

Comparison of Blue Dye and Probe-Assisted Intraoperative Lymphatic Mapping in Melanoma to Identify Sentinel Nodes in 100 Lymphatic Basins

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Objective: To determine whether combining isosulfan blue dye with a radiopharmaceutical agent will increase intraoperative detection of sentinel nodes (SNs) in patients with early-stage melanoma.

Patients and Design: Clinical trial with a consecutive sample. Eighty-seven patients with clinical stage I melanoma underwent preoperative lymphoscintigraphy with 1 of 3 radiopharmaceutical agents to identify the lymphatic basin and the site of the SN. All patients subsequently underwent intraoperative lymphatic mapping and selective lymph node dissection (SLND) with isosulfan blue dye and a radiopharmaceutical agent. A handheld gamma probe determined the radioactive counts over the draining lymph node basins and individual blue-stained lymph nodes before (in vivo) and after (ex vivo) their removal. An irrelevant body site was used as the denominator of a count ratio by which absolute counts were standardized for comparison. Completion lymphadenectomy was undertaken in patients whose SLND specimen had histopathologic evidence of tumor cells.

Setting: Tertiary care cancer center.

Intervention: Lymph node sampling.

Main Outcome Measure: Accuracy of SN detection

by blue dye and radiopharmaceutical techniques.

Results: Preoperative lymphoscintigraphic images identified 100 lymph node basins and 135 lymph nodes in 87 patients. All 3 radiopharmaceutical agents were equally effective in imaging the SN before surgery. During SLND, we identified and removed 136 blue-stained and radioactive (hot) SNs and 8 additional non-blue-stained hot nodes from 98 basins (98.0%). Of the 144 excised lymph nodes, 132 nodes (91.7%) from 83 basins had either an in vivo- or an ex vivo-background count ratio of 2:1 or more and 125 nodes (86.8%) from 77 basins had a count ratio of 3:1 or more. Twelve blue-stained SNs had count ratios of less than 2:1. Seventeen SNs (11.8%) from 15 basins contained metastases: 16 were identified with blue dye and probe and 1 was identified with blue dye alone. Four (1.1%) of 377 non-SNs excised during completion lymphadenectomy contained metastases. There have been no lymph node recurrences during mean follow-up of 16.3 months (range, 7-42 months).

Conclusions: The blue dye technique remains the criterion standard for SLND in melanoma. The addition of a radiopharmaceutical tracer serves as a useful adjunct to the visualization of blue-stained SNs.

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SURGICAL MANAGEMENT of the regional lymph nodes in patients with clinical stage I melanoma remains controversial. More than 100 years ago, elective lymph node dissection (ELND) was proposed as a method to control the permeation of melanoma from the primary site to the regional lymph nodes.¹ Results of multiple retrospective studies²⁻⁵ support this approach by demonstrating a survival advantage for patients who undergo ELND compared with those being treated by delayed (therapeutic) lymph node dissection. Results of 3 prospective randomized trials⁶⁻⁸ fail to confirm a survival benefit for patients who un-

dergo ELND and raise the question as to the efficacy of this treatment. We suspect that any overall survival advantage for early removal of the tumor-positive regional lymph nodes in these randomized trials was likely obscured because only a few patients were likely to have occult lymph node metastases.

Factors such as the primary melanoma's thickness, Clark level of invasion, ulceration, and anatomic location have clear prognostic significance in clinical stage I melanoma, but they are not adequate criteria on which to base the decision for ELND.^{9,10} Even if ELND is reserved for patients in clinical stage I whose primary melanomas meet the criteria for high risk,

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PATIENTS AND METHODS

Eighty-seven consecutive patients (56 men and 31 women) with American Joint Committee on Cancer stage I and II melanoma underwent preoperative LS followed either immediately or at least 24 hours later by SLND. All primary melanomas were diagnosed by either incisional or excisional biopsy examination, and all were Breslow thickness 0.65 mm or greater and Clark level II or greater or had ominous histological features such as regression or ulceration.^{22,23} None of the patients underwent excision of the primary site with margins of 1.5 cm or greater or a previous operative procedure that disrupted lymphatic drainage to the regional nodal basin.

PREOPERATIVE LS

Preoperative dynamic LS was performed with 1 of 3 technetium Tc 99m-labeled radiopharmaceutical agents: Tc 99m HSA (Amersham-Medi-Physics, Arlington Heights, Ill), technetium Tc 99m albumin colloid (Tc 99m AC, CIS-US Inc, Bedford, Mass), or technetium Tc 99m sulfur colloid (Tc 99m SC, Amersham-Medi-Physics). Of 87 patients, 71 underwent LS at least 24 hours before surgery with either Tc 99m HSA (n = 57) or Tc 99m AC (n = 14) and had SLND performed with concurrent intraoperative injection of isosulfan blue dye and Tc 99m HSA. The remaining 16 patients underwent LS with Tc 99m SC performed no more than 4 hours before surgery.

All radiopharmaceutical agents were prepared and stored according to the instructions of the manufacturers. Both colloids were filtered through 0.20- μ m filters to remove larger-

sized particles, which tend to migrate slowly from the primary site.^{24,25} The primary site was injected with approximately 18.5 to 30 MBq (500-800 μ Ci) of the radiopharmaceutical agent at 4 circumferential points, and a large-field stationary scintillation camera (Siemens ZLC, Des Plaines, Ill) was used to image the primary site, afferent lymphatics, and regional drainage basins. After injection of the radiopharmaceutical agent, continuous monitoring was performed until the first nodes or SNs were visualized. The SNs were defined as the first lymph nodes visualized on LS images. Occasionally, 1 or more lymph nodes would be identified simultaneously, and in some cases 2 or more afferent lymphatic channels would lead to different lymph nodes. Each of these lymph nodes were considered SNs. The transit times of the radiopharmaceutical agents were measured from the injection time until the first regional lymph node was visualized. The sites of the afferent lymphatic channels and the SN were marked on the skin with indelible ink and the body image was outlined to create the radiograph.

SURGICAL PROTOCOL

After induction of general or local anesthesia, we injected patients intradermally with isosulfan blue dye (Lymphazurin 1%, Hirsch Industries Inc, Richmond, Va), 1 to 1.5 cm³, at the primary site. The 71 patients who underwent preoperative LS more than 24 hours before SLND received the dye in a 3:1 ratio with Tc 99m HSA (11-55 MBq); the 16 patients who underwent LS on the day of surgery received blue dye alone. Gentle massage facilitated the passage of the injectate into the lymphatics. Five to 10 minutes after injection, an incision was made at the site marked

up to 60% of patients will not have histopathologic evidence of metastases in the regional lymph nodes and are therefore unlikely to benefit from ELND.¹⁰ On the other hand, although only a small fraction of low-risk patients will ever develop lymph node metastases, excluding them from ELND may ultimately prevent them from obtaining the survival benefit of this procedure.

In 1992, Morton et al¹¹ reported a minimally invasive operative procedure to determine the presence of occult regional lymph node metastases in patients with clinical stage I melanoma. Intraoperative lymphatic mapping and selective lymph node dissection (SLND) was devised as an alternative to ELND. Morton et al¹¹ and Wong et al¹² demonstrated that the lymphatic drainage patterns of cutaneous melanoma were predictable by preoperative lymphoscintigraphy (LS) and that the first tumor-draining lymph node could be identified intraoperatively with a single injection of isosulfan blue dye at the primary site. Excision and histopathologic analysis of the sentinel node (SN) was used to determine the tumor status of the regional lymph node basin. Only patients with demonstrated SN metastases would be subject to a complete lymphadenectomy (LND). In their initial experience, Morton et al^{11,13} identified the SN in 194 (81.8%) of 237 predicted lymph node basins. By performing LND in all patients, they found that the frequency of false-negative, tumor-positive SNs was less than 1%. Several other investigators¹⁴⁻¹⁶ subsequently validated the SN technique and proved its strength as a staging procedure.

Because the success of the technique is limited by the surgeon's ability to identify blue-stained SNs, we modified the procedure by using isosulfan blue dye in conjunction with technetium Tc 99m human serum albumin (Tc 99m HSA). The radiopharmaceutical agent was tracked with a handheld gamma probe into the regional nodes, where higher radioactive counts were found in SNs than in non-SNs.^{17,18} Although other investigators¹⁹⁻²¹ published their results with intraoperative probe-directed mapping, none of these early studies clearly defined the radioactive SN using the blue-stained SN as a standard. In this study, we evaluate the utility of several radiopharmaceutical agents for LS and SLND to improve the accuracy of SN detection.

RESULTS

Preoperative LS demonstrated 28 cervical, 44 axillary, and 28 inguinal lymph node basins from the 17 head and neck, 32 torso, and 38 extremity primary sites. Of 87 patients, 13 (15%) had dual pathways of lymphatic drainage, but no patient had more than 2 drainage basins or an ambiguous drainage pattern. Lymphoscintigraphy imaged 135 SNs (mean, 1.35 per basin) within the first hour of injection of the radiopharmaceutical agent. There was no difference ($P = .35$) among the 3 agents in the interval (minutes) between injection and identification of the SN: 9.8 ± 9.8 minutes (range, 1-36 minutes) for Tc 99m HSA; 10.4 ± 7.2 minutes (range, 1-30 minutes) for Tc 99m AC;

by preoperative LS; the exact time of this incision depended on the radiopharmaceutical agent used.²⁴ The lymphatics at the edge of the basin nearest the primary melanoma were identified and were followed by meticulous dissection to the first blue-stained node in the basin. Sentinel nodes were defined as the first blue-stained lymph nodes or radioactive and non-blue lymph nodes. The site and number of lymph nodes identified as SNs was usually closely correlated to the LS findings. The area several centimeters around the SN was carefully explored for additional blue-stained nodes. Injection was repeated every 20 minutes as necessary to identify an SN. If more than 1 lymph node basin was demonstrated by LS (n = 13) we explored the basins in the order they were found by LS.

A handheld gamma particle probe (Neoprobe 1000, Neoprobe Corp, Dublin, Ohio) was used to determine the radioactivity (counts per second) over the regional lymph node basin at the site marked during LS and over each blue-stained SN before (in vivo) and after (ex vivo) its excision. If a blue-stained lymph node was not identified, the nodes with the highest radioactivity were excised until the counts approached measured background levels. Background counts were obtained from 3 neutral body sites (10-20 cm from the primary injection site and lymph node basin) to avoid confusion with either the primary site or the lymph node basin. In cases in which the primary site was adjacent to the dissected basin, the probe crystal was positioned to diminish the artifact from the primary site. In these cases, radioactivity did not reach the neutral background levels. Triplicate counts were recorded from all sites.

While excised SNs were examined for evidence of metastases, the primary melanoma was excised with radial mar-

gins of 1 to 3 cm (depending on the thickness and site of the primary lesion). If results of pathologic examination of the SN were positive for metastases either by frozen or permanent section, LND was performed in the standard fashion.

PATHOLOGIC EXAMINATION

The techniques of pathologic examination have been described previously.^{12,26} Briefly, each SN was bisected from the hilum to the periphery. Each face of the cross section of the specimen was processed by frozen-section examination using conventional hematoxylin-eosin staining; the remaining portion of the lymph node was embedded in paraffin and sectioned for reexamination by hematoxylin-eosin and immunohistochemical staining with antibodies for S-100 and the melanoma-specific protein HMB45.²⁶ Additional lymph nodes removed during LND were examined by hematoxylin-eosin staining alone.

STATISTICS

Preoperative LS transit times of the radiopharmaceutical agents and the first identification of the SN were compared using analysis of variance, and significance was based on $P < .05$. Mean \pm SD radioactivity counts were calculated, and count-background ratios were determined from the preincision lymph node basin, the in vivo and ex vivo lymph nodes, and the postexcision lymph node basin. The difference in mean count ratios associated with immediate (≤ 4 hours after preoperative LS) vs delayed (≥ 24 hours after LS) SLND was assessed using a 2-sample t test with pooled variance.²⁷

and 14 ± 15.1 minutes (range, 2-60 minutes) for Tc 99m SC. There was also no difference ($P = .26$) in the number of nodes identified (1.29 ± 0.49 for Tc 99m HSA, 1.53 ± 0.72 for Tc 99m AC, and 1.33 ± 0.49 for Tc 99m SC) per basin by each of the radiopharmaceutical agents, suggesting that the 3 agents were equally effective for preoperative LS.²⁴

During SLND, SNs were identified in 98 of 100 regional lymphatic basins. One hundred forty-four SNs (mean, 1.4 per basin) were excised. In one third of the lymph node basins, more than 1 SN was identified (29 basins, 2 SNs; 4 basins, ≥ 3 SNs). Sentinel nodes were not found in 2 basins. In 1 patient, preoperative LS demonstrated drainage to the inguinal lymph node basin from a proximal calf primary site. The popliteal space was explored first because of concern that lymphatic drainage to this basin may have been obscured by the artifact from the radiopharmaceutical agent injected at the primary site. After injection of isosulfan blue dye, we suspect that the lymphatic channel leading to the inguinal basin may have been transected in the process of our exploration of the popliteal space. In a second patient, the skin overlying the sternocleidomastoid muscle was marked during LS. Lymphoscintigraphic images led us to believe that the SN was located posterior and lateral to the sternocleidomastoid muscle, but we suspect that the SN was likely deep to the muscle and that the lymphatic channel was cut during dissection through the muscle. In both patients, blue dye was injected concurrently with Tc 99m

HSA, which prevented us from identifying a radioactive SN. Neither of these 2 patients has had a recurrence after 16 and 17 months of follow-up, respectively.

For the 93 basins in which a blue-stained SN was identified, the mean \pm SD absolute radioactivity counts were 104 ± 124 per second (range, 1-690 per second) for the background, 605 ± 834 per second (range, 7-3953 per second) over the basin before incision, 745 ± 1198 per second (range, 5-3136 per second) for the SN in vivo, 551 ± 1104 per second (range, 10-6787 per second) for the SN ex vivo, and 213 ± 440 per second (range, 2-2266 per second) over the basin after excision. The corresponding site-background count ratios (mean \pm SD) were as follows (**Figure 1**): preincision basin, 12.9 ± 20.4 (range, 0.7-123.7); in vivo SN, 27.4 ± 100.6 (range, 0.4-963.0); ex vivo SN, 20.1 ± 77.8 (range, 0.2-711.0); and postexcision basin, 2.8 ± 3.8 (range, 0.1-21.0).

There was a wide distribution of in vivo count ratios measured over blue-stained and non-blue-stained SNs (**Figure 2**). Twelve blue-stained SNs (8.3%) had an in vivo count ratio less than 2:1. By increasing the in vivo count ratio to 3:1 or more to improve the specificity of the technique, the number of blue-stained SNs not defined by the gamma probe technique increased to 21 (14.6%). Count ratios varied from less than 1 to 100. We found a similar wide range of count ratios after the SNs were excised. Twenty-two SNs (16.2%) had ex vivo count ratios less than 2 and 37 SNs (27.2%) had ex vivo counts ratios less than 3:1 (data not shown). To account for all

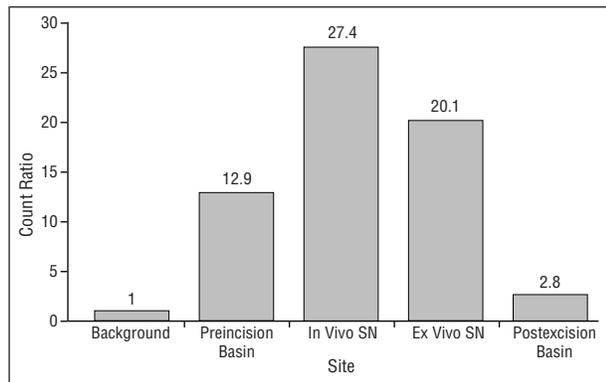


Figure 1. Relative change in count ratios measured during selective lymph node dissection. Absolute counts were determined over each of the sites using a handheld gamma probe and recorded in triplicate. Count ratios were calculated in each case by comparing site counts with those of an irrelevant background location away from the primary site of the lymph node basin. SN indicates sentinel node.

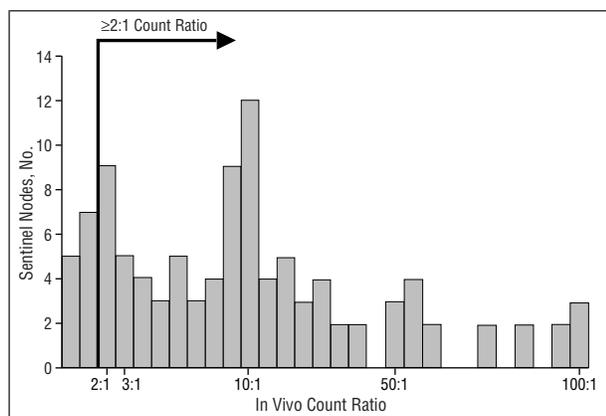


Figure 2. Distribution of in vivo sentinel node-background count ratios. Blue-stained and non-blue-stained sentinel nodes were assessed for the presence of radioactivity using a handheld gamma probe. Count ratios were calculated by comparing in vivo sentinel node counts with those of an irrelevant body site (background). Twelve blue-stained lymph nodes (8.3%) had count ratios less than 2.

blue-stained nodes, in vivo and ex vivo count ratios would have to be 1 or less. We found that a count ratio of 1 or less is impractical for routine use of the gamma probe. We therefore defined a radioactive (hot) SN with an in vivo or ex vivo count ratio of 2:1 or more from a blue-stained node because this ratio is sufficiently large for easy detection with the probe.

As shown in **Table 1**, 124 blue-stained lymph nodes (86.1%) from 83 basins (83.0%) met our definition of a hot SN, defined as an in vivo- or ex vivo-background count ratio of 2:1 or more. Whereas 117 lymph nodes (81.2%) from 77 basins (77%) had count ratios of 3:1 or more. Eight non-blue-stained radioactive SNs (count ratios ≥ 2) were identified in only 5 basins (2 cervical and 3 axillary). None of these lymph nodes contained metastases. The success of the blue dye was 94% and the success of radiopharmaceutical techniques alone was 4%. The combined success rate of the 2 techniques was 98%.

Sixteen patients (18%) underwent LS with Tc 99m SC immediately before surgery (mean time between LS and SLND, 210 minutes; range, 30-240 minutes) and therefore required only a single injection of a radiophar-

Table 1. Results of Intraoperative Lymphatic Mapping*

	No. (%)
Lymph nodes, total SN	144 (100.0)
SN blue stained	136 (94.4)
SN hot (ratio ≥ 2)	132 (91.7)
SN hot (ratio ≥ 3)	125 (86.8)
SN blue stained only (ratio < 2)	12 (8.3)
SN blue stained and ratio ≥ 2	124 (86.1)
SN blue stained and ratio ≥ 3	117 (81.2)
SN hot only (ratio ≥ 2)	8 (5.6)
Lymph node basins, total basins	100 (100.0)
Basins SN identified	98 (98.0)
Basins SN blue stained	93 (93.0)
Basins SN blue stained and hot (ratio ≥ 2)	83 (83.0)
Basins SN blue stained and hot (ratio ≥ 3)	77 (77.0)
Basins SN blue stained only (ratio < 2)	10 (10.0)
Basins SN hot (ratio ≥ 2), not blue stained	5 (5.0)
Basins SN hot (ratio ≥ 3), not blue stained	5 (5.0)

*SN indicates sentinel node.

maceutical agent. In all 16 patients, the SN was successfully identified during surgery. In 15 patients the SN was identified with blue dye and a radiopharmaceutical agent and in 1 patient the SN was identified with blue dye alone (count ratio < 2). An SN was identified in all 18 lymphatic basins (100% success), and 27 of 29 SNs (93.1% concordance) were blue stained and hot (in vivo or ex vivo count ratios ≥ 2). In comparison, in 71 patients who underwent SLND by simultaneous injection of Tc 99m HSA and isosulfan blue dye, the SN was identified in 80 of 82 lymphatic basins (98% success), and 96 of 115 SNs (83.5% concordance) were blue stained and hot. There was no difference in count ratios in vivo (21.6 ± 26 vs 29.5 ± 116.7 ; $P = .60$) or ex vivo (16.3 ± 19.6 vs 20.7 ± 85.6 ; $P = .73$) between patients injected with Tc 99m SC vs Tc 99m HSA, respectively.

Of 87 patients (85 with SNs identified), 14 (16%) had metastases in the SN. Seventeen (11.8%) of 144 SNs removed during SLND were tumor positive. Sixteen (94%) of 17 tumor-containing SNs were blue stained and hot (in vivo or ex vivo ratio ≥ 2); the remaining SN had an in vivo- and ex vivo-background count ratio less than 2 and was identified with isosulfan blue dye alone. The in vivo and ex vivo count ratios of tumor-containing SNs were 18.2 ± 25.6 (range, 0.4-96.0) and 14.1 ± 26.9 (range, 0.2-91.3), respectively. These count ratios were no different than the in vivo ($P = .82$) or ex vivo ($P = .49$) ratios for SNs that were tumor free (**Figure 3**). Four (1.1%) of 377 lymph nodes removed during completion LND contained metastases. There have been no same-basin recurrences in 87 patients (mean follow-up, 16.3 months).

COMMENT

Intraoperative lymphatic mapping and SLND was introduced by our institution as a means of identifying occult regional lymph node metastases in patients with early-stage melanoma.^{12,13} This minimally invasive operative procedure was devised as an alternative to ELND. Results of our initial experience suggest that the blue dye alone was not sufficient. The success of identifying the

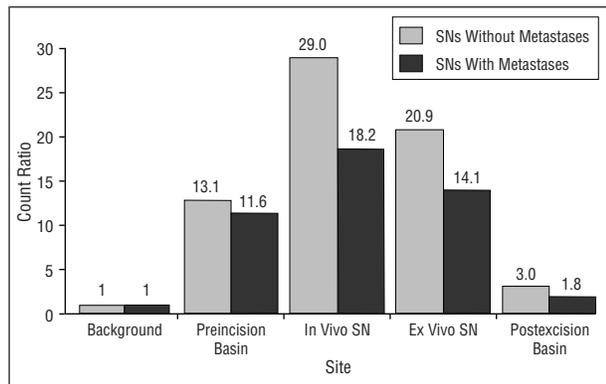


Figure 3. Relative change in count ratios for sentinel nodes (SNs) with and without metastases. Absolute counts were determined over each site using a handheld gamma probe and recorded in triplicate. Count ratios were calculated in each case by comparing counts with an irrelevant background, away from the primary or lymph basin. Relative count ratios were not different for blue-stained SNs in vivo ($P = .82$) or ex vivo ($P = .49$) with or without metastases.

SN is dependent on the surgeon locating a blue-stained afferent lymphatic and lymph node. The first improvement in the technique was the addition of preoperative cutaneous LS in all patients so the surgeon would have a landmark for the location of the SN.²⁸⁻³⁰

Preoperative LS has been adapted by most surgeons as an essential component of the SN technique, but there is no standard method or radiopharmaceutical agent used for this procedure. We performed LS with 1 of 3 agents: Tc 99m HSA, Tc 99m AC, or Tc 99m SC. In our series, LS identified the draining lymphatic basin in all patients and demonstrated dual drainage patterns in 13 patients (15% incidence), a rate similar to that reported by other institutions.^{24,30,31} Lymphoscintigraphy clearly improves localization of the SN, but the intralymphatic kinetics and retention of the various radiopharmaceutical agents in the lymph nodes have not been well studied. We found that Tc 99m HSA had a slightly faster transit time to the SN than did the 2 filtered particulate agents, Tc 99m AC and Tc 99m SC.²⁴ The number of SNs identified by LS was similar for all 3 agents when images were obtained within the first hour. We have not seen demonstration of additional lymph node basins later than 1 hour after injection of any of the 3 radiopharmaceutical agents. Yet, with delayed images (3-4 hours), an increasing number of lymph nodes were identified with Tc 99m AC and Tc 99m SC.²⁴ With longer delay, particulate agents travel from the SN to non-SN, which makes LS unreliable for detecting the site of the SN. Although the concept that particulate agents are trapped in the SN is intriguing, our results suggest that this is not always the case. Transit of the radiopharmaceutical agents in the lymph nodes is time dependent: the longer the imaging period the more nodes that will appear. Our findings indicate that although Tc 99m HSA and particulate agents (Tc 99m AC and Tc 99m SC) have different transit times related to particle size, all 3 are equally effective in identifying the SN by LS if early images are used. The use of a single injection of a radiopharmaceutical agent for both LS and probe-directed SLND would diminish the

cost and radiation exposure to patients and may be the most sensible method for this procedure.

Our second goal for this study was to determine the utility of intraoperative probe-directed mapping for identification of the SN. Several institutions have reported that addition of radiopharmaceutical agents to the blue dye improves localization of the SN, but there are discrepancies in the methods in which the radiopharmaceutical agents are used and in the level of radioactivity that defines an SN. Eighteen hours after injecting Tc 99m AC at the primary site, Pijpers et al³² used a gamma probe to identify the SN but found in most cases high levels of radioactivity in nodes adjacent to the SN. Krag and associates¹⁹ reported successful localization of hot nodes up to 24 hours after injection of unfiltered Tc 99m SC, but they rarely used the blue dye to confirm their findings. Albertini et al²⁰ recommended waiting 3 to 4 hours after injection of Tc 99m SC to achieve radioactive counts 2-fold higher in SNs than in non-SNs. In these studies, the concordance between blue dye and probe ranged from 60% to 100%. Taylor and associates³³ suggested that Tc 99m HSA was not retained in the lymph nodes long enough for effective intraoperative lymphatic mapping. However, in our 71 patients receiving Tc 99m HSA, we identified the SN in 80 (98%) of 82 lymphatic basins, and 96 of 115 SNs were blue stained and hot (in vivo and ex vivo counts ratios $\geq 2:1$, 83.5% concordance). In 16 patients receiving Tc 99m SC, the SN was identified in all 18 lymphatic basins (100%), and 27 of 29 SNs (93% concordance) were blue stained and hot (in vivo and ex vivo counts ratios $\geq 2:1$). These findings suggest that SLND may be undertaken immediately after injecting Tc 99m HSA or up to 4 hours after injecting filtered Tc 99m SC. We are currently investigating the efficacy of preoperative LS and SLND with filtered Tc 99m SC injected 4 hours or longer before the operative procedure.

An in vivo or ex vivo lymph node count ratio of 2:1 or more identified 132 (91.7%) of 144 lymph nodes excised during SLND (Table 1). Although we are not the first to define an SN by a count ratio, other investigators have used count ratios subject to variability.^{19,20,32-38} Albertini et al²⁰ initially suggested defining the SN by an in vivo count ratio of 3:1 or more and an ex vivo SN–non-SN ratio of 10:1 or more. However, background counts were obtained from the dissected basin and thus would be expected to vary significantly according to the kinetics of the radiopharmaceutical agent and the distance of the gamma probe to the hot SN. More recently, this group of investigators redefined the SN by an in vivo count ratio of 2:1 or more.³⁹ Only 220 (80.0%) of 275 SNs demonstrated this level of radioactivity. They did not report on the concordance of blue dye and radiopharmaceutical techniques, but because only 165 SNs were blue stained, the concordance between the 2 techniques may have been as low as 60%. Krag et al¹⁹ defined an SN by an absolute count of at least 15 per 10 seconds and a count ratio of 3:1 or more. Sentinel nodes were identified in all 118 patients, but because blue dye was not used in all cases and LND was not performed, the true accuracy of the probe cannot be determined by this approach. Mudun et al³⁶ defined an SN by an absolute count of 300 to 3000 per 10 seconds and a count ratio of 30:1

Table 2. Results of Probe-Assisted Selective Lymph Node Dissection

	No. (%)	
	Glass et al ³⁹	Present Study
Total SNs*	275	144
Blue dye success	165 (60.0)	136 (94.4)
Probe success	220 (80.0)	132 (91.7)
SN hot only	110 (40.0)	8 (5.6)
SN blue stained only	55 (20.0)	12 (8.3)
Combined basin success	175/180 (97.2)	98/100 (98.0)

*SNs indicates sentinel nodes.

or more. However, true SNs with ratios less than 30:1 were probably missed, and absolute counts are time dependent and not easily reproducible using the gamma probe. Both Loggie et al³⁷ and Wong et al³⁸ defined the SN by the level of radioactivity alone. Using absolute counts for defining the SN may be misleading because the hottest lymph node may vary with the amount of radiopharmaceutical agent injected and the kinetics of the agent.^{24,40}

We prefer to use a count ratio as our method of choice for defining the SN, with an irrelevant body site as the background. Before making a skin incision in the lymph node basin (before incision) we found mean count ratios of 12.9 ± 20.4 . The count ratio over the blue-stained SN increased approximately 2-fold before (in vivo, 27.4 ± 100.6) and after (ex vivo, 20.1 ± 77.8) its excision. The postexcision ratio (2.8 ± 3.8), determined after excision of all hot blue-stained SNs, was less than the preincision ratio in 96% of patients. In the remaining patients, the postexcision ratio did not drop below preincision levels. We suspect that the elevated counts may relate to the gamma probe detecting counts in the afferent lymphatics or residual non-SNs.

The blue dye technique remains the criterion standard for SN localization, and the gamma probe serves as a useful adjunct. In their initial series, Morton et al^{12,13} used blue dye alone to identify the SN in 194 (81.8%) of 237 lymphatic basins. The accuracy of the procedure was determined by performing LND in all cases. After refining the blue dye technique with LS in all cases, Morton et al²⁹ achieved a 90% success rate in 72 patients. Other institutions^{15,16} reported success rates ranging from 90% to 93% using blue dye alone. In this study, the addition of a radiopharmaceutical agent increased our overall basin success to 98%, a rate similar to that reported by others^{19,20,32,33} using the combined technique. Although some of our improved success may relate to our experience with SLND, we believe that the use of the gamma probe expedites the SN localization process and diminishes the chance of the surgeon transecting the blue-stained afferent lymphatics.

Although the overall SN detection rates reported by other institutions are similar to ours, one cannot be certain that the SNs removed by the probe alone are true SNs. In comparing our series with the study by Glass and associates³⁹ (Table 2), the overall basin success was similar. In our study, 94.4% of the SNs were blue stained, 91.7% were hot, and only 8 (5.6%) were identified with

the probe alone. In contrast, Glass et al found that 60% of the SNs were blue stained, 80% were hot, and 110 (40%) were identified with the probe alone. Because the radiopharmaceutical agents cannot be visualized, we assume the hottest node is the SN, yet this may not be the case. The blue dye allows for visual confirmation of the afferent lymphatics and SN. We believe the lack of concordance between the 2 techniques may lead to higher rates of missed SNs.

Several studies^{19,33,39} using blue dye and probe reported an increased incidence of metastases in SNs detected by the probe alone and raise the question of their clinical importance. None of the 17 SNs containing metastases in our study were identified with the probe alone; 16 (94%) of 17 were blue stained and hot and 1 (6%) of 17 was blue stained and did not meet our definition of a radioactive SN. The increased detection of SNs with metastases by the probe alone reported by other investigators^{20,39} can be partially explained by their relatively low success rate (60%-70%) with blue dye. We found no difference in the count ratios of SNs with or without metastases (Figure 3). Based on the results of this study, we propose that the following guidelines be used for LS and SLND:

1. Preoperative LS for all patients considered for SLND regardless of the site of the primary melanoma. The radiopharmaceutical agents Tc 99m HSA, Tc 99m AC, and Tc 99m SC are equally effective for localizing the sites of the SN. Dynamic imaging is essential for accurate identification of the SN.

2. Selective LND can be performed by combining blue dye with Tc 99m HSA during surgery or by injecting filtered Tc 99m SC up to 4 hours before administration of blue dye. The SN is identified by a blue-stained afferent lymphatic channel leading into a lymph node. The gamma probe is used as an adjunct for guiding the surgeon to the first and any additional blue-stained SNs.

3. If a blue-stained SN cannot be identified, all lymph nodes with an in vivo or ex vivo count ratio of 2:1 or more are removed until the count ratios in the basin are less than 2:1.

The blue dye and gamma probe techniques are complementary. Probe-directed SLND is a useful adjunct to the blue dye technique and improves the rate of SN detection.⁴⁰ Although the radioactivity in the regional lymph nodes is easily detected by the gamma probe technique, the blue dye allows for visual confirmation of the afferent lymphatics leading into the SN. Yet, the blue dye can be difficult to locate, especially in the axilla or when the skin site is not well marked by LS. To improve the rates of SN detection and to standardize the use of the gamma probe technique, further studies are necessary to evaluate the kinetics of the radiopharmaceutical agents through the lymphatics and the retention of these agents in the lymph node basins.

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REFERENCES

1. Snow H. Melanotic cancerous disease. *Cancer*. 1892;2:872-875.
2. Milton GW, Shaw HH, McCarthy WJ, et al. Prophylactic lymph node dissection in clinical stage I cutaneous malignant melanoma: results of surgical treatment in 1,319 patients. *Br J Surg*. 1982;69:108-111.
3. Morton DL, Wanek L, Nizze JA, Elashoff RM, Wong JH. Improved long-term survival after lymphadenectomy of melanoma metastatic to regional nodes: analysis of prognostic factors in 1134 patients from the John Wayne Cancer Clinic. *Ann Surg*. 1991;214:491-499.
4. Das Gupta TK. Results of treatment of 265 patients with primary cutaneous melanoma: a five-year prospective study. *Ann Surg*. 1977;186:201-209.
5. Day CL Jr, Mihm MC Jr, Lew RA, et al. Prognostic factors for patients with clinical stage I melanoma of intermediate thickness (1.51-3.99mm): a conceptual model for tumor growth and metastasis. *Ann Surg*. 1982;195:35-43.
6. Sim FH, Taylor WF, Ivins JC, Pritchard DJ, Soule EH. A prospective randomized study of the efficacy of routine elective lymphadenectomy in management of malignant melanoma. *Cancer*. 1978;41:948-956.
7. Veronesi U, Adamus J, Bandiera DC, et al. Inefficacy of immediate node dissection in stage I melanoma of the limbs. *N Engl J Med*. 1977;297:627-630.
8. Balch CM, Soong SJ, Bartolucci AA, et al. Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. *Ann Surg*. 1996;224:255-266.
9. Balch CM, Murad TM, Soong SJ, et al. Tumor thickness as a guide to surgical management of clinical stage I melanoma patients. *Cancer*. 1979;43:883-888.
10. Balch CM, Milton GW, Cascinelli N, Sims FH. Elective lymph node dissections: pros and cons. In: Balch CM, Houghton A, Milton GW, Sober AJ, Soongs SJ, eds. *Cutaneous Melanoma*. 2nd ed. Philadelphia, Pa: JB Lippincott; 1992:345-366.
11. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;127:392-399.
12. Wong JH, Cagle LA, Morton DL. Lymphatic drainage of skin to a sentinel node in a feline model. *Ann Surg*. 1991;214:637-641.
13. Morton DL, Wen DR, Cochran AJ. Management of early-stage melanoma by intraoperative lymphatic mapping and selective lymphadenectomy: an alternative to routine elective lymphadenectomy or "watch and wait." *Surg Oncol Clin North Am*. 1992;1:247-259.
14. Reintgen D, Cruse CW, Wells K, et al. The orderly progression of melanoma nodal metastases. *Ann Surg*. 1994;220:759-767.
15. Thompson JF, McCarthy WH, Bosch CM, et al. Sentinel lymph node status as an indicator of the presence of metastatic melanoma in regional lymph nodes. *Melanoma Res*. 1995;5:255-260.
16. Karkakousis CP, Velez AF, Spellman JE, et al. The technique of sentinel node biopsy. *Eur J Surg Oncol*. 1996;22:271-275.
17. Essner R, Foshag L, Morton DL. Intraoperative radiolymphoscintigraphy: a useful adjuvant to intraoperative lymphatic mapping and selective lymphadenectomy in patients with clinical stage I melanoma. Paper presented at: Annual Meeting of the Society of Surgical Oncology; March 19, 1994; Houston, Tex.
18. Essner R. The role of lymphoscintigraphy and sentinel node mapping in assessing patient risk in melanoma. *Semin Oncol*. 1997;24:54-60.
19. Krag DN, Meijer SJ, Weaver DL, et al. Minimal-access surgery for staging of malignant melanoma. *Arch Surg*. 1995;130:654-660.
20. Albertini JJ, Cruse CW, Rapaport D, et al. Intraoperative radiolymphoscintigraphy improves sentinel lymph node identification for patients with melanoma. *Ann Surg*. 1996;223:217-224.
21. Leong SPL, Steinmetz I, Habib FA, et al. Optimal selective sentinel lymph node dissection in primary malignant melanoma. *Arch Surg*. 1997;132:666-673.
22. Ronan SG, Eng AM, Briele HA, et al. Thin malignant melanomas with regression and metastasis. *Arch Dermatol*. 1987;123:1326-1330.
23. Soong SJ, Harrison RA, McCarthy WH, et al. Factors affecting survival following local, regional or distant recurrence from localized melanoma. *J Surg Oncol*. 1998;67:228-233.
24. Glass EC, Essner R, Morton DL. Kinetics of three lymphoscintigraphic agents in patients with cutaneous melanoma. *J Nucl Med*. 1998;39:1185-1190.
25. Hung JC, Wiseman GA, Wahner HW, et al. Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy. *J Nucl Med*. 1995;36:1895-1901.
26. Cochran AJ, Wen DR, Herschman HR. Occult melanoma in lymph nodes detected by antiserum to S-100 protein. *Int J Cancer*. 1984;34:159-163.
27. Campbell MJ, Machin D. *Medical Statistics: A Commonsense Approach*. 2nd ed. New York, NY: John Wiley & Sons Inc; 1995.
28. Fee HJ, Robinson DS, Sample WF, et al. The determination of lymph shed by colloidal gold scanning in patients with malignant melanoma: a preliminary study. *Surgery*. 1978;84:626-632.
29. Morton DL, Wen DR, Foshag LJ, Essner R, Cochran A. Intraoperative lymphatic mapping and selective cervical lymphadenectomy for early-stage melanomas of the head and neck. *J Clin Oncol*. 1993;11:1751-1756.
30. Lamki LM, Logic JR. Defining lymphatic drainage patterns with cutaneous lymphoscintigraphy. In: Balch C, Houghton A, Milton GW, Sober AJ, Soong SJ, eds. *Cutaneous Melanoma*. 2nd ed. Philadelphia, Pa: JB Lippincott; 1992:367-375.
31. Norman J, Cruse CW, Espinosa C, et al. Redefinition of cutaneous lymphatic drainage with the use of lymphoscintigraphy for malignant melanoma. *Am J Surg*. 1991;162:432-437.
32. Pijpers R, Collet GJ, Meijer S, Hoekstra OS. The impact of dynamic lymphoscintigraphy and gamma probe guidance on sentinel node biopsy in melanoma. *Eur J Nucl Med*. 1995;22:1238-1241.
33. Taylor A Jr, Murray D, Herda S, Vansant J, Alazraki N. Dynamic lymphoscintigraphy to identify the sentinel and satellite nodes. *Clin Nucl Med*. 1996;21:755-758.
34. Wong JH, Truelove K, Ko P, Coel MN. Localization and resection of an intransit sentinel lymph node by use of lymphoscintigraphy, intraoperative lymphatic mapping, and a hand-held gamma probe. *Surgery*. 1996;120:114-116.
35. O'Brien CJ, Uren RF, Thompson RF, et al. Prediction of potential metastatic sites in cutaneous head and neck melanoma using lymphoscintigraphy. *Am J Surg*. 1995;170:461-466.
36. Mudun A, Murray DR, Herda SC, et al. Early stage melanoma: lymphoscintigraphy, reproducibility of sentinel node detection, and effectiveness of the intraoperative gamma probe. *Radiology*. 1996;199:171-175.
37. Loggie BW, Hosseinian AA, Watson NE, et al. Prospective evaluation of selective lymph node biopsy for cutaneous malignant melanoma. *Am Surg*. 1997;63:1051-1056.
38. Wong JH, Terada K, Ko P, et al. Lack of effect of particle size on the identification of the sentinel node in cutaneous malignancies. *Ann Surg Oncol*. 1998;5:77-80.
39. Glass LF, Messina JL, Cruse W, et al. The use of intraoperative radiolymphoscintigraphy for sentinel node biopsy in patients with malignant melanoma. *Dermatol Surg*. 1996;22:715-720.
40. Bostick P, Essner R, Sarantou T, et al. Intraoperative lymphatic mapping for early-stage melanoma of the head and neck. *Am J Surg*. 1997;174:536-539.

Announcement

The Archives of Surgery will give priority review and early publication to seminal works. This policy will include basic science advancements in surgery and critically performed clinical research.