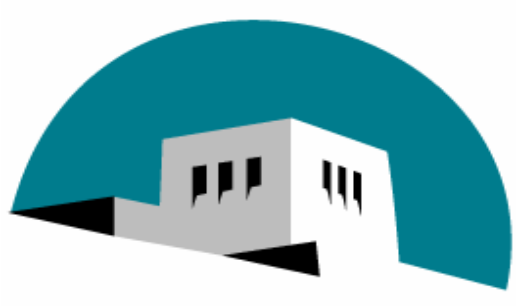

THE UNIVERSITY OF NEW MEXICO
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Correspondence Continuing Education Courses
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Lymphoscintigraphy and Sentinel Node Biopsy in the Staging of Cancer

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LYMPHOSCINTIGRAPHY AND SENTINEL NODE BIOPSY IN THE STAGING OF CANCER

STATEMENT OF OBJECTIVES

The purpose of this lesson is to increase the reader's knowledge and understanding of the basic concepts of lymphoscintigraphy and intraoperative sentinel node biopsy used for the clinical staging of various cancers and the historical development of radiopharmaceuticals used for this technique.

Upon completion of this lesson, the reader should be able to:

1. describe the lymph system and the sentinel node concept of metastatic spread of cancer.

2. discuss skin and breast cancer, including the risk factors, disease spread and importance of sentinel node biopsy for both.

3. discuss the importance and development of the use of sentinel node biopsy in other cancers.

4. describe the injection procedures, nuclear medicine imaging and intraoperative sentinel node biopsy techniques including patient preparation and radiation exposure concerns.

5. discuss the historical development of radioactive materials used for lymphoscintigraphy.

6. discuss the preparation, clinical use and availability of radiopharmaceuticals currently used for lymphoscintigraphy and sentinel node biopsy.

7. discuss the ideal properties and future development of radioactive compounds for lymphoscintigraphy and sentinel node biopsy.

8. discuss the basic properties of intraoperative probe detectors used for sentinel node biopsy.

9. describe the interventional agents and their clinical use for lymphoscintigraphy and sentinel node biopsy.

10. discuss the radiation safety concerns for the use of radioactive materials in the operating room and the handling of sentinel node biopsy tissue specimens.

COURSE OUTLINE

I. INTRODUCTION

A. Development of the Sentinel Node Concept

B. Skin Cancer

C. Technique

II. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF RECURRENT MALIGNANT MELANOMA: PATIENT STUDY

A. Breast Cancer

B. Sentinel Node Biopsy in Breast Cancer Patients

III. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF BREAST CARCINOMA: PATIENT STUDY

IV. OTHER CANCERS

V. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF TONGUE CARCINOMA: PATIENT STUDY

VI. RADIOPHARMACEUTICALS FOR LYMPHOSCINTIGRAPHY AND LYMPHATIC MAPPING-HISTORICAL DEVELOPMENT

A. Technetium Tc-99m Dextran

B. Technetium Tc-99m Hydroxyethyl Starch

C. Technetium Tc-99m Antimony Sulfide Colloid

D. Other Agents

VII. RADIOPHARMACEUTICALS FOR LYMPHOSCINTIGRAPHY AND LYMPHATIC MAPPING -RECENT CLINICAL EXPERIENCE

A. Technetium Tc-99m Human Serum Albumin (HSA)

B. Technetium Tc-99m Albumin Nanocolloid (Nanocoll™-Amersham Health)

C. Technetium Tc-99m Sulfur Colloid

D. Filtered Technetium Tc-99m Sulfur Colloid

E. Technetium Tc-99m Sulfur Colloid Compounding Variations

LYMPHOSCINTIGRAPHY AND SENTINEL NODE BIOPSY IN THE STAGING OF CANCER

By
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I. INTRODUCTION

Lymph nodes are part of the lymph system, a very large network of fluid, nodes and nodules, organs, ducts, glands and vessels that continuously and aggressively cleanse the body of waste matter and distribute infection-fighting lymphocytes. Innumerable nodes, some no bigger than a pinhead, others the size of a dime, guard the passages into the body against the intrusion of destructive substances. Except for cartilage, nails and hair, the entire body is bathed in lymph fluid, amounting to three times the blood volume, through a network of vessels over 100,000 miles in length![1]

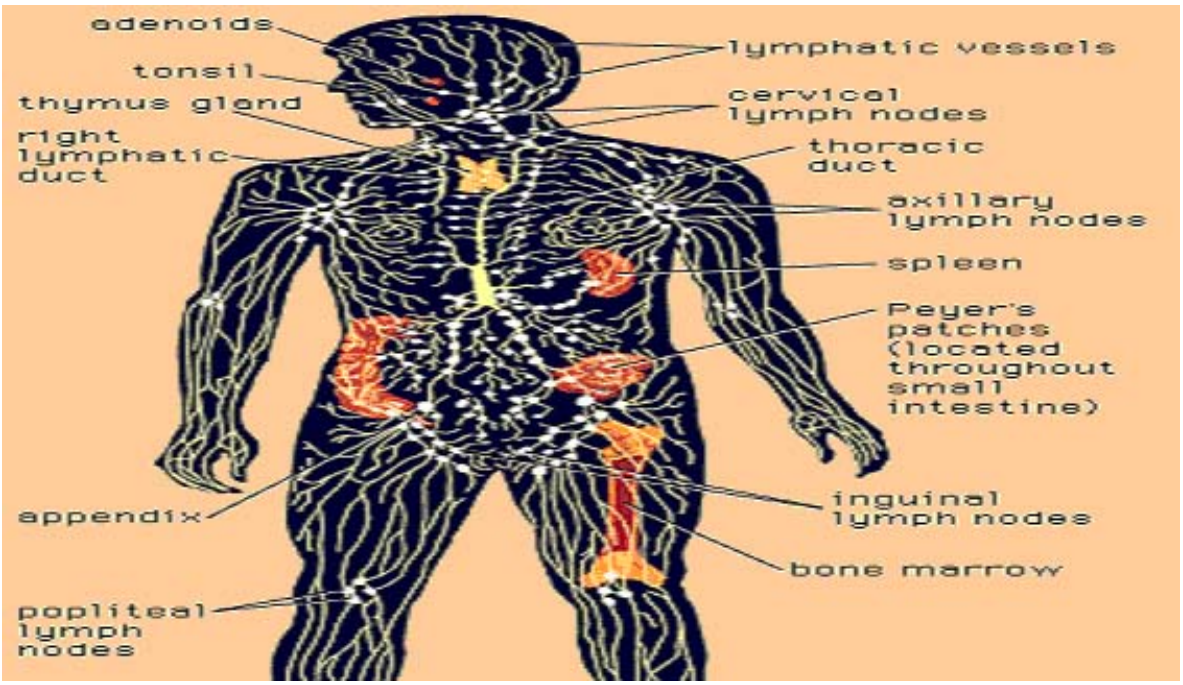
The lymph system carries waste fluid from cells in one direction away from tissues and organs. Due to their role in the movement of materials from organs and tissues, some of the most common tumors are spread via the lymphatic route. Breast cancer, malignant melanoma and colorectal carcinoma tend to spread by this method. Treatment decisions for many malignancies are made only after the nodal status of the patient has been determined.

The metastatic spread of tumor via lymphatic drainage from various areas of the body is often predictable. Breast cancers, for example, tend to spread to either the axillary or retrosternal lymph nodes depending on the site of the primary lesion. However, in patients with truncal melanoma, it may be difficult to determine the drainage path for disease while almost all melanomas that occur on the lower extremities will have lymphatic spread to the inguinal nodes or popliteal nodes behind the knees.

In the past, sampling of multiple regional lymph nodes to determine the presence of tumor in primary tumor draining nodes led to lengthy surgical procedures often involving extensive morbidity for the patient. Although a number of techniques have attempted to predict the drainage of a tumor bed, none have proven reliable. It is desirable to have a reliable method for identifying the lymph node drainage pattern prior to surgery in order to sample the lymph nodes most likely involved with cancer for pathological confirmation of disease status.

The sentinel lymph node (SLN) is defined as the first node draining the primary tumor to which a malignancy is likely to metastasize. Identification of the SLN for biopsy and pathological inspection would simplify the staging of many cancers. If pathological evaluation fails to identify tumor in the SLN, then downstream nodes are most likely free of tumor as well. Unless a palpable lymph node is identified during physical examination, localization of the SLN can be difficult. Historically, delivery of various dye materials into the lymphatic system by injection at the primary tumor site provided some guidance for SLN identification. Typically, these procedures involved extensive surgical sampling in order to make certain the correct node was obtained since exploration of the tumor bed was necessary in order to locate the node containing the dye.

More recently, the use of radioactive materials instead of or in addition to dyes have allowed external imaging and/or radiation detector monitoring for the identification of the sentinel node prior to surgery. In



addition, the intraoperative use of gamma probe detectors permits the surgeon to confirm the external sampling procedures by directly counting the various lymph nodes discovered through a small incision. These techniques dramatically reduces the surgical procedure times from hours to minutes, provides a significant increase in the accuracy of sentinel node identification and sharply decreases the morbidity associated with the staging procedure.

Advantages of Lymphoscintigraphy and Sentinel Node Localization

- Lymphatic drainage from sites on the head, neck and trunk are often not predictable.
- Wide field-of-view, dynamic imaging allows localization of all draining nodal basins in order of appearance from primary tumor.
- A negative excisional biopsy of the sentinel node obviates radical dissection and subsequent morbidity.
- Multiple sentinel nodes in different nodal basins can be sampled for biopsy without significant morbidity.

A. Development of the Sentinel Node Concept

The sentinel lymph node (SLN) is the first lymph node in the afferent lymphatic drainage pathway from a tumor. Assuming the first lymph node draining the tumor has the greatest probability of metastatic lymph node involvement, identification and evaluation of the sentinel node in pathology would serve as a predictor of the nodal status of lymph nodes further along in the drainage pathway. SLN biopsy as a predictor of metastatic spread though lymph channels was originally described in penile carcinoma using lymphangiograms, anatomic dissections and/or microscopic evaluation.[2] Technical details of a clinically useful procedure were first described for staging melanoma.[3] The concept of the SLN biopsy for staging cancer adapted from the use of vital blue dyes by Morton's group. This study describes the injection of one of two dyes (patent blue-V or isosulfan blue) around the primary lesion in a series of 223 patients. The study reported the identification of the sentinel node in 82% of lymphatic basins with less than 1% of patients with non-sentinel nodes identified as

the initial site of metastasis. The technique of SLN identification or selective lymphadenectomy for pathological review allowed a diagnosis of nodal metastasis in melanoma patients providing a rational and practical alternative to broad lymph node dissection or wait-and-watch treatment of patients with melanoma. Although the clinical use of SLN biopsy has grown rapidly involving a variety of both metastatic and benign diseases, the technique is most commonly used in melanoma and breast cancer where it has quickly become the standard of care as a staging method.

B. Skin Cancer

The skin is the body's largest organ. It covers the internal organs, protecting them from injury and serving as a barrier against infection. The skin also helps regulate body temperature and prevents the loss of excessive water and other fluids.

The outer layer of skin, or epidermis, contains melanocytes, which are skin cells that produce a protective pigment called melanin. Melanin production provides the "healthy tan" upon exposure to the sun. Actually, the darkening of the skin is a protective measure to prevent damage to deeper layers of the skin.

The skin's middle layer, or dermis, contains hair follicles, sweat glands, blood vessels and nerves that are held in place by collagen, a protein that gives the skin its strength and resilience.

Following the dermis is the subcutaneous layer of skin providing the greatest protection for lower layers. It contains a network of collagen and fat cells designed to conserve heat and form a buffer for the internal organs.

Although exposure to x-rays, chemicals (ex. arsenic compounds, pitch, creosote, coal tar, radium), viruses, immunosuppression, certain inherited disorders and chronic scars or skin disorders have all been associated with skin cancer, the most common cause is exposure to ultraviolet radiation (the sun). Cancer develops gradually after the skin suffers repeated damage by the sun's rays. Similar to other types of radiation, radiant energy from the sun can damage the DNA within the skin's cells. Eventually, the cells are unable to repair themselves and they lose the ability to control cell division and growth. Skin cancer occurs when abnormal cells grow and multiply within one of the three layers of skin, mainly the epidermis. It attacks one out of seven Americans each year with an estimated one in every three fair-haired, light-skinned Americans suffering from skin cancer. The total amount of sun exposure received over many years and single overexposures resulting in sunburn both can cause skin cancer. Despite the knowledge that the main cause of skin cancer is overexposure to the sun and that the disease can be prevented with appropriate use of sunscreen, the incidence of the disease, particularly melanoma, is on the rise.

Skin cancer occurs more often than all other cancers combined in the United States with an estimated 55,000 melanomas, 250,000 squamous cell carcinomas and 920,000 basal cell carcinomas each year. One in every three cancers is skin cancer.

Melanoma is a cancer that begins in the melanocytes. Melanoma tumors are most often brown or black because the cells continue to produce the skin pigment, melanin. Although melanoma is rare (55,100 new cases expected in 2004), accounting for only 4% of all skin cancer diagnoses, it leads to 80% of deaths associated with skin cancer. An estimated 10,210 deaths expected this year; almost all (7,910) from melanoma and only 2,300 from other skin cancers.[4]

Similar to other skin cancers, melanoma is almost always curable in the early stages of the disease. However, it is much more likely to metastasize than basal or squamous cell cancer. Rarely, melanomas can form in parts of the body not covered by skin such as the eyes, mouth, vagina, large intestine and other internal organs.

Greater than half of all cases of melanoma are seen in people older than 50 with half of melanoma deaths occurring in white men of that age group. Due to exposure patterns, women usually have lesions on the lower legs while men are more likely to have lesions on the back or trunk. The risk for developing melanoma is about twenty times higher for Caucasians than for African Americans due to the protective effect of darkly pigmented skin. White, fair skin that freckles or burns easily along with skin with moles or birthmarks are at greater risk for developing melanoma.

More common skin cancer types include basal cell and squamous cell carcinomas. Basal cell carcinomas, affecting nearly a million Americans annually, rarely leads to death when diagnosed and treated appropriately. Typically located on the face, neck and other areas that receive direct exposure to the sun, these lesions are easily identified by regular physician visits. Accounting for 20% of all skin cancers, squamous cell carcinomas are highly curable when diagnosed early. Again, sun-exposed areas of the skin (face, ears, neck, lips and back of the hands) are more likely to be involved.

Any change on the skin, especially in the size or color of a mole or other darkly pigmented growth or spot can herald cancer. Further development of the disease can include bleeding, oozing, scaliness or simply a change in the appearance of a nodule. Tenderness, itchiness even pain can be experienced. Early detection is common with self-examination of the skin and follow-up with a health care professional for suspicious lesions. Removal of the cancer has long been the method of treatment for localized disease.[5]

Like all cancers, long-term survival depends on early diagnosis and treatment before penetration into deeper layers of the skin and eventually metastasis to other organs and tissues occurs. Initially, treatment for all skin cancers is the same. Physicians excise a small sample of the lesion's cells through a biopsy. Following pathological investigation of the sample cells to confirm the presence of cancer, the entire lesion is then removed to determine the level of penetration of the cancer into the skin. Pathological evaluation, including the Breslow Thickness, is used to determine the therapeutic option of further surgery, radiation therapy or chemotherapy. The thinner the melanoma, the better the prognosis and less invasive the therapy. Generally, melanomas <1 mm thick have a very small chance of spreading. The pathology term, Clark's Level, describes the thickness of the melanoma relative to the layers of skin surrounding the lesion. The Clark's Level of a melanoma uses a scale of I–V to describe whether the cancer has spread to the upper dermis, lower dermis or subcutis (fatty layer of the skin) with higher numbers indicating deeper penetration and corresponding greater chances of metastases.

Risk Factors for Skin Cancer

- Outdoor occupation.
- Cumulative sunlight exposure.
- Light skin complexion (light hair, fair skin, light eyes).
- Older more than younger
- Men more than women.

C. Technique

Preoperative lymphoscintigraphy is completed in nuclear medicine to identify the lymphatic drainage of the tumor basin including the nodes at risk for metastatic spread of the primary tumor. The images guide the surgeon to the lymph node(s) most likely representing the sentinel lymph node(s). Most likely the patient will present with a lesion or scar where an initial biopsy specimen was removed. Although injection techniques vary, typically they include intracutaneous or intradermal injections around the primary melanoma site or injections deep into the breast near the site of lumpectomy. A large

field of view (LFOV) camera is used allowing more lymphatic channels and drainage basin area to be included in the images. A flow study, started immediately after injection of the radioactive material, allows imaging of the lymphatic channels as the material is removed from the injection site and flows towards the lymph node basin. A dual-head camera enables the collection of anterior and posterior images simultaneously.[6]

Preoperative lymphoscintigraphy creates an image that can be used to determine which of the lymph node basins tumor cells most likely migrated to and where the SLN will be found. This procedure often provides drainage patterns different from the expected based on anatomical guidelines increasing the accuracy of staging during SLN biopsy at surgery.[7-10]

Intraoperative lymphatic mapping is started after the patient is anesthetized and prepared for surgery. Blue dye in injected in a manner similar to the administration of radioactive material for lymphoscintigraphy. Approximately ten minutes is allowed for the dye to be removed from the injection site into the lymphatics often with hand massaging of the injected area. A small incision is made for the SLN biopsy. Afferent lymphatic channels stained blue are identified and followed to the SLNs which also stain pale blue. If blue lymphatic channels and/or blue lymph node(s) are not obvious, the intraoperative probe detector can be used to identify "hot" lymph node(s) previously seen on the nuclear medicine images. The SLN is harvested and submitted to pathology as a separate specimen for pathological examination. All blue staining nodes or "hot" nodes are harvested and labeled SLNs. The intraoperative probe detector is then used to survey the surgical field to confirm the radioactivity remaining in the nodal basin has fallen to background levels. This ensures that all SLNs have been removed for staging.

II. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF RECURRENT MALIGNANT MELANOMA: PATIENT STUDY

This patient, a 63 y/o white female, had a past medical and surgical history for malignant melanoma excised from the right ankle three years earlier. The thickness of the melanoma at the time of surgery was unknown. A year after surgery, upon seeking a second opinion, she was treated for a two year period with low dose alpha-interferon. The patient now presents with a palpable nodule located at the previous excision site.

The nodule found at previous site of excision was removed and determined to be recurrent malignant melanoma. Again, the thickness of melanoma is not known. Further evaluation including CT of the abdomen, pelvis, and chest were negative. The patient was scheduled for SLN biopsy and wide excision of the site of melanoma.

Lymphoscintigraphy was planned for day of surgery. Four intracutaneous injections of filtered Tc-99m sulfur colloid, each containing 100 μCi, were injected 1 cm–2 cm from the previous surgical excision in the four quadrants around the site. Over a period of 60 minutes, images from the pelvis downward were obtained. Radioactivity rapidly ascended to the right groin and centralized in the SLN as seen in Figure 1. Although it didn't persist throughout the study, a transient node in the right popliteal fossa was also seen. Finally, secondary nodes were also identified in the right groin. Using indelible ink, the SLN was marked on the patient's skin and the patient sent to surgery.

Prior to surgical incision, 1.25 mL of isosulfan blue dye was given in four divided dosages into the four quadrants at the recent surgery scar. Using a gamma-detecting probe, radioactive "hot" areas were located prior to surgical incision in both the right popliteal area and in the right groin below the inguinal ligament. An incision was made in

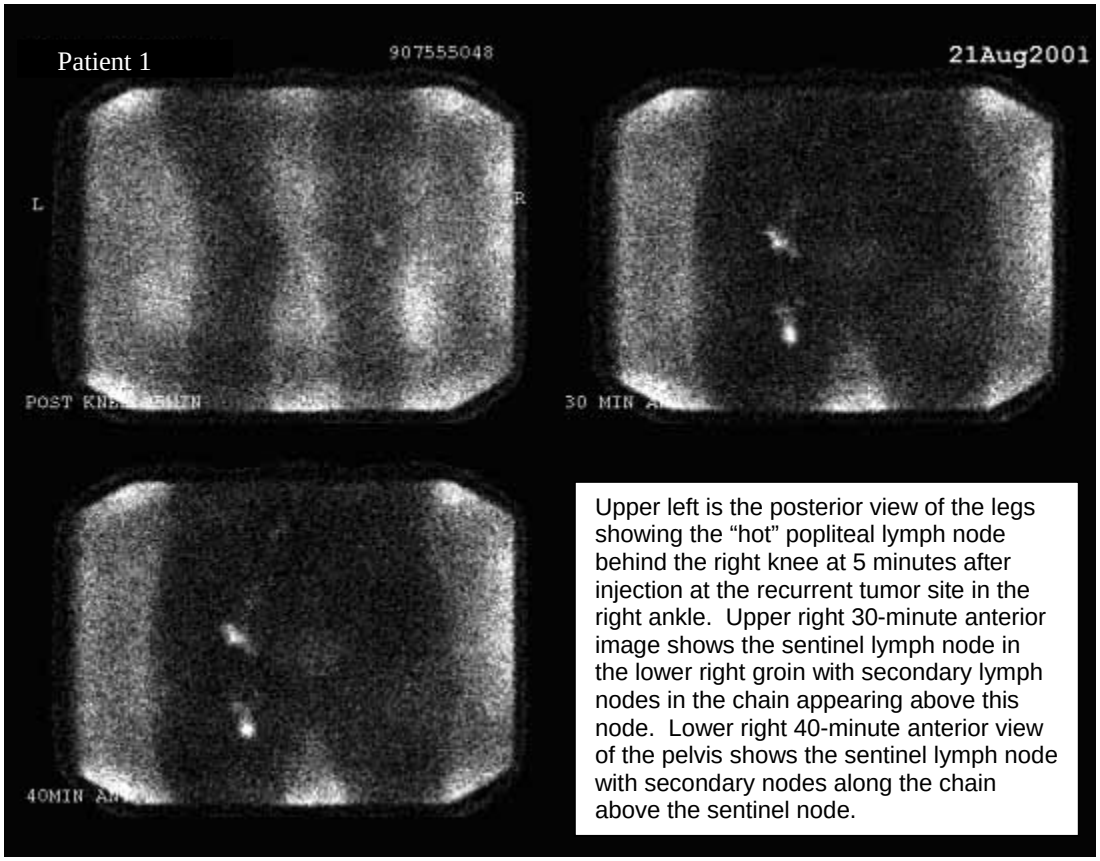
the popliteal fossa and dissection made using the intraoperative gamma probe for guidance until a "hot" lymph node was located. This node was radioactive but not blue in color. The node was removed and sent to pathology labeled as SLN #1. Gamma probe counting of the popliteal fossa area provided no additional "hot" areas.

Dissection of the right groin proceeded after the gamma probe detector revealed a "hot" area below the inguinal ligament. After transverse incision through the dermis, the gamma probe detector helped locate a large, "hot" lymph node, again with no blue dye coloration visible. The lymph node was excised and sent to pathology labeled as SLN #2. Surgery continued with a wide excision of the site of the recurrent melanoma over the right Achilles tendon. The lesion was removed with 1 cm wide border around the scar area and sent to pathology. Microscopic examination completed in pathology revealed no tumor in either of the SLNs submitted. The specimen removed from the patient's right ankle contained malignant melanoma cells.

A. Breast Cancer

Breast cancer is a major health issue in the United States with 215,990 new invasive cases in women and 1,500 cases in men predicted for 2004.[4] It is the most frequently diagnosed non-skin cancer in women with breast cancer incidence rates continually increasing since 1980, mostly in women age 50 and over. In addition to invasive breast cancers, almost 58,000 new cases of in situ breast cancer are expected to occur in women

Figure 1. Lymphoscintingraphy of Malignant Melanoma Patient



Upper left is the posterior view of the legs showing the "hot" popliteal lymph node behind the right knee at 5 minutes after injection at the recurrent tumor site in the right ankle. Upper right 30-minute anterior image shows the sentinel lymph node in the lower right groin with secondary lymph nodes in the chain appearing above this node. Lower right 40-minute anterior view of the pelvis shows the sentinel lymph node with secondary nodes along the chain above the sentinel node.

during 2004. This increase in the number of in situ cases is thought to be due to the increased use of screening mammography which often finds cancer before the mass becomes palpable.

Although mortality rates have declined by 1.4% per year during 1989–1995 and by 3.2% afterwards, probably the result of earlier detection and improved treatment measures, an estimated 40,560 deaths (40,110 women, 450 men) are anticipated from breast cancer in 2004. Breast cancer ranks second among cancer deaths in women.

Early breast cancer can be discovered as an abnormality on a mammogram even before it can be felt by the patient or health care provider. Breast cancer appearing as a breast lump, thickening, swelling, distortion or tenderness is typically the stage of discovery. Pain in the breast is not usually the first symptom of breast cancer.

The risk of being diagnosed with breast cancer increases with age. Higher risk has also been associated with personal or family history of the disease, atypical hyperplasia, increased breast density, a long menstrual history, obesity after menopause and estrogen/progestin use.

Considering patient preferences and medical circumstances, treatment may involve lumpectomy with removal of the lymph nodes draining the lesion if biopsy indicates the cancer has spread to the nodes. Mastectomy; radiation therapy; chemotherapy or hormone reduction efforts may be offered in some cases depending on the extent of disease at diagnosis.

It is estimated that 80% of patients with early stage breast cancer have no lymph node involvement.[11] Surgical treatment for stage I and II disease, accounting for 80% of patients, involves lumpectomy and axillary dissection. Axillary dissection of the lymph nodes is done primarily for staging purposes since the presence of lymphatic spread is associated with decreased survival rates.[12-14] Therapeutic decisions are made with consideration of the presence and location of metastatic spread of the disease.

Breast cancer is slow growing with a 100-day doubling time. This is one of the contributing factors in the difficulty in detecting the disease for 8–10 years from inception. Breast cancer can be invasive or non-invasive although the non-invasive type is thought to preclude a change to invasive cancer. Staging of breast cancer describes the primary tumor size and the extent to which it has spread.

Risk Factors for Breast Cancer

- Female gender.
- Increasing age.
- Family history.
- Age at first birth or lack of children.
- Race (Latinos may have increased risk and severity of breast cancer)
- Alcohol intake.
- Estrogen use.

Confirmation of axillary involvement by physical examination leads to high false-positive and false-negative rates.[15-16] Suspicious palpable axillary nodes frequently have histological evidence of metastatic disease. Conversely, it has been shown that approximately 30% of non-palpable axillary nodes also have histological evidence of cancer. These shortcomings in physical examination, as well as other diagnostic tools, results in the large number of stage I and II disease patients undergoing axillary dissection. Only 25 to 50% of these patients will prove to have positive lymph nodes leaving 50% to 75% of patients (more than 75,000 patients) undergoing unnecessary surgery. Improved selection of patients for axillary dissection procedures would reduce the incidence of morbidity (lymphadema of the arm, upper arm weakness and/or loss of mobility, nerve damage-sensory loss, arm and/or axillary numbness, wound infection, chronic pain, susceptible to infection in arm, seroma, hematoma, post-

operative fluid collections), decrease medical costs and lead to earlier adjuvant therapy in those patients requiring follow-up care.

Sentinel lymph node biopsy is a minimally invasive technique used to determine the status of the axillary nodes without the need for a full axillary node dissection. Lymphadema, swelling of the arm, is common following an axillary node dissection. However, SLN biopsy carries less than 1% likelihood of lymphadema.[17]

Researchers have confirmed that lymphatic drainage of breast cancer can be identified and traced to the first draining lymph node, the SLN. This node accurately predicts the status of the entire axilla. If the sentinel lymph node is negative, then the remainder of the lymph nodes in the axilla may be cancer free.[11] The lymph node status of the patient with breast cancer remains the most powerful factor for predicting recurrence and survival.

B. Sentinel Node Biopsy in Breast Cancer Patients

Axillary lymph node drainage of breast cancer was first illustrated in 1972 with the injection of a vital blue dye during mastectomy.[18] Over twenty years later, SLN biopsy has proven a minimally invasive technique useful in determining the status on the axillary nodes without the need for a full axillary node dissection.

As identified earlier, the SLN is the first node(s) in the body to come into contact with the cancer cells as they leave the organ or origination and begin to spread into the rest of the body's tissues. Identification of the SLN in breast cancer patients allows the removal of the sentinel node only for presentation to the pathologist for a more closely scrutinized specimen aiding in the detection of cancer. There is typically a smaller incision, which may result in shorter recovery time and less postoperative pain compared with complete axillary lymph node dissection.

Women who have undergone a breast biopsy and diagnosed with invasive breast cancer are candidates for SLN biopsy as an alternative to traditional axillary lymph node dissection. On the other hand, patients who have obviously involved lymph nodes should have a complete axillary node dissection in an effort to remove all cancer accessible during surgery. Patients who have had large open surgical biopsies or lumpectomies may have disrupted lymphatic drainage which may interrupt the flow of lymph from the injection site into the axilla. These patients should be very carefully examined with SLN biopsy techniques as the sentinel node may be difficult to locate.

III. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF BREAST CARCINOMA: PATIENT STUDY

A 43 year old female presented with newly diagnosed upper outer quadrant left breast cancer with nipple retraction. The mass was identified by mammogram and ultrasound. Prior to mammogram, patient noticed thickening of the skin in the upper outer quadrant area of the mass with skin retraction. Biopsy indicated an infiltrating mammary carcinoma with associated microcalcifications. The specimen stained 22% estrogen receptor positive and 69% progesterone receptor positive. Patient reports no other previous problems with respect to her breast health care. She does do breast self-exams. After menarche at age 12, the patient reports normal, regular and continuing menstrual periods. First child was born at age 19. She has never used hormone replacement therapy but has a history of oral contraceptive use. She reports no family history of cancer. No alcohol use but does smoke one pack a day. No medications, no allergies, some fatigue lately and a history of mitral valve prolapse.

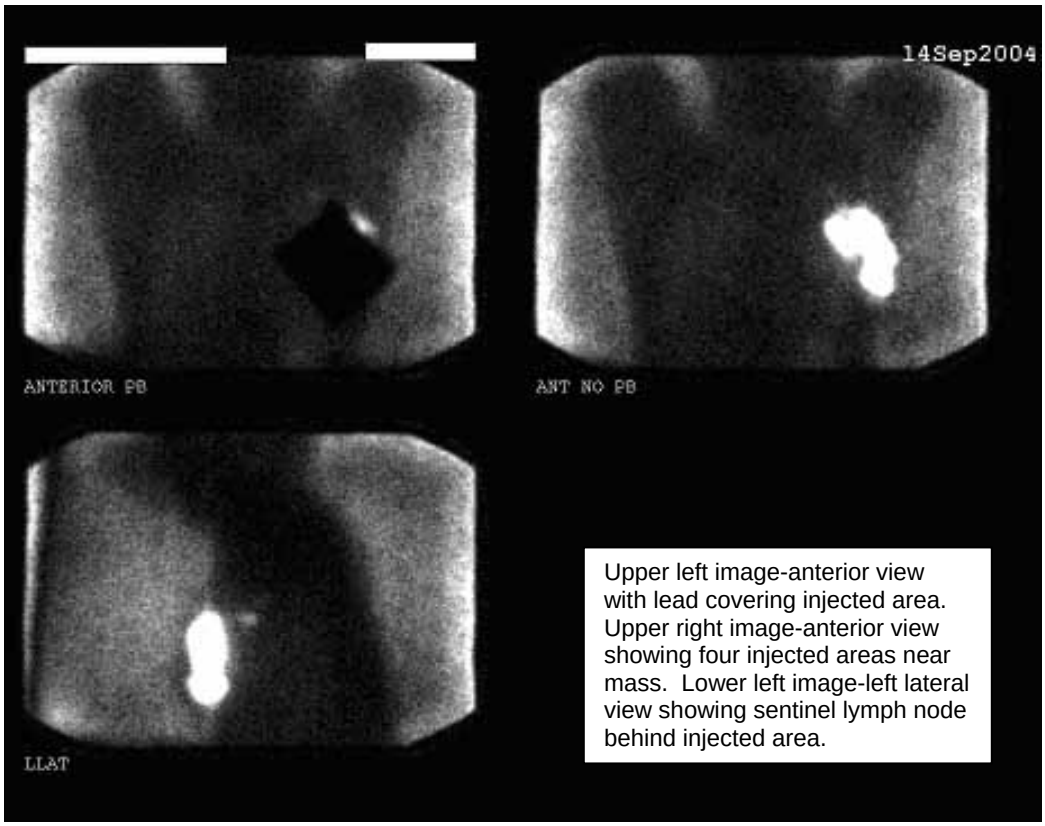
Physical exam revealed a normocephalic, atraumatic woman with normal findings except on the left side where a mobile, firm, 1 cm node in the breast. There is obvious

nipple retraction and some bruising in the upper outer quadrant and an indistinct mass in the upper outer quadrant. Ultrasound shows this as a 3 cm mass.

The patient underwent lymphoscintigraphy in the nuclear medicine department. She was given four injections of 100 μCi each of filtered Tc-99m sulfur colloid in 1 ml saline each. The injections were made near the site of the previous biopsy. Intermittent images were obtained over 60 minutes and SLNs were identified on images seen in Figure 2 sent to the surgery suite.

The preoperative administration of filtered Tc-99m sulfur colloid was given near the site of the known breast mass. Prior to incision, the patient received injections of isosulfan blue dye in the four quadrant areas where the radioactive material was infused. The area was massaged for ten minutes to help disbursement from the injection sites. A 3 cm incision was done in the left axilla near the location of the previously imaged lymph node. Dissection was completed through subcutaneous tissue and with the aid of the intraoperative gamma probe detector a SLN was identified and removed. In total, two "hot" nodes were removed and sent to pathology for examination. No additional "hot" or blue nodes were identified. Surgery continued with the planned mastectomy. The pathology report revealed invasive ductal carcinoma of intermediate grade in the primary mass removed as well as the SLNs.

Figure 2. Lymphoscintigraphy of Breast Cancer Patient



IV. OTHER CANCERS

Cabanas first used sentinel lymph node identification, dissection and pathologic examination in the treatment planning of penile carcinoma patients. Detailed preoperative lymphangiography with contrast material guided this surgeon in selecting the lymph nodes for removal and evaluation. Ninety percent of patients with sentinel nodes negative for carcinoma survived five years or longer. When the SLN alone was involved with cancer, the 5-year survival dropped to 70%. Five-year survival was 50% for patients with both SLN and downstream (inguinal) nodal involvement. This was the first indication the SLN could be used to predict the spread of tumor through the lymphatics.[2]

Sensitivity of the SLN biopsy in determining the presence of ipsilateral regional lymph node involvement in penile cancer patients was shown to be 88% using similar diagnostic studies.[19] However, further studies in penile carcinoma patients using lymphangiography procedures only indicated a false-negative rate of 25% was associated with extended SLN dissection.[20] More recently, dynamic lymphoscintigraphy, followed by intraoperative mapping and sentinel node harvesting was used to detect early metastatic penile carcinoma to identify the patients with micrometastasis for an appropriate regional lymph node dissection.[21]

Using rectal submucosal injections of technetium Tc-99m sulfur colloid, rectal lymphoscintigraphy was used to identify lymph node drainage in patients with rectal carcinoma.[22] This evaluated differences between normal patients and those with different stages of rectal carcinoma in the distribution and intensity of uptake in regional lymph nodes. Although neither preoperative or intraoperative probe evaluations were part of this study, the authors reported rectal lymphoscintigraphy may play a role in the preoperative diagnosis of stage C lesions of the rectum based on the distribution of radioactivity in draining lymph nodes seen on nuclear medicine images.

For many years, the curative surgical procedure for vulvar cancer included radical vulvectomy and bilateral inguinofemoral lymph node dissection. Since the early 1970s, surgery to remove the primary tumor followed by adjuvant radiation therapy to treat lymph node metastases was generally practiced. The use of isosulfan blue dye to identify inguinal lymph nodes, commonly referred to as the sentinel nodes of vulvar cancer, was reported in 1994.[23] The radioactive tracer and intraoperative probe technique proved to be a valuable addition to this procedure leading to improved accuracy and ease of dissection of sentinel nodes.[24] In all ten patients involved in the study, the sentinel inguinofemoral lymph nodes were identified using preoperative nuclear medicine imaging and an intraoperative probe at the time of surgery. According to the authors, blue dye injected at the time of surgery was only useful for confirmation of identification with the radioactive material and hand-held probe detector.

Prostatic lymphoscintigraphy using technetium-99m antimony trisulphide colloid ($^{99m}TcSb_2S_3$) after superficial perianal injection was used to demonstrate lymphatic drainage on external imaging with no histological proof of nodal disease having any bearing on lymphatic clearance. The authors noted intraprostatic injection failed to show any nodal uptake.[25]

One of the few studies reporting a difference in lymph nodes involved with metastatic disease and non-involved nodes was reported in a study of 21 patients with testicular cancer.[26] Injection of $^{99m}TcSb_2S_3$ in the perianal region of patients reportedly demonstrated decreased or absence of uptake of radioactivity in consecutive nodes of a lymphatic chain or in an entire lymphatic chain in nodes involved with metastatic disease suggesting a possible means of determining the

presence of metastatic disease in lymph nodes of testicular cancer patients. This was not seen in numerous studies involving breast cancer patients.

Recently, the SLN biopsy methodology has been used in a study of oral squamous cell carcinoma,[27] head and neck tumors,[28] non-small cell lung carcinoma[29] and gastric cancer.[30] Although the number of metastatic and benign diseases in which SLN biopsy proves helpful will continue to grow, this procedure has already proven invaluable as a staging method for melanoma and breast cancer.

V. LYMPHOSCINTIGRAPHY AND SLN BIOPSY OF TONGUE CARCINOMA: PATIENT STUDY

A 60 year old male diagnosed with Stage II (T2N0M0) squamous cell carcinoma of the left lateral tongue two months earlier was scheduled for a left partial mandibuloglossectomy and neck dissection. Patient reports a 41 pack-year history of tobacco use and binge alcohol use consisting of at least 12 beers per day every other week. He also has a history of hypertension and peripheral vascular disease of the legs. His mother died at age 68 of lung cancer.

At the time of pre-operative evaluation, patient complained of dysphagia and soreness while chewing. He reported no significant weight changes in the past two months. Physical exam revealed a 3 cm mass on the left underside of the tongue. Palpation of the neck revealed a 1 cm hard, mobile, mildly tender lymph node under the left mid-mandible. Also at this time, his blood pressure (180/100 mmHg) and heart rate (100/minute) were elevated. He was prescribed atenolol 50 mg po qAM including the day of surgery. The plan was to maintain his blood pressure below 140/90 mmHg and his heart rate below 70/minute throughout the perioperative period with beta-blocker therapy. He was advised to abstain from alcohol for the time remaining prior to surgery.

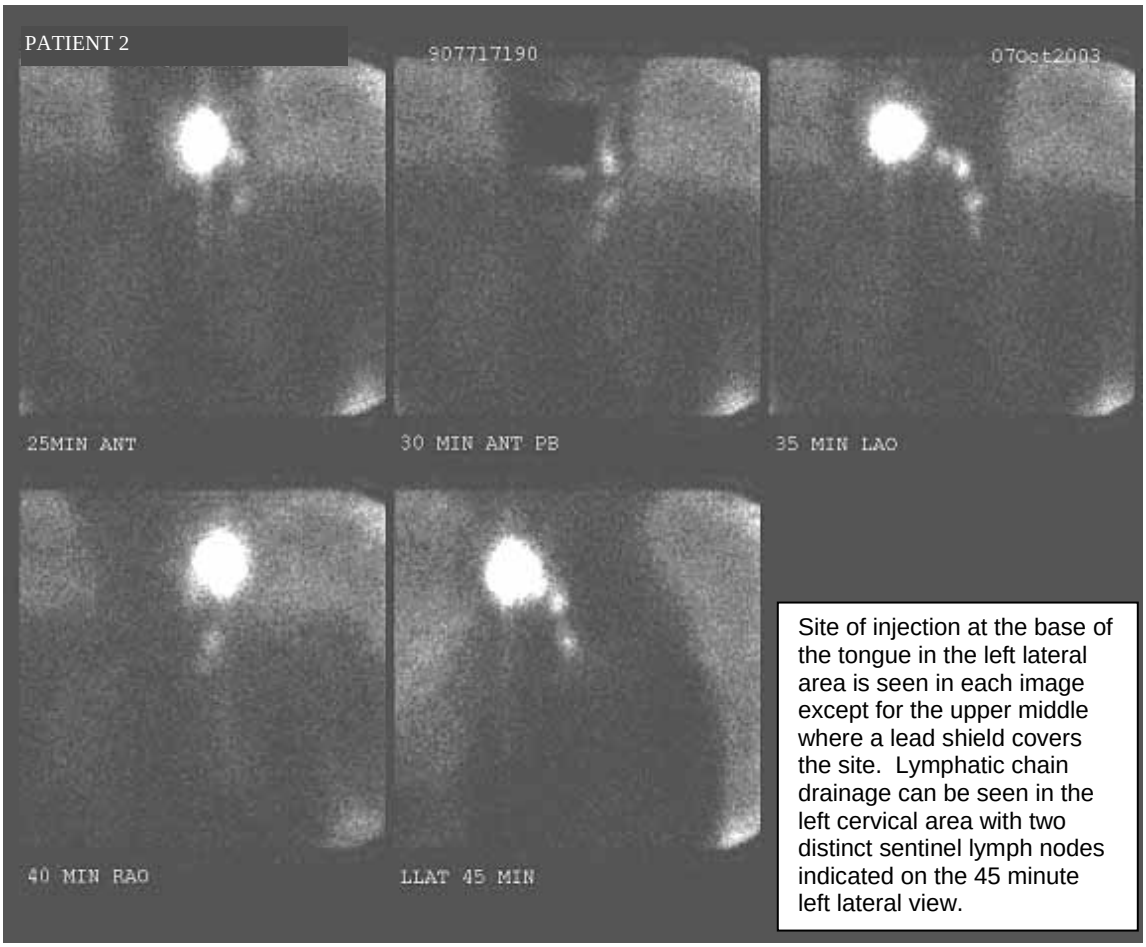
CT study of the neck did not show any abnormal soft tissue mass within the neck or any significant lymphadenopathy. The patient's known tongue mass could not be identified by CT and there was no evidence of lymphadenopathy within the neck to suggest metastatic disease.

The patient underwent lymphoscintigraphy where five intracutaneous injections of 100 μCi filtered Tc-99m sulfur colloid in 0.1 ml saline were made at the 2, 4, 7, 9, and 12 o'clock positions at 1 cm–2 cm from the margin of the tumor in the base of the tongue. Intermittent images were obtained over a 60-minute period (see Figure 3) and SLNs were marked. It was determined that the radioactivity localized within the left cervical chain and two nodes with significant radioactivity were marked prior to surgery.

Two hours later, the patient was taken to surgery for a resection of the primary tumor, excision of the SLNs, and dissection of the neck. The lymph node with the highest radioactivity was excised. It was a level II lymph node with an *in vivo* count of 250 and an *ex vivo* count of 240. The lymph node with the next highest radioactivity was excised. It was a level III lymph node with an *in vivo* count of 184 and an *ex vivo* count of 161. These were the two SLNs identified by lymphoscintigraphy. Two more SLNs were identified by the surgeon and excised.

The pathology report revealed moderately-differentiated squamous cell carcinoma of the tongue and mandible specimens. The four SLN specimens revealed six benign lymph nodes. The neck dissection provided 26 additional lymph nodes, all of which were benign, for a total of 32 benign lymph nodes.

Figure 3. Lymphoscintigraphy of Tongue Carcinoma



VI. RADIOPHARMACEUTICALS FOR LYMPHOSCINTIGRAPHY AND LYMPHATIC MAPPING-HISTORICAL DEVELOPMENT

Colloidal gold-198 was the first radiocolloid used for lymphoscintigraphy. The material reportedly had an ideal particle size of 0.002 μm–0.01 μm permitting rapid clearance from the injection sites into lymphatic drainage channels.[31]

Early studies demonstrated the clinical utility of nuclear medicine imaging of intradermally injected gold colloid (Au-198) transported by the cutaneous lymphatic vessels to regional lymph nodes.[32] Colloidal gold-198 was used to identify anatomic lymphatic drainage in disease-free states and in patients with neoplastic disease[33] including melanoma[34] and breast.[35-38] The product was ultimately abandoned because of the long T_p of Au-198 (2.7 days) and negatron radiation which reportedly caused radiation necrosis at the injection site.[39]

A. Technetium Tc-99m Dextran

Early approaches to the use of Tc-99m labeled dextran shows the ability to stably bind Tc-99m to the macromolecule and achieve some degree of lymphatic clearance from intradermal and subcutaneous injections sites in animals.[40-42] Dextran, a polysaccharide used clinically as a plasma substitute, was successfully radiolabeled with Tc-99m and evaluated for lymphoscintigraphy.[43] Comparison with $^{99m}TcSb_2S_3$ resulted in

faster uptake from the injection site in animal studies.[44] However, pass through to downstream nodes was significantly higher along with more rapid absorption into the bloodstream with distribution to liver and spleen.

Technetium Tc-99m dextran was mentioned as the radiolabeled agent for lymphoscintigraphy used to identify areas of primary drainage for melanomas located in ambiguous sites, such as the midline of the trunk or the shoulders, in Morton's series of patients.[45] Although never an approved agent, Tc-99m dextran reportedly produced good quality images of lymphatic drainage and lymph node uptake. Evidence of early entry into the vascular space was shown with high liver uptake potentially interfering with images involving the abdominal area. More recently, this agent has proven useful in lymphoscintigraphic procedures to demonstrate lymph fluid leakage in chylous ascites.[46]

B. Technetium Tc-99m Hydroxyethyl Starch

In an effort to develop a radiolabeled compound with higher clearance rate from the injection site and lower urinary bladder and liver background activity, an even higher molecular weight, non-particulate substance than dextran or human serum albumin was radiolabeled with Tc-99m and investigated in rabbits[47] and humans.[48] The authors reported an easy and rapid procedure with excellent radiolabeling efficiencies and high specific activities. Animal and human study results indicated this non-colloidal, non-particulate polymer (average molecular weight of 450,000 daltons) led to higher uptake in lymph nodes compared with Tc-99m dextran (average molecular weight of 70,000 daltons). Uptake values at 90 minutes post injection were equivalent to those obtained with Tc-99m human serum albumin (average molecular weight of 69,000 daltons) and Tc-99 m sulfur microcolloid (4 nm–12 nm particle size range) although downstream nodes were seen on the studies completed with the non-colloidal preparations at the same time while the microcolloid material was only found in the first lymph node.

C. Technetium Tc-99m Antimony Sulfide Colloid

Perhaps no other Tc-99m compound has been evaluated for use in lymphatic mapping or lymphoscintigraphy more than technetium Tc-99m antimony sulfide colloid ($^{99m}TcSb_2S_3$). This radiopharmaceutical spurred a significant advance in the clinical investigation of lymphoscintigraphy after the early use of colloidal Au-198. This radiolabeled pre-formed colloid with a small, uniform particle size of 0.003 μm–0.03 μm. reportedly provided optimal mobilization and dispersion from the interstitial injection site.[49-50] $^{99m}TcSb_2S_3$ provided diagnostic quality images within 3 hours post injection using simple techniques that were repeatable and noninvasive.

Clinical investigation of this agent during 1975-1985 demonstrated the importance of lymphoscintigraphy to the management of breast cancer.[50-56] The information provided to clinicians influenced the choice of treatment in the management of breast cancer on an individual basis. An effort to use the technique to predict breast cancer metastasis to lymph nodes did not prove effective which contributed to the loss of interest in the clinical study.[57-58]

In addition, $^{99m}TcSb_2S_3$ provided a means of nuclear medicine imaging of lymphatic drainage from cutaneous melanoma identifying the most probable site for occult nodal disease in at risk patients who might otherwise undergo elective radical lymphadenectomy.[40,59-61] The agent proved reliable in providing information regarding the direction and possible predominance of lymph flow away from the primary tumor site into the skin and subcutaneous tissues. A comparison of $^{99m}TcSb_2S_3$ with a Tc-99m dextran preparation indicated the colloidal form of Tc-99m had less nodal pass through compared with

the high molecular weight soluble dextran material. The appearance of downstream nodes with the Tc-99m dextran material early in the study could potentially lead to confusion during an attempt to identify sentinel nodes.[62]

Available as an investigational agent from Cadema Medical Products, Inc. into the mid-1990s, the multi-dose vial preparation was never approved by the FDA for commercial distribution and all clinical investigations in the U.S. were terminated when the product was abandoned by the distributor. Recently, a report from Australia outlined the properties of this agent commercially available as Lymph-Flo™ and used extensively throughout Australia and New Zealand for lymphoscintigraphy.[63]

D. Other Agents

Limited reports appearing in the literature describe attempts at lymphoscintigraphy and/or sentinel node localization involving Tc-99m rhenium colloid[64-65], Tc-99m stannous phytate[66-68], Tc-99m sestamibi[69] and radiolabeled antibodies.[70-76]

VII. RADIOPHARMACEUTICALS FOR LYMPHOSCINTIGRAPHY AND LYMPHATIC MAPPING -RECENT CLINICAL EXPERIENCE

A. Technetium Tc-99m Human Serum Albumin (HSA)

Based on earlier work with iodinated albumin, the Tc-99m labeled protein molecule was popular for a time.[77] Regional lymph nodes were visualized as early at 10 minutes post intradermal injection of technetium Tc-99m albumin in studies evaluating this radiopharmaceutical for use in lymphoscintigraphy.[78] The dynamic flow patterns seen with this non-particulate agent allowed earlier diagnostic information to be obtained.[79] Improved definition of the position and number of lymphatic vessels were reportedly due to increased quantities of the radioactive material entering the lymphatics in a shorter period of time.[39] Subcutaneous injections led to significant blood pool uptake which can interfere with SLN localization in some anatomical areas.

Low cost and ease of preparation made this radiopharmaceutical popular with a number of physicians as an alternative to Tc-99m sulfur colloid in the localization of SLN in melanoma patients with some reporting higher concordance between "hot" or radioactive nodes and blue nodes seen with injected blue dye material.[80] However, some physicians reported poor retention in lymph nodes resulting in failure to select the sentinel node before downstream nodes became prominent at the time of surgery.[81] Removal of the kit for the preparation of technetium Tc-99m albumin from commercial distribution eliminated this product from clinical use.

B. Technetium Tc-99m Albumin Nanocolloid (Nanocoll™-Amersham Health)

This radiopharmaceutical kit contains a pre-formed colloid of human serum albumin for radiolabeling with Tc-99m. It provides a small particle size with >95% labeled colloid particles smaller than 80 nm according to the manufacturer. After injection, rapid clearance into the lymphatics similar to that seen with $^{99m}TcSb_2S_3$ allows visualization of the dynamics of lymphatic drainage in patients. Clinical studies in cancer of the vulva patients provided good identification of SLNs when used in conjunction with blue dye administration.[82] Varying injection techniques in breast cancer patients with this product led to different lymph nodes being identified indicating injection technique is critical when using radiolabeled compounds for intraoperative mapping of lymph node drainage of breast cancer.[83]

Tc-99m albumin nanocolloid was also used in a study of SLN localization in cutaneous melanoma patients to evaluate the additive effect of using intraoperative probe detection along with blue dye administration

at the time of surgery to visually trace the lymphatic drainage into the lymph nodes. Lymphoscintigraphy completed to identify the position of the SLNs was completed prior to the patient going to the operating suite. At the time of surgery, patent blue dye was injected at the same site as the radio-nanocolloid. When a blue node was discovered during surgery, the level of radioactivity was measured with an intraoperative probe detector. In the absence of blue dye coloration, the probe could be used to locate the SLNs. Study results indicated the use of blue dye alone localized only 84% of SLNs while combining this method with the intraoperative gamma probe detection methodology increased this value to 99.5% of all sentinel nodes.[84]

Further characterization of the original formulation and investigation of the optimization of the preparation techniques to improve the count rates in lymph nodes involved a study in 98 patients.[85] Removal of a portion of the albumin colloid prior to radiolabeling provided different specific activities, the highest being 50 MBq/μg colloidal albumin, with no decrease in labeling efficiency, radiochemical purity or stability. Although the authors reported nine times higher count rates in lymph nodes, there was no significant difference in the rate of successful identification of the sentinel lymph node across study groups. More significantly, improved particle sizing methods indicated the commercially available Tc-99m albumin nanocolloid preparation contained >95% of colloidal albumin particles between 7 nm and 23 nm.

Although currently available in Europe and other parts of the world, this product is not commercially available in the U.S.

C. Technetium Tc-99m Sulfur Colloid

The most commonly used radiopharmaceutical that has aided the development of lymphatic mapping and sentinel lymph node identification has been the imaging agent originally developed for evaluating diseases of the liver, spleen and bone marrow reticuloendothelial system. Although not indicated for this use, technetium Tc-99m sulfur colloid remains the drug of choice for lymphatic mapping or sentinel node localization due to its widespread availability and proven reliability.

Only one manufacturer of the kit for the preparation of technetium Tc-99m sulfur colloid (CIS-SULFUR COLLOID™-CIS-US, Inc., Bedford, MA) markets this product in the United States. Similar to other manufacturers' kits formerly available, CIS-SULFUR COLLOID™ forms particles during its preparation involving heating several ingredients in a boiling water bath (see below).

Preparation of CIS-SULFUR COLLOID™

1. Add 1 mL–3 mL of technetium Tc-99m sodium pertechnetate (not more than 500 mCi/mL) to reaction vial containing:
   - 2.0 mg sodium thiosulfate anhydrous
   - 2.3 mg edetate disodium
   - 18.1 mg gelatin
2. Add 1.5 mL of solution from vial A containing 0.148N HCl
3. Heat in a vigorously boiling water bath for 5 minutes.
4. Remove the vial from the water bath and allow to cool for 3 minutes.
5. Add 1.5 mL of solution from vial B containing phosphate buffer solution.
6. Complete quality assessment of final product. Discard vial 6 hours after compounding.

Sodium thiosulfate provides sulfur atoms for the generation of colloidal particles while edetate disodium and gelatin both act as stabilizing agents. Variations in heating and cooling times, quantities of ingredients added and other changes can result in a variety of particle size ranges with different degrees of stability.[86-88]

Tc-99m sulfur colloid is formed by the reaction of thiosulfate under acidic conditions and heat initially forming sulfur, bisulfite and polythionates which eventually lead to high

molecular weight sulfur polymers. The nature of these reactions, their rates and yields depend on concentration of thiosulfate, temperature of the reaction mixture and acidity. Insoluble sulfides and stable sulfide complexes with Tc-99m are thought to form as secondary compounds.[89] Reaction rates lead to faster formation of Tc-99m colloid compared with the sulfur colloid which can be formed around the original Tc-99m particle. For this reason, smaller particles should contain relatively lower quantities of sulfur and higher levels of Tc-99m compared with larger particles.[89] Gelatin coats the formed particles preventing them from continued growth into larger globs of sulfur. Addition of the buffer solution provides greater stability while bringing the pH of the final preparation closer to neutral range.

In an effort to improve upon the pharmacokinetics and SLN detection efficiency, variations in the compounding techniques used to prepare technetium Tc-99m sulfur colloid have appeared in the recent literature.

D. Filtered Technetium Tc-99m Sulfur Colloid

Concerns about optimizing the radiopharmaceutical for lymphatic mapping and SLN detection are primarily related to the particle size of the radiotracer and timing of the diagnostic imaging and intraoperative probe identification of the SLN. Rate of transport from the injection site and movement through lymphatic pathways is primarily related to the particle size of the colloidal material.[90-91] Particles larger than 0.005 μm are preferred since smaller particles reportedly penetrate capillary membranes allowing them to pass into the general circulation leading to reduced radioactivity levels migrating through the lymphatic vessels into lymph nodes. Particles smaller than 0.1 μm allow rapid removal from the interstitial space into the lymphatic channels and still provide significant retention in the lymph node for surgical procedures to be completed. Colloidal particles >0.5 μm, found in the traditional Tc-99m sulfur colloid preparation, have a much slower rate of clearance from the interstitial space with significantly less accumulation in lymph nodes.[92]

Filtering the standard Tc-99m sulfur colloid preparation through a 0.1 μm, sterile membrane filter reduced the average particle size from approximately 0.3 μm to 0.01 μm with a small (<0.1%) number of particles in the range of 0.09 μm–0.17 μm.[93] Lymphoscintigraphy was successful in a group of patients with lymphedema using a similarly filtered Tc-99m sulfur colloid preparation.[94] Lymphoscintigraphic imaging studies obtained with a modified preparation that includes ultrafiltration through a 0.22 μm, sterile filter provide substantially more definitive SLN identification and improved kinetics.[95]

E. Technetium Tc-99m Sulfur Colloid Compounding Variations

Efforts to further improve the handling of Tc-99m sulfur colloid by the lymphatics and its use in lymphatic mapping and SLN detection, include changes in the method of compounding the radiopharmaceutical to maximize the number of particles small enough to optimally visualize the lymphatic drainage quickly after injection while maintaining sentinel node retention. This was the goal of Eshima, et al in a study utilizing different types of technetium Tc-99m sodium pertechnetate solution and radiolabeling conditions to prepare technetium Tc-99m sulfur colloid.[96] This study was a careful examination of the effects of changing the heating time and the characteristics of the generator eluate used for the kit preparation. In order to provide a larger number of technetium atoms for the nucleation process that initiates the formation of the colloidal particles, generator eluates obtained from $^{99}Mo/^{99m}Tc$ radionuclide generators that had daughter ingrowth times exceeding 72 h. were used for com-

pounding. Evaluation of the particle size distribution and stability of the prepared kit over 6 hours using conventional polycarbonate filtration techniques along with routine radiochemical purity assays provided an optimal compounding procedure as follows.

Optimal Preparation of Technetium Tc 99m Sulfur Colloid (Eshima, et al[96])

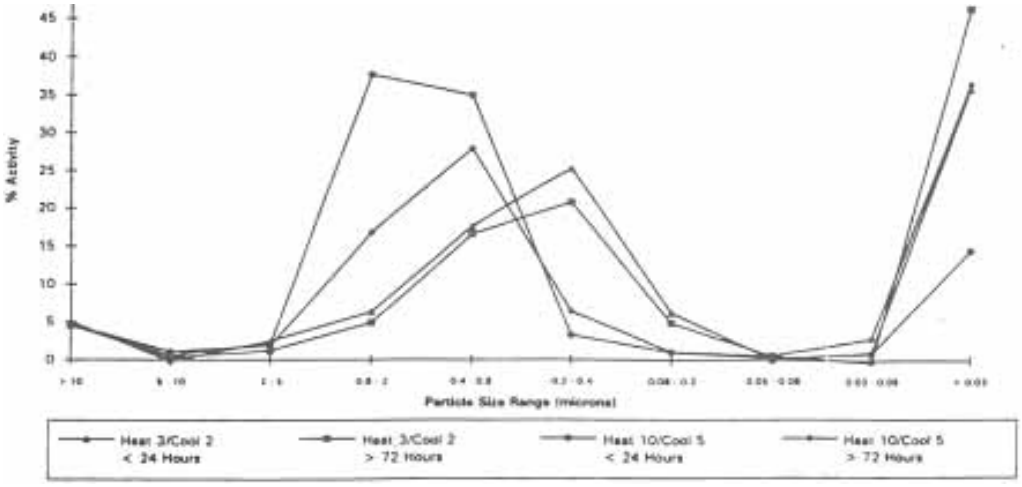
1. Add 3 mL of technetium Tc-99m sodium pertechnetate eluate (not more than 50 mCi/mL) from a radionuclide generator that had a 72-hour ingrowth of Tc-99 to the vial containing:
   - 2.0 mg sodium thiosulfate anhydrous
   - 2.3 mg edetate disodium
   - 18.1 mg gelatin
2. Add 1.5 mL of solution from vial A containing 0.148N HCl
3. Heat in a vigorously boiling water bath for 3 minutes with occasional agitation.
4. Remove the vial from the water bath and allow to cool for 2 minutes.
5. Add 1.5 mL of solution from vial B containing phosphate buffer solution.
6. Complete quality assessment of final product. Discard vial 6 hours after compounding.

As seen in Figure 4 below, the use of this reduced heating time protocol results in a significant increase in the percentage of particles smaller than 0.4 μm (70.86%) regardless of the age of the generator eluate. Prolonged heating significantly decreased the percentage of small particles, 20.1% with fresh eluate and 47.67% with old eluate. These studies also demonstrated a bimodal distribution of labeled particles in a Tc-99m sulfur colloid preparation.

This study illustrates the effects of heating and cooling times and the mass of technetium on the particle size of the technetium Tc-99m sulfur colloid generated during compounding. Radiopharmaceutical compounding variations can be used to maximize the clinical utilization of lymphatic mapping and sentinel lymph node detection.[92,97]

Additional approaches to optimize the preparation of technetium Tc-99m sulfur colloid for lymphatic mapping include evaluating the use of $^{99}Mo/^{99m}Tc$ radionuclide generator eluates with daughter in-growth times exceeding 7 days and utilizing stable rhenium as a point of nucleation for the colloidal particles to form.[98]

Figure 4. Effect of compounding changes on particle size distribution of Tc-99m sulfur colloid (Reprinted by permission of the Society of Nuclear Medicine from Eshima, et. al[96])



RADIOPHARMACEUTICALS FOR LYMPHOSCINTIGRAPHY AND LYMPHATIC MAPPING -RESEARCH AND DEVELOPMENT

Ideal properties of a radiopharmaceutical for sentinel node localization and lymphatic mapping include rapid migration from the injection site into the lymphatic vessels, prolonged retention in normal or extensively diseased lymph node, either all positive nodes or one negative node identified, no migration beyond the sentinel lymph node into downstream nodes and the material should allow both visual (color recognition) and gamma probe identification (radiation detection) of the material. Although no agent has been identified that meets all criteria, there are a number of developments that may bring an ideal radiotracer to the clinic.

F. Technetium Tc-99m DTPA-Mannosyl-Dextran (Lymphoseek™)

The goal of the development of this product was the synthesis of a non-particulate, high molecular weight structure composed of the molecular backbone, dextran, with DTPA-conjugated to this structure followed by the stable attachment of the lymph node receptor substrate, mannose. Initial work proved the material could be prepared in a stable, kit formulation that allows radiochemically pure radiolabeling with Tc-99m using stannous reduction techniques.[99] Although rabbit biodistribution studies indicated a faster clearance from the foot pad injection site of Technetium Tc-99m DTPA-Mannosyl-Dextran compared with filtered Tc-99m sulfur colloid, there was no significant difference in sentinel node uptake between the receptor-binding agent and filtered Tc-99m sulfur colloid.[100]

Lymphoseek™ is the proprietary name for this radioactive tracing agent being developed for use in Intraoperative Lymphatic Mapping (ILM). In an effort to develop a radiopharmaceutical specific for sentinel node localization with a faster injection site clearance and less pass through from the sentinel node to distal nodes thought to be due to the high ratio of mannose attachment sites within lymphoid tissues and the hydrophilic nature of the molecule. This investigational drug was investigated in breast cancer patients, in which Lymphoseek™ demonstrated rapid clearance of the injection site and less "pass through" from the sentinel lymph node.[101] Lymphoseek™ is being investigated in other solid tumor cancers besides breast and melanoma.[102]

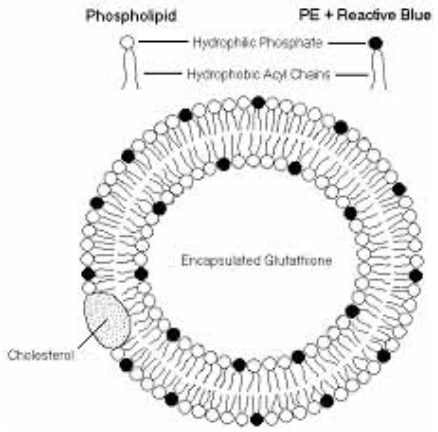
G. Technetium Tc-99m Liposomes

Liposomes are bilayer phospholipid vesicles originally investigated as biological membrane models simulating cellular structures.[103] Spontaneous formation of liposomes occurs when a combination of certain lipids is dispersed throughout an aqueous solution. Materials dissolved in the aqueous solution become entrapped in the enclosed aqueous compartments, which form in an alternating, concentric fashion with the lipid bilayers. In addition, lipid-soluble materials may be incorporated in the formed liposomes if added to the lipids forming the bilayer structures.[104] Positively or negatively charged liposomes with narrow particle size ranges, including colloidal, with various other surface properties can be prepared by altering the conditions during preparation.

Numerous investigations into the use of multi-layer (multilamellar) and single-layer (unilamellar) liposome structures as carriers of drug molecules eventually led to the investigation of this technology for the safe delivery of chemotherapy agents (alkylating agents, methotrexate, bleomycin, ara-C, actinomycin D and anti-infectives.[105] Liposome encapsulated drugs often have biodistributions and toxicities that can differ greatly from the free drug. Commercially available liposomal amphotericin B (AmBisome), liposomal cyclosporin A (Cyclospire), liposomal daunorubicin (Daunoxome) and liposomal

recombinant interleukin-2 are just a few of the drugs resulting from this effort.

Figure 5. A unilamellar liposome vesicle showing the encapsulation of glutathione and reactive blue dye in the lipid bilayer structure.



Early reports of the use of liposomes as carriers of radionuclides led to efforts to develop these agents for diagnostic imaging including lymphoscintigraphy.[106-107] Studies involving radiolabeled liposomes for the detection of tumors, abscesses and inflammatory sites involving ischemic and infarcted regions of the myocardium and arthritic joints were reported.[108-118]

Figure 5 illustrates a method of incorporating Tc-99m along with a blue dye inside a liposome carrier. This methods, reported as a kit for labeling with Tc-99m, combined the radioactive component with the blue color.[119] Combining the two allows one injection for both lymphoscintigraphy or lymphatic mapping in nuclear medicine and SLN localization/biopsy in the surgical suite. Other reports on the use of radiolabeled liposomes for lymphoscintigraphy and SLN biopsy shows promise for this method as a reliable targeting procedure.[120-122]

VIII. INTRAOPERATIVE PROBE DETECTORS

An intraoperative probe consists of a detector, collimator, digital or analog display and an audio signal generator. Originally developed for a procedure known as radioimmunoguided surgery (RIGS) where surgical exploration of the distribution of radiolabeled antibodies allowed a more complete resection of diseased tissue[123], the technique became employed in the intraoperative localization of neuroblastoma, pheochromocytoma, prostate cancer, bone cancer and during parathyroidectomy.[124] Combining intraoperative probe detection with SLN biopsy or lymphatic mapping led a number of manufacturers to develop and market instruments with minimal variations in the design. A number of reports detailing performance parameters of intraoperative probes describe different models available and the specific attributes of each.[125-130]

The physical performance of intraoperative probe detectors primarily involves the sensitivity of the detector, energy resolution and spatial resolution. Sensitivity is a measure of the efficiency of the probe or the ability of the probe to convert incident radiation into an electrical signal contributing to the information received by the surgeon (cps/dps). Energy or spectral resolution relates the statistical uncertainty of the detection process and is inversely related to the number of electrons produced by a radiation in the detector.[130] Energy resolution is particularly important when windowing out scatter radiation or noise. Spatial resolution or angular sensitivity is important when localizing a small radioactive source, such as a sentinel node, in a volume being explored such as the axilla. Spatial resolution is evaluated by determining the detected counting rates as a function of the lateral distance from the central axis of the detector.[130] Collimation or shielding of the detector, limiting the probe detector's field of view, is the most critical factor in determining the spatial resolution of the system.

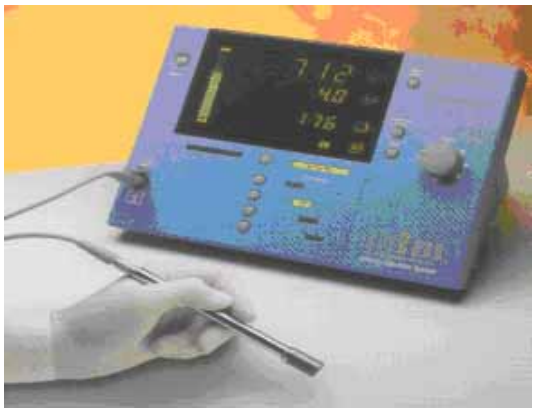
Beyond probe performance, user friendliness of the system is the next consideration

when evaluating a system for use in surgery. The size and shape of the probe, the probe's weight and the audio signal produced are all important characteristics involving the ergonomics of the system. An audio signal which is pleasant to the ears, allowing the surgeon to detect lesions without the need to direct attention away from the surgical field is critical. Variable tone audio signals which increase in pitch as the count rate increases are more popular than the threshold-only systems. Sterility requirements, longevity of the battery pack, need and cost of consumables and overall cost of the system contribute to the selection criteria.

A. neo2000™ (Neoprobe Corporation)

Neoprobe Corporation's neo2000™ (seen in Figures 6 and 7) is a microprocessor-based platform instrument available with a variety of radiation detecting probes for external counting and intraoperative use. The unit incorporates automatic windowing technology providing improved counting statistics for most common radionuclides including Tc-99m, In-111 and I-125.

Figure 6. Neoprobe Corporation's neo2000™ intraoperative probe detector base unit.



The base unit has a lighted display readable at all viewing angles. The cadmium-telluride (CdTe) solid-state detector is precalibrated requiring no field calibration for most detection methodologies. Sound is emitted when the detector encounters a source of radioactivity above a set threshold or background level that alerts the surgeon to the need for careful inspection of the surgical field. The software system automatically defaults to continuous digital count display with fixed counts and target-to-background ratios available.

Figure 7. Intraoperative probe detector being used for external counting of lymph node and residual tissue removed from patient.

B. Node Seeker-720™ (IntraMedical Imaging, LLC)

The electronics of this system is controlled by a laptop computer running software provided by the manufacturer of the probe detector. Visual display of cps (refreshed every 0.1 second), timed average cps and peak count rate during the last five seconds is found on the laptop screen. The audio signal has variable frequency tones and peak activity count alert. Battery pack provides up to five hours of continuous operation. The unit has preset windows for Tc-99m and In-111 with available additional probes for F-18 and I-131.

C. C-Trak® (Care Wise Medical Products)

The C-Trak® Surgical Guidance System was designed for use by a surgeon in identifying tissues containing radionuclide. The probe component is capable of detecting

gamma energies up to 364 keV and is designed to detect small sites of radionuclide in the high scatter, highly variable backgrounds associated with In-111 and Tc-99m in tissue samples. Snap-on collimators allow the probe's field of view to be narrowed from 25 mm–15 mm to match tissue/background levels found in surgical fields. Although the probe can be sterilized using ethylene oxide or glutaraldehyde, a disposable, sterile sheath is recommended for enclosing the probe for each surgical case. An LCD, six-digit display allows cps or timed counts to be viewed. In addition, a variable tone generator coupled to a ratemeter provides the surgeon with a sound increasing in pitch as the quantity of radioactivity encountered by the detector increases.

D. The Gammed IV Surgical Probe System (Capintec)

The Gammed IV Surgical Probe System (shown in Figure 8) is Capintec's latest addition to the surgical probe field. This unit comes standard with two probe detectors: a CdTe detector for low energy (15 keV–170 keV) radionuclides such as Tc-99m and I-123 and a CsI detector for higher energies (150 keV–1,000 keV) for use with In-111 and I-131. Minimal detectable activity of the low energy probe based on 1 cps at 0.5 cm from the source ranges from 0.005 μCi–0.01 μCi for Tc-99m. The control unit employs microprocessor technology yielding an all-digital display with cps, total counts, thresholding, integrated spectrum measurement and radionuclide identification capabilities. The system boasts internally collimated, gas-sterilizable probes with add-on collimators optional. The system is battery operated with seven hours of operation on each charge. User can select from two types of audible signals that are proportional to count rate.

Figure 3. Capintec's Gammed IV surgical probe detector base unit shown along with high energy and low energy probe detectors.

Intraoperative probe detector systems mentioned in the literature for use during sentinel node biopsy procedures include the **Navigator GPS™ (Tyco Healthcare)**. This instrument, initially popular with surgeons with a number of units in service, is no longer produced and marketed. Other devices used for lymphatic mapping/sentinel node localization include the mini-imaging camera, **LumaGEM™ (GammaMedica)**, which actually produces a digital image of the distribution of radioactivity in a small area of the body. The small, hand-held device can be used during surgery to create images of lymphatic drainage and lymph node uptake of radioactivity as it is cleared from the injection site. Less commonly used is the **CTC-4™ (Radiation Monitoring Devices)** intraoperative probe detector. Similar to other commercially available devices, this instrument utilizes a CdTe solid-state detector and analyzer for use during surgery. Table 1 compares the sensitivity, spatial resolution and minimal detectable activity for the more popular intraoperative probe systems.

IX. INTERVENTIONAL AGENTS

A. Injectable Dyes

Commercially available, FDA-approved dyes, including methylene blue injection, USP 1% for treatment of drug induced

Table 1. Performance Characteristics of Commercially Available Intraoperative Probes*

SENSITIVITY (cps/kBq)	neo2000®	Gammed IV	C-Trak®	Navigator	CTC-4	Node Seeker™
No collimation/Co-57 energy window/in air @1 cm	9.2	5.2	9.5	3.31	0.83	10
With collimation/Co-57 energy window/in air @1 cm	4.4	NA	5.0	NA	NA	5.0
Spatial resolution (FWHM in mm)/Tc-99m energy window/in water @ 1 cm	13	14	NA	17	NA	14
Minimum detectable lymph node to injection site separation in mm/Tc-99m energy window/2.5 MBq	69	72	NA	112	NA	NA

*Summary of information found in Zanzonico P, Heller S.[130]

methemoglobinemia, indigotindisulfonate sodium injection, USP for localizing ureteral orifices during cystoscopy and ureteral catheterization and fluorescein injection have all been used to trace the flow of lymph through lymphatic vessels.[132] However, the most commonly used drug is isosulfan blue (patent blue dye, vital blue dye, Lymphazurin® 1%-Tyco International/U.S. Surgical Corporation, Norwalk,CT) since it is the only material indicated for subcutaneous administration in the delineation of lymphatic vessels.[133-136]

Isosulfan blue is a sterile, pyrogen-free, phosphate-buffered, aqueous solution for subcutaneous administration. The material contains no preservative. Following subQ administration as seen in Figure 9, the drug is selectively picked up by the lymphatic vessels. Thus, the lymphatic drainage is delineated by a bright blue color making the channels discernible from surrounding tissue as shown in Figure 10. Approximately 10% of the subQ dose is excreted unchanged in the urine in 24 hours and patients should be cautioned about the urine having a blue coloration. Presumably, the remaining drug is excreted through the biliary system.

The admixture of isosulfan blue with local anesthetics (lidocaine) in the same syringe prior to administration results in an immediate precipitation of up to 10% of the drug complex. If a local anesthetic is planned for concurrent administration, it is suggested a separate syringe be used. The suggested maximum dosage of isosulfan blue is 3 ml (30 mg). Typical dosages reported in the literature include up to 1 mL for melanoma and up to 5 mL for breast cancer patients. Typically, these injections are completed in the operating suite since skin incision usually follows 10–20 minutes post injection due to the fast clearance of the dye material from the injection site into the lymphatic vessels.

Figure 9. Intradermal injection of isosulfan blue in four quadrants around the melanoma resection site.

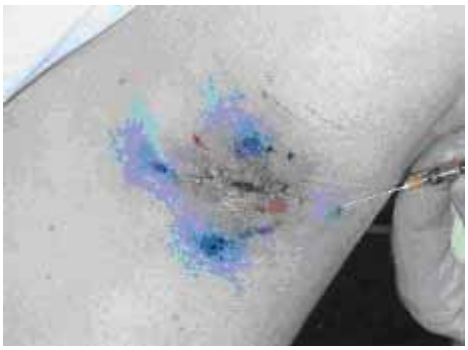


Figure 10. Isosulfan blue in lymphatic vessel and axillary lymph node of breast cancer patient.

Lymphazurin blue dye is often viewed as the gold standard of concordance for identifying the true SLN during surgery.[80] In this study, the authors report the detection of the SLN increased from 75% using blue dye alone to 95% when blue dye was combined with gamma-probe detection using Tc-99m HSA; similar to reports of other researchers. It is generally agreed the success in identifying the SLN is significantly enhanced by the addition of gamma-probe use to blue dye injection at the time of surgery.

B. Lidocaine

Although not universally used, some physicians utilize the topical anesthetic lidocaine, as a separate injection or mixed with the radiopharmaceutical, to reduce the pain associated with the intradermal injections in melanoma patients.[6] Adding a small volume of 1% lidocaine HCl to the syringe containing the Tc-99m sulfur colloid preparation, being careful to leave the anesthetic material in the tip of the syringe near needle, allows the nerve block to begin during the first injection and reduces the pain experienced with the remaining injections. Our studies have shown the radiochemical purity and particle size is not altered by the admixture of the lidocaine to the syringe.

X. RADIATION DOSIMETRY, EXPOSURE IN THE OPERATING ROOM AND PATHOLOGY

Sentinel lymph node biopsy has proven a valuable clinical tool and enjoys widespread use in a large population of patients diagnosed with skin cancer, breast cancer and a number of other malignant disorders. The literature supporting the use of radiocolloids for lymphoscintigraphy along with the use of well-established FDA-approved radiopharmaceuticals for the procedures have contributed to the paucity of literature concerning radiation dosimetric determinations for the patient.

Studies comparing the subcutaneous administration of radiopharmaceuticals with external beam radiotherapy cumulative radiation effect (CRE) values have shown the use of technetium-99m radiopharmaceuticals to be very safe. Estimates for Tc-99m provide a value of 5% of the skin tolerance dose in which damage to the connective tissue of the skin and skin necrosis is likely to occur.[137] Dose estimates can vary considerably depending on the radiopharmaceutical preparation, injection site and technique which play an important role in determining the kinetics of radioactivity removal from the injection site, transit through the lymph channels and retention in lymph nodes. Radiation dose estimates for intradermally injected 0.1-μm filtered Tc-99m sulfur colloid in volumes ranging from 0.2 mL–0.5 mL include 24 rad/mCi–114 rad/mCi at the injection site and 0.04 rad/mCi–3.32 rad/mCi in lymph nodes.[94]

Reportedly, radiation dose to the skin contributes little to the effective dose associated with an intradermal injection of Tc-99m sulfur colloid. A calculated effective dose of 0.0071 rem/mCi has been estimated assuming 20% absorption of the administered dosage into the general circulation which will deliver a critical dose of 0.057 rad/mCi to the spleen.[138]

In summary, the use of radiocolloids for lymphatic mapping/sentinel lymph node biopsy results in a large amount of the radioactivity administered being retained at the injection site. Radiation dose to the skin and underlying tissue can be minimized by administering the minimal amount of radioactivity providing good clinical information.[97]

Radiation safety considerations have become commonplace for operating room personnel and pathologists in addition to the nuclear medicine personnel involved in the radiopharmaceutical administration and clinical imaging. Based on actual exposure measurements, the decision to require personal dosimeters for pathologists during examination of surgical specimens and operating room personnel is typically a decision of the institution's radiation safety officer. Actual exposures from the levels of radioactivity routinely used for SLN biopsy and lymphatic mapping procedures are sufficiently low to not warrant the need to badge the institution's staff working outside of nuclear medicine.

Assuming an average of three hours from administration of 0.4 mCi of Tc-99m sulfur colloid to the time of surgery, the physical decay would reduce this amount to 0.28 mCi (70% of administered dosage). Biological clearance further reduces the quantity remaining at the injection site to 0.2 mCi. Assuming a distance of approximately 1 ft to the nearest exposed person in the operating room, the individual could receive 0.002 mrem in one hour. Using TLD ring dosimeters, radiation dose to the hands of the surgeon range from 0.5 mrem–10 mrem for each SLN biopsy procedure in a patient with melanoma. Up to 60 melanoma SLN biopsy surgeries could be completed before the radiation exposure to the hands approached the 300 mrem effective whole body radiation dose received each year from background radiation. Contamination of the skin from direct contact with the radioactive specimens from surgery should not be a concern since required operating room garb and universal precautions will prevent this from occurring.

Although surgical specimens routinely exceeded the radiologic control level (RCL) of 0.002 μCi/Gm, the hands of the surgical team in a study of 24 breast cancer and melanoma SLN biopsy procedures were exposed to less than 10 mrem per case. This study resulted in the institution implementing a policy of not employing personal dosimeters for the surgical team in addition to storing surgical specimens for 72 hours after an initial frozen-section analysis before handling in the customary manner observed in pathology.[139] Others recommend processing surgical specimens without delay since radiation exposure to pathology personnel who handle radioactive tissues for a limited time period would be no greater than that received by the surgeon.[140]

XI. CONCLUSION

Since the early 19th century, the practice of medicine has recognized the predictable spread of most solid tumors to regional lymph nodes. Routine oncological practice utilizes staging techniques that include a careful pathological examination of lymph nodes removed from the area of the solid tumor. Knowledge of the status of metastatic spread to regional lymph nodes is paramount for decisions concerning prognosis and treatment planning to assure optimum clinical results.

Evaluation of solid tumor spread to regional lymph nodes reached a new level of refinement with the concept of sentinel lymph node identification for pathological staging of metastatic disease, particularly melanoma and breast cancer.[141-143] The pioneering work that helped establish the predictable spread of solid tumors to nearby lymph nodes (the sentinel node concept) was carefully planned with many researchers from various fields of medicine building upon the work of others.[92,142] After introduction of the sentinel lymph node biopsy for

staging into clinical practice, validation of the technique was first established in malignant melanoma[143-145] and breast cancer[10,17,146-151] before moving into the staging and therapy planning of other tumors.

Clinical utility of lymphoscintigraphy and the sentinel node biopsy technique is closely related to the development of radiopharmaceuticals and the intraoperative probe detectors used during surgery.[39,97] Improvements in compounding of the radioactive materials will continue to improve the techniques used for identification of sentinel lymph nodes.

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XIII. QUESTIONS

1. Which of the following best describes the sentinel lymph node?
   a. Any lymph node containing tumor markers that would signal the presence of cancer.
   b. Any lymph node of a particular lymph node chain.
   c. The lymph node most likely to develop B-cell lymphoma.
   d. The first node draining the primary tumor.

2. Which of the following is often used, in addition to radioactive material, to help identify the sentinel lymph node during surgery?
   a. cyalume
   b. flourescein
   c. isosulfan blue
   d. lidocaine HCl

3. Which of the following IS NOT a risk factor for skin cancer?
   a. Being a male.
   b. Outdoor occupation.
   c. Light skin complexion.
   d. Exposure to chemicals found in deodorants/anti-perspirants.

4. Which of the following cancer types has not benefited from sentinel lymph node biopsy procedures?
   a. Brain tumors.
   b. Prostate cancer.
   c. Vulvar cancer.
   d. Head and neck tumors.

5. Which of the following radioactive compounds HAS NOT been used for lymphoscintigraphy?
   a. gold Au-198 colloid
   b. technetium Tc-99m antimony sulfide colloid
   c. technetium Tc-99m dextran
   d. technetium Tc-99m aggregated albumin

6. Which of the following cancer types occurs most often in the United States?
   a. Lung cancer.
   b. Skin cancer.
   c. Breast cancer.

d. Colorectal cancer.

7. Which of the following has the fastest clearance rate from an intradermal injection?
   a. technetium Tc-99m sulfur colloid
   b. technetium Tc-99m antimony sulfide colloid
   c. isosulfan blue dye
   d. 0.22μm filtered technetium Tc-99m sulfur colloid

8. Which of the following IS NOT a risk factor for breast cancer?
   a. Age at menopause onset.
   b. Estrogen use.
   c. Family history of the disease.
   d. Age at first birth or lack of children.

9. Which of the following IS NOT considered an ideal property of a radiopharmaceutical used for lymphatic mapping and sentinel lymph node biopsy?
   a. Fast clearance from the sentinel lymph node into downstream or secondary lymph nodes.
   b. Fast migration from the injection site into lymphatic vessels.
   c. Prolonged retention in lymph nodes.
   d. Visual and gamma probe identification of material in lymph nodes.

10. Which of the following problems was reported with the use of technetium Tc-99m human serum albumin for sentinel lymph node biopsy?
   a. Low count rates encountered with most gamma probe detectors.
   b. Slow clearance from site of injection.
   c. Non-specific binding to blue dyes used at the time of surgery.
   d. Poor retention in lymph nodes.

11. Which of the following is generally recognized as determining the rate of transport from the injection site and movement through lymphatic pathways for radioactive materials used for lymphatic mapping?
   a. Protein binding properties.
   b. Particle size.
   c. Affinity for macrophages.
   d. Ionic charge.

12. Using a Mo/Tc-99m radionuclide generator eluate from a generator in transient equilibrium (> 72 hours since last elution) will lead to which of the following parameters for compounding technetium Tc-99m sulfur colloid?
   a. Less Mo-99 breakthrough.
   b. Higher Tc-99m:Tc-99 ratios.
   c. Greater molar quantity of technetium in the preparation.
   d. Greater radioactive concentration.

13. Which of the following radioactive materials used for lymphoscintigraphy and lymphatic mapping has the smallest particle size?
   a. 0.22μm filtered technetium Tc-99m sulfur colloid
   b. technetium Tc-99m dextran
   c. technetium Tc-99m antimony sulfide colloid
   d. technetium Tc-99m hydroxyethyl starch

14. Which of the following radioactive compounds reportedly has less pass through from the sentinel lymph node to distal nodes?
   a. technetium Tc-99m human serum albumin
   b. technetium Tc-99m DTPA-mannosyl-dextran
   c. technetium Tc-99m albumin nanocolloid
   d. technetium Tc-99m hydroxyethyl starch

15. Which of the following would receive the highest radiation absorbed dose from the subcutaneous administration of tech-

netium Tc-99m sulfur colloid for lymphoscintigraphy and sentinel lymph node biopsy?
   a. Lymphatic channel
   b. Sentinel lymph node.
   c. Injection site.
   d. Liver

16. Which of the following is the most common dosage of technetium Tc-99m sulfur colloid used for lymphoscintigraphy and sentinel lymph node biopsy of malignant melanoma?
   a. 0.05 – 0.1 mCi
   b. 0.1 – 0.5 mCi
   c. 0.5 – 2.0 mCi
   d. 2.0 – 5.0 mCi

17. Intraoperative probe radiation detecting systems were originally developed for which of the following procedures?
   a. Radioimmunoguided surgery (RIGS) for colorectal cancer surgeries.
   b. Radiation area surveys for contamination.
   c. Tracking nasolacrimal drainage during dacryoscintigraphy.
   d. Determination of radiopharmaceutical therapy patient's release criteria radiation exposure levels.

18. Which of the following is generally recognized as most influential on the particle size of technetium Tc-99m sulfur colloid during compounding?
   a. Time of cooling.
   b. Mass of technetium added to kit prior to heating.
   c. Time of heating.
   d. Radioactive concentration of technetium Tc-99m sodium pertechnetate added to kit.

19. Which of the following is the most critical factor in determining the spatial resolution of gamma detecting probe systems?
   a. Angle of the detector in the probe assembly.
   b. Energy resolution of the system.
   c. Sensitivity of the counting system.
   d. Detector head collimation or shielding.

20. Which of the following is most commonly used for sentinel lymph node biopsy procedures in the United States?
   a. technetium Tc-99m sulfur colloid
   b. technetium Tc-99m human serum albumin
   c. technetium Tc-99m albumin nanocolloid
   d. technetium Tc-99m dextran

21. Which of the following is the only material indicated for subcutaneous administration in the delineation of lymphatic vessels?
   a. technetium Tc-99m sulfur colloid
   b. methylene blue injection, USP
   c. isosulfan blue
   d. indigo carmine injection, USP

22. Although a small number of patients (1.5% incidence) demonstrate an allergic type adverse reaction (localized swelling and mild pruritus of hands, abdomen and neck) following administration of isosulfan blue (LYMPHAZURIN® 1%), all patients receiving this drug as part of a sentinel lymph node biopsy procedure should be advised of which of the following precautions with this drug?
   a. Blue coloration of the tissue surrounding the injection site and urine.
   b. Nausea and vomiting
   c. Intense burning at the site of injection.
   d. Respiratory depression.

23. Which of the following IS NOT true concerning radiation exposure of operating room personnel and pathology staff handling surgical specimens.
    a. Actual whole body badge and TLD ring dosimeter measurements indicate exposure levels requiring occupational radiation monitoring by regulation.
    b. Greater than 50 melanoma sentinel lymph node biopsy procedures typically result in less radiation exposure to the hands of the surgeon than the typical whole body radiation dose received each year from background radiation.
    c. Surgical specimens routinely exceed the radiologic control level of 0.002 μCi/Gm.
    d. Actual TLD ring dosimeter measurements of radiation exposure to the hands of surgeons range from 0.5 – 10 mrem for each sentinel lymph node biopsy.

24. Which of the following materials is undergoing evaluation for lymphoscintigraphy and sentinel lymph node biopsy because of the ability to compound with both radioactive and color properties?
    a. technetium Tc-99m microspheres
    b. technetium Tc-99m dextran
    c. technetium Tc-99m hydroxyethyl starch
    d. technetium Tc-99m liposomes

25. Which of the following is the primary reason for completing diagnostic imaging (lymphoscintigraphy) after subcutaneous administration of radioactive colloid around the primary tumor site?
    a. Provide evidence the radioactive material is draining into the lymphatic channels from the injection site.
    b. Make certain adequate count rates will be obtained during surgery.
    c. Guide the surgeon to the sentinel lymph node(s).
    d. Estimate the radiation levels to be encountered in the operating room.