Sentinel lymph node biopsies

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Introduction

This chapter is based on the author’s previous publications on this topic.1–4 Management of patients with primary melanoma in vertical growth phase who have no evidence of regional nodal metastases on palpation or ultrasound has been controversial for many years.

Until recently, the treatment options were:

• observation with lymphadenectomy delayed until regional metastases became clinically evident (watch and wait),
• elective (prophylactic) regional lymph node dissection after microscopic identification of a primary melanoma (Fig. 28.1).

Neither option is ideal. While observation spares the approximately 80% of patients who never develop regional nodal metastases from morbid lymph node surgery:

• it delays lymphadenectomy beyond the optimum time for the 20% of patients who develop regional metastases,
• it allows melanoma metastases to spread to nonsentinel nodes (NSNs),
• it permits the evolving melanoma to achieve a higher AJCC stage.

To resolve this dilemma, Morton, Cochran, and colleagues at UCLA and The John Wayne Cancer Institute developed the techniques of lymphatic mapping (LM) and sentinel node biopsy (SNB).5

Studies of melanoma patients early in the evolution of regional nodal spread showed that most had metastatic melanoma in a single node, the remaining lymph nodes in the regional basin being free of tumor.6 From this observation, we hypothesized that if it were possible to identify the individual node that was susceptible to these earliest metastases for surgical excision and histologic scrutiny, it would be possible to accurately stage the regional basin on the basis of a relatively minor surgical procedure. Lymphoscintigraphy, in which a radioactive isotope, such as 99mTc antimony sulfide, is injected at the site of a primary tumor, reliably identifies the lymph node basin(s) that receive lymph from that specific site.7 If the lymphoscintigram is read “early”, it is possible to identify the individual lymph node that first receives the isotope (Fig. 28.2).8 The location of that node can then be marked on the overlying skin. Animal experiments had previously shown that marker dye injected in anatomically definable areas of the skin drained reliably to a specific lymph node (Fig. 28.3).9 Combination of lymphoscintigraphy and intraoperative insertion of vital dye and isotope permitted the reliable identification of the first lymph node on the direct lymphatic drainage pathway from a primary melanoma, which, because it ‘guards’ the remaining lymph nodes of the group, we called the sentinel node (SN) (Fig. 28.4).7 In a study of 223 melanoma patients who had an SNB followed by completion lymph node dissection (CLND) regardless of whether or not the SN contained melanoma, we showed that if the SN was tumor free there was a less than 1% chance that NSNs would contain metastases. If the SN contained tumor, there was a 16% chance that NSN would contain metastases.7 These findings led to the development of an NIH-funded international randomized clinical trial (MSLT-I) in 2001. This compared (1) patients with intermediate-thickness primary melanoma (1 mm and thicker or Clark level IV of any thickness), without clinically detectable regional or disseminated metastatic melanoma, who had wide excision and SNB with immediate CLND if the SN contained tumor, followed by observation, with (2) CLND only if the patients developed clinically detectable nodal metastases during observation. Analysis of the results of MSLT-I has shown that SNB is a low-morbidity procedure and is the most accurate staging technique available for melanoma.10 Patients treated by SNB with immediate CLND if the SN contained tumor had significantly longer disease-free survival relative to observed patients. Patients who had CLND delayed until nodal metastatic disease became clinically detectable, had significantly more tumor-positive nodes, were staged at a higher AJCC level, and had a significantly less favorable survival than patients with tumor in the SN who had an immediate CLND.11

LM and SNB, in addition to wide use in the management of patients with cutaneous melanoma, have been applied to nonmelanoma skin cancers, including Merkel cell carcinoma, squamous carcinoma, and appendageal carcinomas. The techniques are also used to manage patients with cancers of organs other than the skin. SN procedures have been widely performed for breast cancer. The approach is also used for patients with cancers of the upper and lower gastrointestinal tract, vulva and cervix, lung, and thyroid.

Accurate identification of the true SN and its precise histopathological assessment are essential for accurate staging, individualized prediction of the likelihood of metastases in NSNs, subsequent extranodal metastases,
Fig. 28.1
Approaches to managing patients with intermediate-thickness primary melanoma without clinically detectable lymph node metastases:
- Elective (prophylactic) lymph node dissection (left panel). All lymph nodes in the draining lymph node group are excised immediately after microscopic identification of a primary melanoma (preferably removed by excision biopsy). By this approach, the 80% of patients who have no nodal metastases are subjected to a morbid operation that is unlikely to confer clinical benefit.
- Observation after wide excision of a primary melanoma with lymphadenectomy delayed until regional metastases are clinically evident (right panel). This spares the approximately 80% of patients who never develop regional node metastases morbidity lymph node surgery, but delays, beyond the optimum time, lymphadenectomy for the 20% of patients who eventually develop nodal metastases, with highly negative clinical consequences.
- Lymph node mapping with sentinel node biopsy (middle panel). This low-morbidity technique selectively identifies the lymph node(s) most susceptible to early metastases. Only patients with tumor in the SN receive complete lymph node dissection.

Fig. 28.2
Identification of a left axillary sentinel node (SN) by lymphoscintigraphy in a patient with a primary melanoma on the left mid back: two 20 MBq, 0.1 mL injections of 99mTc antimony sulfide were made on either side of a small excision biopsy site (IS). On the left is an anterior view of the shoulders, axilla, and chest showing the prominent SN (white arrow) and limited spread of tracer to a second-tier node above the SN. On the right is an anterior view of the pelvis showing no evidence of drainage to groin nodes. By courtesy of R. Uren, MD, Sydney, Australia.

and death from melanoma. SN tumor status also assists in determination of the need for additional surgical and nonsurgical therapy. The responsibility for correct identification of the SN lies with the nuclear medicine physicians and surgeons. Correct determination of the presence or absence of tumor in the SN is the responsibility of the pathologist.

Fig. 28.3
Animal study: this demonstrates that dye injected in specific areas of the skin drains reliably to predictable lymph nodes. Dye injected in the lower lateral abdomen, the suprapubic area, and the upper medial thigh passed reliably to the lateral, medial, and middle lymph nodes, respectively. By courtesy of Wong et al.

Fig. 28.4
Diagram and photograph of the sentinel node (SN) technique as originally applied: blue dye (lymphazurin) is injected intradermally around the primary melanoma or biopsy site. The surgeon then explores the lymph node area identified by prior lymphoscintigraphy as the lymph drainage destination from the site of the primary tumor. Blue-colored afferent lymphatics (lower two-thirds of the right panel) are identified and followed to the blue-colored sentinel node (upper mid area of the right panel). Note that the SN is not necessarily the node that is anatomically closest to the primary site.

**Laboratory management and gross evaluation of sentinel nodes**

SNs arrive at the laboratory, fixed or fresh, as a single cleanly dissected lymph node, a single node in a mass of fat, or as multiple nodes embedded in fat. Surgeons may mark SN with clips or stitches to indicate where afferent lymphatics enter the node, to localize blue-stained segments of the node (evidence supporting SN status) and to highlight areas suspicious for tumor. Requisition forms usually refer to the nodes as SNs, less often as ‘blue nodes’, and occasionally simply imply SN status by appending the radioactive count detected intraoperatively.

Most patients have a single SN, but two and three nodes claimed to be SNs may be submitted. The number of nodes claimed as sentinel may reflect:
- the timing of isotope introduction at lymphoscintigraphy,
- whether additional isotope is injected at the beginning of surgery,
the time taken by the surgeon to detect all lymph nodes that demonstrate increased radioactivity.

If many lymph nodes are submitted, the specimen is probably the product of a partial regional lymph node dissection rather than a true SNB. SNB, as originally described, provides a limited specimen that contains critical metastasis-susceptible nodal tissue, permitting a more detailed examination than is practicable for the multiple lymph nodes included in lymphadenectomy specimens. Surgeons need to be aware that there are significant labor and expense issues when SN evaluation is required for specimens that contain multiple lymph nodes.

SN specimens are most informative if they are free of artifacts due to crush or cautery. SN may be partially dissected free of associated fat by the pathologist to facilitate processing, but it is good practice to leave a rim of fat so that entering lymphatics can be assessed for the presence of tumor. During dissection, the SN should be examined for blue coloration (Fig. 28.5) (although dye has usually dispersed by the time the specimen arrives at the laboratory). SN are measured (maximum length × width × thickness in millimeters) and exactly bisected through the longest meridian because melanoma cells first reach the subcapsular sinus via the afferent lymphatics, which mostly enter the node in the plane of the central meridian. Cut surfaces are scrutinized for metastases, collections of melanin, or carbon pigment (from tattoos or deliberately introduced by the surgeon). This inspection is improved by use of a hand lens or dissecting microscope. Imprints can be prepared at this stage for cytological evaluation (see below). The SN halves are placed cut face down in cassettes and fixed in formalin (Fig. 28.6), ideally for 12–24 hours, although a lesser period may be acceptable if the specimen arrived at the laboratory in an appropriate volume of formalin. Larger nodes may need to be cut more extensively, but additional slices should be cut parallel to the initial cut and parallel to the meridian.

**Confirmation of the sentinel status of submitted lymph nodes**

Correct identification of the SN is primarily the responsibility of the nuclear medicine physicians and surgeons. Because of technical problems during nuclear medicine and surgical procedures, lymph nodes submitted as sentinel may not be true SN. Tumor within an SN can obstruct and divert lymph flow to another node, leading to incorrect designation of that node that contains blue dye and shows enhanced radioactivity as sentinel. Preoperative ultrasonography can identify nodal metastases larger than 5 mm (and some claim that smaller deposits are detectable by ultrasound). If putative tumor deposits detected by ultrasound are confirmed as melanoma by fine needle aspiration, the patient may be considered for CLND instead of SNB.

The accuracy of currently used mapping agents is time dependent. The blue dye is not usually visible by the time that the SN is examined in the laboratory, and may have passed on to second-tier (nonsentinel) nodes. The radioactive isotope decays rapidly from the peak emission values measured in the operating room, and few laboratories have the equipment or expertise necessary to measure radioactivity. There is a need for a stable, inert marker that, after intradermal injection with the blue dye/isotope mixture, would selectively accumulate in SN and be readily visible on standard microscopic examination. Such a reagent would allow pathologists to confirm the actual status of nodes claimed to be sentinel.

Carbon particles injected intradermally with blue dye accumulate preferentially and usually exclusively in SN. Particles accumulate in subcapsular sinuses and lymphoid tissues around the entry point of afferent lymphatics (Fig. 28.7). The location of these particles fortuitously indicates the area of the lymph node most likely to harbor metastatic tumor cells, since the carbon particles are delivered to the SN via the same lymphatics as tumor cells shed by the primary melanoma. This approach cannot be used in patients with carbon-based permanent black tattoos of skin in the catchment area of the SN because tattoo pigment frequently tracks to regional lymph nodes. Drug regulations vary from country to country, and widespread use of this interesting and potentially valuable technique must await the clearance of substantial regulatory hurdles.

In Australia, antimony sulfur colloid is routinely used for lymphoscintigraphy prior to SNB. Scolyer and coauthors report the use of inductively coupled plasma mass spectrometry of tissue sections to confirm SN status by detection of increased amounts of antimony; however, this approach is still in development and is not presently available for routine use.

**Can tissue from a sentinel node ethically be made available for research?**

Accurate determination of the presence or absence of tumor in the SN is essential for correct staging of patients and to allow planning of optimal management. Identification of tumor status of an SN may be difficult as the
Tumor cells are most likely afferent lymphatic where the point of entry of the sentinel status and indicate the node, confirming its tattoo. From the site of a black node macrophages in a lymph node to exit and rapidly passes through delivered (AL). The differential diagnosis of passage of the tumor and focus the search for sentinel status of a lymph node to confirm the sentinel. Use Fig. 28.7.

Intraoperative evaluation of sentinel nodes

Early in our development of LM/SNB, we evaluated the SN by intraoperative frozen sections and rapid immunohistochemistry to make possible immediate CLND if the SN contained tumor. This had the advantage of sparing patients a second anesthetic and surgical procedure. However, experience has shown that evaluation of SN from melanoma patients on the basis of intraoperative frozen sections is relatively unreliable. Preparation of a full-face frozen section is necessary to allow complete evaluation of the subcapsular sinus where early tumor is often detected. To obtain a section that includes the entire subcapsular sinus from a frozen block often requires that many of the initial sections are discarded, leading to loss of substantial nodal tissue. Since SN metastases are frequently very small and selectively located in the area of the nodal meridian, limited diagnostic tissue may be entirely lost with these initial discarded sections. Additionally, identification of single melanoma cells or small clusters of melanoma cells or nevocytically differentiated melanoma cells is more difficult in frozen sections than in well-stained slides from fully fixed tissues. We and other authors strongly believe that melanoma-draining SNs should be evaluated using thin sections cut from well-fixed paraffin-embedded tissues (Fig. 28.8). If intraoperative assessment is requested, visual assessment of the cut face of the node for deposits of tumor is recommended, using a hand lens or dissecting microscope if necessary. Cell smears may be obtained by scraping the nodal cut surfaces, or tumor imprints prepared by pressing the cut surfaces of SN onto glass slides for cytological evaluation (Fig. 28.8). Interpretation of such preparations can be challenging if the melanoma cells are few in number or small and nevocytically differentiated, and the opinion of an experienced cytopathologist is likely to be necessary.
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The need to evaluate multiple levels of the sentinel node

Sectioning

There is wide agreement on the general principles that govern acceptable histologic examination of SN from melanoma patients, but limited consensus on optimum sectioning and staining protocols. Multiple sections should be cut from each half of the SN and stained with hematoxylin and eosin (H&E) and immunohistochemically, using antibodies directed to melanoma-associated epitopes. The number of sections to be stained conventionally and by immunohistochemistry and the interval between sections for evaluation remain subject to debate.

In initial studies of nodal micrometastases, we observed that early melanoma metastases are usually located in a relatively narrow band of tissue adjacent to the longest nodal meridian. Thus carefully sampling the tissues adjacent to the nodal meridian should detect the subgroup of melanoma patients with early nodal metastases. In this study, additional sampling of more peripheral areas of the node did not increase the proportion of melanoma-positive lymph nodes. This is in contrast to other tumors, such as breast cancer, where the tumor cells spread more widely across the nodal surface, and thus more extensive sampling progressively increases the proportion of tumor-positive lymph nodes. On the basis of these findings in lymph nodes from melanoma patients, we have consistently recommended examination of 10 full-face serial sections cut from both faces of the node and stained by H&E (sections 1, 3, 5, and 10), S100 (section 2), HMB-45 (section 4), MART-1 (section 6), and SOX-10 (section 7). Sections 7–9 are retained as spares. If tumor cells are not found in the initial 20 sections cut from both halves of an SN of a patient with a primary melanoma thicker than 1.2 mm (and thus significantly at risk for nodal metastases), additional sections may be prepared and examined, although the yield from this additional evaluation is, in our experience, low. This approach detects melanoma in 16–20% of SNB specimens, a figure that varies according to the thickness of the primary melanomas included in the study group and that is closely similar to the rate of development of clinically detectable metastatic melanoma in the ipsilateral regional lymph nodes of patients observed for up to 10 years after wide excision of a primary melanoma. It is arguable that this relatively simple focused sampling protocol detects most or all of the clinically significant melanoma deposits in SN.

Recent studies, however, have reported that more extended sampling to allow evaluation of sections cut from deeper areas of SN identifies melanoma metastases in some patients where sections closer to the nodal meridian were tumor free. The clinical significance of these additional melanoma cells detected by extended sampling remains unknown, and elucidation of their biology will require that patients be followed for up to 10 years.

Cook and coworkers have reported that examination of six pairs of sections cut at 50-µm intervals and stained respectively with H&E and S100 detects melanoma in up to 33.8% of SNs. Spare sections are cut at 50-µm intervals and stained by H&E (sections 1, 3, 5, and 10), S100 (section 2), HMB-45 (sections 4 and 6), MART-1 (section 8), and SOX-10 (section 9). The right column depicts the sampling technique used by the EORTC Melanoma Group. This uses H&E staining and S100 protein (spare sections may be used for additional immunomarkers). It samples more peripheral portions of the two halves of the sentinel node and is reported to detect melanoma in up to 33.8% of sentinel nodes. The clinical significance of the additional positive nodes detected in this way will become clear from extended follow-up studies that are in progress. This approach calls for some additional technical effort and a significant increment in pathologist work.

Fig. 28.8
Imprint of sentinel node showing melanoma cells: inguinal node from a patient with a vulvar primary melanoma. Most imprints are less obvious than this and show a minority of tumor cells admixed with abundant lymph node cells. Specialist cytopathological evaluation is often required.

Fig. 28.9
Guidelines (left panel, UCLA sampling; right panel, EORTC sampling): the left column depicts long-standing recommendations from UCLA that the two halves of the sentinel node are intensively sampled using H&E and immunohistochemistry on sequential full-face sections. This approach intensively samples the parameridional tissues, uses three separate immunomarkers of varying sensitivity and specificity, but does not evaluate the more peripheral parts of the node. This technique detects melanoma in 16% to 20% of sentinel nodes, a frequency identical to the ipsilateral failure rate of patients treated by wide excision alone. The right column depicts the node sampling technique adopted by the EORTC Melanoma Group. This uses H&E staining and S100 protein (spare sections may be used for additional immunomarkers). It samples more peripheral portions of the two halves of the sentinel node and is reported to detect melanoma in up to 33.8% of sentinel nodes. The clinical significance of the additional positive nodes detected in this way will become clear from extended follow-up studies that are in progress. This approach calls for some additional technical effort and a significant increment in pathologist work.
In choosing a protocol for sampling SNs, pathologists, in consultation with their surgical colleagues, should balance the need for accuracy with cost and workload considerations.9

The role of immunohistochemistry in detecting tumor in sentinel nodes

It may be extremely difficult to identify single melanoma cells or small clusters of melanoma cells (micrometastases) in H&E-stained sections. Without the assistance of immunohistochemistry, even experienced pathologists may overlook small amounts of tumor in up to 12% of SN specimens (Fig. 28.10). For this reason, and to minimize delays in reporting, immunohistochemical studies are best ordered at the time of initial processing. It is important to be selective in choosing the antibodies to be used in this evaluation. Antibodies to S100 protein detect nuclear and cytoplasmic epitopes in virtually all melanomas, including desmoplastic melanomas (100% sensitive for melanoma), but are relatively non-specific, staining dendritic leukocytes in the nodal paracortex (Figs 28.11 and 28.12), some sinus histiocytes, fat cells within and outside lymph nodes, Schwann cells of node-associated nerves, and capsular/trabecular nevus cells (Figs 28.13–28.15). Some pathologists dislike antibodies to S100 because of this non-specificity, but with experience it is usually relatively easy to separate clusters of dendritic cells (DC) that tend to lie toward the periphery of paracortical nodules from melanoma cells, which are usually larger and nondendritic and are characteristically located in the subcapsular zone and deeper parenchymal tissues (Figs 28.16 and 28.17). Dendritic cells may be encountered in the subcapsular and other nodal sinuses, in which case they are regarded as immature DC (originally Langerhans cells) migrating from the skin to the nodal paracortex. Melanoma cells can be dendritic, but this morphology is usually observed in the radial growth phase of lentiginous melanomas and is exceedingly rare in vertical growth phase or metastatic melanoma. Paradoxically, nodal dendritic leukocytes in some SNs may be poorly dendritic or nondendritic. This is considered to indicate down-regulation of the paracortical dendritic cells as part of the tumor-induced immune suppression that affects SNs.2 Nondendritic cells are recognized by their location in the nodal paracortex and characteristic immunophenotype: S100 positive, MART-1, SOX-10, and HMB-45 negative.

The epitopes detected by MART-1 and HMB-45 are located in the cytoplasm of melanoma and other melanocyte-derived cells. Such epitopes are more specific for cells of melanocytic lineage than S100, but are not expressed by the cells of up to 25% of melanomas, particularly metastatic melanomas.1 Antityrosinase antibodies are also relatively specific, but of comparably limited sensitivity. There are reports of the use of combinations of antibodies (antibody cocktails), but these seem to be no more sensitive than S100 and do not allow the critical separation of melanoma cells from nevus cells on the basis of immunophenotype (see below).

Pathologist error accounts for some, but not all, cases of false-negative SNB, where – despite a reportedly negative SN – the patient later develops metastatic melanoma in the ipsilateral nodal basin (3–5% in most published series). Such cases may also reflect incorrect identification of the SN by nuclear medicine or at surgery, or a quirk of biology in which, at the time of SNB, the eventually metastatic tumor cells have departed the primary site, but have not yet arrived at the nodal basin. It is highly important to minimize the frequency of false-negative SN as patients in this category have an unfavorable prognosis, comparable to that of patients who develop nodal metastases during a period of observation after wide excision of a primary melanoma.3 False-positive assessment of an SN is relatively uncommon, but may lead to patients being subjected to immediate CLND, an operation that is associated with considerably more morbidity than SNB alone and that confers no benefit on patients without nodally metastatic melanoma.1 False positivity is usually due to misinterpretation of benign cells in the lymph node as melanoma cells. Misidentification often involves macrophages (Figs 28.18 and 28.19), particularly melanin-containing macrophages and macrophages that have phagocytosed melanosomal fragments that express the epitopes of MART-1/Melan-A or HMB-45 released from disrupted melanoma cells, dendritic leukocytes, and capsular and trabecular nodal nevus cells. Confusion between melanin-containing macrophages and immunopositive melanoma cells may be reduced by using a colored chromogen, such as aminoethylcarbazole or alkaline phosphatase (followed by ‘fast red’), in place of brown diaminobenzidine that may be misinterpreted as melanin (Fig. 28.20). However, since colored chromogens are less frequently used, pathologists may
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find such preparations more difficult to interpret. Other potential sources of false-positivity are S100-positive Schwann cells of intranodal and perinodal nerves (Fig. 28.21), ganglion cells, and mast cells. Distinction of these potential confounding cells is usually achieved by scrutiny of their cytology, their location in or adjacent to the lymph node, and immunophenotype. SNs, particularly inguinal SNs removed from older patients, may show extracellular HMB-45 reactivity associated with calcified foci in the collagen of the nodal trabeculae.

We have encountered an unusual potential cause of a false-positive interpretation of an SN in patients where a small piece of melanoma is included on the slide as a positive control. In rare instances, cells from the positive control tissue may detach and float over to settle on top of the nodal section under evaluation. The keys to identifying these ‘internal floaters’ are to recognize that the tumor cells are not in the plane of the section and then to confirm that the ‘floater’ tumor cells are morphologically and immunophenotypically identical to the cells of the positive control tissue.

**Microscopic evaluation of sentinel nodes for metastatic melanoma**

It is useful to examine the immunohistochemically stained sections first since MART-1/Melan-A, HMB-45, SOX-10, and S100 protein stains can detect truly small numbers of melanoma cells that are difficult to identify in H&E preparations. The entire slide is scanned at low power, and the

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**Fig. 28.11**
Lymph node stained for S100 protein: the illustration shows a paracortical nodule with an abundant population of polydendritic dendritic cells (DCs) that tend to cluster in a ring around the periphery of the paracortical tissue. This classic distribution of DCs is best seen at moderate magnification. DC may also be encountered in the lymph node sinuses and less abundantly in B-cell areas. In addition to their distribution, the presence of multiple complex dendritic processes, relatively small size, and lack of a prominent S100 positive nucleus allows separation of these cells from melanoma cells. Additionally, DC do not express MART-1/Melan-A or HMB-45. In some SN, DC may lose their dendrites, and this may present more of a diagnostic challenge.

**Fig. 28.12**
Poorly dendritic and nondendritic DC in a sentinel node: reduced expression of dendrites by DC is viewed as evidence that they are down-regulated by tumor-induced immune suppression. Nondendritic DC are recognized by their scattered distribution in nodal paracortex and sinuses and their characteristic immunophenotype: S100 positive, MART-1 and HMB-45 negative.

**Fig. 28.13**
Capsular nevus: (A) shows a nevus that is clearly confined to a slightly expanded nodal capsule (H&E). Nevus cells are smaller than most melanoma cells, have limited cytoplasm, usually show only traces of cytoplasmic melanin, and may resemble large lymphocytes. Their nuclei are bland and lack large nucleoli or mitoses. (B) A capsular nevus stained for S100 demonstrating epitope in nuclei and cytoplasm.
Separation of nevus cells and metastatic melanoma in sentinel nodes

The great majority of nodal nevus cell collections derive from cutaneous nevi in the catchment area of the lymph node, the nevus cells apparently reaching the node via afferent lymphatics. Careful scrutiny will often show that benign nevi abut and deform adjacent dermal lymphatics. In rare instances, nodal nevi may be the result of aberrant migration of neural crest-derived melanocyte precursors (melanoblasts) during embryogenesis or even of melanocyte stem cells. Accurate discrimination of nodally located benign nevi from melanoma cells is very important. This requires careful consideration of the location of the melanocyte-derived cells in the nodal architecture and detailed assessment of their cytology and immunophenotype. Melanoma cells are mostly larger than nevus cells (although the occasional nevoid melanoma may present a considerable diagnostic challenge) and are commonly located in the subcapsular sinus and deeper lymphoid tissues of the node. Unlike nevus cells, melanoma cells are seldom present in the nodal capsule other than within afferent lymphatics. Cytological features that may be used to distinguish melanoma from nevus cells include large cell size, high nuclear to cytoplasmic ratio, prominent nucleoli, and mitotic figures (especially atypical mitoses). Both melanoma and nevus cells
may contain finely dispersed small melanin granules (single melanized melanosomes that are just visible under the microscope) that indicate melanin synthesis within the cell (Fig. 28.23). The amount of melanin in melanoma cells is widely variable, but in most instances (with the notable exception of the cells of heavily melanized cellular blue nevi) is greater than that encountered in the cells of melanocytic nevi. Coarse melanin granules (aggregates of melanosomes that are readily visible under the microscope) are characteristic of melanin-containing macrophages (melanophages), but may be seen, admixed with smaller melanin granules, in some melanoma cells. Melanoma cells are almost always S100 protein-positive (staining of nuclei and cytoplasm), SOX-10 (nuclear staining), most (up to 85%) stain positively for MART-1/Melan-A and HMB-45, and Ki67 reactive nuclei are present at relatively high frequency.

Conventional nodal nevus cells are smaller than most melanoma cells, have limited cytoplasm, usually show only traces of cytoplasmic melanin, and may resemble large lymphocytes. Their nuclei are bland and lack prominent nucleoli or mitoses. They stain positively for S100 protein, SOX-10, MART-1/Melan-A, and P164, but weakly or negatively for Ki67 and HMB-45.5 Up to 25% of melanoma patients have capsular or trabecular nevus cells in one or more regional node(s). Location of nevi in connective tissue is usually obvious, although it may be necessary to use a connective tissue stain to rule out extension into the subcapsular parenchyma. Extension of nevi into perivascular stroma or ultrafine reticulations of the trabeculae can make interpretation difficult, because on initial appraisal the nevus cells may appear to be located in the nodal parenchyma, a location that is more characteristic of melanoma metastases. Connective tissue stains such as Masson trichrome and reticulin may help by disclosing the complex arborizing pattern of nodal stroma.

**Is there a role for SNB in the evaluation of melanocytic lesions of uncertain metastatic potential?**

The morphology and immunophenotype of some primary cutaneous melanocytic lesions may be insufficiently typical to allow their certain assignment to the category of melanoma or nevus: lesions of uncertain malignant potential.1–5 The majority of lesions falling into this category are atypical cellular blue nevi, atypical spitzoid lesions, and deep penetrating nevi. In such cases, the surgeon will usually excise the lesion with a margin of local clearance appropriate for a primary melanoma of similar thickness, and
Fig. 28.16, cont’d

(C) Detail of the subcapsular melanoma in (B); (D) shows subcapsular melanoma expressing HMB-45. This lesion also expressed MART-1/Melan-A with a similar cytoplasmic pattern of staining (not shown).

Fig. 28.17

Parenchymal melanoma metastases: these panels show a metastasis of melanoma located deep in the nodal parenchyma. (A) While visible in an H&E-stained section, the fact that (B) the amelanotic lesion expresses S100 protein, (C) MART-1/Melan-A, and (D) HMB-45 confirms that it is a melanoma.
review the pros and cons of SNB with the patient. If an SNB is performed and the SN shows no evidence of a melanocytic lesion, this may represent a nevus without capacity to extend to the nodes or a melanoma that has not (yet) metastasized to the regional nodes, but which may later develop clinically detectable metastases in distant sites. In such cases, CLND is not indicated. If a nodal melanocytic lesion is identified but is confined to the lymph node capsule or trabeculae, there is again no indication for immediate completion lymphadenectomy. If lesional cells are confined to the nodal capsule (other than the special case of melanoma cells in an afferent lymphatic) and

**Molecular biology techniques in the assessment of sentinel nodes from melanoma patients**

Since it is not possible histologically to examine the entire SN, it is possible that conventional microscopy may fail to detect some melanoma metastases in SNs of patients with limited tumor burden. This argues for more extensive nodal sampling and/or the introduction of novel approaches such as the RT-PCR. Molecular staging is currently standard in the management of hematological malignancies. The possibility that RT-PCR might identify melanoma cells in SNs in which extensive histologic and immunohistological evaluation reveals no evidence of tumor has attracted considerable interest. However, there is currently no compelling reason to abandon microscopy and analyze SN exclusively by RT-PCR. The techniques commonly used to extract mRNA for evaluation by RT-PCR destroy the tissue and prohibit identification of the specific cell from which the enhanced signal was derived. A molecular signal for a melanoma-associated marker, in addition to originating from a melanoma cell, might also derive from capsular and trabecular nevi, Schwann cells of intranodal nerves or macrophages that have ingested melanosomes or other organelles from melanoma cells. Concerns have been expressed that overinterpretation of RT-PCR results carries the

**Fig. 28.18**

Care must be taken to avoid the overinterpretation of nodal macrophages as melanoma cells. In panel (A), relatively large epithelioid macrophages are scattered among lymphocytes in a subcapsular position. These cells were negative for S100, MART-1/Melan-A, and HMB-45. Panel (B) shows a collection of relatively large macrophages containing abundant coarsely granular melanin (melanophages). These cells were negative for S100, MART-1/Melan-A, and HMB-45. Panel (C) shows a small granuloma-like collection of epithelioid macrophages. These cells were negative for S100.

**Fig. 28.19**

Nodal macrophages: a further problem with nodal macrophages is that they may ingest fragments of melanosomes that express melanoma-associated epitopes from disrupted melanocytic cells and react positively in immunohistochemical studies: (A) shows a MART-1/Melan-A-stained section with metastatic melanoma at the left and toward the right scattered macrophages that contain MART-1/Melan-A positive granules; (B) shows a collection of macrophages that contain coarse HMB-45 positive granules.
Prediction of outcome based on the extent of nodal replacement by tumor and its distribution within the sentinel node

Clinical outcome and the likelihood of death from melanoma correlate with the number of lymph nodes that contain melanoma metastases, but pathologists may further refine that assessment by measuring the size of nodal metastases and specifying their location within the SN. Building on earlier (pre-SN) studies of the morphometric assessment of the area and micrometer-assessed diameter of nodal melanoma metastases (Fig. 28.26), Starz and coworkers reported that the micrometer-measured depth of tumor penetration from the inner surface of the SN capsule correlated directly with the likelihood of metastases in NSNs in the same nodal drainage basin (Fig. 28.27). Wagner and coworkers correlated SN tumor volume risk of overtreatment. Mocellin and coworkers, in a careful meta-analysis, have shown that a positive PCR reaction in the SNs of melanoma patients is significantly correlated with TNM stage, disease recurrence, and overall and disease-free survival. These authors did, however, find extensive heterogeneity between the results obtained in the 22 sizeable studies that they analyzed. They conclude that ‘the available evidence is somewhat conflicting and probably is not sufficient to conclude that PCR status is a prognostic indicator reliable enough to be implemented clinically in the therapeutic decision-making process’. However, they consider that their findings ‘justify additional investigations of the prognostic power of PCR analysis of sentinel lymph node (SLN) in patients with cutaneous melanoma’. The efficacy and clinical relevance of molecular analysis of SN are being studied in the second Multicenter Selective Lymphadenectomy Trial (MSLT-II), sponsored by the National Cancer Institute in the United States.

Fig. 28.20
Melanoma: this small collection of melanoma cells stains positively for HMB-45 and is highlighted by the red chromogen aminoethylcarbazole. Note the granuloma-like collections of melanin-containing macrophages that surround these cells. Traces of AEC-highlighted, HMB-45 positive material are present in some of the macrophages.

Fig. 28.21
Perinodal nerve: the Schwann cells are stained with an antibody to S100. While the neural nature of such structures is usually (as in this case) readily apparent, there may occasionally be a problem in distinguishing neural structures from melanoma cells in a lymphatic or free in the tissue. This type of problem is most likely to occur when the nerves are intranodal or if the tissue section is thick or from poorly fixed material. Nerves stain negatively with antibodies to MART-1/Melan-A and HMB-45.

Fig. 28.22
Metastatic tumor in afferent lymphatics: (A) melanoma in the intracapsular segment of an afferent lymphatic of a sentinel lymph node (H&E); (B) MART-1/Melan-A. In the presence of these appearances, the sentinel node should be reported as positive.

Prediction of outcome based on the extent of nodal replacement by tumor and its distribution within the sentinel node

Clinical outcome and the likelihood of death from melanoma correlate with the number of lymph nodes that contain melanoma metastases, but pathologists may further refine that assessment by measuring the size of nodal metastases and specifying their location within the SN. Building on earlier (pre-SN) studies of the morphometric assessment of the area and micrometer-assessed diameter of nodal melanoma metastases (Fig. 28.26), Starz and coworkers reported that the micrometer-measured depth of tumor penetration from the inner surface of the SN capsule correlated directly with the likelihood of metastases in NSNs in the same nodal drainage basin (Fig. 28.27). Wagner and coworkers correlated SN tumor volume
Fig. 28.23
Metastatic melanoma in the nodal parenchyma: (A) early extension of metastatic melanoma from the subcapsular sinus into the nodal parenchyma (HMB-45); (B) subcapsular metastatic melanoma with subjacent parenchymal melanoma metastases arranged as single tumor cells and a micrometastasis (MART-1/Melan-A); (C) single melanoma cells in the nodal parenchyma (MART-1/Melan-A); (D) macrometastasis in the nodal parenchyma (S100 protein-red chromogen-aminobenzacarbazole).

Fig. 28.24
Extracapsular extension of melanoma from a sentinel lymph node: tumor extends through the capsule and into the adjacent perinodal fat. By courtesy of R.R. Huang, MD, UCLA, California, USA.

Fig. 28.25
Nodal metastatic melanoma: the great majority of cells in this illustration are melanoma cells, but a minority have coarse granules of melanin and are regarded as melanin-containing macrophages (melanophages).
Fig. 28.26
Assessment of clinical outcome and the likelihood of death from melanoma correlate with the number of lymph nodes that contain melanoma metastases, but pathologists may further refine that assessment by measuring the size of nodal metastases. This can be recorded either as the proportional area of the lymph node occupied by tumor or using a micrometer to relate tumor maximum diameter to nodal maximum diameter.

Fig. 28.27
Approaches to the prediction of clinical outcome based on tumor burden and distribution in the sentinel node: (A) shows the Starz approach, in which a micrometer measures the depth of penetration of the metastasis from the internal aspect of the nodal capsule to the deepest invasive tumor cell; (B) the measured area of the metastatic tumor is related to the area of the lymph node (proportional area). (C) A simpler approach is to record the diameter of the largest metastatic deposit; (D) the Dewar approach, in which it is determined whether the tumor is confined to the subcapsular sinus (relatively favorable) or also involves the parenchymal tissues (less favorable).

with outcome, and Cochran and coworkers, using computer-linked morphometry, calculated the percentage area of the SN replaced by melanoma to predict the likelihood of NSN metastasis, recurrence, and death from melanoma. Dewar and coworkers reported that the anatomical location of melanoma metastases in the SN strongly predicted NSN metastases. In their study, metastases confined to the subcapsular zone were not associated with additional metastases, whereas patients with metastases in the lymph node parenchyma more often had additional nodes that contained metastases. Van Akooi and coworkers confirmed that the maximum dimension of the largest nodal tumor deposit is related to prognosis. They found that metastases <0.1 mm in diameter were infrequently associated with metastases in NSNs or unfavorable clinical outcomes during short-term follow-up. Govindarajan and coworkers also reported that tiny SN metastases (<0.2 mm in diameter in their study) were not associated with further disease, but Scher and coworkers have shown that even very small SN metastases can be associated with reduced survival. These important issues remain under extensive study and should yield clearly applicable and clinically relevant criteria by which nodal tumor burden can be assessed.

It is therefore likely that critical decisions regarding the need for CLND and deployment of adjuvant therapy will depend on the pathologist’s assessment of tumor burden and location in the SN. Currently, none of the available measures of tumor burden or disposition in the SN, individually or in combination, is sufficiently accurate that it can serve as the sole basis for treatment decisions. Such observations can effectively assist in the placement of patients in high- and low-risk categories for recurrence and death from melanoma. Pathologists may therefore wish to provide information on the Starz micrometer depth diameter of the largest metastasis and whether tumor is confined to the subcapsular zone or extends into the nodal parenchyma.

**Pathological evaluation of nonsentinel nodes**

Metastases are identified in the NSNs of 17–25% of patients found to have metastatic melanoma at SNB. Such metastases are usually limited to a single NSN and usually replace less of that node than does the tumor identified in the SN. While SNs are studied in detail, using multiple sections and immunohistochemistry, most NSNs undergo a relatively superficial examination: bisection with both halves stained by H&E. There has been some interest in whether the routine use of immunohistochemistry to evaluate NSNs would improve patient management, but data from Scolyer and coworkers and Wen and coworkers suggest that the return from such studies would be low. Immunohistochemical study of NSNs might be more appropriate in the relatively few patients where the amount of tumor in the SN is substantial, but this possibility requires further study.

**The impact of complete lymph node dissection after positive sentinel lymph node biopsy in melanoma-specific survival**

Since the last edition of this book, we completed the initial phase of the MSLT-II trial, an international, multicenter trial randomizing subjects with SLN metastases diagnosed by standard pathology or a multimarker molecular assay to immediate CLND or observation with nodal ultrasound. The primary endpoint was melanoma-specific survival (MSS), and the secondary endpoints disease-free survival and cumulative rate of nonsentinel nodal metastases. Immediate CLND was not associated with improved MSS in the 1934 evaluable subjects (CLND 76% vs. observation 78%, at 5 years, median follow-up of 43 months, p = 0.42). Disease-free survival was better in the CLND group (61% vs. 57% at 5 years, p = 0.048) due to better control of regional nodal disease (89% vs. 74% at 5 years, p < 0.001). In the CLND group, 11.5% had nonsentinel nodal metastases that were a strong independent negative prognostic factor. Cumulative nonsentinel nodal metastases affected 26% of the observed group at 5 years, versus 22.9% in CLND recipients (p = 0.005). Immediate CLND does not improve MSS in patients with SLN metastases, but does improve regional tumor control and provides significant prognostic information. Careful regional nodal observation is an acceptable alternative management approach in patients with SLN metastases. These findings are similar to the observations of Leiter et al. As these findings are entirely new, oncologists will need to review current recommendations for the management of regional lymph nodes in melanoma to determine how to use this new information in treatment planning. In the future, pathologists may be asked to review fewer CLNDs and patients will be spared the substantial side effects of extensive excision of regional lymph nodes.
**Lymphatic mapping and sentinel node biopsy for nonmelanocytic neoplasms**

The SN approach is widely used for nonmelanocytic tumors, and early attempts to develop the concept were based on studies of squamous cell carcinoma (SCC) of the penis. Since then, there have been numerous reports of the use of these approaches for the management of patients with cutaneous SCC, particularly SCC of the male and female genitalia. There are also several reports of the successful application of SNB in patients with Merkel cell carcinoma and carcinomas of the skin appendages, and it has been proposed that this approach may also find use in managing patients with sebaceous carcinoma or extramammary Paget disease. The techniques are applied to patients with nonmelanoma skin cancer in exactly the same way as for melanoma, although each class of tumor requires the use of antibodies specific to the class of tumor that is being evaluated.

**Reporting the sentinel node**

Pathologists may find it useful to develop a pro forma worksheet to facilitate the inclusion of all clinically relevant information obtainable from examination of SNs. Table 28.1 shows an example of such a worksheet that could be modified to fit local practices.

Access ExpertConsult.com for the complete list of references.

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### Table 28.1

Standardized form for reporting characteristics of the SN. This template may be modified to fit local practices and requirements

<table>
<thead>
<tr>
<th>Worksheet for evaluation of sentinel node pathology</th>
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<tbody>
<tr>
<td>Patient name: ___________________________ Date of birth: ___________________________</td>
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<tr>
<td>Hospital number: __________________________ Pathology number: ___________________________</td>
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<td>Anatomic site of sentinel node: ___________________________</td>
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<td>Specimen type: ___________________________</td>
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<tr>
<td>Surgeon-dissected node(s): ___________________________ Number of nodes: __________ Color: ___________________________ Radioactivity*</td>
</tr>
<tr>
<td>Fatty nodule: ___________________________ Number of nodes: * __________ Color: ___________________________ Radioactivity**</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Node 1</th>
<th>Node 2</th>
<th>Node 3</th>
<th>Node 4</th>
<th>Node 5</th>
<th>Node 6</th>
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<tbody>
<tr>
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<td>Node width (mm)</td>
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<td>1 mm slices (number)</td>
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<td>Number of tumor foci</td>
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<td>Extracapsular spread (yes/no)</td>
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<td>Diameter largest deposit (mm)</td>
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<td>Depth of tumor (from capsule) (mm)</td>
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<td>Tumor as % nodal area</td>
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<td>Tumor immunophenotype</td>
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<td>S100 ±</td>
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<td>MART-1 ±</td>
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<td>HMB-45 ±</td>
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<td>Other ±</td>
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<td>Other ±</td>
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<td>Nevus: Capsule ± trabeculum</td>
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</table>

*After dissection in Pathology.
**Radioactive count from requisition sheet.
REFERENCES

Introduction


Laboratory management and gross evaluation of sentinel lymph nodes


Confirmation of the sentinel status of submitted lymph nodes


Can tissue from a sentinel node ethically be made available for research?


Intraoperative evaluation of sentinel nodes


Sectioning


The role of immunohistochemistry in the detection of tumor in sentinel nodes


Separation of collections of nevus cells in sentinel nodes from metastatic melanoma

What is the role of SNB in the evaluation of melanocytic lesions of uncertain metastatic potential?


Molecular biology techniques in the assessment of sentinel nodes from melanoma patients


Prediction of outcome based on the extent of nodal replacement by tumor and its distribution within the sentinel node

7. van Akooi AC, de Wit AC, Verhoef C, et al. Clinical relevance of melanoma micrometastases (<0.1 mm) in sentinel nodes: are these nodes to be considered negative? Ann Oncol. 2006;10:1578–1585.

Pathological evaluation of nonsentinel nodes


Lymphatic mapping and sentinel node biopsy for nonmelanocytic neoplasms


The impact of complete lymph node dissection after positive sentinel lymph node biopsy in melanoma specific survival