Lack of Standards for the Detection of Melanoma in Sentinel Lymph Nodes

A Survey and Recommendations

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● *Context.*—Detection of microscopic melanoma metastases in sentinel lymph nodes drives clinical care; patients without metastases are observed, and patients with metastases are offered completion lymphadenectomy and adjuvant therapy.

Objective.—We sought to determine common elements in currently used analytic platforms for sentinel lymph nodes in melanoma patients.

Design.—An electronic survey was distributed to 83 cancer centers in North America.

Results.—Seventeen responses (20%) were received. The number of sentinel lymph node mapping procedures for melanoma ranged from less than 11 to more than 100 patients per year, with 72% of institutions mapping more than 50 melanoma patients a year. Uniform practices included (1) processing all of the lymph node tissue rather than submitting representative sections and (2) use of immunohistochemical stains if no tumor was identified on the hematoxylin-eosin–stained sections. Significant variability existed regarding the method of sectioning lymph nodes at grossing and in the histology laboratory; most bisected nodes longitudinally (94%) and performed deeper levels into the block (67%), but these were not uniform practices. S-100 was the most commonly used immunohistochemical stain (78%), followed by Melan-A (56%), MART-1 (50%), HMB-45 (44%), tyrosinase (33%), MiTF (11%), and pan-melanoma (6%).

Conclusions.—There is a need for a standardized platform for detecting melanoma in sentinel lymph nodes. Current practices by a majority of laboratories and findings in the reported literature support the following: histologic evaluation of all lymph node tissue, use of immunohistochemical stains, bisecting lymph nodes longitudinally, and performing deeper levels into the tissue block.

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More than 80% of patients diagnosed with primary cutaneous melanoma are without clinical evidence of metastatic disease at the time of diagnosis.[1] Patients with localized disease at presentation have a 5-year disease-free survival that ranges from 50% to 100%, a range that is impacted by primary tumor characteristics, most importantly tumor thickness. Sentinel lymph node mapping is offered to a subset of these patients with clinically localized disease who are deemed to have a high risk of metastasis, for example patients with primary tumors >1 mm or mitogenic and/or ulcerated melanomas slightly less thick.[2]

The clinical value of sentinel lymph node biopsy in the staging of primary cutaneous melanoma is well established; sentinel lymph node status is a principal prognostic factor in predicting disease recurrence and progression.[3–5] In a study by Morton et al,[6] the 5-year disease-free survival rate was estimated at 53% if the sentinel node contained metastases

and 83% if the node was free of metastases; overall melanoma-specific survival rates were 72% and >90%, respectively, at 5 years. Microscopic melanoma metastases are identified in the histopathologic analysis of the lymph nodes in approximately 16% of patients who undergo sentinel lymph node mapping.[7–9] The identification of microscopic metastasis in a sentinel lymph node has a significant impact on patient care; these patients are offered completion lymphadenectomy and stratified on the basis of node status for adjuvant therapy protocols.[10,11] Given the impact that the histopathologic identification of microscopic melanoma metastases has on therapeutic decision making, much effort has been directed at the development of optimal methods for sampling and immunohistochemical analysis of sentinel lymph nodes.[8,12,13] Nevertheless, there is no universally accepted protocol for the processing of sentinel node biopsies in patients with melanoma. Adoption of a common protocol may allow for more uniformity in clinical care, including clinical trial enrollment criteria, and provide for more accurate comparisons in longitudinal multi-institutional studies.

The purpose of the present study was to assess and characterize by means of electronic survey the protocols currently used by cancer centers in North America for detecting melanoma in sentinel lymph nodes.

Sentinel Lymph Node Processing Survey Questions and Answers by Institutions 1 Through 18																		
Institution:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Do you process sentinel lymph nodes from patients with cutaneous melanoma in your laboratory?																		
No																		
Yes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2. How many patients with cutaneous melanoma undergo SLN mapping that is analyzed in your laboratory each year?																		
0–10									x									
11–50	x	x				x								x				
51–100			x	x	x						x				x	x		
>100							x	x		x		x	x				x	x
3. Does your laboratory have a standardized protocol for the processing of sentinel lymph node biopsies for melanoma?																		
No		x							x					x	x			x
Yes	x		x	x	x	x	x	x		x	x	x	x			x	x	
4. Are sentinel lymph node specimens routinely submitted entirely or is only a portion of the lymph node(s) submitted?																		
Entirely	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Portion																		
If submitted entirely, are sentinel lymph node specimens routinely bisected prior to embedding? If so, is this done longitudinally (parallel to the long axis) or transversely (perpendicular to the long axis)?																		
Whole																		
Bisected longitudinally	x	x		x	x		x		*	x	x	x	x		x	x	x	x
Bisected transversely														x				
Sectioned otherwise			x			x		x	*									
5. How many histologic sections are routinely cut and examined for a sentinel lymph node specimen?																		
Total No. of slides stained																		
1		*				*												
2–5	x	*		x		*	x	x	x	x		x		x	x			x
6–10			x		x						x					x		
>10													x				x	
No. of H&E-stained sections																		
1		*				*	x			x		x						
2–5	x	*	x	x	x	*		x			x		x	x	x	x	x	x
>5									x									
If sections are taken at deeper levels, what is the distance between levels?																		
<19 μm										x							x	x
19–100 μm	x			x							x			x	x			
>100 μm			x															
Not specified/DK		x				x	*	x	x			x	*					
6. a. Are immunohistochemical or other stains ordered routinely in the evaluation of sentinel lymph node specimens? b. If so, which ones?[a]																		
No. of immunohistochemical stained slides																		
0		*				*												
1	x					*	*											
2–6		x	*	x	*		x	x	x	x	x	x	x	x	x	x	x	x
>6																		

Abbreviations: DK, don't know; H&E, hematoxylin-eosin; SLN, sentinel lymph node; X, single response to question; *, responses wherein multiple choices were selected or the answer choice was contingent on the histologic findings of the case.

[a] Answers to question 6b are shown in Figure 2.

MATERIALS AND METHODS

A multiple-choice electronic survey was designed to assess protocols used in the processing of sentinel lymph node specimens (Table). A list of 83 candidate institutions was developed from publicly available sources, including the melanoma treatment centers listed at http://www.skincancer.org/melanoma-treatment-centers.html (last accessed 2009) and cancer centers with representatives listed in the American Society of Dermatopathology membership directory at www.asdp.org/ScriptContent/DirectoryPDF.cfm (last accessed 2009).

RESULTS

A total of 17 completed surveys were received from institutions that process sentinel lymph nodes, representing 20% of contacted institutions. These survey results allowed for the comparison of sentinel lymph node melanoma protocols from 18 institutions including our laboratory. The number of sentinel lymph node mapping procedures for cutaneous melanoma at each institution ranged from less than 11 to more than 100 patients per year, with most institutions mapping more than 50 melanoma patients a year (13 of 18; 72%; Figure 1).

Most institutions reported using a standardized laboratory gross dissection protocol for processing sentinel lymph nodes (13 of 18; 72%). All 18 institutions processed all of the lymph node tissue rather than submitting representative sections. One institution reported bisecting lymph nodes transversely (perpendicular to the long axis) prior to embedding; the other 17 institutions reported either bisecting the node longitudinally or sectioning the node longitudinally at 2- to 3-mm intervals if too large.

Regarding tissue sectioning and levels, nearly all institutions (16 of 18; 89%) reported routinely cutting multiple sections for each lymph node, with a majority (10 of 18; 56%) reporting 2 to 5 slides per sentinel lymph node (range of 1 to >10 slides). All institutions reported performing a hematoxylin-eosin (H&E) stain on at least 1 tissue level (Figure 1). Two institutions (11%) reported that the total number of slides examined was conditional on whether melanoma was identified in the initial H&E-stained tissue section.
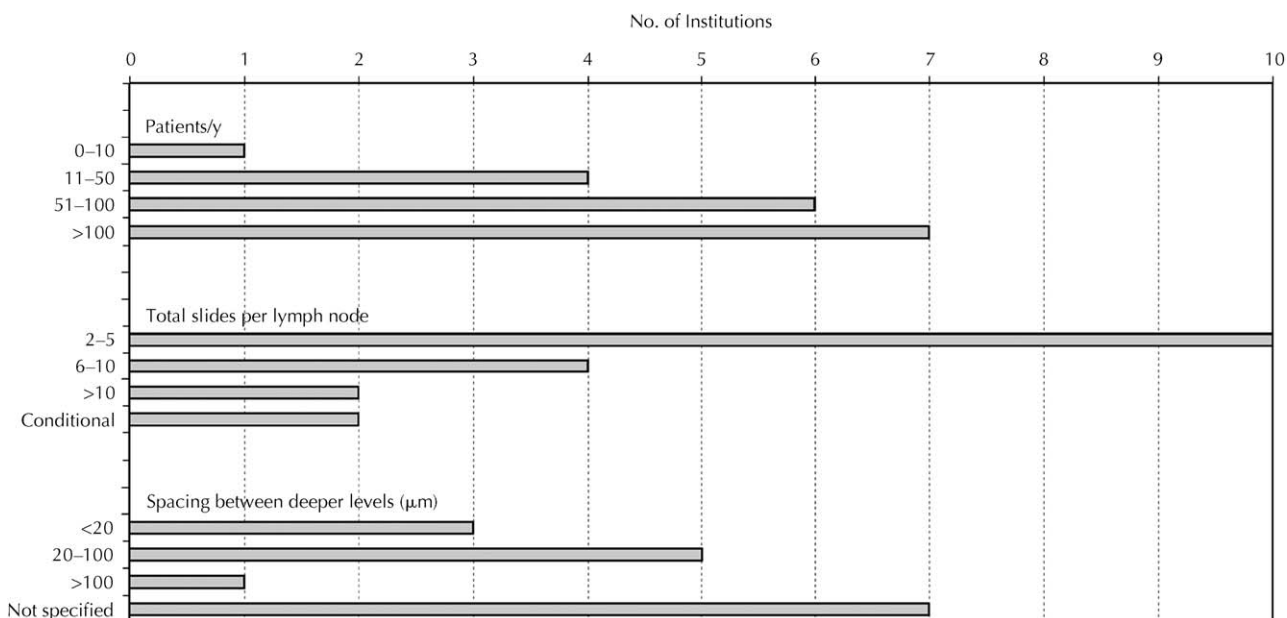
Figure 1. *Number of patients per year undergoing sentinel lymph node mapping for cutaneous melanoma at responding institutions, reported total slides stained, and reported spacing between deeper levels.*

A number of different protocols were reported for the handling of deeper levels into the tissue block (Figure 1). Institutions reported examining deeper levels cut at intervals <20 μm (3 of 18; 17%), or intervals of 20 to 100 μm (5 of 18; 28%). One institution reported intervals between level sections of more than 100 μm. More than a third reported that the spacing between deeper levels was not specified or was unknown (7 of 18; 39%). One institution reported cutting no routine deeper levels on the tissue block; however, this institution reported sectioning the entire lymph node at 2-mm intervals prior to embedding. Another site reported a protocol in which deeper sections were performed "at the discretion of the dermatopathologist and based on the risk assessment of the primary cutaneous melanoma."

All respondents used immunohistochemical techniques in their melanoma sentinel lymph node detection platform. Most reported performing immunohistochemical stains on all sentinel lymph nodes (16 of 18; 89%), whereas 2 (11%) reported that immunohistochemical stains were performed on the lymph node only if the initial H&E-stained slide was negative for metastasis. More than 80% of institutions performed 2 or more immunohistochemical stains (15 of 18; Figure 2). S-100 was the most commonly used immunohistochemical stain (78%), followed by Melan-A (56%), MART-1 (50%), HMB-45 (44%), tyrosinase (33%), MiTF (11%), and pan-melanoma (6%) (Figure 3). One institution reported that immunohistochemical stains were performed based on the depth of invasion of the primary lesion, with 1 slide routinely stained with S100 for primary melanomas with a Breslow thickness of <3 mm, and 2 to 6 immunohistochemical stains with S-100 and Melan-A for primary melanomas >3 mm in thickness.

COMMENT

In this survey we document the absence of a standard protocol for the detection of microscopic melanoma in sentinel lymph nodes. The results demonstrate that although certain basic protocol elements are similar across institutions, there is marked overall variability in protocol specifics. In contrast to carcinoma of the breast, the detection of even small foci of melanoma in sentinel lymph nodes is associated with a substantially increased risk of melanoma-associated death.[7,14] Whereas some authors report a minimal incidence of subsequent metastasis in patients with micrometastases <0.1 mm in diameter, others have reported that patients with only a single metastatic melanoma cell identified in the sentinel node may develop widely disseminated metastatic disease.[15,16] In a recent Australian report, 20% of patients with micrometastases <0.1 mm died with metastatic melanoma.[17] Indeed, in the most recent American Joint Committee on Cancer staging schema, the identification of a solitary melanoma cell in a sentinel lymph node upstages a patient and leads to a shift in clinical management from observation (if the lymph node is negative), to the performance of completion lymphadenectomy and often adjuvant therapy. In this environment there is great interest and progress in the development of protocols for the analysis of melanoma sentinel lymph node samples; nevertheless, no single approach has been generally adopted. A standardized protocol could have many potential benefits, including improvement in cross-institution reproducibility of diagnostic and clinical trial enrollment criteria. Our goal in this study was to characterize the types of protocols currently in use at cancer centers.

Our survey results revealed the following majority practices in the pathologic analysis of sentinel lymph nodes in patients with cutaneous melanoma: (1) gross dissection of the lymph nodes by cutting along the longitudinal axis, (2) submission of the entirety of the lymph node tissue, (3) performance of level sections deep into the block (beyond the initial set of tissue sections), and (4) use of immunohistochemical stains.

Despite the above consistencies, there remained many variables among protocols, including (1) the thickness and

Figure 2. *Immunohistochemical stains used in the analysis of sentinel node biopsies for melanoma at 18 institutions. The set of immunohistochemical stains used by each institution is represented by the z-axis row. Overall use of each immunohistochemical stain is represented on the left of the histogram.*
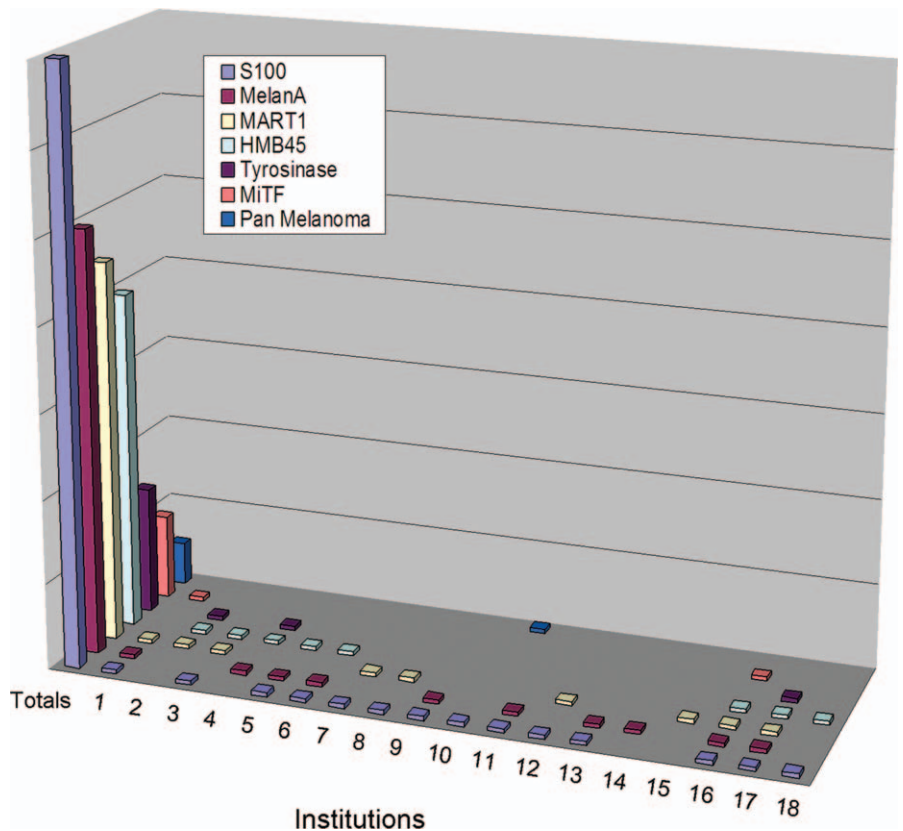
number of level sections (although nearly a third of institutions had 20–100 μm of waste between levels), (2) the type and number of immunohistochemical stains (although a significant majority used S-100), and (3) the conditional implementation of immunohistochemical stains (many stained every lymph node up front; others waited until after failure to identify tumor on the H&E-stained slide).

We previously examined the influence of level sections and immunohistochemical stains on the detection of metastases in sentinel lymph nodes.[8] This investigation examined 235 sentinel lymph nodes reported as negative for metastasis by routine pathologic examination in the early 1990s (a single H&E-stained slide), from 94 patients with American Joint Committee on Cancer stage I and II cutaneous melanoma. We found that deeper sections into the lymph nodes and immunohistochemical staining with antibodies against S-100, HMB-45, NK1C3, and MART-1 detected microscopic metastases in approximately 12% of the cases that had been reported negative by routine pathologic analysis. This work and that of others has established that the addition of deeper levels and immunohistochemical stains to the sentinel lymph node analytic platform increases the sensitivity of pathologic analysis for melanoma micrometastases.[18–21] In a survey of European practices, 66% evaluated step sections; whereas this is a majority practice, it is by no means universal.[22] Immunohistochemical stains are also a standard part of the platform in Europe; in a report from Germany, a 4-slide platform is recommended for the evaluation of sentinel lymph nodes, with Melan-A, S100, and HMB-45 as the possible immunohistochemical reagents.[23]

The finding that all institutions use immunohistochemical staining to enhance the detection of melanoma in their sentinel lymph node platforms is consistent with reports that immunohistochemical stains increase the specificity and sensitivity of detection.[8,24] Nearly 90% of institutions perform immunohistochemical stains on all sentinel lymph nodes; the remaining laboratories examine the initial H&E-stained tissue sections and if they are negative will order immunohistochemical stains. No institution reported the absence of tumor without doing immunohistochemical stains.

Several immunohistochemical stains exist for detecting melanoma metastases; they have markedly variable sensitivities and specificities.[20,25] In our survey, S100 was the most frequently used immunohistochemical stain with more than three-quarters of the institutions reporting that they used it routinely. This is similar to the 74% of laboratories using S100 in a European survey of sentinel lymph node processing.[22] Additionally, nearly three-quarters of the institutions reported either using Melan-A or MART-1; a minority of institutions (17%) reported routinely using both stains. HMB-45 was the next most commonly used immunohistochemical stain (44%), followed by tyrosinase (17%) and MiTF (11%). This use frequency is also similar to that in Europe, with 65% and 62% of laboratories using Melan-A and HMB-45, respectively.[22] One institution reported a protocol in which the choice of immunohistochemical stains was conditional on the depth of invasion of the primary lesion. In this protocol, 1 slide was routinely stained with S100 in patients with primary tumors <3 mm in maximal thickness. If the primary cutaneous melanoma measured >3 mm, then 2 to 6 slides were examined immunohistochemically, using S-100 and Melan-A. Al-
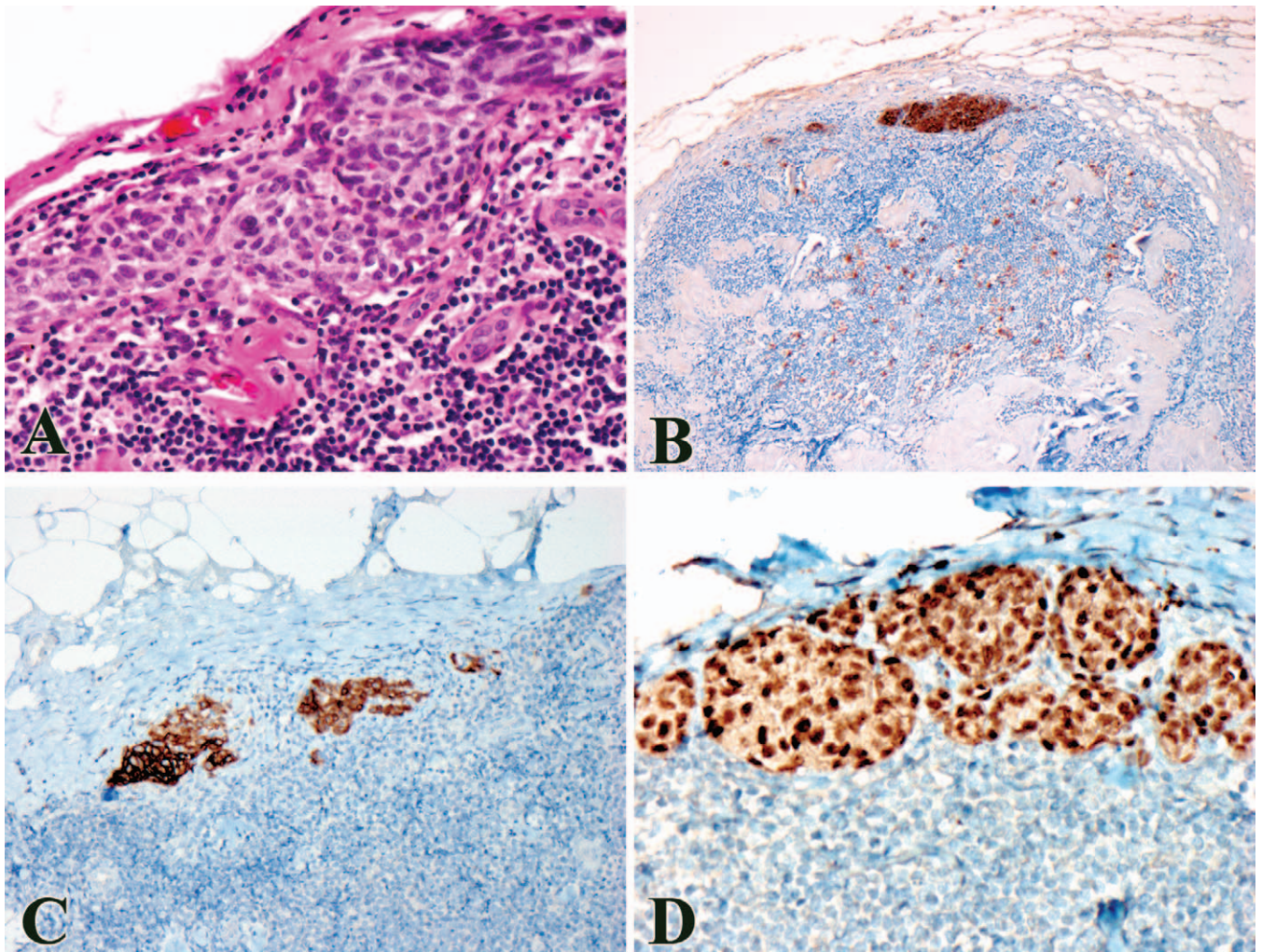
Melanoma Sentinel Lymph Node Protocols—Dekker & Duncan

Figure 3. *Detection of melanoma in a sentinel lymph node. A, Collections of cytologically atypical tumor cells in the subcapsular sinus. B, Subcapsular nests of tumor cells and scattered positive nonmelanocytic dendritic cells. C, Tumor cells in nests and individual cells. D, A nuclear stain highlights the tumor cell pleomorphism (hematoxylin-eosin, original magnification ×400 [A]; S100, original magnification ×40 [B]; MART-1, original magnification ×200 [C]; MiTF, original magnification ×400 [D]).*

though few institutions stain for MiTF routinely, because this is a nuclear stain it has the added benefit of highlighting the size and shape of the nuclei (Figure 3). This may be helpful as a supplement to the H&E staining when distinguishing melanoma from nodal nevus. Staining for HMB-45 may also be helpful in this setting, as it rarely stains nodal nevi, but is detected in approximately 75% of nodal melanoma metastases.[8]

Not only does each reagent have inherent sensitivities and specificities for detecting melanoma in lymph nodes, but also, because standard operating procedures for immunohistochemical staining do not exist, any given reagent is likely to have variable sensitivity and specificity among laboratories. The use of automated staining machines may lead to more consistency among institutions; however, until standard operating procedures exist, it may be best practice to allow pathology laboratories to establish their sentinel lymph node analytic platforms with immunohistochemical stains selected based on the strengths of the reagents in their laboratory.

Although the utility of performing deeper levels into the specimen was clearly recognized by our survey respondents,

there was significant variability in the protocols for cutting deeper levels (Figure 1). The sectioning intervals for deeper levels were reported as <20 μm at 17% of institutions, 20 to 100 μm at 28%, and >100 μm at 1 institution. More than a third of institutions reported that the spacing between deeper levels was not specified or was unknown. We confirmed the importance of level sections in sentinel lymph nodes in our study of sentinel lymph nodes from 475 patients at the Massachusetts General Hospital (Boston, Massachusetts).[26] In this melanoma sentinel lymph node cohort from 2004 to 2008, we found that nearly a third of patients with positive lymph nodes did not have tumor present on all protocol slides. More specifically, of the 78 patients with positive sentinel lymph nodes, 12 had tumor that was not present on the first set of levels, establishing that failure to do levels would have led to a false-negative report in 15% of patients.[26] On the other hand, the Sydney Melanoma Unit reported a false-negative rate of <1% in more than 1000 patients' sentinel nodes evaluated with a platform that included 2 slides with H&E-stained sections, 1 S100-stained slide, and 1 HMB-45–stained slide.[27]

Nearly all the respondents in our survey bisected sentinel lymph nodes longitudinally prior to processing. Nevertheless, this is not a uniform practice, and dissecting the lymph nodes into 2-mm-thick slices, perpendicular to the longitudinal axis of the lymph node, may yield a more thorough evaluation of the subcapsular sinus.[28] Although not a practical technique for all lymph nodes, in the case of sentinel lymph nodes, increased sectioning provides for an increased detection rate of metastatic melanoma.[29] Importantly, these techniques usually generate more tissue blocks per lymph node, which may lead to constraints in analysis in order to comply with the current coding environment in the United States.

It is known that the risk of sentinel lymph node positivity varies among institutions. The factors responsible for this variability may include differences in the analytic platforms. However, other factors, including the prognostic features of the melanoma population at the institution (mean tumor thickness, mean mitotic rate, incidence of ulceration, and lymphovascular invasion), will also impact the rate of sentinel lymph node positivity. In an effort to address the impact of different platforms without the confounding of interinstitutional variability, we evaluated results within an institution with a portion of the protocol.[26] This analysis revealed that elimination of level sections would have led to a significant number of clinically important false-negative reports.

These findings raise important issues with regards to arriving at the optimal detection platform. It will be critical to balance cost with sensitivity of the assay. In the United States, reimbursement is allowed for 1 of each type of immunohistochemical stain per tissue specimen. If a lymph node is submitted in multiple blocks, S100 will be covered for only 1 tissue block. There are significant technical and professional costs associated with the processing and histopathologic analysis of multiple tissue sections with multiple stains for each lymph node. These costs must be balanced with the known increase in sensitivity of performing more tissue sections of every lymph node. Additional techniques may also add to the costs associated with sentinel lymph node analysis. Intraoperative frozen section has a low sensitivity for detecting melanoma in sentinel nodes.[30] On the other hand, the use of molecular techniques such as polymerase chain reaction is associated with a high false-positive rate. As a predictor of subsequent metastasis or overall survival, molecular analysis of the sentinel node to date has not been shown to be superior to immunohistochemical analysis.[31,32]

Although this survey did not focus on the interpretation of the slides, a few notes are in order given the recent changes in the American Joint Committee on Cancer staging guidelines. Anytime that melanoma is detected with immunohistochemical stains, efforts should be made to identify the tumor on an adjacent H&E-stained tissue section. Nevertheless, the current American Joint Committee on Cancer guidelines allow for the diagnosis of a positive sentinel lymph node if a solitary tumor cell is identified only on an immunohistochemical stain.[2] More specifically, the recommended diagnostic criteria for the diagnosis of melanoma in sentinel lymph nodes include (1) the presence of individual cells or nests of epithelioid or spindle cells foreign to the lymph node, (2) cytologic atypia, defined as large cells with nuclear pleomorphism, prominent nucleoli, or dusty cytoplasmic melanin granules, (3) positive staining for 1 or more melanocytic markers (eg, S-100, MART-1,

Melan-A, HMB-45, MiTF, tyrosinase), and (4) identification of the cells on H&E-stained sections (not required if cytologically atypical cells are confirmed on immunohistochemically stained tissue section).

Based on the results of this survey and the reports in the literature, we put forth the following recommendations for the processing and analysis of sentinel lymph nodes in melanoma patients: (1) submit the entirety of the lymph node tissue, (2) perform at least 1 set of level sections deep into the block (beyond the initial set of tissue sections), and (3) if tumor is not visible on the H&E-stained tissue sections, use immunohistochemical stains. Although it is tempting to dictate a specific protocol for sentinel lymph node analysis, implementing the above common elements will bring laboratories toward a more uniform practice than currently exists. Further studies and the development of standard operating procedures for immunohistochemical staining may lead to more specific recommendations for sentinel lymph node analysis in patients with cutaneous melanoma.

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