STUDY

Prediction of Sentinel Lymph Node Micrometastasis by Histological Features in Primary Cutaneous Malignant Melanoma

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Objective: To develop a prognostic model, based on clinical and pathological data, to estimate the probability of micrometastasis in the sentinel lymph node in patients with malignant melanoma.

Design: Retrospective analytical study.

Setting: University medical center.

Patients: Two hundred fifteen patients with American Joint Committee on Cancer stages I and II cutaneous malignant melanoma underwent sentinel lymph node biopsy.

Measurements: Presence of microscopic melanoma in the sentinel lymph node(s). Clinical attributes recorded included age, sex, and location of the primary melanoma. Pathological attributes recorded before lymph node evaluation included ulceration, microsatellites, angiolymphatic invasion, mitotic rate, tumor infiltrating lymphocytes, and regression.

Results: Forty-six patients (21.4%) overall had a positive sentinel lymph node. Patients with tumor thickness ranging from 3.0 to 3.9 mm had the highest incidence (50%) of nodal involvement, followed by those with tumors 4.0 to 4.9 mm thick (41%). Patients with melanomas measuring greater than 4.9 mm thick and those between 1.0 and 2.9 mm had a similar rate of nodal involvement (16%-17%). Clinical characteristics had minimal correlation with nodal status in multivariate analysis. The total number of histological high-risk features was significantly correlated with sentinel lymph node involvement. Important pathological risk factors included ulceration, high mitotic rate, angiolymphatic invasion, and microsatellites. Patients with tumor thickness greater than 1.0 mm but lacking these features had a 14% risk of occult metastases.

Conclusion: Among patients with clinically node-negative primary melanoma, the presence of 1 or more high-risk histological features significantly increases the incidence of microscopic nodal involvement and can be used to predict the likelihood of a positive sentinel lymph node biopsy.

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APPROPRIATE treatment and counseling of patients with malignant melanoma depends on an understanding of the individual patient's prognosis. The prognosis of primary cutaneous clinical stage I malignant melanoma has been shown to correlate most closely with vertical thickness of the primary tumor.[1] Anatomical location and patient sex are 2 other modifying factors that consistently appear to influence prognosis.[1-7] Age of the patient and pathological features, such as ulceration, microsatellitosis, lymphatic invasion, and extensive regression, may likewise adversely affect prognosis.[1-7]

The presence of regional or distant metastasis is the most powerful prognostic factor available[1,8-12] and takes precedence over primary tumor characteristics. Melanoma has been shown to metastasize to the regional lymph nodes in a systematic fashion, with "skip" metastases within a nodal group being rare.[13] The historical experience with elective lymph node dissection for patients with intermediate- or high-risk primary cutaneous melanomas has shown that approximately 20% of clinically node-negative patients harbor microscopic lymph node metastases in the regional draining lymph node beds.[7,13,14]

It has recently become possible to identify this subset of patients with occult microscopic regional lymph node involvement[14] without subjecting all patients at risk to a lymph node dissection, which involves the formal resection of all lymph nodes in a regional basin. The use of lymphoscintigraphy for presurgical mapping enables the qualified surgeon to

PATIENTS AND METHODS

From October 1, 1993, through April 30, 1997, patients referred to the UCSF/Mount Zion Melanoma Center who had intermediate- or high-risk disease (defined as an estimated 5-year survival of less than 95%)[5] were offered SLN biopsy in addition to standard reexcision of the primary site. Clinical characteristics of the patients studied are listed in **Table 1**. All patients under consideration had primary cutaneous malignant melanoma and were clinically without evidence of regional lymph node involvement or distant metastasis. Patients were examined clinically by members of the multidisciplinary UCSF/Mount Zion Melanoma Center Tumor Board, including a pathologist (R.W.S.), internists (including S.M.-G.), dermatologists (including M.K.-S.), and medical and surgical oncologists (including S.P.L.L.).[20] All patients were given a thorough physical examination, plain chest radiograph, complete blood cell count, and serum chemistry studies including liver function tests. Any patient with an abnormal physical, radiological, or serological finding suggestive of metastatic disease underwent further tests as individually indicated. Patients with clinical evidence of lymph node metastasis were ineligible for SLN biopsy.

All patients underwent SLN biopsy within 10 weeks of their original biopsy. Most patients had a Breslow thickness of 1.0 mm or greater. Exceptions included patients with scalp and upper-back lesions with a Breslow tumor thickness of 0.6 to 0.9 mm, since the risk associated with melanoma on these locations belies the tumor thickness alone.[21-23] The original eligibility criteria were derived from the National Cancer Institute–sponsored multicenter selective lymphadenectomy trial in which UCSF participates. Several patients received SLN biopsy off the multicenter selective lymphadenectomy trial protocol and were included in our analysis. Included were patients older than 75 years (10 patients), patients with intermediate-risk melanomas less than 1.0 mm thick (9 patients), and those who declined randomization to wide reexcision without SLN biopsy (19 patients). Wide reexcision performed before evaluation represented a criterion for exclusion from SLN study in selected cases, since the anatomical disruption from surgery has been shown to make the lymphatic drainage pattern analysis less reliable when in a location other than a distal extremity.[24] Patients with ocular melanomas or with a melanoma located on the ear, nose, or any mucous membrane were excluded from study. Pregnancy was another contraindication for SLN biopsy, because the effects of radiolabeled technetium on the fetus are not known.

HISTOLOGICAL ASSESSMENT

Slides of the primary tumor were reviewed by a single pathologist (R.W.S.) before clinical evaluation. Patients considered eligible for SLN biopsy had primary cutaneous melanoma that was evaluated as *intermediate* or *high risk,* defined as having a 5-year survival of less than 95%. The systematic approach to the pathological assessment and description of melanoma specimens reviewed at the UCSF/Mount Zion Medical Center has been previously described.[5,25]

In addition to tumor thickness in millimeters (Breslow measurement), the following were evaluated as histological high-risk attributes: (1) presence of ulceration, (2) high mitotic rate (defined as greater than 5 mitoses per 1 mm^2), (3) presence of microsatellites, (4) presence of identifiable angiolymphatic invasion, (5) absence of tumor infiltrating lymphocytes in the vertical growth phase, and (6) presence of regression involving greater than 50% of the surface length of the tumor measured on the slides.

SURGICAL PROCEDURE

All patients gave informed consent, aware that alternatives to SLN biopsy included regional lymph node dissection or observation. All surgical procedures were performed by 1 of 2 surgical members of the UCSF/Mount Zion Melanoma Center multidisciplinary tumor board (S.P.L.L. or Robert E. Allen, Jr, MD). Details on the surgical method are described elsewhere.[15]

The SLNs were analyzed histologically by means of level sections and hematoxylin-eosin staining. Immunohistochemistry staining for HMB 45 and S-100 was performed if the hematoxylin-eosin preparation was not definitive. Frozen sections were not used. The patient was considered to have a *positive* SLN if 1 or more lymph nodes contained 1 or more melanoma cells. A *negative* result was defined as no histological evidence of tumor cells in any of the SLNs identified.

STATISTICAL ANALYSIS

The principal statistical tool used to analyze the data was the Fisher exact test of frequency tables based on attributes with numeric values, such as tumor thickness and number of high-risk features. A 1-tailed significance test was executed for each distinct value of each numeric attribute. The improvement in probable prognostic correctness was calculated for each distinct value of each numeric attribute, with an associated *P* value. A 2-tailed test was used to calculate the significance of differences in rates of positivity between tumor thickness ranges.

identify for selective biopsy the few anatomical lymph nodes at risk for metastatic spread.[13-19]

Factors such as the cost of the procedure, the psychological stress to the patient, and the risk of uncommon but potential complications dictate that patients be selected appropriately for sentinel lymph node (SLN) study. Additionally, certain clinical, geographical, or psychosocial circumstances may preclude a patient from undergoing an SLN biopsy. The ability to first select and then properly counsel patients on whether to consider SLN biopsy requires knowledge about the probability of lymph node metastasis in a given individual. In this regard, a model predicting an individual's likelihood of harboring micrometastatic disease may help guide decisions regarding SLN biopsy and potential adjuvant therapies in select settings.

To examine the factors associated with micrometastasis, we retrospectively reviewed the results of 215 consecutive SLN biopsies performed at the University of California, San Francisco (UCSF)/Mount Zion Melanoma Center. Patients were selected to undergo an SLN biopsy by means of clinical and pathological character-

Table 1. Clinical Characteristics of Patients Undergoing Sentinel Lymph Node Biopsy

Characteristic	Finding
Age, y	
Range	17-88
Mean	51
Sex, No. (%)	
Male	117 (54)
Female	98 (46)
Tumor location, No. (%)	
Trunk	98 (46)
Extremity	90 (42)
Scalp	20 (9)
Face	4 (2)
Acral	3 (1)

istics that are readily available in most cases at the time of diagnosis. The clinical characteristics used to select patients were tumor location, patient sex, and, to a lesser extent, age. Tumor thickness of 1.0 mm or greater was used as a general guideline for eligibility. Patients were individually assessed, taking into account clinical characteristics, a psychological evaluation, and discussion, which resulted in exclusion of some patients with melanomas thicker than 1.0 mm and inclusion of a small subset (n = 9) of patients with melanomas ranging in thickness from 0.6 to 0.9 mm. Analysis was directed at identifying clinical or histological characteristics that best predict an individual's probability of having a positive SLN.

RESULTS

The overall incidence of finding 1 or more positive SLNs (per patient) was 21.4% (46 positive of a total of 215). Tumor thickness alone was significantly correlated with SLN positivity (**Figure 1**). Patients with the greatest incidence of clinically occult SLN metastases were those with primary tumor thickness ranging from 3.0 to 4.9 mm. Fifty percent of patients with tumor thickness between 3.0 and 3.9 mm and 41% of those with tumor thickness between 4.0 and 4.9 mm had a positive SLN. In contrast, those with tumor thickness ranging from 1.0 to 2.9 mm and those with tumor thickness greater than 5.0 mm had a less than 20% incidence of SLN positivity overall, with little heterogeneity found in subgroup analysis. For example, patients with tumor thickness in the 1.0- to 1.9-mm range had a 16% overall incidence of SLN positivity. An 18% incidence of SLN positivity was observed among those with tumor thickness ranging from 1.0 to 1.4 mm (n = 50), and a 14% incidence of SLN positivity was seen among those with tumor thickness ranging from 1.5 to 1.9 mm (n = 43).

We next examined the relationship between histological attributes and SLN status. The number of high-risk histological features present in the primary tumor correlated positively with the probability of lymph node metastasis. Patients with no histological high-risk features (HRFs) had a 14% incidence of SLN positivity overall. This incidence increased to 31% in patients with 1 HRF ($P = .002$). Those with 2 HRFs had a 47% incidence of SLN positivity ($P < .001$), and those with at least 3 HRFs had a 75% incidence of harboring a positive node ($P = .005$). The total number of histological HRFs corresponded to an increased probability of occult micrometastatic disease with statistical significance for all values of HRF (**Figure 2**). Individual histological HRFs that were significantly predictive of occult micrometastases included microsatellitosis, angiolymphatic invasion, ulceration, and a high mitotic rate. Ulceration was present more than twice as often in those with a positive SLN than in those with a negative SLN ($P < .001$). A high mitotic rate was almost 2 times as frequent among those with a positive SLN result than in the negative SLN group ($P = .03$). Microsatellitosis was 13-fold more common in the positive SLN group than in the negative SLN group ($P = .001$). Finally, angiolymphatic invasion was more than 6-fold more common with a positive SLN than with a negative result ($P = .003$) (**Table 2**). Absence of tumor infiltrating lymphocytes and presence of regression were found to have no individual predictive value for the presence of occult regional lymph node involvement. Clinical factors of age, location, and sex were likewise without predictive value, both when analyzed separately and in multivariate analyses (data not shown).

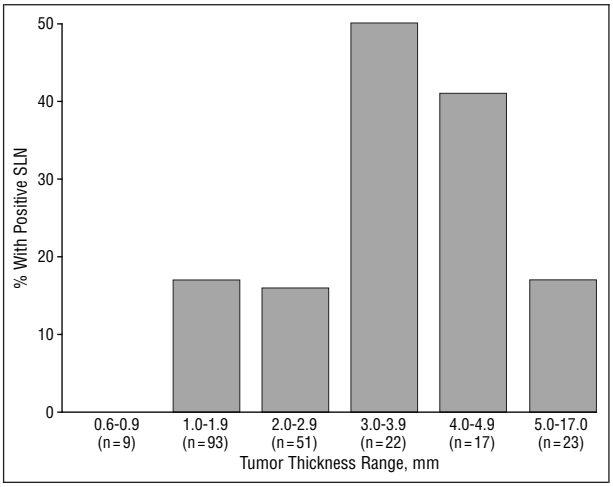

Figure 1. *Incidence of sentinel lymph node (SLN) positivity by tumor thickness. The groups with tumor thickness of 1.0 to 2.9 and 5.0 to 17.0 mm combined had significantly different rates of positivity from those with thickness of 3.0 to 4.9 mm (P<.001).*

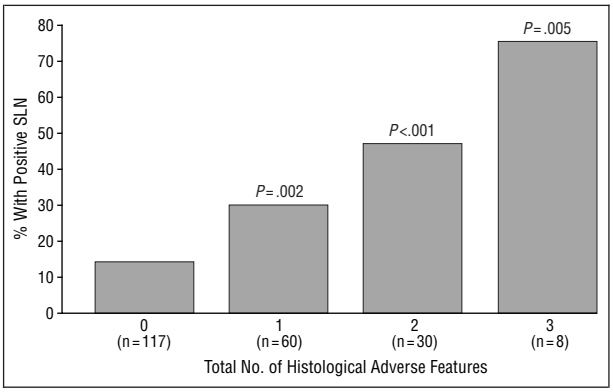

Figure 2. *Incidence of sentinel lymph node (SLN) positivity by number of adverse histological features (excluding tumor thickness).*

Table 2. Presence of HRF by SLN Status*

HRF	% SLN+	% SLN−	P
Ulceration	47	22	<.001
Microsatellites	13	1	.002
Vascular invasion†	13	2	.004
Mitotic rate‡	30	17	.03

*HRF indicates high-risk features; SLN, sentinel lymph node; plus sign, positive; and minus sign, negative.
†Includes microscopic angiolymphatic invasion.
‡Six or more mitoses per 1 mm^2.

COMMENT

The status of the SLN is of importance to the clinician for 3 major reasons. First, it helps assess individual prognosis with greater accuracy.[1,26-28] Second, it identifies with minimal morbidity the subset of patients who may benefit from subsequent therapy (ie, a lymph node dissection and adjuvant therapy, such as with interferon alfa-2b[29]). Third, the procedure itself may have a therapeutic effect and improve a patient's probability of long-term survival.[26,27]

In our series, approximately 22% of patients with intermediate- and high-risk primary cutaneous melanoma had microscopic lymph node metastases, a finding consistent with other studies.[7,13,14] Select subsets were found to have a much higher or slightly lower incidence. These observations are important both for patient counseling and for understanding the relationship between primary tumor characteristics and regional metastasis.

The presence and total number of histological HRFs show a direct relationship with SLN positivity. Many previous studies have shown ulceration, high mitotic rate, lymphatic invasion, and microsatellitosis to be associated with a worse prognosis.[4,5,7,11] The tumor's ability to form microsatellites and growth characteristics that enable it to visibly invade small vessels or cause ulceration may be closely tied to the factors that enable it to undergo lymph node metastasis.

The observation that histological HRFs are associated with an increased incidence of microscopic lymph node metastasis has been previously demonstrated. Callery et al[11] found that among clinical and histological node-positive patients, the prognosis was worse for patients with an ulcerated primary tumor, satellitosis, or a high mitotic rate. To our knowledge, this is the first study to show the predictive value of the total number of histological HRFs to SLN metastasis. The implication of this finding is that the presence of 1 or more histological HRFs is an important biological tumor marker that can be used in addition to tumor thickness to predict the probability of a primary tumor's metastatic potential.

The relationship between tumor thickness alone and SLN positivity is an interesting one. A distinct and statistically significant peak association with microscopic regional lymph node metastasis was seen with patients who had a Breslow thickness between 3.0 and 4.9 mm. Interestingly, there was a similar rate of SLN positivity among the group of patients with tumors between 1.0 and 2.0 mm thick with the use of 1.5 mm as a cutoff, suggesting that selecting patients with tumor thickness greater than 1.0 mm for SLN biopsy is appropriate. Moreover, the overall incidence of a positive SLN in patients with Breslow thickness between 1.0 and 2.9 mm is similar to that of patients with tumors greater than or equal to 5.0 mm thick. We cannot exclude the possibility that a selection bias may contribute to this finding. Patients with thick tumors may be more likely than those with thinner tumors to present with clinically palpable lymph nodes and, therefore, undergo a therapeutic lymph node dissection or other treatment. Still other patients with thick primary tumors may have evidence of distant metastasis. Both of these subsets would be excluded from consideration for SLN biopsy. The data presented in this study do not address the relationship of SLN metastases to long-term survival. Long-term survival and recurrence patterns between SLN-negative patients with thick melanomas and those with tumors ranging from 1.0 to 2.9 mm in thickness will be interesting to compare.

Few data are available on the subset of patients with T4 tumors (melanomas measuring 4.0 mm or greater in maximal depth). Such data indicate that elective lymph node dissection for this group shows a greater than 50% incidence of clinical and occult metastasis to the regional lymph node bed and that overall mortality for this group is greater than 50% at 5 years.[30-34] The mortality appears to be significantly lower for those found to have pathologically confirmed negative lymph nodes.[30-34] Our analysis shows that among the subgroup of patients with melanomas between 4.0 and 4.9 mm thick and no clinical or radiological evidence of metastasis, 41% harbor occult regional lymph node metastasis. In contrast, only 17% of those with melanomas greater than 5.0 mm thick and no clinical or radiological evidence of metastatic disease have regional lymph node involvement. This finding may be caused by a selection bias in studying only patients with primary melanomas without metastasis at initial examination or may be a reflection of the SLN status being a poor predictor of long-term survival for patients with thicker tumors.

Similarly unclear is the predictive value on survival of a negative SLN biopsy result in a patient with 2 or 3 HRFs. These patients may still have a high risk of mortality through distant metastasis and could potentially benefit from adjuvant therapy.

In conclusion, this study provides data that may assist physicians in the counseling of patients considering SLN biopsy with information regarding the probability of harboring occult lymph node metastases based on histological tumor characteristics.

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Robert E. Allen, Jr, MD, is a surgical oncologist on the UCSF/Mount Zion Melanoma Tumor Board, San Francisco, Calif, and performed approximately half of the SLN biopsies.

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