Sentinel node biopsy. Evolving from melanoma to breast cancer
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CHAPTER 4

RELIABILITY OF LYMPHOSCINTIGRAPHY
IN INDICATING THE NUMBER OF
SENTINEL NODES IN MELANOMA
PATIENTS

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SUBMITTED
Background: This study was undertaken to establish the reliability of lymphoscintigraphy in indicating the number of sentinel nodes in patients with melanoma.

Methods: Lymphoscintigraphy was performed with dynamic imaging after injection of 60 MBq $^{99m}$Tc-nanocolloid (1.6 mCi) and static imaging after two hours in 200 patients with clinically localised primary melanoma of the skin. The following day, sentinel nodes were retrieved with the blue dye technique and a gamma detection probe (Neoprobe® 1000/1500). The discrepancies between the number of sentinel nodes indicated by lymphoscintigraphy and the actual number of sentinel nodes as established by the surgeon were evaluated.

Results: Lymphoscintigraphy showed drainage to 393 sentinel nodes in 255 lymphatic fields in 199 patients. In 48 lymphatic fields (19%) in 46 patients (23%), the number of sentinel nodes was different from the number that was visualised with scintigraphy. Additional sentinel nodes were found by the surgeon because a lymphatic vessel was not seen on the lymphoscintigraphy (43%), because a sentinel node was not visualised separately from other hot nodes or vessels or the injection site (36%), or because a sentinel node was blue and not hot (4%). Fewer sentinel nodes were found than suggested by scintigraphy because a lymphangioma was mistaken for a sentinel node (4%) or because a single elongated node was depicted as two hot spots (6%).

Conclusions: Although lymphoscintigraphy is indispensable for lymphatic mapping, the predicted number of sentinel nodes is accurate in only 81% of lymph node fields. The limited discriminating power of the gamma camera is an important cause of discrepancies.
INTRODUCTION

Sentinel node biopsy is a reliable method for staging regional lymph nodes in patients with melanoma of the skin. Morton introduced the technique in 1992, using a blue dye for intraoperative lymphatic mapping. He performed lymphoscintigraphy only for melanoma on the trunk and head and neck, anticipating ambiguous drainage from these sites. Soon afterwards, the gamma probe was introduced by various investigators for intraoperative identification of the sentinel node. Nowadays, most groups combine the three techniques. The sentinel node can thus be identified in 95-100% of patients with cutaneous melanoma.

Lymphoscintigraphy, gamma probe and patent blue dye are complementary techniques. Lymphoscintigraphy provides a road map for the surgeon, the gamma probe guides the dissection towards a sentinel node before the surgeon can see it, and the patent blue dye provides detailed insight in the anatomic configuration of lymphatic structures. To optimise the sentinel node procedure, it is important to know the shortcomings of each of the techniques. Although some studies compared the blue dye technique with the gamma probe, we have not found a report which describes possible discrepancies between preoperative imaging and intraoperative lymphatic mapping. In this paper, such an analysis is presented to establish the reliability of lymphoscintigraphy in indicating the number of sentinel nodes in patients with melanoma.

PATIENTS AND METHODS

From October 1993 through October 1998, all patients presenting at our institution with melanoma of the skin of at least 1.0 mm Breslow were asked to participate in a prospective study validating the sentinel lymphadenectomy procedure. From August 1995, patients were entered in
the randomised Multicenter Selective Lymphadenectomy Trial, initiated by Donald L. Morton, Santa Monica, CA. Patients were not enrolled when regional lymph nodes were palpable, when there was clinical evidence of distant metastases or when the patient was pregnant. The study protocol was approved by the Institutional Review Board and informed consent was obtained from all patients.

A total of 200 patients was enrolled (93 men, 107 women). The mean age was 48 years (range 16-78). The primary tumour was located on the trunk in 72 patients (36%), the leg in 82 (41%), the arm in 33 (16%) and head and neck in 13 (6%). The median Breslow thickness was 2.1 mm (mean 2.7, range 1.0 - 11.9). The primary tumour had been excised with narrow margins or was still present at the time of lymphoscintigraphy in 178 patients (89%). In 13 patients (7%), the melanoma had been excised with a margin between 0.5 and 1.9 cm, in 9 patients (5%) with a margin between 2.0 and 3.0 cm.

One day before surgery, lymphoscintigraphy was performed with $^{99m}$Tc-labeled human albumen colloid (Nanocoll®, Amersham Cygne, Eindhoven, the Netherlands). A dosage of 60 MBq (1.6 mCi) in a mean volume of 0.3 ml was injected at four to ten sites intradermally closely around the tumour or the biopsy site. Immediately after injection, dynamic scintigraphy was performed during 20 minutes, using a dual-head gamma camera with low-energy, high-resolution collimators. Sixty images were obtained, each of 20 seconds, simultaneously in an anterior and a lateral view. Anterior and lateral static images were made during five minutes, at 30 minutes and two hours after injection. Oblique images were made occasionally if they provided a better view on the draining lymph node basins. A hot spot was regarded to represent a sentinel node if an afferent lymphatic vessel coming from the injection site was visualised. If no afferent vessel was identified, the first hot spot in each basin that appeared on the lymphoscintigraphy images was considered to represent the sentinel node. Hot spots appearing subsequently were regarded as non-sentinel nodes. Faint non-sentinel nodes were also counted. The location of the sentinel node(s) was then marked on the skin with indelible ink. The results of imaging were discussed with the surgeon before the operation.
The 200 operations were performed by one of two surgeons (OEN, BBRK). After induction of general anaesthesia, 1.0 ml patent blue dye (Bleu Patenté V, Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected intradermally completely surrounding the tumour or biopsy site. Measurements were made over the skin marks with a gamma ray detecting probe (Neoprobe® 1000/1500, Neoprobe Corporation, Dublin, Ohio) to confirm the location of the sentinel nodes as seen on scintigraphy. Through an incision of a few centimetres, a blue-stained lymphatic vessel was sought. Such a blue vessel was carefully dissected up to the site where it drained into a lymph node. The probe was used to confirm that a blue node was radioactive. If no blue-stained lymphatic vessels were found, the probe was used to guide the dissection. After removal of the sentinel node(s), the wound was scanned with the probe to confirm that no sentinel node had been left behind. Remaining hot nodes were not removed if the level of radioactivity in and the location of these nodes was in concordance with the visualisation of non-sentinel nodes on lymphoscintigraphy, but only if no additional blue vessels were found.

The findings of the surgeon were considered to indicate the true number of sentinel nodes ("golden standard"). A lymph node was considered to be a sentinel node when a blue afferent lymphatic vessel was identified coming from the injection site and leading to this node. If no blue dye was seen during exploration, which occurs in about 9% of the lymphatic basins, the nodes were sought that were identified as sentinel nodes on scintigraphy. All discrepancies between the findings of the surgeon and the prediction of the nuclear medicine physician were registered prospectively. If the surgeon claimed to have found a sentinel node that was not recognised on the preoperative images, the node was counted as an additional sentinel node only if a separate blue afferent lymphatic vessel was identified.
RESULTS

Lymphoscintigraphy showed drainage to 255 basins in 199 of 200 patients (groin 109, axilla 101, neck 21, other sites such as popliteal fossa, triangular intermuscular space, flank 24). A total of 393 sentinel nodes (mean 2.0 per patient, median 1, range 1-4) was recognised. There were 516 additional hot spots, often faint, which were regarded as non-sentinel nodes (mean 2.6 per patient, median 2, range 0-10).

Nine sentinel nodes that were depicted on the images were left behind by the surgeons. In one of these nine patients, a cervical sentinel node that was visible on the images could not be identified by the surgeon. No blue lymphatic duct was found and the node was situated so close to the injection site that the gamma probe could not be used to localise it. In the remaining eight patients, a sentinel node was visualised but not explored: in four early cases a node in the parotid gland was not removed for fear of damaging the facial nerve, in two cases a sentinel node was not removed because it was located paravertebrally and in two cases in the internal mammary chain. These nine cases were not included in the following analysis because they do not provide information on the reliability of lymphoscintigraphy.

The number of sentinel nodes as established with blue dye and gamma probe was different from the number that was visualised by lymphoscintigraphy in 48 lymphatic basins (19%) in 46 patients (23%). There were 53 discrepancies in these 48 basins. The incidence was similar for each biopsy site: 20% in the groin, 18% in the axilla, 19% in the neck and 17% at other sites. Discrepancy occurred significantly more often in the second half of the study population (31%) than in the first 100 cases (17%) (Fisher’s exact test, 3 DF, \( P = 0.016 \)).

There were several reasons for discrepancy and these are summarised in the Table. In 44 cases of discrepancy, more sentinel nodes were present than recognised on the preoperative images. In 23 of the 53 instances of discrepancy (43%), the surgeon identified a separate blue lymphatic vessel to
Table Reasons for inaccuracy of lymphoscintigraphy

<table>
<thead>
<tr>
<th>Reason</th>
<th>No. of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>More sentinel nodes than seen on scintigraphy</strong></td>
<td></td>
</tr>
<tr>
<td>Lymphatic vessel not visible on image</td>
<td>23 (43%)</td>
</tr>
<tr>
<td>Obscured by another vessel or node</td>
<td>14</td>
</tr>
<tr>
<td>Containing too little radioactivity</td>
<td>5</td>
</tr>
<tr>
<td>Unexplained</td>
<td>4</td>
</tr>
<tr>
<td>Sentinel node not visible on image</td>
<td>21 (40%)</td>
</tr>
<tr>
<td>Obscured by another node</td>
<td>16</td>
</tr>
<tr>
<td>Obscured by injection site</td>
<td>3</td>
</tr>
<tr>
<td>Sentinel node blue and not hot</td>
<td>2</td>
</tr>
<tr>
<td><strong>Fewer sentinel nodes than seen on scintigraphy</strong></td>
<td></td>
</tr>
<tr>
<td>One elongated node, two hot spots</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Lymphangioma</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Radioactive tissue contained no lymph node</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Hot node was non-sentinel node</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

a (sentinel) node that was considered to be a non-sentinel node by the nuclear medicine physician because no separate lymphatic vessel was visible on the images. Most often (14 cases) the lymphatic vessel had probably been obscured by another vessel or another node. Sometimes (five cases) the lymphatic vessel may have been visualised on the scan if it had contained more radioactivity (Fig. 1). In the remaining four cases, it remains unexplained why the blue vessel that the surgeon found could not also be seen on the images. In 21 of the 53 instances of discrepancy (40%), the surgeon found an additional sentinel node that was not seen on the images. In most of these cases (16), a sentinel node was found so close to another sentinel node that the nodes could not be visualised separately (Fig. 2) and were shown as one hot spot. In three cases, a hot sentinel node was obscured by the overlying injection site. This occurred twice in the neck and once in the axilla. In the remaining two cases, a blue non-radioactive sentinel node with a separate afferent lymphatic duct was found in addition to hot sentinel nodes.

In the nine remaining cases of discrepancy, fewer sentinel nodes were removed than expected after lymphoscintigraphy. In three of those nine cases (6% of 53 discrepancies), two hot spots seen on scintigraphy were found to represent two hot and blue parts in a single elongated lymph node.
Fig. 1 This case illustrates that lymphatic vessels which do not contain a lot of activity may not be visualised on the scintigraphy and that removing only the hottest nodes may not be sufficient. Within one minute after injection of the radioactive tracer around the biopsy scar on the left lower leg, one sentinel node was visualised in the left femoral triangle with two lymphatic vessels (1). Only three minutes later, three less hot nodes were seen without separate lymphatic vessels. The hottest node was indeed a sentinel node with an afferent blue lymphatic vessel (1). But the second lymphatic vessel bypassed this node and drained onto a second blue sentinel node just caudal to the inguinal ligament (2).

as a result of separate lymphatic ducts entering the node at opposite ends (Fig. 3). In two other cases (4%), the hot spot on the lymphoscintigram represented retained radioactivity in a lymphangioma (Fig. 4) as was confirmed by the pathologist. In two cases (4%), the radioactive and blue tissue excised did not contain a lymph node but parotid gland tissue or connective tissue. In the remaining two cases (4%), the nuclear medicine physician marked a node as sentinel node which turned out to be a non-sentinel node: the vessel, seen on lymphoscintigraphy, did not come from the injection site but from another (sentinel) node.

The fact that the number of sentinel nodes differed from the lymphoscintigraphy was discovered by following the course of all blue lymphatic vessels in 57% of the discrepancies. The remaining 43% of the
Fig. 2 This case shows that hot nodes which are close together may not be visualised separately on the scintigraphy images. It also shows that lateral images may provide additional information. The images were obtained in a patient with a melanoma on the right forearm. The anterior view shows one hot spot. When one looks carefully at the lateral view, there may be two sentinel nodes back to back. Actually, there were three sentinel nodes. When scanning the wound after removal of two adjacent hot and blue sentinel nodes, there was still a definite hot focus in the axilla. Exploring further, a third sentinel node was found with its own blue lymphatic channel.

Fig. 3 Sometimes the radioactive tracer accumulates at opposite ends of an elongated sentinel node which is then seen on the scintigraphy image as two hot spots, like in this patient with a melanoma at the right knee. We expected to find two sentinel nodes close together in the right groin (1 and 2). A 3 cm long lymph node was found which was blue and hot at both ends.
Occasionally, a hot spot may be due to prolonged accumulation of radioactive colloid in a lymphangioma. This is the lymphoscintigraphy image of a patient who had a melanoma on the medial side of the right calf. The surgeon explored the hot spot on the thigh (1) some 20 hours after injection of the tracer and found some tissue which was clearly hot and blue but which did not look like a lymph node. The node in the groin to which the lymphatic vessel was leading (2) was then also removed. An afferent blue lymphatic vessel was seen. The tissue removed from the upper leg (1) was a lymphangioma. We concluded that the node in the groin (2) was the true sentinel node.

Discrepancies were discovered with the gamma probe: when the expected sentinel nodes were removed and no additional blue vessels were found, an area with remaining radioactivity was explored and an additional sentinel node with a separate blue lymphatic vessel was retrieved subsequently.

Ultimately, 435 sentinel nodes were removed from 247 basins (groin 109, axilla 101, neck 21, other 16) in 199 patients (mean 1.8 sentinel nodes/basin, 1.2 basins/patient, 2.2 sentinel nodes/patient). Eighty-six percent of the sentinel nodes were both blue and radioactive, 13% were only radioactive and 1% was only blue. In almost all blue sentinel nodes, a blue afferent vessel was also identified. Eighty-six non-sentinel nodes were removed (mean 0.4 non-sentinel nodes/patient).

The discrepancies did not lead to upstaging. Forty-eight patients (24% of 200) had metastases in a sentinel node. In nine of them, more sentinel nodes were removed than seen on scintigraphy, but the additional nodes were not tumour-positive or both the first sentinel node(s) found and the additional sentinel nodes contained metastasis.
One patient was understaged when a sentinel node was left behind. This was one of the patients who were excluded from analysis of the reliability of scintigraphy. Three tumour-negative cervical sentinel nodes were removed in this patient. A fourth sentinel node which was seen on scintigraphy, could not be found. Six months later, a single metastatic lymph node was found at that exact site. Five other patients had a recurrence in the mapped basin after removal of tumour-negative sentinel nodes. In all five cases, the same number of sentinel nodes was found by the surgeon as was seen on lymphoscintigraphy and all sentinel nodes were blue and hot and had a separate afferent lymphatic vessel. When revising these cases with the present experience, a sentinel node may have gone unrecognised on the images and was then left behind by the surgeon in three of these five.

**DISCUSSION**

Lymphoscintigraphy accurately predicts the number of sentinel nodes in only 81% of the lymph node basins. Intraoperative use of both blue dye and a gamma probe is of crucial importance to compensate for the limitations of scintigraphy. Nevertheless, lymphoscintigraphy remains indispensable for lymphatic mapping. Sentinel nodes are visualised in 99.5% of patients. Knowledge of the strong and weak points of the three techniques will lead to more accurate sentinel node biopsy.

The limited discriminating power of scintigraphy was the most frequent reason for discrepancies (33 of 53 cases, 62%): in 14 cases a lymphatic vessel could not be seen separately from another vessel or node, in 16 cases two or three sentinel nodes were close together and were depicted as one hot spot and in three cases a hot node was obscured by the injection site. The collimator is an important factor determining the discriminating power of a gamma camera. We use low-energy, high-resolution (LEHR) collimators which have ninety thousand parallel holes of 33 mm long, 1.4 mm in diameter and 0.15 mm apart. With this collimator, it is just possible to discriminate between two small equally intense sources at a distance of about 7 mm from each other with the camera 10 cm away. The distance
between two hot lymph nodes which is needed to visualise them separately increases quickly if one of them is stronger than the other. The discriminating power also decreases when the camera is further away, when the nodes are covered by a thicker layer of tissue, when less time is taken to obtain an image or when the patient moves during acquisition. The LEHR-collimator provides the best discriminating power of the collimators available at our institution. Other collimators, such as general purpose collimators, are more sensitive but have less discriminating power. Because sensitivity appeared to be a problem in only five cases and discriminating power was a problem in 33 cases, we will continue the use of low-energy, high-resolution collimators.

The surgeons at our institution carefully perform blue dye mapping before they use the gamma probe to identify the sentinel nodes. They also check carefully whether hot nodes are left behind after removal of a sentinel node. This may explain why such a high incidence of discrepancies was found. The fact that discrepancy was found more often later in the study may reflect a learning curve for the blue dye mapping.

The blue dye technique provides a lot of information that may easily be missed when using the gamma probe to take the shortest route to the nodes indicated by lymphoscintigraphy. The blue dye indicates which nodes are sentinel nodes because it makes the afferent lymphatic vessels visible, coming from the injection site. The gamma probe cannot make a distinction between sentinel nodes and non-sentinel nodes if more than one node contains radioactivity. Non-sentinel nodes can also become blue when they receive the blue dye from a sentinel node, so the sole fact that a node is blue does not prove that it is a sentinel node. Once mastered, the blue dye technique does not take much time: the median time needed to identify the first sentinel node was seven minutes (range 0-63 min; L. Jansen, unpublished data). Our approach helps to remove as few nodes as necessary. We removed a mean number of 2.2 sentinel and 0.4 non-sentinel nodes per patient whereas an average of 4.5 hot nodes were seen on the images. Removing a lot of non-sentinel nodes that also have become radioactive may cause unnecessary surgical trauma and possible morbidity.
Clinical impact of the shortcomings of lymphoscintigraphy may seem limited: staging was not influenced by the discrepancies. Larger numbers of patients are needed to prove that our careful mapping technique is worth the effort, because thus far only nine of 48 patients with lymph node metastases had a discrepancy.

Based on this study, we have formulated some recommendations.

- Dynamic imaging from the moment of injection of the radioactive tracer should be performed because it provides useful information. During the first 47 procedures, this technique was not used at our institution. It is routinely included now. The lymphatic vessels are usually best visualised shortly after injection. Compressing the dynamic images, which means that the 60 images made during the first 20 minutes are superimposed, further enhances the depiction of the vessels.

- High-resolution collimators should be used and the camera should be positioned as close to the patient as possible to achieve optimal discriminating power. For the same purpose, the acquisition time can be extended to for example 10 minutes. It is then important that the patient lies perfectly still.

- It is important to make images in two projections because two adjacent hot nodes are often seen as one in either of the two views. If possible, the dynamic study should be performed simultaneously in two directions. An oval or irregular shaped hot spot may represent either a large node with multiple lymph vessels entering at different sites, or more than one node close together (Figs. 2 and 3).

- If lymphatic vessels cannot be followed up to the hot nodes on the scintigraphy images and more than one node is visible within a few minutes after injection, one should be aware that there may be several sentinel nodes (Fig. 1). In such cases, the surgeon should be advised to search carefully for blue vessels bypassing a sentinel node and leading to another sentinel node.
• Removing just the hottest nodes is not enough. Nodes that appear to be less hot on the images may also be sentinel nodes as shown in Figure 1. A small sentinel node may accumulate less radioactive colloid than a large node. A node that is located deeper and further away from the gamma camera appears less hot than a superficial node with the same amount of radioactivity.

• It is important to reinsert the probe in the wound after removal of the expected sentinel node because an additional sentinel node may be present (Fig. 2).

• If the sentinel node is close to the injection site, the surgeon has to rely on the blue dye technique and if this fails, the injection site should be excised before proceeding with lymphatic mapping.

• Last but not least, good communication between the surgeon and the nuclear medicine physician is important both before and after the operation, to improve the accuracy of interpretation of the images.

Sentinel node biopsy will remain an experimental procedure until a survival benefit is proven or effective adjuvant therapy becomes available for patients with involved sentinel nodes. Lymphoscintigraphy, the gamma probe and patent blue dye should be combined to optimise the potential benefit from the procedure and limit the number of non-sentinel nodes that is removed.
REFERENCES


