Sentinel Lymph Nodes in Cutaneous Melanoma
Handling, Examination, and Clinical Repercussion
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Context.—Within the last 15 years, evaluation of sentinel lymph nodes (SLNs) has become the most popular method of early staging of several malignancies, including melanoma. Sentinel lymph nodes are usually examined on formalin-fixed, paraffin-embedded sections and by routine histology/immunohistochemistry (research protocols have used other techniques such as polymerase chain reaction). Approximately 20% of patients with cutaneous melanoma have metastasis in the SLN. In most studies, detection of positive SLN conveys a poorer prognosis for patients with cutaneous melanoma.

Objective.—To review the morphologic patterns of melanoma metastasis in the SLN, the differential diagnosis, and the quantification of tumor burden as a prognostic factor.

Data Sources.—Personal observations and review of the pertinent literature.

Conclusions.—Evaluation of sentinel lymph nodes is certainly becoming a widespread technique and most authors agree on its prognostic power for staging patients with cutaneous melanoma. Current studies are evaluating the possible therapeutic value of removal of positive SLNs. (Arch Pathol Lab Med. 2010;134:1764–1769)

Most authors seem to agree that evaluation of sentinel lymph nodes (SLNs) has become the most popular method of early staging of several malignancies, including melanoma. Sentinel lymph nodes are reportedly those nodes most likely to contain metastatic deposits. Therefore, to detect early metastasis, such lymph nodes should be examined histologically in a more intense manner than that used for standard lymphadenectomy specimens (usually a single hematoxylin-eosin slide per cassette). Although it has been suggested that removal of SLNs may improve overall survival, the main goal in examining SLNs remains the determination of an accurate staging of early lesions to more accurately define the prognosis of patients and provide more consistent grouping in clinical trials.

This article will discuss the main clinical, macroscopic, histologic, and immunohistochemical features of SLNs from patients with cutaneous melanoma.

CRITERIA FOR RECOMMENDATION OF SENTINEL LYMPHADENECTOMY

Most protocols recommend SLN examination for patients with melanomas with Breslow thickness of 1 mm or more, or with ulceration, and traditionally Clark level IV. These criteria will probably change in the near future because Clark level has been replaced in the new American Joint Commission on Cancer (AJCC) classification in favor of mitotic count.1 At our institution, other criteria are vertical growth phase (in particular those cases with dermal mitotic figures), vascular invasion (particularly highlighted with anti–D2-402,3), and satellitosis. At least 1 study4 has indicated that paucity of lymphocytic infiltrate is associated with higher positivity rate for SLN. It is unclear if the presence of regression correlates with a higher rate of positive SLNs,5,6 such that most protocols do not consider regression as a criterion for SLN analysis. A tumor subgroup that may not benefit from the examination of SLN is that of pure desmoplastic melanoma, since such lesions only rarely metastasize to the SLN.7,8

PROCESSING OF SLN

Some authors recommend the use of frozen sections to examine SLNs, particularly in breast carcinoma, to try to render an immediate diagnosis of metastatic disease during the surgical procedure. Patients with positive SLNs by frozen section then undergo completion of the lymphadenectomy in the same surgical procedure. However, frozen sections provide a suboptimal morphology and may lack the subcapsular region of the lymph node (which is the area most likely involved in microscopic metastases). Furthermore, because processing of the frozen tissue requires embedding in paraffin, facing off the block, and new sectioning of the tissue, micrometastases may be lost in the discarded, unexamined sections.9 Therefore, at least for SLN from melanoma patients, most authors consider the gold standard to be the examination of routinely processed material (formalin fixed, paraffin embedded) and discourage the use of frozen sections.

A possible alternative is touch preparations/cytologic specimens10,11; however, it is not a widely used technique, since evaluation of such specimens requires expertise in cytologic smears, particularly for distinguishing between melanoma cells and pigmented macrophages.

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Regarding the grossing technique, there is no complete agreement on how to process SLNs. However, the classic processing method used for lymph nodes, that is, bivalving of the node and examination of a single, routine hematoxylin-eosin (H&E) slide, misses a number of small metastases. In an early study from our institution, of 243 patients whose SLN was initially diagnosed as negative when examining 1 H&E slide per block, 10 patients (4.1%) presented with a recurrence in the same lymphatic basin. When the original SLN was reexamined by using new serial sections or immunohistochemistry, 8 (80%) of these 10 patients with recurrence were reclassified as having a positive node. In another study, 3 of 7 patients with recurrent disease had metastatic melanoma in the originally “negative” SLN, after reexamination with serial sections and immunohistochemistry. Based upon these studies, most current protocols in SLN require more than 1 H&E section, usually with the addition of immunohistochemistry.

A recent survey in Europe shows that several protocols are used to process SLNs. The original protocol proposed by Cochran called for bivalving the SLN through the hilum, with the intent to allow examination of the lymphatic vessels of the lymph node. At our institution we recommend “breadloafing” the SLN to allow examination of a large area of the subcapsular region (Figure 1). Then, we study 1 H&E slide; if the result is positive we report it as such. If the result is negative, we submit the block again to the laboratory to obtain a new, deeper H&E section slide (about 200 μm deeper in the block) and 2 unstained slides. One slide will undergo reaction with a pan-melanocytic cocktail (HMB-45, anti-MART-1, and anti-tyrosinase). The other unstained slide is used, if needed, to perform additional immunohistochemical analysis (see below) (Figure 2).

Regarding other studies, some clinical trials call for preserving a portion of the node for polymerase chain reaction (PCR) analysis to try to detect messenger RNA associated with melanocytic differentiation (see also below).

**Microscopic Features**

Approximately 20% of patients with cutaneous melanoma show deposits of melanoma cells in the SLN. Metastatic melanoma cells may display a large variety of morphologies, epithelioid or spindled, pigmented or amelanotic. Most commonly, metastatic melanoma cells resemble the cells in the primary lesion. Thus, when examining an SLN, it may be very important to study the original melanoma and to compare the morphologic features, particularly to distinguish metastatic cells from macrophages or nevus cells (see also below the section on differential diagnosis). It may be difficult to distinguish pigmented melanoma cells from melanophages; however, pigment granules are usually coarser and larger in macrophages than in melanoma cells.

In general, melanoma cells in the SLN are located in the subcapsular sinus, as single cells, small nests, or large, expansile clusters (Figure 3, A through C). Less frequently, the metastasis is located within the parenchyma. Very rarely do melanoma cells involve the fibrous capsule, and in such cases, it is likely secondary to involvement of intracapsular lymphatic vessels (see also below the section on differential diagnosis). As with other solid tumors, there may be extracapsular extension into the perinodal fibroadipose tissues (<5% of cases).

Immunohistochemical studies are very helpful when trying to detect small metastatic deposits and also when differentiating metastasis from nodal (capsular) nevus. Of the approximately 20% of cases with positive SLNs, 16% are detected in the initial hematoxylin-eosin slide and the remaining 4% are detected with serial sections or immunoperoxidase. Some authors propose the use of anti-S100 protein. However, because S100 labels lymph node dendritic cells in addition to melanocytes, in our opinion, it is difficult to distinguish single melanoma cells from a background of dendritic cells. Therefore, other markers may be more useful. Among the different options, we recommend a pan-melanocytic cocktail (HMB-45, anti-MART-1, and anti-tyrosinase) (Figure 3, A through C). In addition, since MART-1 can be expressed by macrophages, we sometimes use HMB-45 by itself when trying to differentiate between macrophages and melanoma cells (HMB-45 usually does not label macrophages). For spindle cell melanomas in which the tumor cells do not express MART-1 or gp100 (with HMB-45), we may use anti-S100.

The differential diagnosis also includes capsular nevi. Up to 20% of lymphadenectomies from the axilla or groin contain such benign collections of melanocytes (Figure 4, A through C). The characteristic capsular location of these nevus deposits is different from the common subcapsular location of metastatic melanoma. Therefore, clusters of...
melanocytes in the capsule are usually benign (nodal nevus), while subcapsular/intraparenchymal clusters are malignant. However, a potential problem is the presence of vascular metastasis in the intracapsular lymphatic vessels of the node, thus mimicking a capsular nevus (in our experience, it is extremely rare to detect nevus aggregates within the vessels of the SLN capsule). Immunohistochemistry against vascular markers (CD31, CD34, or D2-40) may be helpful in detecting the rim of endothelial cells, thus confirming the intravascular location of the melanoma cells.

Rarely, capsular nevi may extend into the underlying node parenchyma. In general, those lymph nodes contain similar melanocytes in the capsular region, lack gp100 expression (with HMB-45), and show very low Ki-67 expression; thus, their profile is consistent with benign melanocytes. To facilitate the identification of proliferating melanocytes, we have recently developed a homegrown cocktail that includes anti–MART-1 and MIB1 (against Ki-67). Since these 2 markers are expressed in different cellular components (Ki-67 in the nucleus and MART-1 in the cytoplasm), it is relatively easy to determine how many of the melanocytes (ie, cells expressing MART-1) are proliferating (ie, expressing Ki-67) (see also section on use of immunohistochemistry in melanocytic lesions).

**TUMOR BURDEN**

We recommend issuing a pathology report that includes the number of positive nodes and the total count, both spelled out and expressed as numbers, to avoid possible typographic errors (eg, “one of three lymph nodes [1/3]”). Sentinel lymph node positivity is associated with decreased survival, along with Breslow thickness, and ulceration. Quantification of melanoma metastasis size in SLNs correlates with subsequent involvement of non-sentinel lymph nodes from the same anatomic region and with prognosis. Some authors recommend a modification of Breslow thickness (measurement of the distance between the capsule and the most deeply located deposit). On the basis of our results, we measure the tumor burden in the SLN as determined by the size of the largest tumor deposit (in 2 dimensions, in millimeters), the location (subcapsular versus other), and presence or absence of extracapsular extension (Figure 5). Not all studies have detected an association between tumor location (subcapsular/intraparenchymal) and survival, but at any rate, most responders to a recent survey in Europe also report the size of the largest tumor deposit in the SLN.

Our preliminary data, based on 237 patients with positive SLNs (of 1417 patients), suggest a stratification for patients with melanoma into 3 groups with progressively worse prognosis, as follows: (1) involvement of 1 or 2 SLNs and metastasis size of 2 mm or smaller (in the largest nest), and no ulceration (in the primary lesion); (2) ulceration in the primary lesion or any metastatic nest larger than 2 mm; (3) involvement of 3 or more SLNs or ulceration in the primary lesion and any metastatic nest larger than 2 mm. Additional studies are necessary to determine if such a stratification scheme will provide clinically significant prognostic information.

Regarding the relationship between tumor size and prognosis, an unexpected finding in our study was the lack of a definite cutoff. Unlike breast carcinomas, in which SLN with tumor deposits smaller than 0.2 mm are not considered positive by some authors, we have seen at least 2 cases in which only a single melanoma cell was identified in the SLN, and both lesions had recurred as multiple distant metastases within 4 years of diagnosis.

Regarding additional techniques, some studies have indicated that PCR detection of melanocytic messenger RNA in SLN correlates with decreased survival, a finding not shown by other authors. A possible explanation for these differences may be the presence of nodal nevi in some SLNs that would result in positive PCR results. Therefore, unless messenger RNA specific for melanoma cells becomes available for PCR studies, it seems that histologic examination will remain the gold standard in evaluating SLN for melanoma.
Figure 3. A, Metastatic melanoma involving the subcapsular region. B, Note the cytologic atypia. C, Melanoma cells react with a pan-melanocytic cocktail (hematoxylin-eosin, original magnifications ×10 [A] and ×40 [B]; pan-melanocytic cocktail, diaminobenzidine with light hematoxylin, original magnification ×10 [C]).

Figure 4. A, Nodal nevus. Notice the benign-appearing melanocytes located in the capsule. B, These cells show uniform nuclei with small nucleoli. C, These cells express MART-1 with low proliferation rate (hematoxylin-eosin, original magnifications ×4 [A] and ×40 [B]; anti–MART-1, aminoethylcarbazole, anti–Ki-67, and diaminobenzidine with light hematoxylin, original magnification ×20 [C]).
lymphovascular invasion with D2-40 in melanoma correlates with sentinel survival, while others have failed to show such an effect.

Prognostic power for staging patients with cutaneous melanoma using a widespread technique and most authors agree on its value. However, some studies have suggested improved results in patients with positive sentinel nodes. A, Measurement of a single subcapsular nest. B, Subcapsular and intraparenchymal nests. The larger is measured. C, For the purpose of measuring, when small aggregates are located in clusters in the same region of the sentinel lymph node, we consider them to be a single nest. D, Extension beyond the lymph node.

**THERAPEUTIC EFFECT OF REMOVAL OF POSITIVE SLN**

Regarding a possible therapeutic effect secondary to the removal of a positive SLN and subsequent lymphadenectomy, some studies have suggested improved survival, while others have failed to show such an advantage. Additional studies are necessary to determine whether such therapeutic value exists.

**CONCLUSIONS**

Evaluation of sentinel lymph node metastasis is certainly becoming a widespread technique and most authors agree on its prognostic power for staging patients with cutaneous melanoma.

**References**


**Sentinel Lymph Nodes in Cutaneous Melanoma—Prieto**


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