Sentinel Node Lymphoscintigraphy in Cutaneous Malignant Melanoma
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Citation

Abstract
Until fairly recently radical nodal dissection was the only procedure available to identify and remove micrometastatic foci of nodal spread of malignant melanoma. A less invasive technique, biopsy of the first draining node, the sentinel node, has to been shown to accurately stage lymphatic spread.

Identification of the sentinel node is possible using intradermal injection of a blue dye (such as isosulfan blue) and/or radionuclides (sentinel node lymphoscintigraphy). Sentinel node lymphoscintigraphy is advantageous because multiple nodal basins can be "simultaneously" imaged, and the sentinel node can be relatively easily localized with an intraoperative probe.

Successful lymphoscintigraphy requires careful injection technique, rapid imaging, and in many cases acquisition of special views to localize nodes. In this article we will discuss the rationale for, the use of, and the methods for performing lymphoscintigraphy.

INTRODUCTION
Lymphoscintigraphy has been in use for decades to map lymph nodes in several tumors including cutaneous malignant melanoma (1,2). The recent application of lymphoscintigraphy to map sentinel nodes draining cutaneous melanoma was patterned after the use of blue dyes. Surgical biopsy of the sentinel node can replace extensive nodal dissections for staging nodal spread of melanoma. This is because the lymphatic spread of melanoma is orderly (3); the pathological status of the sentinel node is predictive of regional nodal involvement (4,5).

In the following text we will review the basic physiology of the lymph system, as relevant to this technique, and the fundamentals of lymphoscintigraphy, including technique, and will briefly discuss the function and use of the intraoperative probe. We will also briefly review relevant aspects of the pathology and spread of malignant melanoma the technique and current status of lymphoscintigraphy as a tool for the staging of malignant melanoma.

THE LYMPHATIC SYSTEM
The lymph system has developed in higher level animals to drain fluid, cells, and proteins which have leaked from the vascular system into the interstitium. Lymph channels parallel veins, and like the venous system the flow of lymph is from the smallest to the largest channels.

The smallest channels (the initial lymphatics or terminal lymphatic capillaries) are composed of a single layer of endothelial cells that are tethered to the interstitium by anchoring filaments. The edges of the endothelial cells overlap, but are not adhered to one another. This architecture ensures a unidirectional flow of lymph from the interstitial tissue into the lymphatic lumen (lymph movie, figure 1).
Figure 1

Figure 1 (lymph movie): In a resting state the lumen of the capillaries are collapsed and the edges of the cells overlap. When the interstitium is distended with lymph the anchoring filaments pull the endothelial cells apart allowing the fluid, protein and cells to enter the lumen. As the lumen fills the edges of the cells are pushed together and overlap, blocking the egress of lymph back into the interstitium.

When injecting lymphoscintigraphy agents it is important to distend the interstitial tissues to ensure the entrance of these compounds into the capillary lumen. The initial lymphatics interconnect in a fine plexus (network) (channels, figure 2) allowing local multidirectional flow of lymph. In the skin this plexus is located in the dermal layer. The initial lymphatics empty into the collecting lymphatics. The plexus of collecting lymphatics, located in the subcutaneous tissue, is relatively loose and the endothelium lining these larger lymphatics have fewer open cellular junctions (6). Elastic and muscular tissue surrounding the vessels contracts and moves the lymph; internal valves ensure unidirectional flow. A looser plexus and fewer open endothelial junctions results in poorer migration of injectate from a subcutaneous injection compared to an intradermal injection.

Figure 2

Figure 2 (lymph channels): The intradermal network of initial lymphatics drains into the subcutaneous network of collecting lymphatics. The intradermal network has many more openings for the ingress of lymph.

Lymph channels empty into lymph nodes which contain a plethora of protein ingesting macrophages on a reticular matrix. Smaller particles, such as blue dye, pass through the node without retention. Tumor cell emboli travel through the lymph channels and become trapped in the reticular matrix.

MALIGNANT MELANOMA

The lifetime risk of malignant melanoma is rapidly increasing. In 1934 1 in 1500 people developed melanoma; the predicted lifetime risk in 2000 is 1 in 70 people.

PRIMARY LESIONS

Melanomas develop from malignant transformation of the melanocyte, a cell of neural crest origin that produces melanin. There are four major types of malignant melanoma. Superficial spreading (spread, figure 3) accounts for 70% of cases; this malignancy is characterized by lateral spread, and occurs mainly on the trunk and extremities. Nodular (16% of cases) (deep, figure 4) is characterized by vertical spread, occurs mainly on the trunk and extremities, and has a poor prognosis. Lentigo maligna (5% of cases) (lentigo, figure 5) is a facial lesion which is more prevalent in the elderly with a strong history of sun exposure, and can remain relatively stable for 5-15 years. Acral-lentiginous (5-10% of cases) (nail, figure 6) occurs on the hands and feet and can be subungal.
Primary lesions are graded by deepest layer of invasion (clarks, figure 7) or thickness of lesion (Breslow thickness grading—see subdivisions in Table 1). Poorer prognostic features include thicker lesions, male sex, truncal, head and neck location, higher mitotic rate, and ulceration of lesion.

**Figure 3**
Figure 3 (spread): Superficial spreading melanoma.

**Figure 4**
Figure 4 (deep): Nodular melanoma.

**Figure 5**
Figure 5 (lentigo): Lentigo maligna.

**Figure 6**
Figure 6 (nail): Acral-lentiginous melanoma.
Figure 7
Figure 7 (clarks): Clark levels for grading cutaneous malignant melanoma. Clark level I includes tumors restricted to the epidermis (E). Clark level II refers to tumors that have invaded to the level of the papillary dermis (PD), level III describes those tumors reaching the junction of the PD and the reticular dermis (RD). Level IV and V tumors are those which have invaded the RD and the subcutaneous tissue (SC) respectively. Underlying muscle is denoted by the letter M.

Figure 8
Table 1: MELANOMA STAGING American Joint Committee on Cancer

<table>
<thead>
<tr>
<th>STAGE</th>
<th>BRESLOW TUMOR THICKNESS (mm)</th>
<th>CLARK'S LEVEL</th>
<th>TNM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt;0.75</td>
<td>II</td>
<td>T1N0M0</td>
</tr>
<tr>
<td>II</td>
<td>0.75 - 1.5</td>
<td>III</td>
<td>T2N0M0</td>
</tr>
<tr>
<td>III</td>
<td>&gt;1.5</td>
<td>IV</td>
<td>T3N0M0</td>
</tr>
</tbody>
</table>

+ If thickness and Clark's level do not agree thickness is used.
* In-transit: metastatic cells entrapped within dermal lymphatic between primary lesions and regional nodes.

METASTATIC DISEASE
Malignant melanoma spreads via lymphatics (more commonly) as well as hematogenously. Staging of melanoma is defined by the Joint Commission on Cancer and is summarized in Table 1. The likelihood of lymphatic spread is directly proportional to the thickness of the tumor. Lesions less than 0.76 mm thick are unlikely to have metastasized whereas lesions between 2.5 and 4.0 mm have a 24% likelihood of metastatic lymph node involvement; lesions greater than 4 mm thick have a greater than 60% likelihood of regional and 70% likelihood of distant metastatic spread (6).

Palpable nodal involvement in the absence of distant metastatic disease is treated with radical excision of the involved nodal bed. Radical nodal dissection to eradicate micrometastatic nodal disease is controversial in patients with lesions between 1 and 4 mm thick, and is therefore referred to as “elective lymph node resection”. It is not used for patients whose lesions are less than 1mm thick as these are unlikely to have metastasized. Conversely the extent of disease in patients with lesions thicker than 4mm is most often too advanced to benefit from this treatment.

The benefits of elective lymph node resection (ELND) have been controversial (7-9). However, a prospective randomized study has shown lymph node dissection to improve survival in patients less than 60 years, with 1-2mm thick or non-ulcerative lesions (10). Accurate nodal staging has become more important as adjunct therapy with high dose interferon alfa-2b confers a survival benefit for patients with stage III disease. (11).

Radical nodal dissections, either to remove micrometastatic disease or to stage nodal involvement, have significant associated morbidity. These include side effects from general anesthesia, wound complications, lymphocele formation, and persistent limb edema.

PREDICTABILITY OF PATH OF LYMPH DRAINAGE
The pathway of lymphatic drainage from a skin injection is unpredictable, particularly from a wide swath centered on the midline of the torso, pelvis, and head and neck, and around the waistline (12). Clinical prediction of the draining nodal bed in patients with malignant melanoma is incorrect 37% of the time in lesions of the head, neck, in 25% of lesions of the trunk, and in 14% of extremity lesions (13). The use of lymphoscintigraphy, in contrast to vital dye studies, allows visualization of multiple nodal beds. Specialized gamma probes allow intraoperative localization of the sentinel node(s) for removal in lieu of radical node dissection. Multiple nodal beds (>10% of cases demonstrate drainage to sentinel nodes in more than one nodal basin) can be sampled because of the relatively low morbidity of the limited surgical intervention. Staging is more accurate as more
sensitive histopathology (14) can be applied to the limited number of sentinel nodes than can be feasible used with an entire nodal bed.

LYMPHOSCINTIGRAPHY BACKGROUND

In an attempt to narrow the patient population who would benefit from radical nodal dissection, Morton and colleagues (1) introduced intraoperative identification of the sentinel node using blue dye injection. In this technique dye is injected intradermally in close proximity to the primary lesion. This dye travels in the lymphatics and stains nodes. Surgery is timed to coincide with the dye arriving in the nodal basin that is to be explored, approximately 15 minutes after injection. The blue dye technique is quite successful in skilled hands. The primary drawbacks of this approach are the need for fairly precise timing of injection relative to harvesting, and to the difficulty in clinically predicting the correct nodal draining basin.

Lymphoscintigraphy, the intradermal injection of radiolabeled colloid followed by imaging permits identification of the correct nodal draining basin(s) as well as identification of the sentinel node within this(these) basin. Alex and Krag (15) supplemented imaging results with the use of intraoperative radiation probe to guide localization and verify successful removal of the sentinel node(s).

RADIOPHARMACEUTICALS

An ideal radiopharmaceutical for lymphoscintigraphy is characterized by rapid migration from the site of injection, and prolonged retention in lymph nodes. Small particles (<.005 microns in diameter) cross into the blood capillaries. Mid-sized particles (>0.01 and <0.1 microns) readily enter the openings in the lymphatics. Larger-sized particles (>0.5 microns) are slower to enter the lymphatics and have prolonged retention at the site of injection. (16)

Sulfur colloid (particle size 0.1 to 1.0 microns) is approved for use in the United States. A greater yield of smaller particles is ensured by fresh preparation and shortened heating time (both reduce colloidal clumping). Filtration through micropore filters (0.1 or 0.22 micron pore filters) removes larger particles.

TECHNIQUE FOR DERMAL LYMPHOSCINTIGRAPHY.

Small gauge (25 or 27) needles are attached to two to four 1.0 cc syringes containing approximately 200 uCi of ultrafiltered 99mTc-sulfur colloid in approximately 0.1 cc of sterile saline. The injection site is cleaned with a povidine-iodine solution, followed by isopropyl alcohol. Bending the needle 30-45o can facilitate handling of the syringe during injection (bent needle, figure 8). The injection is placed approximately 1 cm away from intact tumors, and 2 cm from previously biopsied sites. Lymphoscintigraphy is not recommended for patients with prior wide local excision of the primary tumor because of potential disruption of local lymph drainage. If, however, the study is deemed necessary the injections should be placed several centimeters away from the wound to avoid fibrotic tissue.

Intradermal injection, approximately 1 cm from the wound or tumor, characterized by a tight painful blanched wheal with accentuation of the hair follicles, is crucial to the success of lymphoscintigraphy (wheal 2, figure 9). The epidermis is approximately 0.1 to 0.2 mm thick except on the palms (0.3 mm) and soles (0.5-0.7 mm). Protection against skin contamination, of both the patient and the physician, can be accomplished by draping the area surrounding the site of injection, and holding a gauze pad over the injection site prior to removal of the needle.
Figure 10

Figure 9 (wheal 2): A proper intradermal injection will result in a small tense blanching wheal (generally with accentuated hair follicles. Subcutaneous injections (not shown) meet with little resistance and yield relatively flat, poorly marginated swellings.

Figure 9 (probe movie): Semiconductors can be thought of as functionally equivalent to gas detectors but with all the advantages of a solid material. Gamma radiation separates the gases in a gas detector into positive ions and negative electrons. Instead of positive ions, positive “holes” are created within the semiconductor (“+”). These “holes” and electrons (e-) are attracted to the anode and cathode on either side of the semiconductor, thereby creating a current, which is detected by the preamplifier. Since the electrons are less tightly bound in the semiconductor material than they are to the molecules of gas there is a greater yield of charges for a similar volume. As a consequence, a relatively smaller volume of solid material is needed, which allows the production of much smaller detectors (19).

Immediate imaging is crucial as the migration of the injectate is generally very rapid (the sentinel node is usually visualized within a few minutes of injection). Lymph channels are followed to the site of the sentinel node; alternate nodal beds are checked frequently for the appearance of other sentinel nodes.

With the patient in surgical position the skin overlying the sentinel node(s) is marked with an indelible marker (outside, figure 10). Lateral, oblique, and sometimes SPECT cine images are recommended for more accurate nodal localization.

Figure 11

Figure 10: The probe is used prior to surgery to plan the incision. The location on the skin with the greatest number of counts detected from the radioactive node is marked for the incision site. Figure 10 (outside): The skin over the sentinel node is marked with blue dye for aid localization with the intraoperative probe.

CASES

The following cases are selected to demonstrate lymphoscintigraphy in different areas of the body, as well as the variability of drainage patterns from nearly identical lesions on different individuals (figures 11-15).
Figure 12
Figure 11: Intraoperatively the probe is used to locate the node within the wound. Care should be taken to direct the probe tip away from the primary injection site while searching for the radioactive node. Prior to use the probe should be sterilized in an autoclave or covered with a plastic sleeve to prevent contamination of the surgical wound.

Figure 13
Figure 12

Figure 14
Figure 13

Figure 15
Figure 14: This 55 year old female presented one month after resection of malignant melanoma, unidentified type, Clark level IV (1.85 mm) on the lower right side of her neck.
Figure 16
Figure 15: This 25 year old patient presented three months after excisional biopsy of a Clark level IV (1.9 mm) melanoma (alternate pathology was spindle and epitheloid neoplasm) from the anterior surface of her ankle.
INTRAOPERATIVE GAMMA PROBES

Several manufacturers produce intraoperative probes for localization of radioactive nodes. The probes are lightweight, hand held detectors connected to rechargeable control units with visual and audio signal output. The probe contains a crystal, such as NaI, or a solid state semiconductor (probe movie, figure 16, 17), such as cadmium telluride, adjacent to a charge sensitive preamplifier. Accurate localization of the radioactive nodes is possible due to very effective lateral collimation (shielding) so that only emissions directly in front of the probe are counted. The tungsten shielding blocks 99-99.9% of $^{99m}$Tc photons.

**Figure 17**

Figure 16

Generally there is more than one type of probe available for use with each control unit (17). Specialized probes available for use with higher energy photons are designed with thicker shielding and detector material (crystal or semiconductor). Probes with smaller fields of view can be useful for cases in which the nodes are in close proximity to the injection sites, such as in breast lymphoscintigraphy.

The energy window for the semiconductor probes is set from above 80 kEv (the energy of tungsten fluorescence) up to approximately 200 kEv (or higher). Sensitivity is approximately 60-70%. Modification of auditory output allows differentiation of count rates up to a few thousand cts/sec. These modifications generally are such that count rates are converted into oscillating frequencies or warbles in which the pitch frequency is proportional to the number of counts.

A modicum of training is necessary to properly use the probe. Users must be taught to direct the face of the probe away from the injection site while scanning the skin or operative site for radioactive nodes.

Once located (inside, figure 18), the sentinel node is excised and its counts are measured (away from the patient). The node should be at least two to three times as radioactive as the background tissue, generally it is 10 to 100 times as radioactive. The wound is then checked with the probe for residual radioactive tissue.
ACCURACY OF SENTINEL NODE FOR STAGING REGIONAL METASTASES

More than 95% of lymphoscintigraphy procedures successfully identify a sentinel node. In earlier studies in which the entire nodal bed is resected the false negative rate (cases in which the sentinel node is pathology negative, but another node within the nodal basin is pathologically positive) for the sentinel node is up to 5%. (18). For studies in which the sentinel node alone was harvested and was pathologically negative the recurrence rate in the same nodal basin is up to 11% (19,20). Several authors note that the false negative rates declined as the studies progressed; higher initial rates are presumed to be due in part to inexperience, missed in-transit nodes, and sub-optimal initial pathology. In addition, a higher relapse rate is seen in patients with thicker primary lesions.

SUMMARY

Minimally invasive biopsy of the sentinel node is becoming a more widely used procedure in place of radical nodal dissection for the staging of nodal spread of cutaneous malignant melanoma. Lymphoscintigraphy for identifying the sentinel node, used alone or in conjunction with blue dye injections, is an effective and relatively easily performed procedure. Careful, early imaging, skin marking, communication of results to the referring surgeon, and knowledgeable use of the intraoperative gamma probe are essential for the successful application of this technique.

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