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

Peter Bostick, MD; Richard Essner, MD; Edwin Glass, MD; Mark Kelley, MD; Terry Sarantou, MD; Leland J. Foshag, MD; Karen Qi, MS; Donald Morton, MD

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. Results of multiple retrospective studies 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 trials fail to confirm a survival benefit for patients who undergo 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.

Even if ELND is reserved for patients in clinical stage I whose primary melanomas meet the criteria for high risk,
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-µm 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 µCi) 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 primary site was injected with approxi- mately 18.5 to 30 MBq (500-800 µCi) 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.

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.14 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 time marked during LS and over each blue-stained basin 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 margins 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.12,20 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 HMB45.20 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 \( P < 0.05 \). 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.21

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 (14.6%). Count ratios varied from less than 1 to 100. We defined by the gamma probe technique increased to 21 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 (16%). 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 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 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 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 an in vivo count ratio
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–background count ratio less than 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 ≥3). 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 ≥3); 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).

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
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 al23 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 al30 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 ≥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 ≥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 al30 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 30:1.
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 al2,13 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 al22 achieved a 90% success rate in 72 patients. Other institutions23,33 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.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 associates, we found that 60% of the SNs were blue stained, 80% were hot, and 110 (40%) 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 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 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. 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.

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

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>Glass et al39</th>
<th>Present Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SNs*</td>
<td>275 (100)</td>
<td>144 (100)</td>
</tr>
<tr>
<td>Blue dye success</td>
<td>165 (60.0)</td>
<td>136 (94.4)</td>
</tr>
<tr>
<td>Probe success</td>
<td>200 (80.0)</td>
<td>132 (91.7)</td>
</tr>
<tr>
<td>SN hot only</td>
<td>110 (40.0)</td>
<td>8 (5.6)</td>
</tr>
<tr>
<td>SN blue stained only</td>
<td>55 (20.0)</td>
<td>12 (8.3)</td>
</tr>
<tr>
<td>Combined basin success</td>
<td>175/180 (97.2)</td>
<td>98/100 (98.0)</td>
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</tbody>
</table>

* SNs indicates sentinel nodes.

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REFERENCES