number of histologically positive SLNs harvested per patient is the most significant indicator of overall survival (Table 4). Additionally, the
number of histologically positive SLNs, Clark level of the primary tumor, and the number of basins mapped per patient were significant predictors of disease-free survival by multivariate analysis. However, of the significant prognostic indicators, the number of positive SLNs identified per patient was the most significant. These data confirm previous reports indicating SLN status as the most powerful prognostic indicator for survival in patients with primary cutaneous melanoma, and once nodal metastases occurs, prognostic information based on the primary tumor lends little additional information to prognostic models.

In this study, we identified three patients who developed nodal metastases after demonstrating histologically negative SLNs. If these patients represent true false negatives, the incidence for this study would be 0.8%. Three mechanisms have been described to explain regional nodal failure: technical, biologic, and pathologic.

Technical failure results from an inability of the surgeon to adequately identify and remove all of the SLNs in the regional nodal basin. Using the blue dye technique alone, it may not be possible to visualize all of the SLNs without an extensive dissection. However, when lymphoscintigraphy and intraoperative gamma probes are used in conjunction with the blue dye, the surgeon is provided with a road map identifying the presence of all possible SLNs that results in improved success rate of SLN identification and thus more accurate staging. Another mechanism of technical failure occurs if the lymphatic mapping procedure does not accurately describe the drainage pattern of the primary melanoma. Changes may occur in lymphatic flow following a disruption in the lymphatic channels secondary to wide local excision of the primary tumor. If this is the case, the SLN with metastatic disease may not be identified by lymphatic mapping, and an alternate node without disease may be harvested.

Biologic failure results from the secondary spread of metastatic melanoma to the nodal basin from a local recurrence, an in-transit or systemic site of disease. In this situation, the SLN(s) harvested accurately represented the status of the regional basin; however, nodal failure would subsequently occur as a result of metastases from other metastases.

Pathologic failure occurs when occult disease is present in an accurately identified SLN but is not detected by the analysis performed. For example, bivalving a lymph node specimen and analyzing a few sections may not identify disease localized to a specific section of the lymph node. In order to overcome pathologic failures, more intensive methods of analyzing lymph nodes are required. The combination of serial sectioning and immunohistochemical staining has been shown to improve the detection of microscopic disease. More recently, a molecular biology assay for identifying nodal metastases has been introduced. Using RT-PCR to identify the presence of tyrosinase mRNA, a gene product specific to melanin-producing cells, a single melanoma cell can be identified in a background of 10^6 lymphocytes. Although recent data suggest PCR-based prognostic evaluations may be clinically relevant, additional follow-up is required to properly evaluate the role of RT-PCR in patient care and staging.

Interestingly, three of 357 patients in this study developed regional nodal metastases after a negative SLN biopsy. RT-PCR analysis was performed on the SLNs of two of the patients, and this analysis demonstrated evidence of "submicroscopic" metastatic disease in both patients. Samples were not available for the third patient. If patients with RT-PCR-positive SLNs are considered to have clinically relevant disease, CLND would have been performed and these recurrences would have been prevented. The data presented suggest that utilization of specialized pathologic techniques, such as RT-PCR for tyrosinase mRNA, may help to identify melanoma patients with the greatest risk of developing nodal recurrence. This hypothesis is being evaluated in the Sunbelt Melanoma Trial, in which patients with histologically negative but RT-PCR-positive SLNs will be randomized into three arms (observation, CLND, or CLND with adjuvant interferon alfa-2b) and compared with patients whose SLN is histologically negative and RT-PCR-negative. In this way, the clinical significance of the RT-PCR assay will be studied, but if the disease is confirmed to be clinically relevant, the proper treatment of these patients will be determined.

Overall, the data presented in this report strongly support the hypothesis that melanoma metastases occur in an orderly fashion and provide additional evidence that the number of histologically positive SLNs is the most powerful indicator of patient survival. Finally, these data support the contention that SLN status can accurately stage the regional lymph node basin, sparing patients with histologically negative SLNs the morbidity and expense associated with a CLND.

References

From the Cutaneous Oncology Program (EMR, DK, AB, AS, VK, CWC, FG, JM, NF, CB, DR) and the Division of Cancer Control: Biostatistical Core (AC, DC) of the H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, Tampa, Fla, and the Department of Surgical Oncology (MIR) at M.D. Anderson Cancer Center in Houston, Tex.

Address reprint requests to Dr Reintgen at the Department of Cutaneous Oncology, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Dr, Tampa, FL 33612.