Impact of Thorough Block Sampling in the Histologic Evaluation of Melanomas

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Objective: To determine if changes in histologic parameters obtained from intermittent sampling of the entire block correlated with differences in prognosis and management.

Design: Prospective analysis of skin biopsy specimens.

Setting: Skin pathology laboratory.

Patients: One hundred consecutive patients with an unequivocal diagnosis of melanoma.

Interventions: Two initial slides were prepared from serial sections of 5-µm thickness. When evaluation of the initial slide revealed melanoma, 5 additional slides were obtained by sectioning at levels through the entire block. Breslow depth, Clark level, ulceration, tumor infiltrating lymphocytes, vascular invasion, regression, presence of a precursor lesion, and histologic type of melanoma for the first slide and the additional 6 slides were analyzed and compared.

Results: Review of the additional 6 slides from level sectioning revealed a greater maximum tumor thickness than was evident from the original slide in 43% of the cases. In 10 of these cases, the new maximum tumor thickness measurements changed the surgical management of the patients. Ulceration was observed in 6% of cases on the initial slides, and an additional 3% of lesions were found to have ulceration on levels. The level of invasion was deeper than originally found in 10% of the cases.

Conclusions: Level sectioning through an entire block of a melanoma specimen provides additional information in the classification and management of melanomas. Extensive block sampling will result in more accurate information regarding histologic parameters of melanoma, but the yield must be balanced with the extra cost of materials, time, labor, and the potential disadvantage of not retaining tissue for future use.

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A dermatopathologist’s assessment of the histologic features of a melanoma provides information critical to staging, treatment, and prognosis. In assessing the prognosis of a patient with a primary melanoma, tumor thickness and ulceration are the first and second most important prognostic determinants, respectively.1 Other relevant histologic factors are Clark level, vascular invasion, tumor vascularity, mitotic index, microsatellitosis, and regression.1,4 Despite the importance of accurately assessing the histologic features of a melanoma, few studies address the issue of appropriate block sampling.2,8

A survey of dermatopathology laboratories found that there are no recognized standards for how many initial slides or deeper sections dermatopathologists obtain.9 We have encountered consultations on cases in which the histologic evaluation of melanoma was made using 1 slide containing 1 level. Suspecting this to be inadequate, we decided to study the impact of more thorough block sampling on determining the histologic characteristics of melanoma.

METHODS

We prospectively reviewed 100 consecutive skin biopsy specimens from patients with unequivocal diagnoses of melanoma. The specimens were collected between August 2002 and May 2003. The biopsy techniques used to obtain specimens were shave (56%), punch (28%), and fusiform excision (16%). All specimens were fixed in formalin, embedded in paraffin, and then routinely processed for hematoxylin-eosin staining. Specimens larger than 4 mm were sectioned transversely at intervals no greater than 4 mm.

Two initial slides were prepared from serial sections of 5-µm thickness, done rou-
tinely for all specimens in our laboratory. The interval between the 2 slides varied from 50 µm for smaller specimens up to 100 µm for thicker specimens. When evaluation of the initial slide revealed unequivocal melanoma, 5 additional slides were obtained by sectioning at levels through the entire block. Levels were estimated to range from 300 to 500 µm apart, depending on the thickness of the tissue.

Histologic parameters assessed were Breslow depth, Clark level, ulceration, tumor infiltrating lymphocytes, vascular invasion, regression, presence of a precursor lesion, and histologic type of melanoma for the first slide and the additional 6 slides. Five dermatopathologists assessed the specimens (S.S., J.B., J.P., C.J., and J.S.). For each specimen the dermatopathologist assessed the initial slide also reviewed the subsequent slides. Diagnostic concurrence by at least 2 dermatopathologists was required for all specimens.

## RESULTS

The patients ranged in age from 24 to 95 years. The sites of the melanomas were the head and neck, trunk, and upper and lower extremities (Table 1). The melanomas were of superficial spreading (46%), lentigo maligna (37%), unclassified (14%), nodular (2%), and desmoplastic types (1%). The tumor (T) stages of the melanomas according to the TNM staging system were T1 (88%), T2 (7%), T3 (1%), and T4 (3%).

The tumor thickness after review of the initial slide for the 100 cases ranged from in situ melanoma to 4.9 mm. Review of the additional 6 slides from level sectioning resulted in an increase in measured maximum tumor thickness for 43% of the cases. The increase in measured thickness ranged from 0.02 mm to 0.45 mm (median, 0.16 mm). Ulceration was observed in 6% of cases on the initial slides, and an additional 3% of lesions were found to have ulceration on levels. Clark level of invasion was deeper in 10% of the cases. Regression was noted in 14% of cases on the initial slide, and an additional 3% of cases were found to have regression on deeper sections. Precursor nevi were noted in 15% of initial slides; an additional 4% of cases were found to have precursor nevi on deeper sections. Vascular invasion was noted in 1% of cases on both the initial slide and deeper sections (Table 2). The type of specimen obtained and the corresponding changes in histologic parameters of melanoma observed on deeper levels are outlined in Table 3.

## COMMENT

The objective of this study was to determine if changes in histologic parameters obtained from intermittent sampling of the entire block correlated with differences in prognosis and management.

Review of the initial slide failed to show the maximum tumor thickness in 43% of melanomas. In 10 of those cases the new maximum tumor thickness measurements changed the surgical management of patients. In 9 cases, the diagnosis changed from in situ melanoma to invasive T1 melanoma (thickness, 0.22-0.45 mm). The Clark level of invasion was level 2 for 7 cases and level 3 for 2 cases. (Our findings suggest that disease-free survival rates lower than 100% among patients with in situ melanomas may result from invasion that goes undetected because of incomplete sampling.) In the remaining case, the thickness changed from 0.99 mm to 1.30 mm (stage T1 to stage T2). The National Institutes of Health Consensus Panel recommendations for wide local surgical excision of primary melanomas are different for in situ and invasive melanomas. Even though these margins can be modified for anatomic or cosmetic reasons, in general, higher staging results in a larger wide local excision. The present single case that changed from stage T1 to stage T2 placed the patient into a higher risk group according to the new American Joint Committee on Cancer staging system. Even though the remaining cases with changes in tumor thickness were not reclassified into higher risk groups, the prognoses of these patients might have worsened, since risk group divisions are arbitrary and death rate is a linear function of thickness.
The change in Clark level from 2 and 3 to 4 in 2% of the cases changed patient surgical management. Clark level of invasion is considered in assessing the management of thin melanomas.1

Ulceration not observed on the initial slides was observed on deeper levels in 3% of the cases. Two of the 3 cases had subtle histologic features suggestive of adjoining ulceration. The presence of ulceration changed the 5- and 10-year survival projections of these patients.1

There are no standardized methods for sampling tissue in dermatopathology; however, it has been shown that maximizing specimen sampling leads to a more accurate diagnosis.2,5,9,12 A survey of 82 dermatopathology laboratories indicated that there were no recognized standards in the number of initial slides prepared and the methods used to search for tumors where lesions suspected clinically were not seen in the initial sections.9 Another study concluded that in 37.3% of cases, additional sections provided more diagnostic information to the pathologist. The additional diagnostic information most commonly assisted in the diagnosis of malignant skin lesions.7 A study evaluating step sections in cases showing actinic keratosis on initial sections found malignant lesions in 20% of cases after evaluation of deeper levels.12

Tissue sampling for microstaging of a melanoma is an issue seldom addressed in the literature. Breslow5 discussed this problem in the measurement of tumor thickness and Clark level for melanomas. He concluded that since the treatment of a patient with a melanoma depends on the accurate assessment of the thickness and the level of invasion of the lesion, “the entire tumor must be embedded and step-sectioned at 1- to 2-mm intervals so as to be certain that the thickest portion of the tumor or the deepest level of invasion has been found.” Another study demonstrated that an increase in the maximum tumor thickness was discovered in 100% of specimens evaluated with serial sections through the entire specimen.6

Although it is well known that the histologic features of a primary melanoma are important survival prognosticators, most authors do not include the tissue sampling method used to determine these features. The recent American Joint Committee on Cancer report4 on the prognostic factor analysis of melanoma patients does not discuss the methods used to determine the histologic features of a melanoma. The data were obtained from 13 institutions and cooperative study groups without any indication that a standardized method of tissue sampling was used.

We have presented evidence that level sectioning through an entire block of a melanoma provides additional information in the classification and management of melanomas. We did not set out to establish optimum sampling standards, but our data support the utility of obtaining multiple levels in the histologic evaluation of melanoma. Extensive block sampling will result in more accurate information regarding histologic parameters of melanoma, but the yield must be balanced with the extra cost of materials, time, labor, and the potential disadvantage of not retaining tissue for future use.

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REFERENCES