A Phase I/II Trial of ^{125}I Methylene Blue for One-Stage Sentinel Lymph Node Biopsy

Jason David Cundiff, MD, Yi-Zarn Wang, MD,* Gregory Espenan, MS,† Thomas Maloney, BS,‡ Arthur Camp,‡ Laura Lazarus, MD,* Alan Stolier, MD,* Randy Brooks,§ Bruce Torrance,* Shawn Stafford,* James P. O'Leary, MD,* and Eugene A. Woltering, MD**

Background: Sentinel lymph node biopsy can be associated with delays in operating room schedule and with significant pain during the preoperative ^{99m}Tc colloid injection. To avoid these problems, we developed a novel radiolabeled blue dye that can be injected intraoperatively.

Methods: We performed a phase I/II trial (IND#70627) of sterile pyrogen-free ^{125}I-methylene blue to identify sentinel nodes in patients with breast cancer. Twelve women were studied. Two women each were given peritumoral or circumareolar injections of 100, 200, 300, 400, 500, or 1000 μCi of ^{125}I methylene blue.

Results: Sentinel nodes were detected in 11 of 12 patients, with a low-dose 200 μCi patient being the single exception. The number of sentinel nodes detected per patient ranged from 0 to 3 (mean = 1.66 nodes/case). Radioactivity at the tumor injection site [counts per second (cps) averaged over 10 seconds] ranged from 3346 to 47,300 cps and was highly dose-dependent ($r = 0.90$, $P = 0.0002$). In contrast, the in vivo node counts ranged from 0 to 1228 cps, while ex vivo counts ranged from 0 to 1516 cps. The in vivo nodal counts were dose-dependent ($r = 0.67$, and $P = 0.0231$). Radiation was carefully monitored inside the operating room and in pathology. Even with the 1-mCi dose, the radioactive blue dye produced significantly lower personnel exposure than historically seen with ^{99m}Tc.

Conclusions: This method eliminates the painful preoperative injections of ^{99m}Tc colloid, is performed by the surgeon in the operating room, is associated with lower radiation exposures for personnel, and avoids the delays caused by nonoperating room personnel. These observations warrant a more extensive trial of this method using the 1000-μCi dose of ^{125}I methylene blue dye for sentinel lymph node biopsies.

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Sentinel lymph node biopsy was initially developed as a technique for the detection of regional lymph node metastasis in patients with melanoma by Morton et al.[1] The evolution of the procedure as a means to detect the presence of axillary lymph node metastasis in patients with breast cancer developed shortly thereafter. In less than 10 years following the initial reports utilizing this technique, dozens of studies have appeared in the scientific literature validating use of sentinel node biopsy as an accurate means of detecting metastatic disease in the axillary lymph nodes in patients with breast cancer.[2–5]

Almost simultaneously, reports appeared in the literature documenting the success of sentinel node localization using either isosulfan blue dye alone or using isosulfan blue dye in conjunction with technetium^{99m} labeled sulfur colloid (^{99m}TcSC). While most authors use a combination of blue dye and ^{99m}TcSC, Guiliano et al continue to report excellent results using isosulfan blue dye alone.[6] Conversely, Krag et al report similar excellent results using only radiotracer.[2,3] Currently, the majority of breast surgeons prefer to use both dye and radiotracer for their evaluation of sentinel nodes. In recent years, others have published studies suggesting that sentinel node accuracy and yield could be duplicated with the use of methylene blue dye as opposed to isosulfan blue dye.[7–9] This change in dye preference has found its way into the practice of a significant population of breast surgeons. Injection of small quantities (0.1–0.5 mL) of methylene blue into the breast during needle/wire breast cancer mammographic localization procedures has been done for years and has been associated with no reported adverse effects.[10] In a similar fashion, injection of methylene blue in the web space between the toes has been used widely as a method to detect lymphatic channels for their cannulation for lymphangiograms.

With the development of the sentinel node biopsy method of evaluating axillary lymph node status in women with breast cancer came some unanticipated consequences for both surgeon and patient alike. Patients must undergo a separate preoperative injection of radiocolloid prior to their surgery. The radiocolloid injection is commonly performed a minimum of 2 hours preoperatively, often the afternoon prior to surgery or more typically the morning of surgery. Patients uniformly note that the radiocolloid injection is very painful. The injection is painful whether it is injected in small quantities intradermally or in larger quantities around the periphery of a tumor.[11,12] With the advent of sentinel lymph node biopsy as an alternative standard of care for breast conservation candidates, there has been an increase in patient and referring-physician demand for this less invasive

From the Departments of *Surgery and †Radiology, Louisiana State University Health Sciences Center, New Orleans LA; ‡Iso-Tex, Friendswood, TX; and §General Electric Health Care New Orleans, LA.

Reprints: Jason D. Cundiff, MD, 30 Chateau Pontet Canet, Kenner, LA 70065. E-mail: jcundi@lsuhsc.edu or jasoncundiff@cox.net.

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procedure. Surgeons have often been forced to deal with major delays in their surgical schedules based on the requirement for an additional preoperative procedure that is performed at the discretion of nonoperating room personnel, specifically nuclear medicine physicians and technicians.[13]

A single, simultaneous injection of blue dye and radiocolloid in the operating room at the time of breast cancer surgery would obviate the need for an additional preoperative procedure for the patients and would prevent delays in the surgical schedule. Unfortunately, ^{99m}Tc sulfur colloid has high-energy gamma emissions and a significant amount of activity (1–10 mCi) is commonly injected to ensure adequate node uptake. Much of this activity must clear from the injection site before the hand-held gamma probe can effectively discriminate individual nodes in the axilla from the injection site

We have devised a simple, economically feasible way to produce a sterile, pyrogen-free ^{125}I-labeled methylene blue dye. Preliminary animal experimentation showed rapid transit to regional nodes and limited systemic biodistribution.[14] When absorbed systemically, the radiolabeled dye is rapidly cleared in the urine. We hypothesized that admixing small quantities of the ^{125}I labeled methylene blue dye (0.1–0.5 mL) in a much larger (4.5–4.9 mL) quantity of unlabeled methylene blue dye should offer the surgeon all the specificity of a 2-stage sentinel lymph node biopsy procedure, enhanced intraoperative 2-point discrimination based on the low energy of ^{125}Iodine's gamma emissions, and potentially offer increased safety based on ^{125}Iodine's low-energy (35 Kev) gamma emissions. More importantly, the use of a 1-step intraoperative procedure would be painless and would obviate the need for a second procedure performed outside of the operating room by nonsurgical personnel.

METHODS AND MATERIALS

To test these hypotheses, we performed a prospective phase I/II trial of ^{125}I methylene blue for the intraoperative detection of sentinel nodes in 12 women with invasive breast cancer.

Drug Information

Methylene blue (1% USP) used in the protocol was obtained commercially as a sterile pyrogen-free product (Faulding Pharmaceutical Co., Paramus, NJ). This drug is commonly used in clinical practice and has documented low risk and incidence of adverse side effects. Allergic or adverse reactions attributed to methylene blue are rare and usually occur when much higher doses of this drug are administered. This blue dye was labeled with ^{125}Iodine (^{125}I) using a proprietary method, purified, and its sterility and pyrogenicity determined prior to distribution by Iso-Tex, Friendswood, TX. ^{125}Iodine was commercially obtained from Nordion, Kanata, Ontario, Canada or from McMasters University, Hamilton, Ontario, Canada. The identity of ^{125}I was confirmed on a multichannel analyzer. Purity of the final product was confirmed using mass spectrometry of ^{127}I-labeled (by identical parallel reactions) product and HPLC. Each batch was tested for radiostability. The final product has a shelf-life of 120 days.

The decay-corrected radioactive content of the final admixture of cold (methylene blue) and hot (^{125}I methylene blue) products ranged from 100 μCi/5 mL to 1000 μCi/5 mL. The physical half-life of ^{125}I is 60 days; however in preliminary animal experiments, the biologic half-life of the radiolabeled dye is about 6 hours.[14] This correlated well with the known plasma half-life of nonlabeled methylene blue (5.4 hours).

Clinical Study Design

Patients

Twelve women with pathologically proven invasive breast cancer were enrolled into this trial between December 2004 and May 2005. Clearance to use the experimental drug ^{125}I methylene blue in this phase I/II pilot trial was granted following submission of an Investigational New Drug (IND #70627) application through the FDA and approval by the IRB for the Louisiana State University Health Sciences Center New Orleans, LA. Participating surgeons in this trial were considered to be experts in sentinel lymph node biopsy techniques for breast cancer. In general, the surgical techniques used for sentinel node biopsy in this series were based on the National Surgical Adjuvant Breast and Bowel Program (NSABP) guidelines. The inclusion criteria for this study included female subjects with stage 0, I, or II invasive breast cancer with a clinical axillary node status of N0. The exclusion criteria for this study precluded participation of subjects known to be pregnant or nursing, incarcerated prisoners, subjects under the age of 18 years, and subjects with a known allergy to shellfish, iodine, or methylene blue dye.

Preoperative Preparation

To reduce the potential for ^{125}I uptake in the thyroid, all patients were given 10 drops a day of a saturated iodine solution (Lugol's solution) orally for 2 days prior to surgery, the day of surgery, and for 3 days thereafter.

Intraoperative Technique

Following induction of general anesthesia, patients were injected with a combination of unlabeled methylene blue and ^{125}I methylene blue in doses ranging from 100 μCi to 1000 μCi. Two patients each received doses of 100, 200, 300, 400, 500, and 1000 μCi of ^{125}I methylene blue dye combined with unlabeled methylene blue dye in a total volume of 5 mL. Patients were injected in the peritumoral or subareolar location using 1.25-mL aliquots in the 3-, 6-, 9-, and 12-o'clock positions of breast tissue surrounding the tumor or areola. Decisions regarding peritumoral or subareolar injection were at the discretion of the operating surgeon. Counting of the radioactive emissions at the primary injection site [counts per second (cps) averaged over 10 seconds] was performed and recorded immediately following injection. Manual massage and compression of the injected breast was then performed for 10 minutes following injection. A hand-held gamma detector (Neoprobe, Model 1000, Dublin, OH) was used to scan the axilla once each minute beginning at 15 minutes postinjection. Transcutaneous "hot spots" in the axilla were defined as radioactive counts (cps) consistently higher than the adjacent background. Failure to elicit a significant "hot spot" in the axilla within 20 minutes

prompted us to perform "flushing" of the primary injection site with 25 to 50 mL of sterile NaCl solution per the NSABP B-32 protocol training manual. The time interval necessary for discovery of the "hot spot" following primary injection was also recorded. An incision was made in the axilla overlying the "hot spot." The hand-held gamma detector was placed within the wound to facilitate more precise detection of the sentinel lymph node. Lymph nodes that were significantly higher in radioactive content than adjacent axillary tissue were considered "hot" sentinel lymph nodes and excised. In vivo and ex vivo nodal counts, and axillary background counts were performed and recorded before and following removal of the sentinel node. Lymph nodes with in vivo counts greater than 10% of the in vivo counts obtained with the first node identified were also considered sentinel lymph nodes. Lymph nodes that were stained blue and/or having afferent lymphatic channels stained blue were also considered to be sentinel lymph nodes and excised. In addition, lymph nodes that contained both significant radioactive counts and were stained blue were categorized as "hot and blue" sentinel lymph nodes. All sentinel lymph nodes identified by the established criteria were labeled, submitted individually, and delivered to pathology where they were stained with hematoxylin and eosin and for the presence of cytokeratin (immunohistochemistry) following serial step sectioning. All information regarding separate sentinel lymph nodes was reported individually.

Postoperative Procedure

All patients received standard postoperative care and continued to take oral Lugol's solution on the day of surgery and for 3 days thereafter. Patients were scanned postoperatively at 7 and 14 days with a hand-held gamma detector for detection of residual radioactivity in the primary injection site, the axilla, and the thyroid gland.

Statistical Analysis

This project was a pilot phase I/II study to determine the feasibility of this technique and to determine the specific doses (ratio of hot to cold dye) and timing from injection to surgical removal of the nodes required for a larger scale trial. Thus, formal statistical analysis of this data was not done.

RESULTS

Between December 2004 and May 2005, 12 patients were enrolled in this study. The mean age (±SD) of these women was 51 ± 8 years. Four of the women were white, 7 were black, and 1 was Hispanic. All patients were originally diagnosed with invasive breast cancer. Eleven patients underwent needle core biopsy and 1 underwent open incisional biopsy to establish a tissue diagnosis. All patients were staged clinically as stage I or II prior to surgery. Two patients underwent neoadjuvant chemotherapy prior to definitive surgery. All of the patients initially underwent lumpectomy and sentinel lymph node biopsy. Three of the patients were found to have metastatic disease in the sentinel nodes and underwent subsequent completion axillary dissection. No other metastatic nodal disease was discovered in these patients. All 3 findings of axillary node metastatic malignancy returned as invasive ductal carcinoma of the breast, which was congruent with the primary breast malignancy in all cases (Fig. 1).

Four of the patients received peritumoral injections and 8 received subareolar injections. The mean radioactive count (cps ± SD) at the primary injection site was 24,050 ± 11,664 and ranged from 3346 to 47,300 (Table 1). The cps at the primary injection site correlated well with the dose injected (Fig. 2, $R^2 = P < 0.001$). All patients were injected with the full 5 mL volume of ^{125}I methylene blue solution. Six patients received a supplemental 25-mL sterile NaCl solution "flush" injection to facilitate lymphatic drainage according to NSABP guidelines. Sentinel nodes were detected in 11 of 12 patients. A low-dose 200-μCi patient was the single exception. The mean number of nodes per patient detected was 1.66. The mean time interval between injection and transcutaneous detection of the sentinel lymph node was 22 minutes. Hot spots in the axilla were transcutaneously identified in 11 of 12 patients. All hot spots involved nodes in the level I axillary node group. A sentinel node was found underneath the dermal hot spot in all instances. Of the 20 total sentinel lymph nodes discovered (Table 1), 16 nodes were classified as "hot and blue" and 4 nodes as "blue only." No nodes met the criteria for "hot only." The mean in vivo counts (±SD) for all "hot and blue" nodes (Table 2) were 391 ± 324 and ranged from 20 to 1228 cps; however, the mean in vivo (±SD) counts for patients receiving doses ranging from 400 μCi to 1000 μCi were 531 ± 289, with values ranging from 55 to 1228 cps. The mean ex vivo counts (±SD) for all "hot and blue" nodes were 264 ± 347 cps and ranged from 14 to 1516 cps; however, the ex vivo counts (±SD) for patients receiving doses ranging from 400 μCi to 1000 μCi (n = 6) were 310 ± 419 cps, with values ranging from 25 to 1516 cps. The mean axilla background counts were 19 ± 29 cps and ranged from 0 to 107 cps.

No adverse local, systemic, or anaphylactic reactions were noted during or following injection of the ^{125}I methylene blue. No evidence of skin necrosis in the vicinity of the primary injection site was observed. One patient receiving a 200-μCi dose developed a local infection in the lumpectomy wound that resolved with local wound care. One patient receiving a dose of 300 μCi developed a seroma 1 week

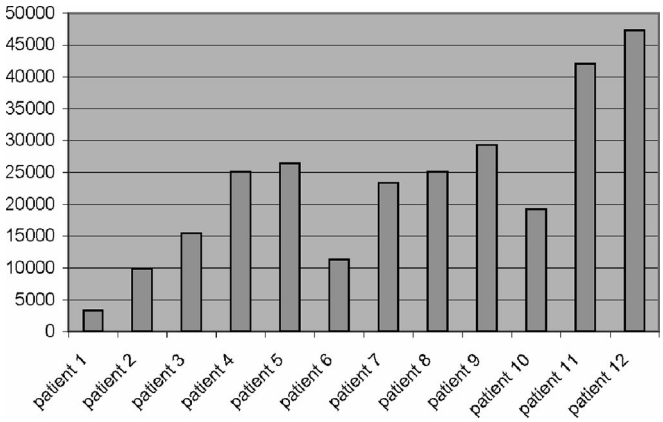

FIGURE 1. Mean cps (averaged over 10 seconds) at the primary tumor injection site.

TABLE 1. Time Interval and Mode of Detection for Discovery of Sentinel Lymph Nodes

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Total (%)
Dose*	100	100	200	200	300	300	400	400	500	500	1000	1000	
Primary injection site†	3346	9872	15,432	25,110	26,438	22,000	23,400	25,000	29,300	19,300	42,100	47,300	
Transcutaneously detected‡	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Time interval (mins)§	24	22	NA††	21	25	25	20	20	18	22	25	20	
Hot only‖	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Blue only¶	0	0	0	2	1	0	0	0	0	0	0	1	4 (20%)
Hot/blue#	1	1	0	0	2	1	3	1	2	2	1	2	16 (80%)
Flushed**	Yes	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	6 (50%)

*Microcuries per injected dose; no sentinel lymph nodes were identified in this patient.
†Mean of 4 counts per second averaged over a 10-second interval using a handheld gamma detector (Neoprobe).
‡Radioactive counts significantly above the background using a handheld gamma detector (Neoprobe).
§Time period from initial injection of ^{125}I methylene blue until transcutaneous detection of sentinel lymph node.
‖Defined as having significant radioactivity, but no blue staining of the node or afferent channels leading to the node.
¶Defined as visibly stained blue or with blue afferent lymphatic channels without detectable radioactivity.
#Defined as having detectable radioactivity and blue staining of the node or afferent lymphatic channel.
**Denotes injection of 25 mL of sterile NaCl solution into primary injection site following injection of ^{125}I methylene blue into breast.
††No sentinel lymph nodes where detected in this patient.

postoperatively. The radioactive count at the breast wound site was recorded as 3100 cps at that time. Drainage of the seroma resulted in dramatic decrease in wound counts (count 77 cps) 1 week later (day 14).

The mean radioactive counts (±SD) of the thyroid gland, axillary wound, breast lumpectomy wound, and background radioactive counts (Table 3) 1 week postoperatively were 26 ± 7, 22 ± 12, 349 ± 833, and 4 ± 2 cps, respectively.

DISCUSSION

The use of sentinel lymph node biopsy has become a standard technique for nodal staging of patients with early-stage breast cancer. The most common application of this technique involves a 2-stage procedure involving a preoperative injection of a ^{99m}Tc sulfur colloid radiotracer and the intraoperative injection of unlabeled isosulfan blue dye.

The use of isosulfan blue as the injectable dye in sentinel lymph node biopsy procedures stems from feline studies and studies comparing isosulfan blue to different dyes indicating effective lymphatic uptake and nodal binding of isosulfan blue in the primary node groups.[15,16] Successful use in sentinel lymph node biopsy with melanoma patients made isosulfan blue a logical choice when the technique was extended to breast cancer patients.[17] Subsequent clinical studies have shown that methylene blue is equivalent to isosulfan blue when used alone or in conjunction with a radiotracer.[7–9] A review of the literature reveals an incidence of allergic reactions occurring in 1% to 3% of patients receiving isosulfan blue.[18–20] These allergic reactions range

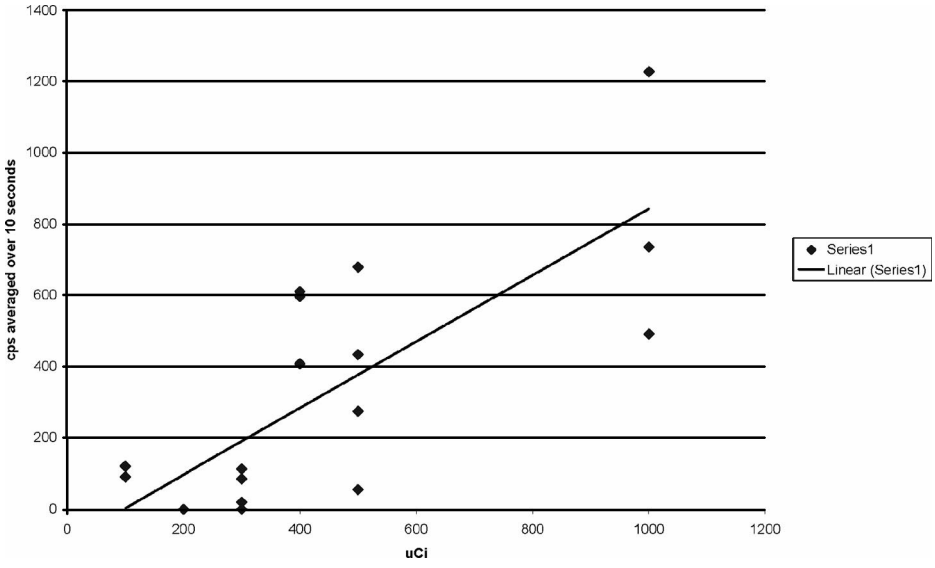

FIGURE 2. Mean in vivo nodal counts in cps (averaged over 10 seconds).

TABLE 2. Primary Site Counts, *In Vivo, Ex Vivo,* and Axilla Counts per Second

Patient No.	Dose (mCi)	Primary Site	SLN (no. per patient)	*In Vivo**	*Ex Vivo*†	Axilla‡
1	100	3346	1	120	150	65
2	100	9872	1	91	66	2
3§	200	15,432	N/A	0	0	0
4‖	200	25,110	1	0	0	0
			2	0	0	0
5	300	26,438	1	112	264	6
			2	85	75	2
			3	0	14	2
6	300	22,000	1	20	39	1
7	400	23,400	1	596	389	107
			2	327	153	66
			3	407	95	48
8	400	25,020	1	610	83	2
9	500	29,313	1	679	659	17
			2	55	35	9
10	500	19,302	1	433	42	1
			2	274	25	2
11	1000	42,100	1	491	391	2
12	1000	47,300	1	1228	244	9
			2	735	1516	5
			3	0	0	3
Mean (SD)		24050 ± 1164	1.66	391 ± 324	264 ± 347	19 ± 29

**In vivo* count per second average over 10 seconds of lymph node still in axilla prior to excision.
†*Ex vivo* count per second average over 10 second of lymph node following excision.
‡Background count per second over 10 seconds of axilla background following excision of lymph node.
§Patient 4 had no sentinel lymph nodes detected.
‖Patient 5 had two blue stained sentinel lymph nodes with no detectable radioactivity.

from hives and wheals to anaphylactic shock.[20] Others have documented cases of severe anaphylaxis in patients following administration of isosulfan blue.[8,12,18,20] Isosulfan blue is a triphenylmethane-based dye and is one of the rosaniline dyes. Triphenylmethane dyes have been used for years in cosmetics, leather, medicine, and textile industries. Since many items used by the general population contain triphenylmethane-related dyes, sensitization to isosulfan blue can occur and may not be elicited by an allergy history or negative skin test.[18,20] The exact mechanism for allergic reaction is unknown but has been theorized to be anaphylactic (ie, IgE-related) or anaphylactoid in nature.[20] Conversely, serious allergic reactions to methylene blue injection are extremely rare, and there have been no reported anaphylactic reactions related to methylene blue use during sentinel lymph node biopsy.[20] There have been several reports of local skin inflammation and even local necrosis associated with subdermal injection of methylene blue; however, peritumoral or subareolar injection, as performed in this study, can be performed at tissue depths that should lessen this risk.[21]

The use of the standard radiotracer 99Tc sulfur colloid is based on collaborative data from multiple studies demonstrating unfiltered ^{99m}Tc as having the highest success rate for labeling sentinel lymph nodes.[2,3,22,23] Other commercially available radiopharmaceuticals; such as Cardiolyte, Dextran 40, or Microlite; have been found to be inferior to unfiltered

TABLE 3. Thyroid, Axilla, Breast Wound, and Background Counts per Second* 1 Week Postoperatively

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Thyroid	29	19	24	40	28	16	21	28	33	38	27	19
Axilla†	25	32	28	15	60	17	11	14	21	16	17	15
Wound‡	300	180	110	220	3100‖	55	49	46	38	44	31	26
Background§	3	3	3	2	4	8	7	3	5	6	8	4

*Counts per second averaged over a 10-second interval.
†Recorded over the axillary wound incision.
‡Recorded over lumpectomy wound site.
§Recorded from contralateral breast.
‖In seroma, counts decreased to background following drainage of seroma.

^{99m}Tc for detection of the sentinel lymph node.[2,24–26] Although effective, the use of ^{99m}Tc sulfur colloid is not without drawbacks. In terms of patient experience, injection of the recommended 5 to 8 mL volume of ^{99m}Tc sulfur colloid into the breast is an extremely painful consequence of this "minimally invasive" procedure. Indeed, the pain related to the injection of the viscous sulfur colloid has been subjectively quoted as being "severe" by our patients. In addition to complaint of pain, vasovagal episodes following injection of ^{99m}Tc colloid have been reported.[27] Although no concrete data exist on the rate of these complications and the degree of anxiety created, we theorized that the pain and anxiety experienced during injection of ^{99m} Tc colloid should be similar to that reported for needle localization procedures. Kelly and Winslow reported the mean anxiety score of 5.3 (scale 0–10) in women undergoing needle localization procedures. Nine percent of patients fainted and other reported complications including pain, stinging, embarrassment, and dizziness.[17] The usual 2-hour interval required for adequate transit of media to the sentinel nodes necessitates that this injection be performed while the patient is awake, often the day before or more typically the morning of surgery. The impetus behind the requisite waiting period revolves around the need for not only adequate time for proper nodal collection of the radioactive media, but also for adequate clearance of the primary injection site to prevent the phenomena known as "shine-through." The relatively high radioactive dose of ^{99m} Tc colloid (1–10 mCi) makes discrimination between primary injection site radioactivity and significant radioactivity in the sentinel nodes difficult, due to overlap in the radioactive signal from the primary injection site and the sentinel node(s) in the axilla (shine-through phenomenon).

Layeeque et al[27] and Zogakis et al[13] have both reported success with intraoperative subareolar injection of ^{99m}Tc colloid; however, more powerful comparative studies must be conducted before a final conclusion on this technique can be reached. The maximum dose of ^{125}I methylene blue (1000 μCi) represents one tenth the radioactivity (10 mCi) for ^{99m}Tc sulfur colloid injected during lymphoscintigraphy procedures done the day before surgery. Obviously, less radioactive exposure provides a higher level of safety for patients, surgeons, and perioperative personnel. In addition, the energy of ^{99m}Tc is 140 Kev as compared with 35 Kev from ^{125}I.

^{125}I methylene blue as a single entity can be injected intraoperatively because the radiolabeled dye quickly transits through the lymphatics to the sentinel node groups. Animal studies using rabbits demonstrated significant radioactive uptake in regional lymph nodes within 10 minutes following injection.[14] In our study at doses of 400 μCi and higher, transcutaneous detection of "hot spots" was achieved approximately 20 minutes postinjection. At lower doses (100–200 μCi), transcutaneous detection of "hot spots" was achieved within the same time period, but only following a 25-mL sterile NaCl "flushing" at the primary injection site to facilitate lymphatic drainage. Flushing procedures were used as an adjunct in 6 subjects. Regardless of the administered dose, 11 of 12 patients demonstrated transit of detectable radioactivity to the sentinel lymph nodes. The remaining subject had no sentinel lymph nodes detected by radiotracer or by the blue dye. The percentage of detected sentinel lymph nodes classified as "hot and blue" was 80%, with the remaining 20% of sentinel lymph nodes observed to be "blue only." Wada et al have reported a similar percentage of "hot and blue" nodes (81%) in their analysis of the traditional combined technique using blue dye and radiotracer.[28] Based on these results, it appears that conjugated dye and radiotracer effectively transit from the primary injection site to the sentinel lymph nodes. The lower emitted energy of ^{125}I gamma particles necessitates an injected dose of at least 300 μCi to allow reliable transcutaneous detection, but injections of 1000 μCi were proven to be more effective. No significant difference in time interval for initial detection of "hot spot" or eventual detection of sentinel node was noted between the patients undergoing peritumoral versus subareolar injection. In general, patients with upper, lateral quadrant tumors were given subareolar injections. No adverse reactions were attributed in this study to injection of ^{125}I methylene blue or unlabeled methylene blue dye. Superficial injections administered in a subareolar location exhibited no incidence of skin necrosis.

The 1- and 2-week postoperative radioactive counts using a hand-held gamma detector revealed no patients with significant thyroid radioactivity above background counts on the contralateral chest. All patients reported complete compliance with use of the thyroid-protective Lugol's solution. The 1 patient with a significant residual radioactive count 1 week postoperatively within the lumpectomy wound site had a large seroma at that time. Subsequent drainage of the seroma lowered the residual activity to background 2 weeks postoperatively. We theorized that the residual injected ^{125}I methylene blue had become trapped in the seroma within the lumpectomy cavity.

Currently, there are no restrictions on intraoperative injection of radiodiagnostic agents listed by the Office of Safety and Health Administration guidelines or International Commission on Radiation Protection. Thus, there are no rules or guidelines that prohibit injection of radioisotope by surgeons in the operating room. Our hospital requires a 4-hour training class on radiation safety prior to handling of such materials. Indeed, injection of ^{125}I methylene blue by a surgeon may be a billable event under the Injection of Contrast Code (CPT Code 38792).

The use of ^{125}I methylene blue for labeling of sentinel lymph nodes in the axilla may prove to be an effective means of reliable detection of sentinel lymph nodes in selected breast cancer patients. Administration of the drug was proven to be safe for humans with no incidence of adverse reactions or residual radioactive uptake by the thyroid gland within this study. An effective dose of 1000 μCi was identified that allows for reliable transcutaneous detection of the sentinel lymph node.

CONCLUSION

This technique eliminates painful preoperative injections of ^{99m}Tc colloid, is performed by the surgeon in the operating room, is associated with lower radiation exposures

for personnel, and avoids the delays caused by nonoperating room personnel. A prospective, adequately powered phase II study will be necessary to prove equivalency to the techniques that have become the current standard of care.

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