Biopsy of the Sentinel Lymph Node

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Abstract

Lymphatic mapping and sentinel lymph node biopsy are standard components of the care of newly diagnosed patients with clinically localized intermediate- and high-risk melanoma. The procedures are the culmination of a long evolution of the management of regional lymph nodes in newly diagnosed patients. Sentinel lymph node biopsy is the most accurate staging method currently available and can be performed with limited morbidity. This staging information is of critical importance in an era of rapidly changing and improving systemic therapy. Alone or with completion lymph node dissection, sentinel lymph node biopsy enhances regional disease control and appears to improve melanoma-specific survival for some patients. The sentinel lymph node also appears to be a potentially rich subject for investigations of melanoma-host interactions.

History and Conceptual Basis of Sentinel Lymph Node Biopsy

The propensity for melanoma to spread via the lymphatic system has long been recognized. Early in the history of modern surgery, observations of lymphatic dissemination led to proposals for immediate surgical removal of all potentially draining nodal sites, even in the absence of clinical evidence of metastasis. This approach became known as elective lymph node dissection, but the morbidity of full nodal dissection and the absence of nodal metastases in most patients at the time of diagnosis of the primary melanoma made this practice controversial. It also created an impetus for devising a means of reliably separating those patients whose nodes contained metastases from those who did not.

Over many years, several observers noted that pathological or physiologic changes (e.g., inflammation, tumors, and tattoos) in specific anatomic locations could alter in lymph nodes at predictable sites. Rudolph Virchow noted drainage of tattoo pigment from specific skin sites to regional lymph nodes (Virchow 1860). Leonard Brathwaite described a "glans sentinel" at the root of the small bowel mesentery that received lymph drainage from the omentum (Braithwaite 1923). Ernest Gould described as "sentinel" a lymph node close to the junction of the facial and jugular veins that was the initial drainage site for parotid tumors (Gould et al. 1960). And after a study including 100 lymphangiograms, Raul Cabanas described as "sentinel" a node adjacent to the superficial epigastric vein at the level of the junction of the femoral

head and the ascending pubic ramus. This node preferentially receives lymph drainage from tumors of the penis (Cabanas 1977). Cabanas demonstrated that this node could provide representative staging information that reflected the status of the entire nodal basin.

A problem with these early descriptions of lymphatic drainage and definitions of "sentinel" lymph nodes (SLNs) was the assumption that the node of interest would always reside at a predictable anatomic location. It is now clear that lymphatic drainage is quite variable from individual to individual, and hence a personalized, functional definition of the "sentinel lymph node" is preferred. The resolution of this issue occurred in the 1980s through an extensive series of investigations led by Donald Morton and Alistair Cochran (Morton et al. 1992).

As mentioned previously, historical clinical observations led some early surgeons to propose that regional lymph nodes should be removed electively by complete dissection prior to the development of regional metastases detectable by palpation. This was first proposed by English surgeon Herbert Snow in 1892 (Snow 1892). His recommendation for "anticipatory gland excision" was debated over much of the twentieth century and eventually led to randomized clinical trials comparing outcomes for patients treated after elective lymph node dissection with patients who were observed, with therapeutic dissection only if they developed clinical nodal recurrences (Veronesi et al. 1977, 1982; Balch et al. 2000). For melanomas arising in certain sites, such as an extremity, the lymphatic drainage path and target nodal basin (albeit not a specific node within that basin) are usually clear. For others, such as truncal melanomas, several basins may receive primary lymphatic drainage, and determining the basin to dissect based on anatomy alone is challenging. In 1977, Morton and colleagues reported a technique to map lymphatic drainage from a primary cutaneous tumor site involving injection of radioactive colloidal gold (Holmes et al. 1977). While this technique identified the draining basin, rather than individual lymph nodes, it helped substantiate the concept that only nodal basins that received drainage of tracer were at risk for metastases and that these draining basins could be reliably identified preoperatively. This enabled a more rational approach to elective node dissection, but still required removal of all the nodes in an at-risk basin.

As radiotracers and imaging technology advanced, it became apparent that lymphatic drainage of a primary tumor site was not to *all* nodes in the basin but was initially directed to one or a small number of nodes within the basin. Subsequent studies identified and excised the node(s) that first received dye and/or radioactive isotope: the "sentinel" lymph node(s). If the SLNs were tumor-free, it was found likely that the remaining nodes in that basin would be tumor-free as well. Such SLN-negative patients could then be spared the substantial morbidity of full regional node dissection.

Studies in a feline model confirmed that mapping of lymphatic drainage to a specific node was technically feasible (Wong et al. 1991). When the technique was initially applied to patients, after SLN identification and removal all patients underwent complete regional node dissection to determine mapping accuracy. These initial experiences were reported at the Society of Surgical Oncology Symposium in 1990 and demonstrated that the status of the SLN status accurately represented the status of the nodal basin. Only 2 of 237 lymphadenectomy specimens (0.8%) had metastases in other nodes within basins in which the SLN was tumor-negative. This report was initially met with considerable skepticism and publication of the results took nearly 2 years (Morton et al. 1992). Extensive accumulated experience has now confirmed the reliability of SLN biopsy for regional disease staging in melanoma. That initial 1992 publication is currently one of the most highly cited surgical oncology papers of all time (Long et al. 2014). The availability of hand-held gamma counters led to the intraoperative use of dual-agent (dye plus isotope) mapping and obviated the need for extensive dissection of lymphatic channels, further decreasing the invasiveness and morbidity of regional node staging (Alex and Krag 1993).

Rationales for SLN Biopsy

SLN biopsy was initially developed purely as a staging tool. It was a means to distinguish node-negative patients who could be observed from node-positive patients who would all undergo radical lymphadenectomy. Staging remains a central purpose for the procedure, but as treatment paradigms for both node-positive and node-negative disease have evolved, other rationales including regional disease control and improvement in melanoma-specific survival remain important considerations even if a positive SLN biopsy does not automatically trigger lymph node dissection for all node-positive patients.

Rationale: Staging

Regional lymph nodes are the most likely initial site of melanoma metastasis. For patients with clinically localized primary melanomas, the absence of disease in regional nodes is the most powerful predictor of long-term melanoma-specific survival. It would be hard to overstate how significant the advent of SLN biopsy has been to the accuracy of melanoma staging. Prior to SLN biopsy, regional nodal staging depended on physical examination (palpation) or assessment of nodes from elective lymph node dissection. Clinical staging per se had a very low sensitivity for detection of disease, demonstrated by the poor outcomes in patients considered node-negative by clinical staging. A large retrospective study of melanomas >1 mm in thickness found that patients with clinically negative lymph nodes had a 5-year survival of 69.8% (Fig. 1) (Dessureault et al. 2001). Patients whose nodes were pathologically negative after elective lymph node dissection had significantly better survival (77.7%), but about a quarter of these "node-negative" patients died of melanoma. Enhanced staging by more intensive nodal evaluation following SLN biopsy identified a node-negative group who achieved 5-year survival of 90.5%. The greatly improved discrimination of favorable outcome groups afforded by SLN staging reflects the pathologists' ability to examine the SLN more thoroughly than is possible when dealing with the multiple nodes retrieved by dissecting the entire basin. Greater accuracy is attributable to step sectioning and extensive immunohistochemistry, performed on one or at most a few SLNs, which would be impractical if required for the multiple nodes of a full dissection specimen.

While modern imaging has steadily improved, even the best current imaging techniques fail to detect a significant number of nodal metastases that are readily detected by SLN biopsy. Nodal ultrasound is currently the most sensitive imaging modality for evaluating regional lymph nodes. Ultrasound characteristics associated with nodal metastases include a length to width ratio of <2, loss of hilar ultrasound echoes, asymmetric thickening of the nodal cortex, and increased peripheral perfusion or vascular density seen on duplex ultrasonography. Ultrasound may be used in two contexts: as a pre-SLN biopsy staging tool or as an adjunct to other modalities during patient follow-up. In the former setting, a very small number of

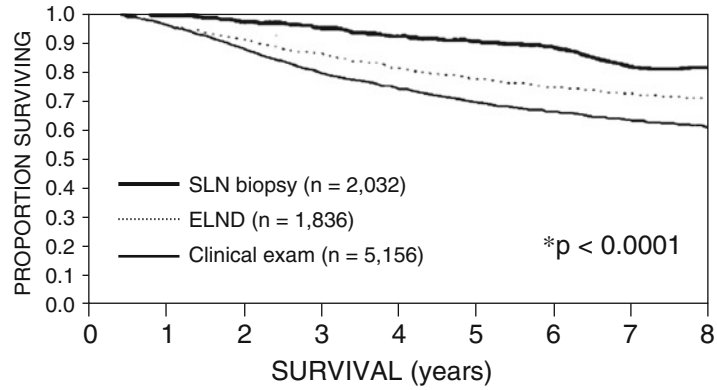

Fig. 1 Analysis of survival for patients deemed free of nodal metastases by either clinical exam, elective lymph node dissection or SLN biopsy. The more intensive pathologic staging possible with SLN biopsy results in more accurate staging and prognostic assessment (Dessureault et al. 2001)

dedicated centers have reported high sensitivity of >90% for ultrasound in detecting SLN metastases, but these results have not been able to be duplicated elsewhere, even with dedicated ultrasonographers and substantial experience (Testori et al. 2005; Thompson et al. 2011). For the melanoma centers that used ultrasound as part of the screening phase of the second Multicenter Selective Lymphadenectomy Trial (MSLT-II), ultrasound detected only 8% of sentinel node metastases. Sensitivities are better in certain groups, including patients with thicker primary tumors (Chai et al. 2012). The high operator-dependency of ultrasound may be responsible for the low sensitivity of the technique across multiple centers. The most significant challenge for ultrasound is the very small size of metastases currently identified in SLNs. In MSLT-II, the median size of SLN metastasis was <1 mm^2, while the median size of ultrasound-detected metastases was 4.8 mm^2. Finally, the central rationale for using pre-SLN ultrasound was to identify nodal disease without surgery and enable patients to proceed directly to radical lymph node dissection. However, as discussed below, radical LND is no longer an automatic consequence of detection of SLN metastases. This change in treatment recommendations diminishes the rationale for undertaking pre-SLN ultrasound.

Staging Value of SLN Biopsy: Relationship to Primary Tumor Thickness

The degree to which SLN staging discriminates melanoma-specific outcomes varies with primary tumor thickness. In patients with intermediate-thickness primaries, in whom 12–20% will harbor SLN metastases, the presence of SLN metastasis was associated with a 2.4-fold increase in melanoma-related death in MSLT-I (Morton et al. 2014). Similarly, the Sunbelt Melanoma Trial found SLN tumor status is the variable most strongly associated with disease recurrence (OR 2.76, 95% CI 1.80–4.25, $p < 0.0001$).

For patients with thick primary melanomas, the high risk of distant metastasis, even for node-negative patients, has the potential to diminish the importance of regional nodal staging and treatment. This competing risk of hematogenous dissemination led to the exclusion of such patients from many elective lymph node dissection trials. Initially smaller series of SLN biopsy applied to this population did not definitively confirm a relationship between SLN status and survival. However, more mature, larger series have demonstrated a consistent and strong relationship has been demonstrated (Table 1). Prospective clinical trial data from MSLT-I showed SLN metastases are associated with an increased

Table 1 Hazard ratio for survival related to SLN metastasis in recent series of thick melanomas

Author	HR SLN^+ for OS	95% CI	p
Robinson (2018)	3.82	1.69–8.64	<0.001
Borgognoni (2017)	3.08*	NR	NR
Bello (2016)	3.85*	2.13–6.97	<0.01
Morera-Sendra (2016)	2.2*	NR	0.002
Gyorki (2016)	2.88	1.75–4.73	<0.001
Ribero (2015)	1.61*	1.04–2.56	0.03
White (2014)	2.91	1.02–4.0	0.02
Pasquali (2013)	2.68	1.70–4.22	<0.0001
Gambichler (2013)	2.8	1.1–7.7	0.029
Fujisawa (2012)	2.14*	1.04–4.43	0.04
Rughani (2012)	4.6	2.22–9.52	<0.0001
Rondelli (2012)	1.44	1.25–1.65	NR
Covarelli (2011)	7.1*	1.8–28.7	NR
Scoggins (2010)	1.68	1.17–2.43	0.009
Goydos (2009)	2.28	1.37–3.77	0.0014
Gutzmer (2008)	2.3	1.2–4.2	0.007

NR Not reported *Melanoma-specific survival

relative risk of melanoma-related death of 1.75 (95% CI 1.07–2.87) for patients with thick (>3.5 mm) primary melanomas. A pooled analysis of multiple studies including over 2100 patients found a hazard ratio for overall survival of 2.3 (95% CI 1.95–2.71) for SLN metastasis from thick primary melanomas (>4 mm), which was the only consistently identified prognostic factor in the relevant studies (Gyorki et al. 2016).

For patients with thin melanomas, the prognostic value of SLN biopsy has been controversial given the generally favorable outcomes of these patients, including those who have SLN metastases. Initial reports of series examining selected patients with thin melanomas who underwent SLN biopsy did not identify prognostic significance (Wong et al. 2006). However, more recently studies of larger series with long-term follow-up have reported a consistent significant relationship between SLN tumor status and melanoma-specific survival (Wright et al. 2008; Ranieri et al. 2006; Mozzillo et al. 2013; Han et al. 2013; Jafari et al. 2016) (Fig. 2). The absolute magnitude of this difference is less (approximately a 10–20% absolute decrease in survival for node positive patients), but the relative importance of SLN metastasis may be even higher than in intermediate or thick melanomas. Events in patients with thin melanomas also tend to occur after a much longer interval, typically starting 2–3 years after initial treatment.

Overall, the relative and absolute impact of SLN status on patient outcome varies by primary thickness. A theoretical consideration of this relationship is described in Table 2. Although the figures in Table 2 are estimates based on literature observations, the relationships of absolute and relative differences in outcome are illustrative of the differing effects across different tumor thicknesses.

Rationale: Regional Disease Control

Regional control of metastatic melanoma is an important goal on its own, even when it does not influence overall survival. Advanced regional disease is difficult to control and may become

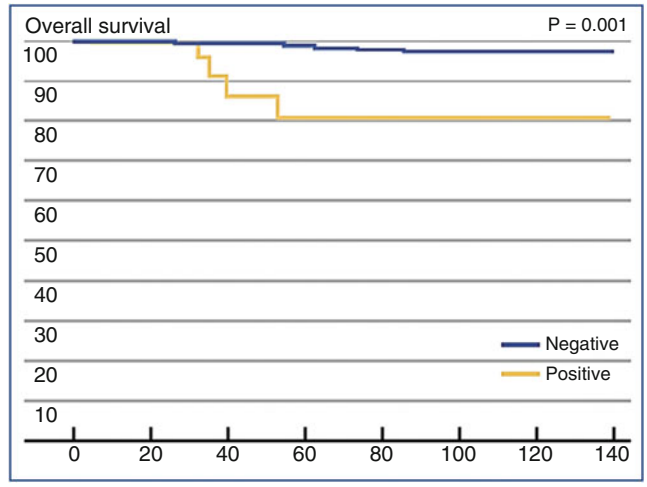

Fig. 2 Overall survival by SLN status for patients with thin (≤1 mm) melanoma. (Data from Italian Intergroup Mozzillo et al. 2013)

Table 2 Illustration of relative and absolute survival impact of SLN status based on primary tumor thickness

	Thin (<1 mm)	Intermediate (1–4 mm)	Thick (>4 mm)
5-year survival with negative SLN	~97%	~92%	~68%
5-year survival with positive SLN	~85%	~70%	~45%
Absolute survival difference	11%	22%	23%
Proportional risk for melanoma-related death	5	3.8	1.7

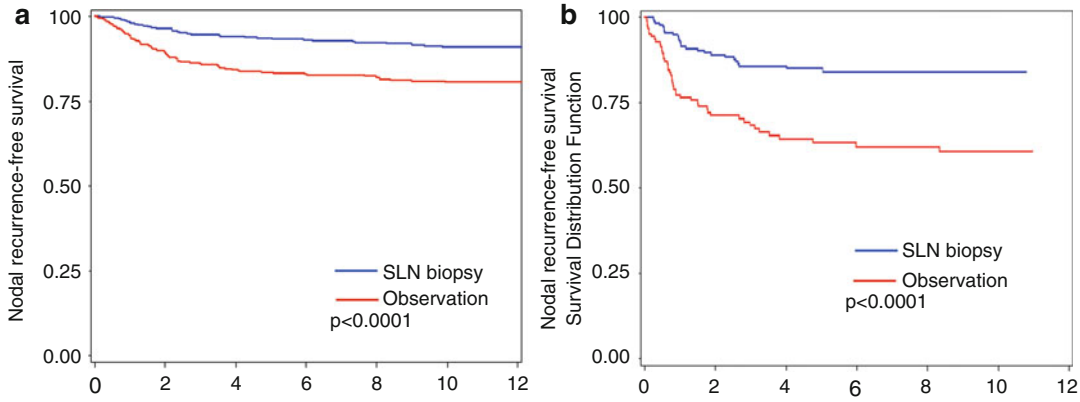

Fig. 3 Nodal recurrence-free survival from MSLT-I by primary tumor thickness. In both intermediate-thickness (1.2–3.5 mm, **a**) and thick (>3.5 mm) cohorts (**b**), management by SLN biopsy results in significantly superior freedom from in-basin nodal recurrence (Morton et al. 2014)

unresectable. Uncontrolled regional metastases can result in pain, infection, edema, and open wounds that markedly diminish quality of life. Uncontrolled regional metastases may also compromise patients' performance status and limit their ability to tolerate systemic therapy or qualify for clinical trials. SLN biopsy allows early identification and thus optimal treatment of patients in whom melanoma has spread to regional nodes. When compared to management by observation, early intervention greatly increases regional node recurrence-free survival. In MSLT-I (Fig. 3) this was highly significant for patients with intermediate-thickness melanomas and those with thick melanomas. Considering the SLN-positive patients in MSLT-II who were managed with completion lymph node dissection, 86.4% were free of nodal recurrence at 5-years. Even for those managed after SLN biopsy with nodal observation, most (73.9%) were free of regional node recurrences at 5-years (Fig. 4).

The subject of "loss of regional control" has not been well studied to this point. Most regional recurrences can be surgically managed, provided the patient has been followed closely. It is often possible to re-establish regional control even after recurrence. However, compared to dissection for clinically occult, SLN-detected disease, lymph node dissection has been shown to carry a higher risk of lymphedema when performed in the setting of clinically detectable macroscopic metastases. In MSLT-I the rate of lymphedema was 20.4% for dissection performed following clinically detected nodal recurrence vs. 12.4% after dissections performed following a positive SLN biopsy (Faries et al. 2010). The issue of maintenance of regional control is also important in considering the future of completion node dissection after a positive SLN, as discussed below.

Rationale: Survival Improvement

The impact of early removal of regional lymph nodes on survival has been debated for over a century. The controversy can be traced back at least to Herbert Snow, who noted the apparently orderly and sequential progression of melanoma from primary site to regional nodes and then to distant sites (Snow 1892). He suggested that "anticipatory" removal of the nodes at the time of the treatment of a primary melanoma would interrupt this metastatic cascade and lead to improved survival. Failure to remove the nodes until metastases became clinically apparent, in his view, would result in increased distant dissemination and death from melanoma.

The survival question was initially evaluated in a series of randomized clinical trials in which patients were received either elective lymph node dissection or nodal observation with additional surgery only in the event of regional

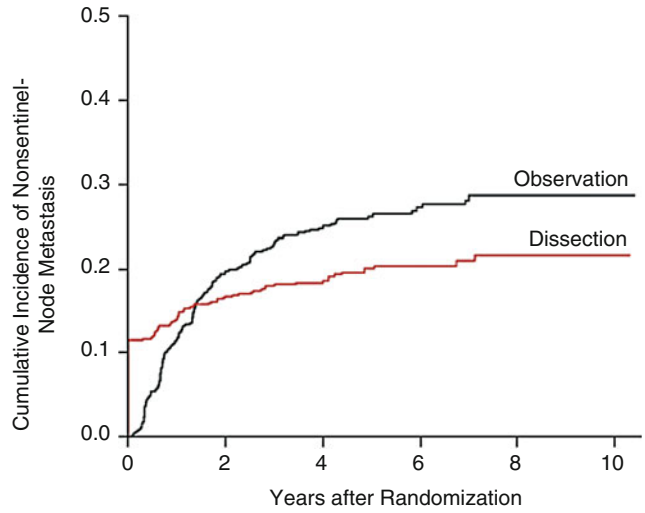

Fig. 4 Cumulative rate of non-SLN metastases in MSLT-II. For the observation group, this is based on nodal recurrence. For the dissection group, it is the sum of nodal metastases detected on completion dissection pathology and subsequent in-basin nodal recurrences. The lower number of patients in the dissection arm with non-SLN metastases is likely due to occult metastases missed on standard pathologic processing of the specimen, which in the observation arm go onto to develop into clinically detectable recurrence (Faries et al. 2017)

Table 3 Meta-analysis of survival in elective lymph node dissection trials

Author	Year	N	OR (95% CI)
Balch et al.	1996	740	0.74 (0.50–1.10)
Cascinelli et al.	1998	240	0.76 (0.46–1.27)
Veronesi et al.	1982	553	1.03 (0.72–1.48)
Total		1533	0.86 (0.68–1.09)

From Lens et al. (2002)

recurrence. These trials had generally similar results: survival was numerically superior in the elective node dissection group, but not at a statistically significant level. A design problem for these elective node dissection trials was that patients with nodal disease, who were most likely benefit from earlier surgical evaluation, could not be identified prior to surgery. As a result, the majority of patients enrolled in these studies did not have nodal metastases and were highly unlikely to derive a survival benefit from nodal surgery. Even when a meta-analysis of the data from three elective node dissection trials was conducted, any impact of elective lymphadenectomy on survival did not reach significance (HR 0.86 95% CI 0.68–1.09, $p = 0.2$) (Table 3) (Lens et al. 2002).

Some trials, however, reported significant survival benefit for subgroups. The Intergroup study found that patients with extremity melanomas, those with nonulcerated melanomas, those less than 60 years old, and those with melanomas 1–2 mm in thickness all had improved outcomes with elective node dissection (Balch et al. 2000). Even though these subgroups were prespecified and stratified in the trial randomization, the superiority of elective node dissection in those subgroups was not considered definitive and has not been independently corroborated to date.

The relationship between primary melanoma thickness and survival benefit is of particular interest. Retrospective analyses of recurrence patterns and outcomes indicated that a potential survival benefit for early nodal surgery was most likely to be confirmed by studying patients with intermediate-thickness melanomas (Balch et al. 1979) (Fig. 5). Patients with thin melanoma did well with or without nodal intervention, since the risk for nodal disease was low. Those with thick melanomas were at high risk for distant metastases even in the absence of nodal involvement, and hence regional intervention was likely to be of limited or no benefit.

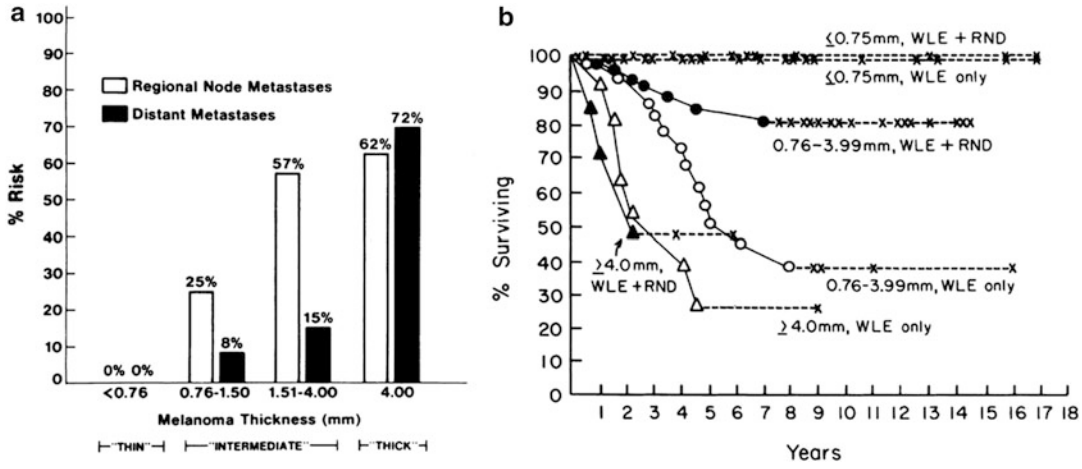

Fig. 5 Impact of nodal intervention is dependent on primary tumor thickness (Balch and Soong 1983). (**a**) Retrospective analysis of metastasis patters for patients with melanoma divided by tumor thickness. This suggests the most likely group to derive detectable benefit from early nodal intervention are those patients with intermediate thickness melanomas. (**b**) Retrospective analysis of survival after treatment with or without elective lymph node dissection for melanoma patients of varying thickness. This type of data was used to help design subsequent prospective trials

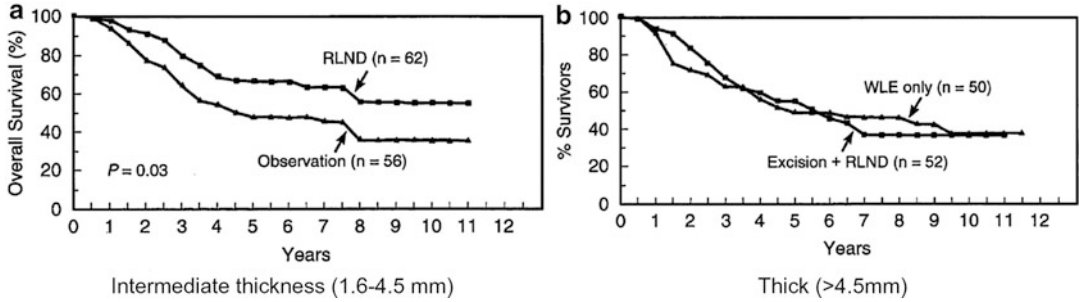

Fig. 6 Survival from WHO #1 Melanoma trial by primary tumor thickness (Balch et al. 2003). This is one example of outcomes that suggest early nodal surgery (in this case elective lymph node dissection) is more likely to be beneficial in intermediate-thickness melanomas. However, these subgroups were not prospectively stratified, making definitive conclusions impossible

The prospective data obtained in clinical trials of elective node dissection also seemed to suggest a predictive value of primary tumor thickness. The WHO Melanoma Trial No.14, which did not demonstrate a significant overall survival benefit for elective lymph node dissection ($p = 0.11$), did show a survival benefit in patients with melanomas 1.5–4.0 mm in thickness, whereas patients with thicker melanomas derived no benefit (Fig. 6).

A similar effect of thickness was apparent in the WHO Melanoma Trial No.1, where intermediate thickness was defined as 1.6–4.5 mm. In this trial, 5-year survival was 8.8% higher with elective dissection (78.5% vs. 69.7%), but with only a 1.2% absolute difference for patients with thicker melanomas (52.9% vs. 51.7%). The Intergroup Melanoma Trial found a significant improvement in survival for patients with melanomas 1–2 mm in thickness, but not for thicker melanomas. Overall, the data from elective lymph node dissection trials could be interpreted as supporting or refuting the therapeutic validity of early surgery.

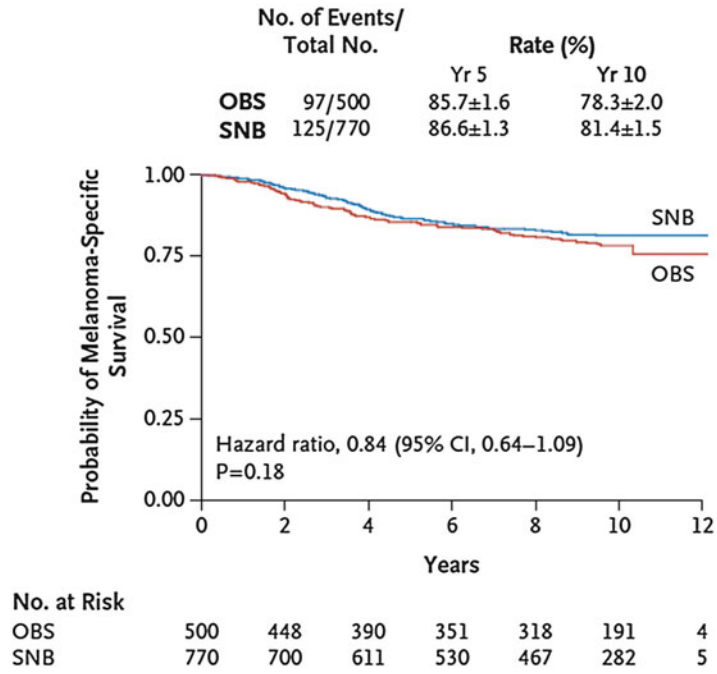

Fig. 7 Melanoma-specific survival for the intermediate-thickness cohort in MSLT-I, comparing patients with melanomas 1.2–3.5 mm in thickness randomized to undergo sentinel node biopsy (SNB) or nodal observation (OBS). No significant difference was observed between the two arms of the trial. Lower than anticipated event rates in the trial led to decreased statistical power to detect a small but potentially clinically meaningful survival difference (Morton et al. 2014)

With the advent of SLN biopsy, elective lymph node dissection was no longer the only means of pathologically staging regional lymph nodes. Indeed, SLN biopsy provided more accurate staging information, while causing less morbidity than radical lymphadenectomy. Most importantly, the impact of radical lymphadenectomy – positive and negative – could be restricted to those most likely to derive benefit from the procedure. Not long after the introduction of SLN biopsy, an international, prospective randomized trial was initiated to evaluate its value in melanoma. The first Multicenter Selective Lymphadenectomy Trial (MSLT-I) randomized patients with melanomas at least 1.2 mm thick to either SLN biopsy (with completion lymph node dissection for patients with nodal metastases) or observation of the regional nodes, with delayed dissection only for those with clinically identified regional nodal recurrence. The primary endpoint compared melanoma-specific survival in patients with melanomas 1.2–3.5 mm in thickness. A similar comparison of survival in patients with thicker melanomas was a secondary endpoint, as was disease-free survival.

At 10 years of follow-up, there was no significant difference in survival between the two randomized treatment groups (Fig. 7). However, there was a lower rate of nodal involvement and a lower event rate in the trial than had been anticipated in the study's statistical plan, leaving the trial underpowered. When the analysis was restricted to patients who had nodal disease, detected either on SLN pathology or by clinical disease recurrence, there was a substantial survival advantage for patients whose nodal disease was detected as a result of SLN biopsy. The survival benefit was only seen for patients with intermediate thickness (1.2–3.5 mm) melanomas (HR, 0.56 (95% CI, 0.37–0.84); $p = 0.006$). No survival difference was seen for patients with melanomas >3.5 mm thick (HR 0.92 (95% CI, 0.53–1.60); $p = 0.78$) (Fig. 8).

Limiting the analysis to node-positive patients is potentially problematic from a statistical point of view, since the comparison is not of two prospectively randomized groups. However, the characteristics of the patients in each group were similar, as was the proportion of patients with nodal disease in each arm of the trial. Despite this, concerns about ascertainment bias in the comparison remain. This type of bias could occur since members of the comparison groups are identified through different means (SLN

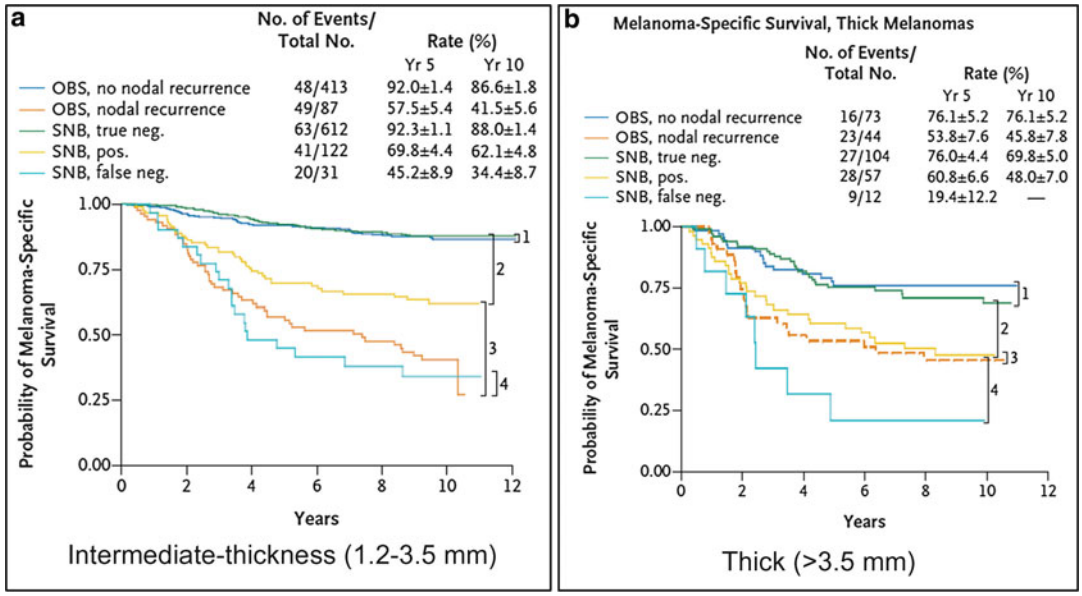

Fig. 8 Melanoma-specific survival in MSLT-I. In patients with intermediate-thickness melanoma, there was a significant survival advantage for patients with positive sentinel nodes (SNB, pos.) compared to observation arm patients who developed clinically detectable nodal recurrence (OBS, nodal recurrence). The same was not true for patients with thick primary melanomas. Observation arm patients with no nodal recurrence (OBS, no nodal recurrence) fared similarly to sentinel node negative patients (SNB, true neg.), while sentinel node-negative patients who developed clinically detectable nodal recurrence (SNB, false neg.) had similar outcomes to observation arm patients who developed clinically detectable nodal recurrence (OBS, nodal recurrence) (Morton et al. 2014)

biopsy detection of metastases vs. clinical recurrence.) To address these concerns, statistical methodology was developed for analysis of MSLT-I data. This methodology, called latent subgroup analysis, utilizes numerous simulations created with trial data to account for unmeasured or unknown potential confounders (Altstein et al. 2011). Latent subgroup analysis confirmed a treatment-related benefit, with a doubling of survival duration in patients with nodal metastases treated by SLN biopsy-guided surgery. The salutary effect was again only seen in patients with intermediate-thickness melanomas. The methodology used was specifically developed to address the issues raised in MSLT-I and remains to be validated in other clinical studies. Until that occurs, these findings remain strongly supportive of a therapeutic effect and a survival benefit of SLN biopsy-guided surgery but are not ultimately definitive.

Across the spectrum of tumor thickness, patients with intermediate thickness melanomas appear likely to derive a survival benefit from early removal of nodal metastases, whereas patients with thick melanoma generally do not. Perhaps the most controversial group, though, are patients with thin (T1) melanomas. The proportion of node-positive patients in this specific population is small (<10%), so a randomized trial would be impractical. Retrospective comparisons of patients with thin melanomas and SLN metastases to those who develop clinical nodal recurrence after nodal observation show a large survival advantage for the SLN group (Karakousis et al. 2017). Even though the retrospective comparisons included multivariable and matched-pair analyses, without randomization, the validity of such comparisons is impossible to assure. SLN biopsy for all patients with thin melanoma is both impractical and cost-ineffective. This makes appropriate selection of patients for SLN biopsy critically important, though as discussed below, areas of uncertainty remain in selection as well.

Selection for SLN Biopsy

Regardless of any controversy regarding a survival benefit from early nodal surgery, the prognostic value and regional disease control benefits of SLN biopsy make it invaluable in the management of many patients. However, not every melanoma requires nodal evaluation and appropriate selection criteria should be applied in ascertaining who is considered an appropriate candidate for SLN biopsy.

For patients with intermediate-thickness (1–4 mm) melanomas, SLN biopsy should be recommended in the absence of specific contraindications. This recommendation is in line with guidelines from most national and professional organizations and is based on the staging, regional control and possible survival benefits of the intervention (Wong et al. 2017; Coit et al. 2016; Chakera et al. 2009; Bichakjian et al. 2011). For patients with thick melanomas (>4 mm), there is little evidence that the sentinel node approach improves survival, but the staging information (see above) and regional disease control benefits of the procedure suggest that it should be offered to these patients as well.

The place of SLN biopsy in managing patients with thin primary melanomas remains controversial. Since most melanoma patients present with thin primaries, the absolute number of such patients is large. In the United States, it is estimated that over 60,000 patients present with thin primary melanomas annually (Sondak et al. 2017).

Determining optimal criteria for selection within the thin melanoma population has proved challenging. Numerous retrospective series have sought features that define patients with thin melanomas who are most likely to benefit harbor occult nodal metastases at the time of primary tumor diagnosis. Some of these examined the results of SLN biopsy in patients with thin melanomas. However, these results may be biased by surgeon selection of perceived "high-risk" patients and are subject to the risk of false-negative biopsy. This latter risk may be greater given the low volume of disease that may be present in these patients. Alternatively, patients with thin melanomas who do not undergo surgical nodal staging can be analyzed for regional nodal recurrences, provided adequate length of follow-up is available (because when thin melanomas do recur, they tend to do so late (Lo et al. 2018).

A meta-analysis of series of SLN procedures undertaken for thin melanomas found an overall rate of nodal positivity of 4.5% (95% CI 3.8–5.2%) Primary characteristics associated with nodal metastases include Breslow thickness (within the $\leq$1 mm range), ulceration, mitoses, regression, Clark level, age, gender, tumor infiltrating lymphocytes, and lympho-vascular invasion. However, there is poor agreement among different studies of the various prognostic factors, with some failing to identify any predictive markers. The most consistently significant variable in thin melanomas is tumor thickness within the $\leq$1 mm range. Thin primary tumors measured as 0.8 mm or greater are now considered T1b, carry a higher risk of nodal involvement than thinner lesions and are recommended for consideration of SLN biopsy (Wong et al. 2017; Lo et al. 2018). For melanomas thinner than 0.8 mm, SLN should be considered if the primary tumor is ulcerated or shows a very high mitotic rate, though those findings are uncommon in thin melanomas. The definition of a "very high mitotic rate" for T1 melanomas is, however, not conclusively defined. A single mitosis should not be enough to alter treatment planning for a patient. Melanoma cells at the deep margin of the initial biopsy may lead to uncertainty in microstaging of the primary. The implications of a positive deep biopsy margin are also not definitively established. Retrospective series of patients with tumor at the deep biopsy margin have yielded mixed results, ranging from no difference in frequency of SLN metastasis (Lowe et al. 2011; Zager et al. 2011a), to a statistically nonsignificant increase in SLN metastasis in the positive deep margin group (OR 1.69, $p = 0.07$), (Herbert et al. 2018) to nodal metastasis rates in the margin-positive group similar to intermediate thickness melanomas (Koshenkov et al. 2012). Transection of the primary tumor base or extensive involvement of the deep margin is likely to be due to a substantially thicker primary and should cause more concern than isolated cells at

the deep margin. Re-biopsy of the area may not be able to adequately determine true depth, due to cauterization artifact and/or inflammation at the biopsy site. This issue emphasizes the need for adequate biopsy technique to ensure optimal treatment of the patient (see chapter ▶ "Biopsy of Suspected Melanoma").

Technical Details of Mapping

The concepts of lymphatic mapping and SLN biopsy are deceptively simple. However, in practice, application of the techniques can be challenging and requires skilled input from multiple disciplines. There are three components to the procedure: lymphoscintigraphy, surgical excision, and finally detailed pathologic analysis. Lymphoscintigraphy has been extensively discussed previously (see chapter ▶ "Lymphoscintigraphy in Patients with Melanoma"), and we here discuss the technique from the start of surgery. This is often scheduled on the same day as lymphoscintigraphy, though may be performed on the day after the scan. Images of the lymphoscintigram are reviewed and should be available during the surgery.

The patient is positioned for surgery in a manner that best provides access to the nodal basin. Often, the nodal site is approached first. However, it may at times be advantageous to perform the wide excision first (see below). Prior to prepping the surgical site, a vital blue dye (Patent Blue V, isosulfan blue, or methylene blue) is injected at the primary tumor site (Fig. 9). Comparisons of these different dyes are limited. There are rare allergic reactions to Patent Blue V and isosulfan blue, including anaphylaxis. The rate of such reactions in melanoma patients appears markedly lower than the rate associated with mapping in breast cancer patients. Reasons for this disparity are not clear, though it may relate to the lower volume of dye used for melanoma. In MSLT-I and the Sunbelt trial, rates of allergic reactions were 0.17% (2/1173) and 0.0003% (1/3600), respectively. No anaphylaxis was reported in either trial (Morton et al. 2005; McMasters et al. 2004). Methylene blue does not carry the same risk of allergic reaction but has been associated with more wound complications than isosulfan blue (Neves et al. 2011).

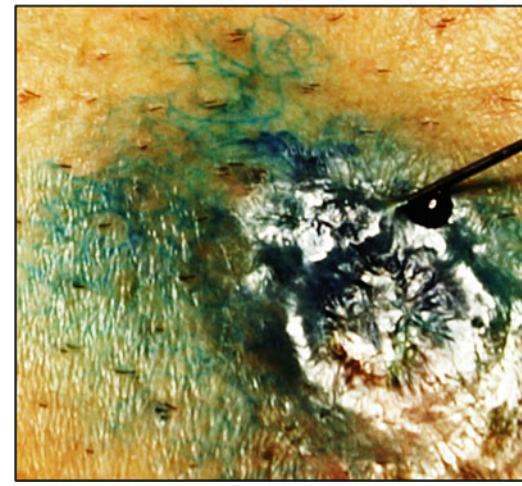

Fig. 9 Care should be taken to inject blue dye and radiotracer in the dermis, not the subcutaneous space. The dermis contains a high density of lymphatic channels which often become visible at the time of a proper injection. The wispy blue lines at the upper portion of the photograph are the visualized dermal lymphatic channels

The injection of radiotracer and blue dye should be intradermal, as close as possible to the melanoma or the excision-biopsy site, and the agents should be injected around the primary site. Often lymphatic channels are visualized at the time of dye injection, offering reassurance that the injection was properly positioned. Massage of the area is not typically necessary if the injection is well positioned. Deeper injections into subcutaneous tissues do not provide the same access to lymphatic channels and are less likely to be effective.

The SLN incision site should be planned with location of the primary site and potential need for eventual lymph node dissection in mind. This includes positioning the incision in the orientation and location of a subsequent dissection incision. Much of the approach to the nodal sites can be performed by blunt dissection, gently separating tissues and avoiding disruptive transection of structures. The fascial or muscular covering of the nodal basin (e.g., platysma in the cervical basin or the preaxillary fascia in the axilla) is opened and spread to access the nodes.

Minimizing division of structures in the nodal area, including other lymphatics, nerves, and blood vessels, may help to limit morbidity and seroma formation. When the SLN is identified by its color and relatively enhanced radioactivity, it is dissected from the surrounding structures. Lymphatic channels entering the node and nodal vessels should be divided between ties or clips. Care should be taken to avoid damaging the nodal capsule or architecture. Since many nodal metastases are limited to the subcapsular sinus, surface damage can obscure critical pathologic findings. Care should be taken not to grasp or pull the capsule or outer surface of the SLN. Rather the node may be gently pushed or held across broad areas of connective tissue along the nodal edge.

After removal of a SLN, it should be closely assessed while it is in the operative field. The location of selective blue staining or of maximal radioactivity may be marked on the node. The location of metastases within a SLN has been shown using carbon particles as tracers to correlate with the location of maximal radioactive tracer deposition (which may not be uniform throughout the node) (Morton et al. 2003). Marking of the node can be done with sterile ink, a metallic clip or a fine suture. Care should be taken if a suture is used not to crush the nodal tissue at the site of the marker. The pathology request form should, in every case, state clearly that the specimen is a SLN, ensuring that it will receive the special handling that such specimens require. If the surgeon uses stitches, clips, or surgical ink to highlight an area of the lymph node, the requisition should clearly explain the significance of these markings. This will ensure that the pathologist will pay appropriate attention to the marked areas. As discussed below, the SLN should be fixed in neutral buffered formalin and sent immediately for permanent pathology.

Frozen section evaluation of SLNs should be avoided, as it provides less accurate assessment of the node and may sacrifice diagnostic material in the course of processing. After removal of each SLN, the basin is assessed again for residual tracer activity. Lymphoscintigraphy images are used to help determine the expected number of SLNs, a number that varies by patient and by basin. It is common to find more draining SLNs in the cervical basin. The "10% rule" has been promoted as a practical guideline used to determine when all true SLNs have been identified and removed. This rule states that all blue nodes and any nodes demonstrating at least 10% of the counts of the "hottest" node should be excised to minimize the possibility of a false-negative dissection. It is supported by data from the Sunbelt Melanoma Trial that show a 0.4% false-negative rate when this rule is used to define a SLN (McMasters et al. 2001). However, there are practical difficulties in applying the rule (e.g., the actual counts of a given node can only be determined accurately after it has been removed (see also chapter ▶ "Lymphoscintigraphy in Patients with Melanoma"). Concern that application of the rule may lead to removal of an excessive number of SLNs has led some surgeons not to adopt the rule. There is broad agreement, however, that clinical judgment is critical in determining the extent of any SLN procedure.

Special Situations: Difficult Sites

Some clinical situations make SLN biopsy more difficult. The head and neck region is considered a more challenging area due to the relatively large number of potential SLNs, the complex lymphatic drainage patterns of the area, the small size of many cervical nodes and the frequent close proximity of the site of the primary tumor to the SLNs, as well as the many critical neurovascular structures in the neck and periparotid area. Smaller doses of tracer and blue dye may be used to avoid excess radioactivity or staining in the soft tissues surrounding the nodes. SPECT/CT imaging has been evaluated to facilitate identification of SLNs in the head and neck. Use of SPECT/CT has been associated with increased identification of positive SLNs and a lower relapse rate, suggesting increased accuracy of staging (Chapman et al. 2016; Stoffels et al. 2012; Doepker et al. 2017). Finally, excision of the primary site may remove much of the radioactivity associated with injection. The nodal basin can then be

reassessed with substantially reduced background radioactivity and any SLNs previously obscured by radioactivity may become apparent.

The same challenges encountered in the head and neck may also exist in other regions where the primary tumor is located close to a nodal basin. Pelvic nodal drainage, from truncal or lower extremity primary sites, may also be complex and difficult to visualize based on routine planar lymphoscintigraphy. Similar techniques to those employed in the head and neck, including the use of SPECT/CT and initial excision of the primary site, may be useful in these situations.

In occasional cases, injected tracer may not pass to any identifiable SLN (see also chapter ► "Lymphoscintigraphy in Patients with Melanoma"). This problem is more common in the head and neck and in older patients. If no SLNs are seen after the initial injection of tracer, gentle massage of the injection site may facilitate drainage. If that is unsuccessful, a repeat injection may be helpful. If there is still no SLN identifiable on imaging, the patient should be assessed with the gamma probe intraoperatively, both before and after wide excision of the primary site. It has been suggested that failure to demonstrate nodal drainage may occasionally be due to obstruction due to the presence of tumor in the draining afferent lymphatic or the SLN itself and that complete node dissection should be performed in those cases. However, recent evaluations of this question have indicated that overt nodal metastases in these situations are infrequent (Schuitevoerder et al. 2017). Nodal basin observation with serial ultrasonography initiated soon after the patient has recovered from the unsuccessful SLN biopsy is therefore a reasonable course when there is no migration of tracer.

Special Situations: Patients Presenting After Wide Excision

At times, patients who would benefit from SLN biopsy present after the wide excision of their primary melanoma has been completed. This may be related to treatment at a center where SLN biopsy is not offered or it may be due to discovery of a higher risk melanoma on final pathology, when the initial biopsy had only found a low-risk T1a or in situ lesion. In these circumstances, the final assessment of the primary tumor suggests a significant chance of nodal metastasis and the need for regional nodal staging after wide excision has been conducted. This raises the question of whether lymphatic mapping is reliable under those conditions, or if there is a substantial risk of inaccurate mapping.

Data regarding this question are mixed. There is support for the feasibility of mapping after prior wide excision. Evans et al. reported successful identification of a SLN in 98.6% of 76 patients in which mapping was attempted after an earlier wide excision (Evans et al. 2003). Similarly, Gannon et al. reported successful identification of a SLN in 99% (103/104) of patients (Gannon et al. 2006). However, the feasibility of mapping and identification of a SLN is not the most critical issue. The key question is whether such identified SLNs are accurate representations of the drainage from an undisturbed primary tumor site.

One study examined results of lymphoscintigraphy performed both before and after wide excision for primary melanomas (without SLN biopsy in these cases). This study found good agreement of the results of lymphoscintigraphy before and after the primary excision. Drainage was reported as unaltered in 13/19 (68%) patients, which showed additional nodes in 21–26% and fewer nodes in 5–10% (Ariyan et al. 2007). The false-negative rate in the Evans study was 21.4%, and a similar study by Keleman et al. reported a 27% false-negative rate (3/11) (Kelemen et al. 1999). These rates are at the high end of the range for SLN biopsy under normal circumstances. However, the study by Gannon et al. reported no false-negative SLN biopsies among their patients with a median follow-up of 51 months. Overall the data suggest SLN biopsy should be performed at the same time as the primary tumor wide excision whenever possible, but attempted mapping is reasonable in carefully selected circumstances where that is not possible.

Special Situations: Nonclassical Nodal Sites

The classical nodal basins of the neck, axilla, and groin are the most common location for SLNs identified at mapping. However, SLNs may be located in a number of additional locations, and some of these are recognized sites where nodes are not rare. Verwer et al. reported a 9.0% incidence of such nodes, although most other series have reported rates of 2–7% (Verwer et al. 2011) (see also chapter ▶ "Lymphoscintigraphy in Patients with Melanoma"). Terminology identifying these nodal locations outside of the traditional major basins has been somewhat inconsistent. The terms "interval" and "in-transit" have been applied. The nodal sites include the popliteal and epitrochlear basins, which are often considered "minor" basins in comparison to the "major" cervical, axillary, and inguinal basins. Zager and colleagues promulgated a definition of interval nodes as nodes directly draining a primary tumor that are outside of a recognized major or minor basin (Zager et al. 2011b). These include nodes of the scalp, costal margin, intermuscular triangle of the back, breast, biceps groove or along the saphenous vein outside of the groin. Their proposed definition of in-transit nodes includes any nodes between the primary tumor site and a classical major drainage basin. This would include both interval nodes and minor basin nodes that fit the definition. One final group of nonclassical nodal locations are "terminal" locations that are not on a route to a major basin. These might include retroperitoneal or intrathoracic nodes draining sites on the back, for example.

Lymph nodes in these nonclassical locations can harbor nodal metastases and should be removed when possible if they are identified on lymphoscintigraphy or by intraoperative localization techniques. Both the risk for nodal metastasis and the prognostic significance of the nodal status appears to be similar for these in-transit nodes as that of lymph nodes in classical basins (Verwer et al. 2011; Caraco et al. 2014). In the past, the treatment of adjacent classical basins in the setting of nodal disease in an in-transit site has been somewhat uncertain, particularly when a negative SLN is identified in the classical basin concomitant with a positive SLN in an in-transit node. Series reporting results of CLND in this scenario have reported very low rates of additional positive nodes. Other series report a relatively high rate of nodal recurrence in the classical basin if that basin is observed, though the number of patients reported with either management strategy is very limited (Steen et al. 2011; Kidner et al. 2012). In light of reports from the MSLT-II and DeCOG-SLT studies, discussed below, dissection of the classical basin in the absence of clinically evident disease is likely unnecessary, though close follow-up of that basin is clearly indicated.

Pathology of the SLN

This section provides an overview of SLN pathologic evaluation. More detailed coverage of the topic is available elsewhere (Cochran et al. 2008) (see also chapter ▶ "Classification and Histopathology of Melanoma"). One advantage of SLN biopsy is that it permits the pathologist to focus microscopic evaluation on a single lymph node or a small number of nodes. The development of immunomarkers (Cochran et al. 1989a; Ohsie et al. 2008) facilitates the detection of small numbers of melanoma cells and even single tumor cells, which would be extremely difficult to identify in hematoxylin and eosin (H&E) stained sections. Evaluation of multiple levels of each SLN by H & E and immunohistochemical staining are important to correctly determine whether a lymph node contains metastases. Routine frozen section evaluation of SLNs is not recommended. Interpretation of frozen sections is generally less accurate than interpretation of sections stained by H&E after formalin fixation and paraffin embedding (Cochran et al. 2008). Rapid immunohistochemistry on frozen tissue is less accurate than immunohistochemistry on fixed tissues. Processing of tissue for preparation of frozen sections requires removal of substantial (potentially diagnostic) tissue to obtain a completely representative tissue section. This process may at times remove all tumor tissue, especially if, as is often the case, that is limited in amount. Most

importantly, the imperative that drove the desire for frozen sections, the need to progress immediately to completion node dissection, is no longer appropriate, since a full discussion of the completed SLN pathology with the patient is essential as a basis for deciding whether to proceed to additional nodal surgery (Faries et al. 2017).

Procedures for processing and staining SLNs vary across specialized centers. All protocols use serial nodal sectioning and immunohistochemical stains. For example, at UCLA after formalin fixation, the SLN is bisected along its long axis, and the two halves are placed face down in a cassette for paraffin embedding. Multiple tissue slices removed from the cut faces of the bisected lymph node(s) are stained in sequence with hematoxylin and eosin, and antibody stains specific for S100-protein, HMB-45, Mart-1, and Sox-10. At UCLA, 10 sections from each half lymph node are routinely prepared. With large lymph nodes, additional tissue is obtained by cutting further 2 mm slices parallel to the first bisecting cut. The multiple tissue slices removed from the cut faces of the bisected lymph node(s) are stained in sequence with hematoxylin and eosin, S100-protein, HMB-45, Mart-1, and Sox-10 (Fig. 10). Other protocols involve a greater number of sections. The optimal extent of sectioning is not currently known.

It has been claimed that extensive sectioning decreases the risk of a pathologic false-negative result (Abrahamsen et al. 2004; Spanknebel et al. 2005). More extensive pathologic approaches are resource intensive and regarded by many as prohibitively expensive, given the generally limited yield of additional sectioning. The cost and effectiveness of different sampling protocols should be formally compared to allow a more logical approach to this key component of SLN assessment. It is possible that emerging molecular and genetic approaches (Gerami et al. 2015; Egger et al. 2018) may be useful as supplements to histology and will change our approach to nodal sampling. This is a promising area of investigation and several institutions are actively seeking gene signatures that predict the likelihood of distant metastasis and death from melanoma.

The extent and location of metastatic disease within SLN are predictive of likely clinical outcome and thus can serve as a guide to appropriate management. These observations relate to risk of metastases in non-SLN, risk of distant metastases, and death from melanoma. The total number of lymph nodes that contain tumor determines the N stage in the AJCC staging system. Both number and extent of involvement of SLNs are associated with clinical outcome. There are reports of evaluation of different techniques to measure extent of SLN metastases as predictors of the likelihood of metastasis to other lymph nodes in the same basin (non-SLN metastasis), subsequent distant metastasis, and melanoma-specific death (Fig. 11).

The presence of any amount of melanoma in a lymph node is sufficient for classification as Stage III. However, the risk of further nodal and distant metastases increases with increasing volume of nodal tumor (Cochran et al. 1989a). From analysis of the AJCC melanoma databases for both the 7th and 8th editions, no minimum threshold has been identified below which additional metastases would be unlikely. Tumor burden, estimated from the maximum diameter of the largest focus of nodal melanoma has been commonly evaluated (Cochran et al. 1989a) and is technically feasible for routine pathology application. Cut points proposed include 0.1 mm, 0.2 mm, 1 mm, and 2 mm.

Other measures of nodal disease burden include measurement of the percentage area of the SLN that tumor occupies (Cochran et al. 1989b). For this measure, cutoff values of 1% and 4% have been proposed, and these correlate with likelihood of non-SLN metastasis and melanoma-related death. The depth of invasion of a melanoma metastasis into a SLN (micrometer-measured thickness of tumor from capsule to deepest contiguous tumor cell) has also been evaluated (Starz et al. 2001). This "Starz thickness" is practical and prognostic, as is the "Dewar classification" based on location of metastases: confined to the subcapsular area, confined to the parenchyma or multifocal and extensive (Dewar et al. 2004). There have been few comparisons of the relative efficacy of these different techniques or of the predictive accuracy obtained by variously combining them. The most practical and

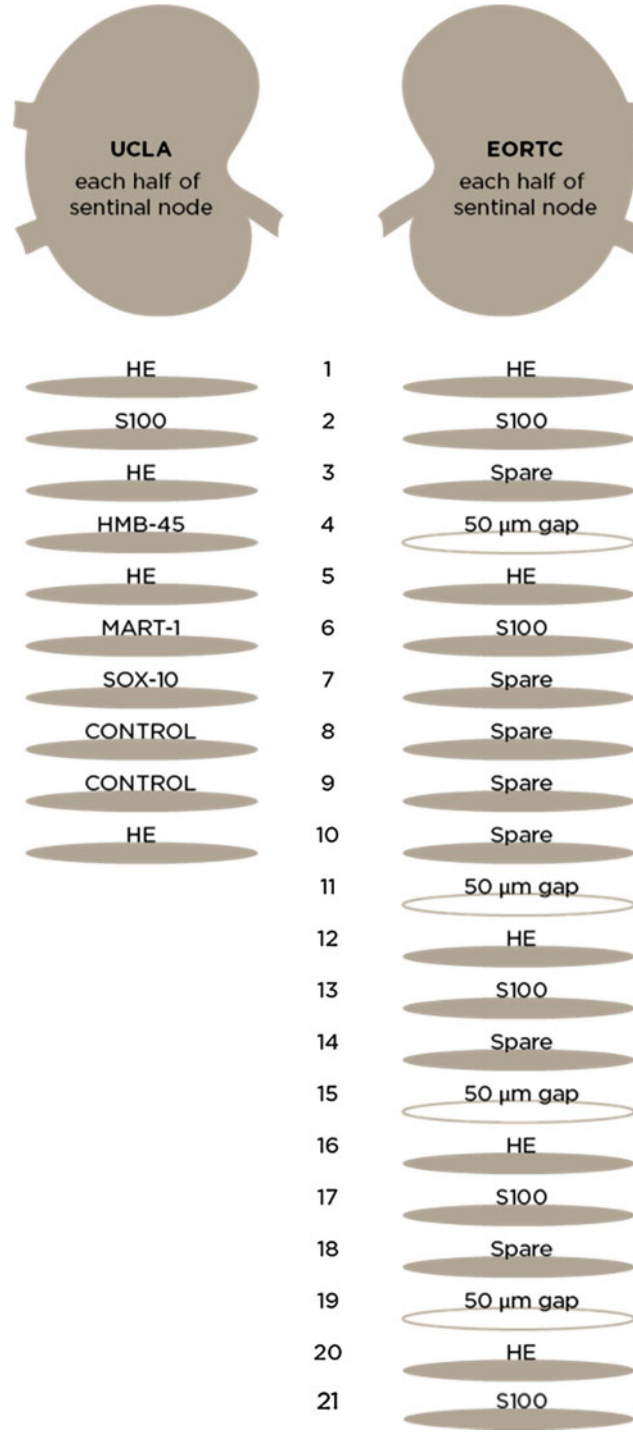

Fig. 10 Comparison of UCLA sentinel node sampling technique with intensive protocol proposed by EORTC. Section numbers are listed in the center. The EORTC protocol at right provides a more exhaustive evaluation of the node but is also more resource and labor intensive. The optimal approach has not been definitively determined

widely used approach is to measure the longest diameter of the largest metastatic focus with a micrometer, typically using a 1 mm cutoff to separate high and low volume disease.

Theoretically, the smallest volume of nodal tumor would be detectable only by molecular analysis, using techniques such as reverse transcriptase polymerase chain reaction (RT-PCR).

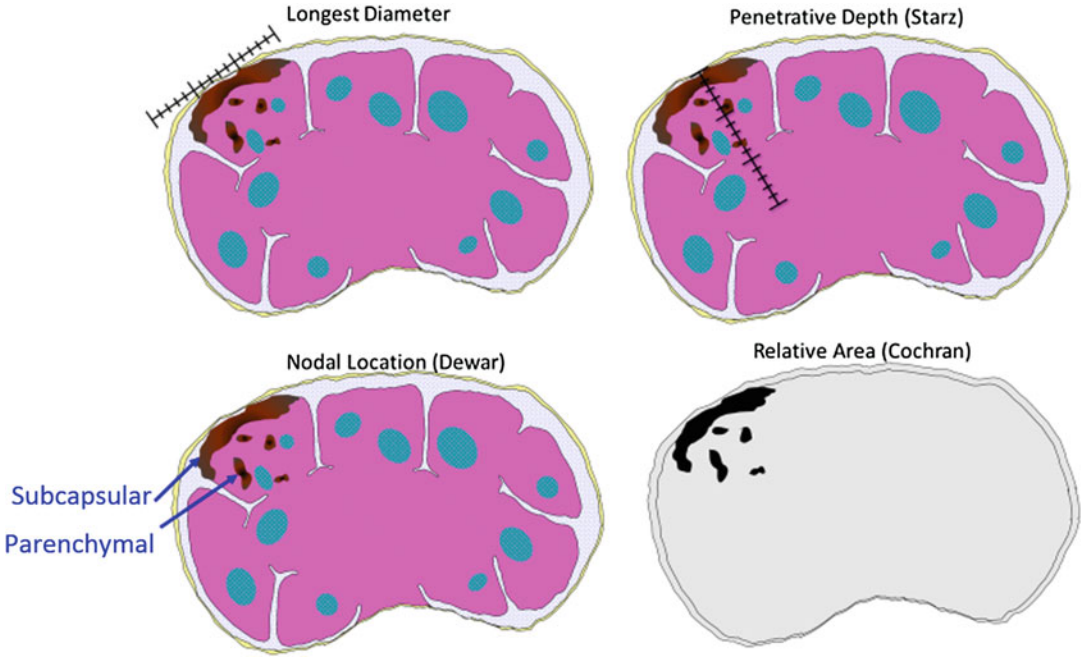

Fig. 11 Multiple quantification methods for SLN tumor burden: Micrometer-measured diameter of the largest metastasis (Top left). Micrometer-measured invasion of tumor invasion from nodal capsule to deepest tumor cell (Starz et al. 2001). (Top right) Measured area of metastasis in node (surrogate for volume) (Cochran et al. 1989a) (Bottom right). Location of metastases in node: Subcapsular only, subcapsular, and parenchymal or parenchymal only (Dewar et al. 2004) (Bottom Left)

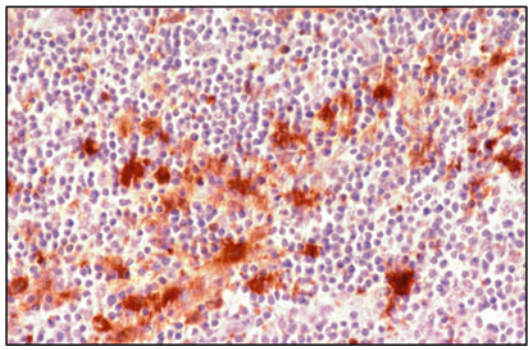

Fig. 12 Photomicrograph of the paracortex of a non-SLN. This node, which has not been affected by lymphatic drainage from a primary melanoma site, demonstrates a rich network of well-formed dendritic processes projecting from dendritic cells, stained brown for S-100 protein. Such networks are often attenuated or lost in SLN, and the dendritic cells are more readily confused with melanoma cells

This approach has been evaluated in multiple retrospective series. A meta-analysis suggested that prognostic information was obtainable from this type of study. However, two prospective evaluations of RT-PCR in the Sunbelt (McMasters et al. 2004) and MSLT-II clinical trials (Faries et al. 2017) have not confirmed that accurate prognostic information is currently obtainable from RT-PCR.

It is possible to misinterpret common benign microscopic features in SLNs as metastatic melanoma. S-100 positive dendritic cells are present in variable numbers in the paracortical tissues (Fig. 12) and may present interpretative difficulty, especially if they are dendrite-poor in immune-suppressed lymph nodes. Phagocytic cells in lymph nodes may ingest debris from melanoma cells. This is most readily seen in melanophages that have ingested melanin-decorated melanosomes from melanoma cells. These cells also phagocytose melanoma-derived epitopes, such as Melan A and HMB-45. Such cells are usually separately identifiable by their cytology and lack of staining with Sox10. Schwann cells of nerves also stain for S100. Benign nevocytes are encountered in normal lymph nodes, where they can form nodal nevi in the nodal capsule, trabeculae, and

(rarely) parenchyma that must be differentiated from metastases.

It is considered likely that these cells arrive in the nodes by migration through lymphatics from cutaneous nevi (Carson et al. 1996). Nodal nevocytes stain positively with S100, MART-1, and Sox-10, but weakly or negatively with HMB45. Separating the cells of nodal nevi from melanoma cells is usually straightforward, but may at times be extremely difficult, especially in the case of nevocytically differentiated melanomas. Features suggesting benign nodal nevi include benign cytology, capsular or trabecular location, proximity to capsular lymphatics, and lack of HMB45 staining (Fig. 13). Sox2 and nestin staining favors melanoma, as these markers are negative in benign nevi (Chen et al. 2013). Molecular or genomic testing has been proposed and may eventually contribute to assessment of these cases.

False-Negative SLNs

Although lymphatic mapping and SLN biopsy are very accurate techniques, false-negative results do occur. The SLN biopsy procedure, though simple in concept, requires expertise from radiologists, nuclear medicine physicians, surgeons, and pathologists. The rate of false-negative SLN biopsies has been estimated in different reports using various statistical methods. The standard calculation should use the number of false-negative cases divided by the total number of positive cases (true positive plus false negative). By this method,

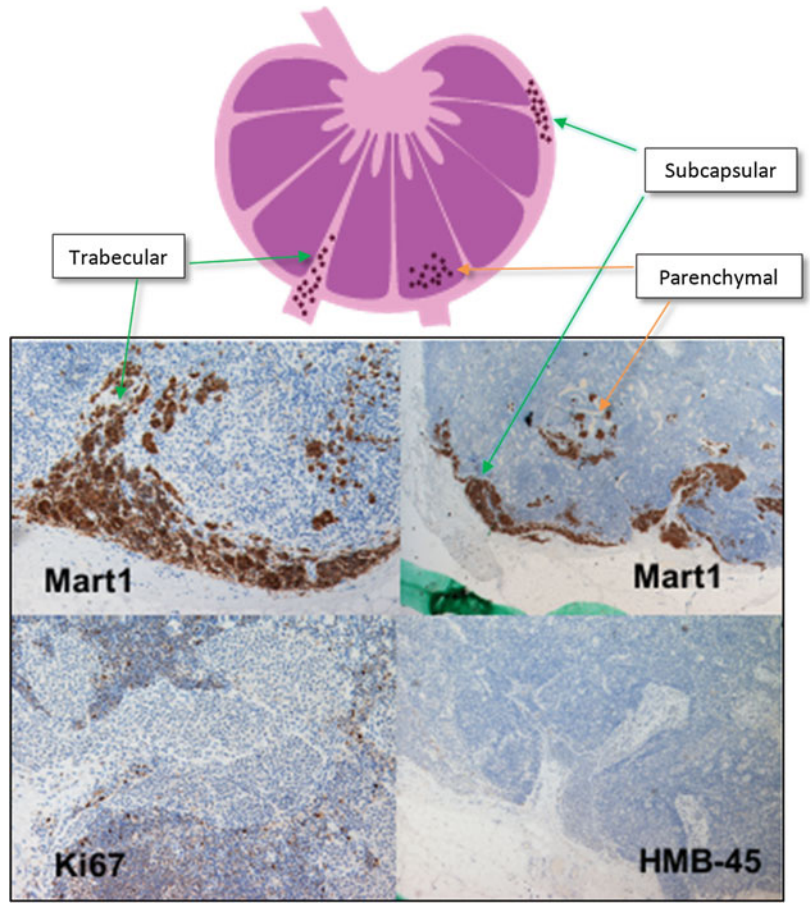

Fig. 13 Nodal nevocytes are located in the lymph node capsule, capsule and trabeculae, and less frequently in the subcapsular parenchyma. Nevocytes appear to reach the lymph node via the afferent lymphatics. (Graphic courtesy of Eric Montgomery) Capsular, trabecular, and parenchymal nevus cells express S-100, SOX 10, and Mart-1. They do not express or weakly express HMB-45 and have a low Ki67 index

reported false-negative rates are between 5% and 21%. The false-negative rate correlates with experience. In MSLT-I, participating centers, despite a required 30-case learning experience before entry of the first patient, had a higher false-negative rate during their first 25 cases in the trial relative to cases later in their experience. Negative predictive value is another useful way of examining the accuracy of SLN biopsy. By this measure, a negative SLN should be reassuring to most patients. For example, if the expected rate of SLN metastasis is 15% for a given population and the surgeon's false-negative rate is 10%, a negative SLN would imply a 1.5% risk of in-basin nodal recurrence or a 98.5% negative predictive value.

There are multiple potential reasons for false-negative SLN biopsy, including technical problems that affect nuclear medicine, surgery, and pathology (Karim et al. 2008) (Fig. 14). The correct node may not be identified due to misplaced isotope or dye injection or failure to identify the node during lymphoscintigraphy. This is particularly likely when drainage is aberrant (e.g., across the midline), or when the node basin is close to the injection site. A SLN may also be missed at the time of surgery. Typically, in this situation, a radioactive or dye-highlighted node is found, but another true SLN is missed. Similar to lymphoscintigraphy errors, these errors may be more common when the SLN is close to the injection site. It may also be due to inadequate probe interrogation of the basin after one SLN has been removed. Pathology-based false-negatives decrease with pathologist experience but can occur because of insufficient nodal sampling, failure to use immunohistology, or misidentification of melanoma cells as nodal nevocytes or macrophages.

An in-basin nodal recurrence may occur even when all "true" SLNs were removed in the initial procedure. In some instances, it appears that melanoma cells transitioning between the primary melanoma wide excision site and the regional nodes are present at the time of SLN surgery and are therefore not removed in the procedure. These foci of tumor cells may subsequently present as regional node metastases and be categorized as false negatives. Up to 40% of false-negative SLN cases are associated with local/in-transit recurrence (Lee et al. 2016). This association is in comparison to those with true positive SLNs, which suggests it is not merely a factor that

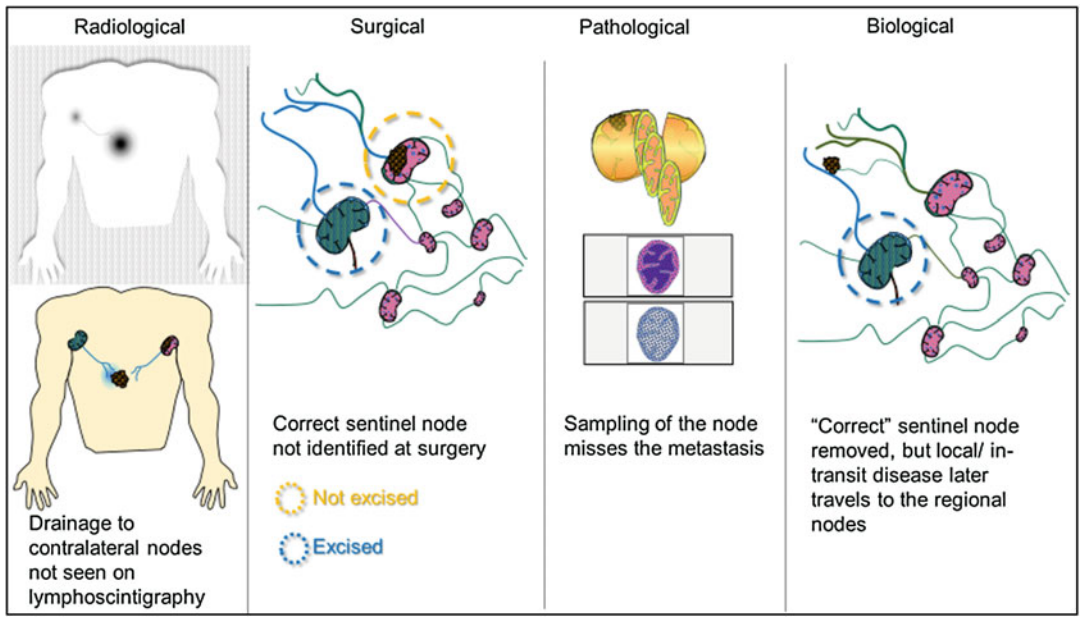

Fig. 14 Sources of false-negative SLNs

predicts nodal metastasis, but rather a specific biological mechanism leading to an increased risk of a false-negative SLN biopsy.

False-negative SLN biopsies can be minimized by being aware of and avoiding potential technical problems. Patients in whom nodal metastases are not correctly identified lose the benefits of accurate staging and any survival benefit from early excision of nodal metastases. Careful performance of the procedure by experienced personnel is key. Emerging technologies are under assessment to facilitate more accurate identification of tumor-affected SLNs. There is interest in imaging by SPECT-CT as well as planar lymphoscintigraphy. The use of an intraoperative gamma camera to re-evaluate the nodal basin after SLN excision has also been proposed, though the impact of this technology has not yet been validated.

Complications

Although SLN biopsy is associated with a low risk of complications, acute and chronic morbidities do occur. There is significant variation in the reported rates of morbidity in the literature, which is likely due to variations in the intensity of surveillance to identify such events. For example, postoperative seromas may be relatively common if assessed by imaging but are rarely manifested in a clinically significant finding or one that requires intervention. Acute problems include rare allergic reactions to blue dye (discussed above), seroma, hematoma, infection, and wound dehiscence. Chronic morbidities include lymphedema and nerve injury. In the Sunbelt Melanoma Trial, 4.6% of patients undergoing wide excision and SLN biopsy suffered major or minor complications. In that trial, most complications affected less than 1% of patients. Seromas and hematomas occurred in 2.3% of Sunbelt subjects, somewhat lower than the rate in MSLT-I of 5.5%. In MSLT-I, the overall complication rate was 10.1%. Lymphatic flow appears mostly preserved after SLN biopsy, though lymphedema is observed in a relatively low number of patients after SLN biopsy (Yokota et al. 2015). There is substantial variation in reported rates of lymphedema, which likely reflects operative technique and the intensity of postoperative surveillance for limb volume changes and the criteria used to diagnose the presence of the morbidity. Lymphedema may also depend on primary tumor location, since edema may be seen after wide excision without nodal surgery with primary tumors in certain locations. In the Sunbelt trial, the rate of lymphedema was 1.7% after SLN biopsy, and in MSLT-I it was 0.6% (vs. 0.3% after wide excision alone). Substantially higher rates of edema have been reported in series using rigorous non-invasive limb volume measurement. It is unclear whether these higher rates reflect increased incidence of the morbidity or simply an increased rate of detection (Hyngstrom et al. 2013). Upper limb lymphedema rates reported after melanoma-related surgery are generally lower than those reported for breast cancers treated by SLN biopsy, though the reasons for this difference are not entirely clear (Voss, Cromwell et al. 2015).

Regional lymph nodes are frequently close to sensory or motor nerves, especially in the head and neck region. With careful dissection, however, the risk of nerve damage should be very low. In the Sunbelt trial, reported rates of sensory and motor nerve injury were 0.14% and 0.09%, respectively (McMasters et al. 2004).

Lymphatic Mapping and SLN Biopsy from Melanoma Metastases

Some patients with recurrent melanoma may be candidates for mapping of the lymphatics that drain these metastases and biopsy of any detected SLNs. Series reporting this intervention have mainly examined mapping from local tumor recurrences and/or in-transit metastases, and the technique is feasible in this setting, allowing successful identification and removal of SLNs in nearly all cases. The rate of occult nodal involvement in these patients is relatively high, between 33% and 47% (Gonzalez et al. 2016; Yao et al. 2003; Read et al. 2015; Beasley and Tyler 2015). Presence of melanoma in SLNs draining local/in-transit metastases has

prognostic significance, being associated with shortened disease-free survival, although the timing of node dissection in these patients is unlikely to have therapeutic importance. The technical aspects of mapping may also be difficult if multiple in-transit metastases are present. Mapping has also been undertaken from pulmonary melanoma metastases (Faries et al. 2004). This anatomic location is challenging due to limited space, the close proximity of hilar nodes, and the frequent anthracotic discoloration of pulmonary lymph nodes. Nodal metastases in this setting, however, were found to be predictive of decreased overall survival.

Completion Lymph Node Dissection

Morton, Cochran, and colleagues originally described SLN biopsy as a method "to identify within the total population of patients with clinical stage I melanoma, those who have nodal metastases, because those are the ones who are most likely to benefit from ELND" (Morton et al. 1992). Under that conception, a positive SLN would result in a full nodal basin dissection in every case. "Completion dissection" is a specific surgical term that refers to a radical lymphadenectomy performed for regional nodal metastases diagnosed by SLN biopsy. Although sometimes referred to in the literature as "immediate" node dissection, in almost all cases the completion dissection is done several weeks after the SLN biopsy. Completion dissection does allow earlier dissection of the regional basin than would take place if the basin was observed without SLN biopsy, with radical lymphadenectomy was "delayed" until the time of clinically detected nodal recurrence. However, extensive experience with completion dissection demonstrated that, in the majority of cases, no additional melanoma-containing lymph nodes were identified by the pathologist in the completion lymph node dissection specimen. Furthermore, it became apparent that patients who did harbor disease identified in the completion dissection specimen (i.e., non-SLN metastases as well as SLN metastases) had a poor prognosis even accounting for the additional number of tumor-involved nodes (Ariyan et al. 2009; Ghaferi et al. 2009; Leung et al. 2013; Reintgen et al. 2013). The prognosis for patients with nodal disease identified in the completion dissection specimen was similar, in at least some series, to that for patients diagnosed with clinically apparent regional node metastases.

So the question of whether a completion lymph node dissection was necessary for patients with SLN metastases became important. Over the years some patients and surgeons opted for observation of the nodal basin after discovery of a positive SLN, and the proportion of patients who did so gradually increased. In 1998 over three-quarters of patients underwent completion dissection. In a series using National Cancer Database data from 2004 to 2005, however, only 50% of patients were reported to have done so (Bilimoria et al. 2008). Since most patients with SLN metastases will not have additional metastases found at the time of completion dissection, efforts have been made to determine which SLN-positive patients are at greatest (or least) risk of non-SLN metastases. Agreement on the most useful criteria has been moderate, at best. Generally, a thicker primary tumor and higher SLN tumor burden correlate with increased risk of non-SLN metastases. Greater Breslow thickness of the primary melanoma has correlated with risk of non-SLN metastasis in most studies. SLN tumor burden has been quantified by several approaches, including greatest diameter of the largest metastatic focus, percent nodal area occupied by metastases, sum of longest diameters of metastases, and micrometer-measured depth of penetration of metastases from the nodal capsule into the node. Other features found to correlate with outcome include patient gender, primary melanoma regression, perinodal lymphatic invasion, SLN dendritic cell area, metastasis location within nodes, number of involved SLNs, and proportion of SLNs involved by tumor. Scoring systems have been developed to improve accuracy of estimation of risk of progression. Validation of these diverse scoring systems has proved difficult when they have been applied to independent patient populations (Cadili et al. 2010; Wevers et al. 2013).

One problem shared by all of these series examining non-SLN metastases is that they were reliant on pathologic identification of metastases in completion dissection specimens. However, those specimens are often large and require the pathologist to search for and find each node within the basin. Those nodes are then generally evaluated only with limited sectioning and without the use of immunohistochemistry, as a more exhaustive approach would not be practical. It appears that this approach fails to identify a substantial number of patients who harbor non-SLN metastases. Evidence for this is found in series in which non-SLNs were subjected to serial sectioning with immunochemical stains. Wen et al. found metastases in an additional 8–10% of patients whose completion dissection had been reported to be negative by standard processing (Wen et al. 2004). Recent evidence from MSLT-II supports this further. In that study, patients who underwent immediate completion node dissection were found to have positive non-SLNs 11.5% of the time. Among those whose basin was observed, nodal recurrence developed at a rate of 26.1% at 5-years of follow-up (Fig. 4). This suggests that a large fraction of patients who had non-SLN metastases had not been identified using routine histologic techniques.

The clinical effectiveness of completion node dissection has been evaluated in two prospective randomized trials, MSLT-II and DeCOG-SLT (Faries et al. 2017; Leiter et al. 2016). Those studies randomized melanoma patients with SLN metastases to completion lymph node dissection or clinical observation with serial ultrasound of the at-risk nodal basin. DeCOG-SLT randomized 483 patients and MSLT-II randomized 1939 patients. The primary endpoint of the De-COG trial was distant metastasis-free survival, and that of MSLT-II was melanoma-specific survival. Neither trial demonstrated a significant benefit in their primary endpoints for patients who were randomized to undergo completion lymphadenectomy (Fig. 15). Subgroup analyses of MSLT-II did not identify any subgroup with a statistically significant benefit from completion dissection, though the trial was not powered to be definitive in all subgroups. Specifically, there was no trend suggesting that "high-risk" groups, such as patients with larger SLN metastases, were more likely to benefit from completion lymphadenectomy, though such

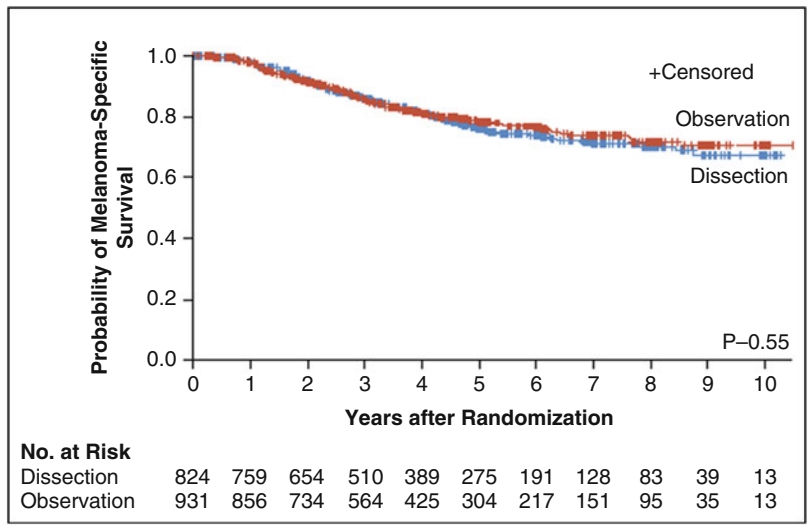

Fig. 15 Primary outcomes of two randomized trials (MSLT-II and DeCOG-SLT) where patients with sentinel-node positive melanoma were randomized to either completion lymph node dissection or nodal observation with serial ultrasonography. There were no significant differences between the two arms for melanoma-specific survival (primary endpoint for MSLT-II) or distant metastasis-free survival (primary endpoint for DeCOG-SLT) (Faries et al. 2017; Leiter et al. 2016)

individuals have a higher risk of non-SLN metastases (Gershenwald et al. 2008) and would be likely to have an increased benefit in terms of regional control and staging.

From the results of these two clinical trials, overall there is a high level of confidence that completion dissection does not offer a survival advantage for patients with tumor-positive SLNs relative to careful ultrasound-based observation of the at-risk regional nodes. Thus, either approach may be applied after appropriate discussion between surgeon and patient. If evidence were to develop during extended follow-up of these trial groups that specific patient populations derive benefit, further confirmatory studies would be needed. Completion dissection remains a reasonable option that some patients may choose. The procedure provides some value, though at the cost of significant complication rates. One value of completion dissection comes from its staging significance. Completion dissection is needed to determine the total number of regional lymph nodes with metastases, which correlates directly with outcome and is part of the current AJCC staging system. Several retrospective studies have shown that melanoma in non-SLNs is an adverse indicator of survival, independent of the total number of involved nodes (Reintgen et al. 2013; Leung et al. 2013; Ariyan et al. 2009). In addition, in MSLT-II, the pathologic status of non-SLNs was an independent prognostic factor for disease-free and melanoma-specific survival. This suggests that, at present, there is important prognostic information to be derived from examination of non-SLNs, information that may not be fully replaced by other variables. This information may be critical for patients who are undecided about whether to proceed with adjuvant systemic therapy. The expense, duration, and potential toxicity of recently approved adjuvant therapies increase the importance of considering each relevant piece of prognostic information.

Completion node dissection decreases the risk of melanoma-specific regional node recurrences, despite there being no difference in the rate of distant metastasis attributable to the procedure. However, any recurrence may be a source of distress for patients and, for some, completion dissection may be considered to reduce the risk of these events. Earlier treatment of nodal metastases has the potential to reduce the frequency of progression to bulky nodal disease and the associated technical challenges involved with surgery to deal with the problem. In addition, there is the question of whether observation and increased nodal recurrence is associated with an increased risk of "loss of regional control." As noted above, this issue has not been well studied. Bamboat et al. examined outcomes among 167 patients with a positive SLN who did not undergo completion dissection. Nodal recurrences were the only site of disease upon recurrence in 15% of patients. Three-quarters of those patients underwent delayed completion dissection, most of whom remained free of disease at a median of 18 months of follow up (Bamboat et al. 2014). In MSLT-II, node-only recurrences occurred in 63 (7.7%) of 820 observed patients and 10 (1.3%) of 744 patients who underwent dissection. There was a nodal component to recurrence in 208 (25%), compared to 9% in the dissection arm of the study. Whether there was a difference in the ability to salvage the nodal basin with delayed surgery remains unknown.

SLN as an Experimental Model for Tumor-Host Interface

The process of melanoma metastasis begins most commonly in the SLN. This is the first interaction point between malignant cells and the normal host defense, including the immune system, with that interaction apparently beginning even prior to the arrival of malignant cells. Tumor cell-free lymphatic drainage from a primary melanoma appears to induce changes in the local and regional nodal microenvironment that facilitate subsequent tumor cell dissemination. The effects, including lymphangiogenesis and immunosuppression, seem likely to be mediated by factors released from the primary site and have been observed to occur even prior to metastasis, as described below.

Theoretically, the lymphatic system is an ideal route for melanoma dissemination. Open-ended lymphatic vessels are abundant in the dermis and

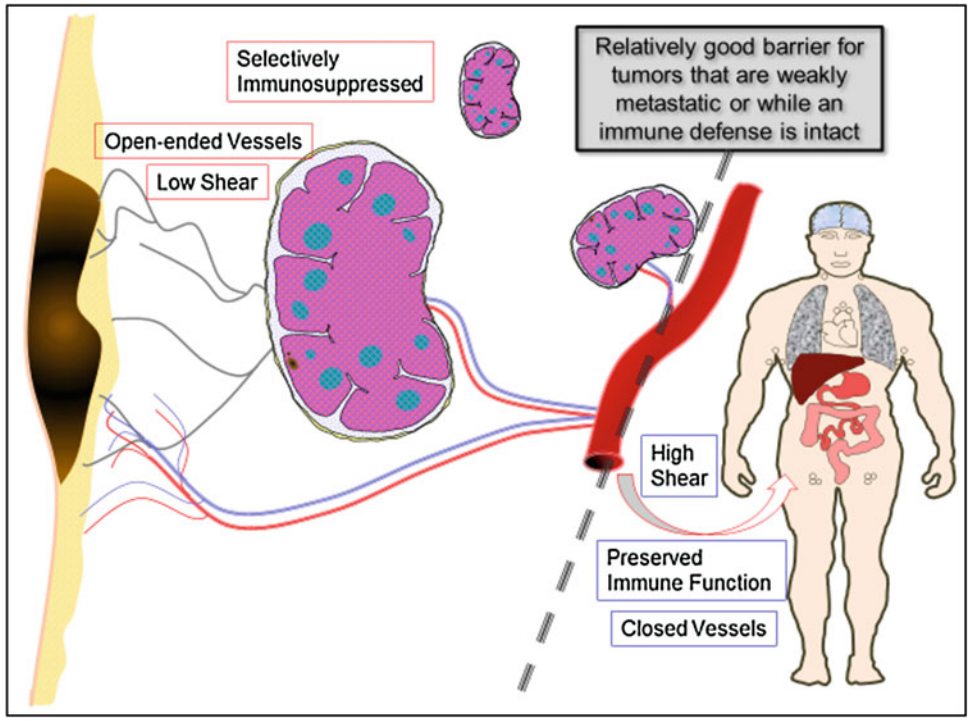

Fig. 16 Theoretically there is a large difference in the threshold for successful metastases to regional lymph nodes compared to distant sites. Tumor cells may traffic to draining nodes without requirement for extensive intravasation or extravasation and without a need to tolerate the high shear forces of the blood circulation. Draining nodes also appear to be relatively immunosuppressed, making immune evasion there easier

permit entry of tumor cells without the need for vascular invasion or survival within turbulent blood flow (Fig. 16). These pathways also appear to provide a route for direct transmission of tumor-derived factors to the first draining lymph nodes. One effect is on the density and proliferation of lymphatic vessels in the skin and in the SLN. These changes and expansion of the lymphatic sinuses in draining nodes are correlated with melanoma metastases and with survival (Dadras et al. 2005; Pastushenko et al. 2016). Tumor-induced changes in SLNs appear to precede the arrival of tumor cells in the nodes, consistent with preparation of the so-called premetastatic niche (Harrell et al. 2007). The speed of lymphatic flow has also been correlated with nodal metastases, with fast flow predicting an increased risk of metastasis and slow flow the converse (Maza et al. 2003; Cammilleri et al. 2004).

The immunological competence of tumor-draining lymph nodes is reduced in many patients, presumably largely through tumor-induced mechanisms. Regional nodal immunosuppression appears to be "zoned," with nodes closest to the primary tumor having the most apparent changes (Cochran et al. 1987). Evidence of tumor-induced immunosuppression includes loss of dendritic cell area and absence of interactive meshworks of mature dendritic cells in SLNs relative to non-SLNs (Cochran et al. 2001). Dendritic cell immune-suppression (Cochran et al. 2006) occurs in a sequential fashion, with the first interaction at the primary tumor site followed by changes in the SLN (van den Hout et al. 2017). There is a decrease of $CD8^+$ T-cell frequency in SLNs compared to non-SLNs, and the $CD8^+$ T-cells that are present exhibit an "exhausted" phenotype with increased expression of the programmed death-1 (PD-1) receptor (Grotz et al. 2015). Other T-cell changes include an increased ratio of CD4:CD8 and a decreased ratio of CD8:Treg (van den Hout et al. 2017). Finally, there is a tendency toward Th2 response

polarization and away from Th1 responses. Increased B cell numbers and cytokine profiles in tumor-draining lymph nodes also appear to be modulated in the presence of melanoma. Decreased interferon-γ, interleukin-2, and granulocyte macrophage-colony stimulating factor have been reported in SLNs that contain micrometastases (Leong et al. 1999; Barbour and Coventry 2003).

Melanoma-secreted factors that may cause immunosuppression include indoleamine 2,3 deoxygenase (IDO) (Speeckaert et al. 2012), interleukin-6, interleukin-10, and TGF-β (Cochran et al. 2006; Botella-Estrada et al. 2005). Melanoma-derived extracellular vesicles are generated by the primary tumor and have a significant role in compromising the immune function of SLNs (Maus et al. 2017). These vesicles include both exosomes and microvesicles and range in size from 30–1000 nm. They have been identified in melanoma-draining tumor lymphatics and exert a suppressive effect on the maturation of dendritic cells in SLNs. Identification of these mechanisms of local and regional immunosuppression may allow for development of novel strategies to reverse these effects and create opportunities for improved systemic therapy.

Considerations for the Future

SLN biopsy is well established as part of the standard evaluation and treatment of many patients with clinically localized melanoma. It provides value to patients in terms of staging and prognosis and aids clinicians in their determination of appropriate surgical and nonsurgical therapies following the initial surgery. SLN biopsy is minimally invasive and generally well tolerated with a low risk of significant acute or chronic morbidity. For patients with intermediate-thickness melanomas, early removal of nodal metastases appears to improve their long-term survival. The SLN also appears to be a useful environment for study of the interaction of melanoma with the immune system, and insights gained from the pathobiology of the SLN may assist the process of designing new targeted therapies for melanoma. Further research is needed to answer many unsolved questions, such as which patients with thin melanomas benefit from SLN biopsy and how best to identify the patients who have metastases in regional nodes beyond the SLN.

Cross-References

- ▶ A History of Melanoma: From Hunter to Morton
- ▶ Biopsy of Suspected Melanoma
- ▶ Lymphoscintigraphy in Patients with Melanoma
- ▶ Melanoma Prognosis and Staging

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