ANNALS OF SURGERY
Vol. 233, No. 5, 676–687

Dermal Injection of Radioactive Colloid Is Superior to Peritumoral Injection for Breast Cancer Sentinel Lymph Node Biopsy: Results of a Multiinstitutional Study

Kelly M. McMasters, MD, PhD,* Sandra L. Wong, MD,* Robert C. G. Martin II, MD,* Celia Chao, MD,* Todd M. Tuttle, MD,‡ R. Dirk Noyes, MD,§ David J. Carlson, MD,‖ Alison L. Laidley, MD,¶ Terre Q. McGlothin, MD,¶ Philip B. Ley, MD,# C. Matthew Brown, MD,** Rebecca L. Glaser, MD,†† Robert E. Pennington, MD,‡‡ Peter S. Turk, MD,§§ Diana Simpson, RN,* Patricia B. Cerrito, PhD,† and Michael J. Edwards, MD,* for the University of Louisville Breast Cancer Study Group

*From the *Division of Surgical Oncology, J. Graham Brown Cancer Center, Department of Surgery, and †Department of Mathematics, University of Louisville School of Medicine, Louisville, Kentucky; ‡Park Nicollet Clinic, Minneapolis, Minnesota; §LDS Hospital, Salt Lake City, Utah; ‖St. Mary's Medical Center and Deaconess Hospital, Evansville, Indiana; ¶Breast Surgeons of North Texas, Dallas, Texas; #Surgical Clinic Associates, Jackson, Mississippi; **Norton Hospital, Louisville, Kentucky; ††Kettering Hospital, Dayton, Ohio; ‡‡General Surgeons Inc., Richmond, Indiana; and §§Presbyterian Hospital, Charlotte, North Carolina*

Objective
To determine the optimal radioactive colloid injection technique for sentinel lymph node (SLN) biopsy for breast cancer.

Summary Background Data
The optimal radioactive colloid injection technique for breast cancer SLN biopsy has not yet been defined. Peritumoral injection of radioactive colloid has been used in most studies. Although dermal injection of radioactive colloid has been proposed, no published data exist to establish the false-negative rate associated with this technique.

Methods
The University of Louisville Breast Cancer Sentinel Lymph Node Study is a multiinstitutional study involving 229 surgeons. Patients with clinical stage T1–2, N0 breast cancer were eligible for the study. All patients underwent SLN biopsy, followed by level I/II axillary dissection. Peritumoral, subdermal, or dermal injection of radioactive colloid was performed at the discretion of the operating surgeon. Peritumoral injection of isosulfan blue dye was performed concomitantly in most patients. The SLN identification rates and false-negative rates were compared. The ratios of the transcutaneous and ex vivo radioactive SLN count to the final background count were calculated as a measure of the relative degree of radioactivity of the nodes. One-way analysis of variance and chi-square tests were used for statistical analysis.

Results
A total of 2,206 patients were enrolled. Peritumoral, subdermal, or dermal injection of radioactive colloid was performed in 1,074, 297, and 511 patients, respectively. Most of the patients (94%) who underwent radioactive colloid injection also received peritumoral blue dye injection. The SLN identification rate was improved by the use of dermal injection compared with subdermal or peritumoral injection of radioactive colloid. The false-negative rates were 9.5%, 7.8%, and 6.5% (not significant) for peritumoral, subdermal, and dermal injection techniques, respectively. The relative degree of radioactivity of the SLN was five- to sevenfold higher with the dermal injection technique compared with peritumoral injection.

Conclusions
Dermal injection of radioactive colloid significantly improves the SLN identification rate compared with peritumoral or subdermal injection. The false-negative rate is also minimized by the use of dermal injection. Dermal injection also is associated with SLNs that are five- to sevenfold more radioactive than with peritumoral injection, which simplifies SLN localization and may shorten the learning curve.

Sentinel lymph node (SLN) biopsy has become increasingly accepted as a minimally invasive alternative to routine axillary dissection. There are two key parameters of successful SLN biopsy: the SLN identification rate and the false-negative rate. The SLN identification rate is defined as the proportion of patients undergoing the procedure who have localization and removal of an SLN. More important, however, is the false-negative rate, because it defines the frequency with which the SLN is pathologically negative when other axillary nodes harbor metastases. A false-negative SLN biopsy may be detrimental to the patient because it could lead to persistent or recurrent axillary nodal disease and result in inappropriate adjuvant therapy decisions.[1]

Although the results of numerous studies have shown that SLN biopsy can accurately determine the axillary nodal status, SLN identification rates and false-negative rates have been variable.[2–8] This may be related to the considerable variations in the techniques used for SLN biopsy. SLN biopsy is performed by injection of a vital blue dye, radioactive colloid, or both around the tumor site. Peritumoral (into the breast parenchyma around the tumor or biopsy site), subdermal, and dermal injection techniques have been reported. Peritumoral injection of blue dye relies on intraoperative identification of a blue afferent lymphatic channel leading to a blue-stained SLN. Injection of radioactive colloid has also been used, with hand-held gamma probe detection of SLNs. Although the vast majority of the published experience with radioactive colloid involves peritumoral injection (with or without peritumoral blue dye injection), these techniques have not resulted in uniformly good results.

It has been proposed that the skin overlying the breast cancer accurately reflects the lymphatic drainage of the tumor beneath it. Veronesi et al[9] found that subdermal injection of radioactive colloid resulted in an SLN identification rate of 98.2% and a false-negative rate of 4.7%. Further, Linehan et al[10] compared dermal injection of radioactive colloid with concomitant peritumoral blue dye injection. In that study, the radioactive colloid and blue dye localized to the same SLN in 95% of cases. However, the current literature regarding the accuracy of the dermal injection technique is limited by the lack of completion axillary dissection to determine the false-negative rate associated with this technique.[10–13]

We performed this analysis to compare peritumoral, intradermal, and subdermal injection of radioactive colloid in a large multiinstitutional study. The results indicate that dermal injection of radioactive colloid, in conjunction with peritumoral blue dye injection, leads to optimal SLN identification and false-negative rates.

METHODS

After informed consent was obtained, patients were enrolled in the University of Louisville Breast Cancer Sentinel Lymph Node Study, a multiinstitutional study involving 229 surgeons. The study was approved by the institutional review board at each participating institution. Patients with clinical stage T1–2, N0 invasive breast cancer were included in the study. Patients with clinical stage T2 tumors that were later found to be T3 tumors on final pathology were also included. All patients in this analysis underwent SLN biopsy, followed by level I/II axillary dissection. A sentinel node was defined as any blue-stained node, or any node with radioactive counts 10% or more of the ex vivo count of the most radioactive SLN. SLNs were examined by hematoxylin and eosin staining at a minimum of 2-mm intervals. Immunohistochemistry for cytokeratins was used for SLN evaluation in approximately 50% of cases. Nonsentinel nodes were evaluated by routine histology.

Peritumoral, subdermal, or dermal injection of technetium sulfur colloid (TSC) was performed at the discretion of the operating surgeon. Our recommended guidelines included peritumoral injection of 0.5 mCi 0.2-μm-filtered TSC in a volume of 6 mL around the tumor or biopsy site, or dermal injection of 0.5 mCi 0.2-μm-filtered TSC in a volume of 0.5 mL in five areas into the skin (raising a wheal) overlying the tumor or biopsy site. Guidelines for subdermal injection were not provided in the protocol. In 32 of the 297 instances in which subdermal injection was used, some radioactive colloid was also reported to be injected in the dermis; these patients were included in the subdermal injection group. Some investigators also used subareolar or periareolar injection techniques.

Preoperative lymphoscintigrams were not obtained routinely. Biopsy of nonaxillary (e.g., internal mammary) sentinel nodes was not required in this study. Peritumoral blue dye injection, either alone or in addition to radioactive colloid, was also used at the discretion of the operating surgeon.

The ratios of the transcutaneous radioactive count to the final background count and the SLN ex vivo radioactive count to the final background count were calculated as measures of the relative degree of radioactivity of the nodes. One-way analysis of variance and chi-square tests were used for statistical analysis. Significance was determined at $P < .05$.

RESULTS

Between August 1997 and October 2000, 2,206 patients were enrolled in the study. Peritumoral blue dye injection as a single agent was used in 239 patients. When radioactive

Supported by the Center for Advanced Surgical Technologies (CAST) of Norton Hospital, Louisville, Kentucky, and the Links for Life Foundation, Louisville, Kentucky.

Presented at the 112th Annual Meeting of the Southern Surgical Association, December 4–6, 2000, Palm Beach, Florida.

Correspondence: Kelly M. McMasters, MD, PhD, University of Louisville, J. Graham Brown Cancer Center, 529 S. Jackson St., Louisville, KY 40202.

E-mail: kelly.mcmasters@nortonhealthcare.org

Accepted for publication December 2000.

Table 1. CLINICOPATHOLOGIC CHARACTERISTICS OF PATIENTS UNDERGOING SENTINEL LYMPH NODE BIOPSY

Characteristic	Blue Dye Alone (n = 239)	Technetium Sulfur Colloid Injection Site			Total (n = 2,121)
		Peritumoral (n = 1,074)	Subdermal (n = 297)	Dermal (n = 511)	
Age, years (median)	60	60	61	58	60
Tumor size					
T1	73.8%	69.4%	68.3%	72.9%	70.6%
T2	24.0%	28.5%	29.4%	24.1%	27.0%
T3	2.1%	2.1%	2.4%	3%	2.4%
Palpable tumor	55.6%	54.8%	50.2%	53.0%	52.6%
Tumor location					
Central	13.0%	13.8%	16.7%	17.0%	14.9%
Upper outer quadrant	56.7%	51.5%	48.8%	50.3%	51.4%
Upper inner quadrant	9.5%	15.2%	16.0%	15.8%	14.8%
Lower outer quadrant	16.0%	13.5%	11.3%	8.5%	12.3%
Lower inner quadrant	4.8%	6.1%	7.2%	8.5%	6.7%
Pathology					
Invasive ductal carcinoma	82.0%	79.1%	84.2%	84.0%	81.3%
Invasive lobular carcinoma	7.9%	10.6%	8.8%	11.0%	10.1%
Other	10.0%	10.2%	7.1%	5.1%	8.5%
Biopsy type					
Excisional	31.8%	33.9%	38.7%	32.7%	34.0%
Needle	68.2%	65.8%	60.6%	67.1%	65.7%
Other	0%	0.3%	0.6%	0.2%	0.3%
Surgery type					
Total mastectomy	28%	28.9%	36.7%	35.8%	31.5%
Partial mastectomy	72%	71.1%	63.3%	64.2%	68.5%
% axillary node metastasis	30.1%	34.2%	36.9%	33.5%	33.9%
Mean # SLN removed	1.66*	2.29	2.47	2.57	2.32
Mean # axillary nodes removed	14.01	15.11	14.09	14.77	14.76
Blue dye injected also	100%	93.1%	95.3%	94.7%	94.6%

SLN, sentinel lymph node.
* $P < .0001$, analysis of variance.

colloid was used, the injection was performed using the peritumoral, subdermal, or dermal technique in 1,074, 297, and 511 patients, respectively. A total of 85 patients underwent either subareolar or periareolar injection of radioactive colloid. Because of the small sample size, the subareolar and periareolar radioactive colloid injection groups were not included in the statistical analysis. Most of the patients (94%) who underwent radioactive colloid injection also received peritumoral blue dye injection. Exclusion of the patients who did not have concomitant blue dye injection did not alter the statistical analysis; therefore, all patients were included. The groups were well balanced with respect to age, tumor size, tumor palpability, tumor location, histologic subtype, type of biopsy, type of surgery, percentage with axillary metastases, and mean number of axillary nodes removed (Table 1). There was, however, a significant decrease in the mean number of SLNs identified with the use of blue dye alone compared with other methods ($P < .0001$, analysis of variance).

Comparison of results using different injection techniques is shown in Table 2. The SLN identification rates were 89.9%, 95.3%, and 98.0% in the peritumoral, subdermal, and dermal groups, respectively (significant difference among all techniques, $P < .0001$, chi-square). The false-negative rates were not statistically significant among the three major radioactive colloid injection type groups. Of the 85 patients who underwent either subareolar or periareolar injection of radioactive colloid, the SLN identification and false-negative rates were 98.8% and 5.9%, respectively. When patients undergoing blue dye injection alone were compared with patients undergoing radioactive colloid injection (all techniques), the SLN identification rates were 87.0% versus 92.9%, respectively ($P = .0012$), and the false-negative rates were 11.3% versus 7.7%, respectively (no significant difference).

Overall, upper outer quadrant tumor location was associated with an increased likelihood of a false-negative result compared with all other locations when all patients were included (11.3% vs. 4.5%, $P = .001$). Table 3 shows the relation between tumor location and radioactive colloid injection technique. Only the use of peritumoral injection was associated with a significant difference in false-negative rate between upper outer quadrant tumors and all other locations (11.4% vs. 4.9%, $P = .016$). The use of blue dye

Table 2. RESULTS OF SENTINEL LYMPH NODE BIOPSY BASED ON INJECTION TECHNIQUE

Injection Technique	SLN ID	SLN ID Rate*	True Positives	False Negatives	False-Negative Rate¶
Radioactive colloid					
Peritumoral	965/1,074	89.9%	311	28	8.3%
Subdermal	283/297	95.3%‡	94	8	7.8%
Dermal	501/511	98.0%§,‖	157	11	6.5%
Blue dye alone	208/239	87.0%†	63	8	11.3%
Overall	2,041/2,206	92.5%	641	55	7.9%

SLN, sentinel lymph node identification.
* $P < .0001$, significant difference among peritumoral, subdermal, dermal, and blue dye alone groups, chi-square.
† $P = .20$ vs. peritumoral injection, chi-square.
‡ $P = .0037$ vs. peritumoral injection, chi-square.
§ $P < .0001$ vs. peritumoral injection, chi-square.
‖ $P = .026$ vs. subdermal injection, chi-square.
¶ No significant differences among peritumoral, subdermal, dermal, and blue dye alone groups, chi-square.

as a single agent was associated with a significantly decreased SLN identification rate for tumors outside the upper outer quadrant compared with upper outer quadrant tumors (90.8% vs. 82.2%, $P = .05$).

Both the transcutaneous radioactive count to final background count ratio and the SLN ex vivo radioactive count to final background count ratio were calculated for each TSC injection technique group (Table 4). Dermal injection was associated with SLNs that were approximately five- to sevenfold more radioactive than with peritumoral injection ($P < .0001$).

DISCUSSION

If SLN biopsy is to replace axillary dissection as the means by which the axillary nodes are staged, it is desirable to identify the SLN in at least 90% of patients. Although failure to identify the SLN indicates the need to proceed with standard axillary dissection, no harm is done to the patient from an oncologic standpoint. However, a false-negative result can be detrimental to the patient because it results in incorrect nodal staging, which could lead to inappropriate adjuvant therapy decisions and the risk of persistent or recurrent disease in the axilla. Therefore, we believe that the false-negative rate is the most critical factor in the evaluation of SLN biopsy as a diagnostic test to stage the axillary nodes. A false-negative rate of 5% or less has been commonly cited as the goal for SLN biopsy.[1,14]

A significant problem has been the lack of standardized methodology for the procedure. Peritumoral injection of blue dye alone has been proposed as a simple technique that offers the advantage of not requiring injection of radioactive isotope.[1,6,15] However, the blue dye alone technique is technically challenging and is associated with a significant learning curve. Our results indicate that the SLN identification rate is significantly decreased when blue dye is used alone compared with the use of radioactive colloid. Al-

Table 3. IDENTIFICATION AND FALSE-NEGATIVE RATES BY INJECTION TECHNIQUE AND TUMOR LOCATION

	Radioactive Colloid Injection				
Tumor Location	Peritumoral	Subdermal	Dermal	Blue Dye Alone	Total
SLN identification rate					
Upper outer quadrant	90.5%	95.1%	97.5%	90.8%	92.9%
Other	89.0%	95.4%	98.5%	82.2%	91.6%
P value	.39	.92	.48	.05*	.27
False-negative rate					
Upper outer quadrant	11.4%	10.9%	9.8%	14.6%	11.3%
Other	4.9%	5.6%	3.5%	6.9%	4.5%
P value	.016*	.33	.10	.32	.001*

P values based on chi-square.
* Statistically significant.

Table 4. DEGREE OF RADIOACTIVITY BASED ON INJECTION TECHNIQUE

Radioactive Colloid Injection Technique	Transcutaneous to Final Background Count Ratio*	Ex Vivo to Final Background Count Ratio†
Peritumoral	51	113
Subdermal	126	550
Dermal	239‡	859§

* As measured by the hand-held gamma probe, the ratio of the transcutaneous counts per second of the most radioactive or "hottest" sentinel lymph node (SLN) in the axilla to the final background count after removal of all SLNs. Transcutaneous counts per second divided by final background counts per second.

† The ex vivo counts per second of the hottest SLN divided by the final background counts per second.

‡ $P < .0001$ vs. peritumoral injection.

§ $P < .0001$ vs. peritumoral injection.

though the false-negative rate associated with injection of blue dye alone in the present study (11.3%) was not statistically different from that of patients who received radioactive colloid injection, it is greater than the false-negative rate associated with any of the radioactive colloid injection techniques. Review of all published literature of SLN biopsy with completion axillary dissection (Tables 5 and 6) also suggests that the use of blue dye alone is associated with a significantly decreased SLN identification rate and a trend toward a higher false-negative rate. Although blue dye alone in expert hands can be used effectively, this technique is not easily applied in widespread surgical practice.

Numerous single-institution and only a few multiinstitutional studies have attempted to answer the question of the optimal technique for SLN biopsy. Peritumoral injection of radioactive colloid has been the most widely accepted method, with or without concomitant peritumoral blue dye injection. However, when the body of literature to support peritumoral radiocolloid injection is analyzed, it is clear that this technique is far from perfect. When peritumoral radioactive colloid injection is used (with or without blue dye), the collective experience results in an SLN identification rate of 87.4% and a false-negative rate of 7.9%. In the current study, the SLN identification and false-negative rates associated with peritumoral injection of radioactive colloid (most with concomitant peritumoral blue dye injection) were 89.9% and 8.3%, respectively. In the only two other large multiinstitutional studies[4,32] using peritumoral radioactive colloid injection, the SLN identification and false-negative rates were 93.2% and 11.4% (no blue dye) and 87.1% and 12.9% (concomitant blue dye used), respectively. Taken together, these results suggest that peritumoral injection of radioactive colloid does not provide acceptable results when applied in multiinstitutional practice. Although the SLN identification and false-negative rates are generally considered to improve with increasing surgeon experience, a technique that provides more consistent and reproducible results would be preferable.

We were intrigued by a pilot study of 33 patients by Borgstein et al[33] in which dermal injection of blue dye and peritumoral injection of radioactive colloid resulted in colocalization of both agents to the same nodes. This suggested that the breast parenchyma and overlying skin share a common lymphatic pathway to the axilla, which has a firm embryologic basis. This was also supported by a report from Veronesi et al[9] using subdermal injection of technetium-labeled human serum albumin, with an SLN identification rate of 98.2% and a false-negative rate of 4.7%. Despite these results, dermal or subdermal injection has not been widely accepted for breast SLN biopsy.

As a result of these reports, we began to study dermal injection for SLN identification. Unlike Borgstein et al,[33] however, we used dermal injection of radioactive colloid injection combined with peritumoral blue dye injection. This was based on several factors. First, blue dye injected into the skin results in significant blue staining that can persist for many months, a concern for patients undergoing breast conservation. Further, dermal injection of radioactive colloid has several theoretical advantages. Drawing on the experience with melanoma, dermal injection of radioactive colloid results in rapid, reliable, and efficient identification of SLNs. Peritumoral injection of radioactive colloid for breast cancer generally results in SLNs that are much less radioactive than the SLNs identified in melanoma patients. Second, peritumoral injection of 4 to 8 mL radioactive colloid results in a large zone of diffusion that can cause difficulty in discriminating the mildly radioactive SLNs in the axilla from the high background. This is especially problematic for upper outer quadrant tumors. Because half of all breast cancers are located in the upper outer quadrant, this is a very practical concern. Dermal injection of a small volume of radioactive colloid into the skin overlying the tumor allows the skin to be retracted away from the axilla and also results in SLNs that are much more radioactive (five- to sevenfold greater radioactive counts per second compared with peritumoral injection in the present study). Therefore, the signal-to-background ratio for transcutaneous localization of the SLNs with a hand-held gamma probe is much more favorable. Indeed, an unequivocal "hot spot" in the axilla can be defined before making an incision in virtually all patients with dermal injection. Finally, dermal injection has the advantage that the radioactivity is concentrated in the skin; thus, partial mastectomy specimens are generally only mildly radioactive and less problematic for immediate pathologic analysis.

Although dermal injection of radioactive colloid has been proposed as the optimal technique,[12,13] published data are lacking to establish the false-negative rate associated with this method. Linehan et al[10] reported that peritumoral blue dye injection and dermal radioactive colloid injection colocalize to the same SLNs in 95% of cases, but backup axillary dissection was not performed to determine the

Table 5. PRIOR PUBLISHED EXPERIENCE WITH SENTINEL LYMPH NODE BIOPSY AND COMPLETION AXILLARY LYMPH NODE DISSECTION

Study	N	Blue Dye Injection	Radioactive Colloid Injection	True Positives	False Negatives	Identification Rate	False-Negative Rate
Studies Using Blue Dye Alone							
Giuliano, 1994[15]	174	Peritumoral	Not used	37	5	114/174 (65.5%)	5/42 (11.9%)
Giuliano, 1997[6]	107	Peritumoral	Not used	42	0	100/107 (94%)	0/42 (0%)
Guenther, 1997[16]	145	Peritumoral	Not used	28	3	103/145 (71%)	3/31 (9.7%)
Flett, 1998[17]	68	Peritumoral	Not used	15	3	56/68 (82%)	3/18 (16.7%)
Subtotal	494	Peritumoral	Not used	122	11	373/494 (75.5%)	11/133 (8.3%)
Current study	239	Peritumoral	Not used	63	8	208/239 (87.0%)	8/71 (11.3%)
Total	733	Peritumoral	Not used	185	19	581/733 (79.3%)	19/204 (9.3%)
Studies Using Peritumoral Radioactive Colloid							
No Blue Dye							
Krag, 1993[18]	22	Not used	Peritumoral TSC	7	0	18/22 (81.8%)	0/7 (0%)
Pijpers, 1997[19]	37	Not used	Peritumoral TCA	11	0	30/37 (81.1%)	0/11 (0%)
Roumen, 1997[20]	83	Not used	Peritumoral TCA	22	1	57/83 (68.7%)	1/23 (4.2%)
Borgstein, 1998[21]	104	Not used	Peritumoral TCA	44	1	104/104 (100%)	1/45 (2.2%)
Miner, 1998[22]	42	Not used	Peritumoral TSC	6	1	41/42 (97.6%)	1/7 (14.3%)
Krag, 1998[4]	443	Not used	Peritumoral TSC	101	13	413/443 (93.2%)	13/114 (11.4%)
Krag, 1998[23]	157	Not used	Peritumoral TSC	39	2	119/157 (75.8%)	2/41 (4.9%)
Offodile, 1998[24]	41	Not used	Peritumoral TD	18	0	40/41 (97.6%)	0/18 (0%)
Snider, 1998[25]	80	Not used	Peritumoral TSC	13	1	70/80 (87.5%)	1/14 (7.1%)
Crossin, 1998[26]	50	Not used	Peritumoral TSC	7	1	42/50 (84.0%)	1/8 (12.5%)
Winchester, 1999[27]	72	Not used	Peritumoral TSC	35	4	58/72 (80.6%)	4/39 (10.3%)
Rubio, 1998[28]	55	Not used	Peritumoral TSC	15	2	53/55 (96.4%)	2/17 (11.8%)
Subtotal	1186	Not used	Peritumoral	308	26	1045/1186 (88.1%)	26/334 (7.8%)
Blue Dye Used							
Albertini, 1996[29]	62	Peritumoral	Peritumoral TSC	18	0	57/62 (91.9%)	0/18 (0%)
Barnwell, 1998[30]	42	Peritumoral	Peritumoral TSC	15	0	38/42 (90.5%)	0/15 (0%)
Nwariaku, 1998[31]	119	Peritumoral	Peritumoral TSC	26	1	96/119 (80.7%)	1/27 (3.7%)
Bass, 1999[5]	186	Peritumoral	Peritumoral TSC	53	1	173/186 (93.0%)	1/54 (1.9%)
Tafra, in press[32]	535	Peritumoral	Peritumoral TSC	122	18	446/535 (87.1%)	18/140 (12.9%)
O'Hea, 1998[7]	59	Peritumoral	Peritumoral TSC	17	3	55/59 (93.2%)	3/20 (15.0%)
Borgstein, 1997[33]	25	Dermal	Peritumoral TCA	14	0	25/25 (100%)	0/14 (0%)
Subtotal	1028	Blue dye used	Peritumoral	265	23	890/1028 (86.6%)	23/288 (8.0%)
Current study	1074	Peritumoral	Peritumoral TSC	311	28	965/1074 (89.9%)	28/339 (8.3%)
Total	3288		Peritumoral	884	77	2900/3288 (88.2%)	77/961 (8.0%)
Studies Using Subdermal Radiocolloid							
Veronesi, 1997[9]	163	Not used	Subdermal TCA	81	4	160/163 (98.2%)	4/85 (4.7%)
Current study	297	Peritumoral	Subdermal TSC	94	8	283/297 (95.3%)	8/102 (7.8%)
Total	460		Subdermal	175	12	443/460 (96.3%)	12/187 (6.4%)
Studies Using Dermal Radioactive Colloid							
Current Study	511	Peritumoral	Dermal TSC	157	11	501/511 (98.0%)	11/168 (6.5%)
Total	511	Peritumoral	Dermal	157	11	501/511 (98.0%)	11/168 (6.5%)
Other Studies							
Veronesi, 1999[3]	376	54 patients with blue dye injection	Either peritumoral or subdermal TCA	168	12	371/376 (98.7%)	12/180 (6.7%)
Canavese, 1998[34]	100	Yes, technique not specified	Either peritumoral or subdermal TCA or TSC	28	5	96/100 (96.0%)	5/33 (15.2%)
Morrow, 1999[35]	139	Peritumoral	42 patients had TSC, technique not specified	28	4	110/139 (79.1%)	4/32 (12.5%)

TSC, technetium 99m sulfur colloid; TCA, technetium 99m colloidal albumin; TD, technetium dextran.
In each study, the false-negative rate, or the percentage of patients with nodal metastases who would be incorrectly staged as negative if only sentinel nodes were removed, was verified as the calculation of the number of false-negative sentinel node results divided by the total number of patients with positive axillary lymph nodes [FN/(TP + FN)]. Only the most current data available from each institution's study were included in this review.

Table 6. SUMMARY OF RADIOACTIVE COLLOID INJECTION TECHNIQUES FROM ALL PUBLISHED LITERATURE

Injection Technique	SLN Identification Rate	False-Negative Rate
Blue dye alone	79.3%	9.3%
Peritumoral radioactive colloid (with or without blue dye)	88.2%	8.0%
Subdermal radioactive colloid (with or without blue dye)	96.3%	6.4%
Dermal radioactive colloid (present study, 94% with peritumoral blue dye)	98.0%	6.5%

SLN, sentinel lymph node.

false-negative rate. In the current study, dermal injection of radioactive colloid resulted in a 98% SLN identification rate, significantly better than the SLN identification rate associated with blue dye alone (87.0%), peritumoral injection of radioactive colloid (89.9%), or subdermal injection of radioactive colloid (95.3%). Although the false-negative rates were not statistically different among the various techniques used in this study because of the relatively small numbers of false-negative results, there was a trend for improved false-negative rates for dermal injection that may be clinically meaningful. Dermal injection was associated with a 6.5% false-negative rate, the lowest of any of the techniques studied. These results with dermal injection of radioactive colloid were obtained despite the fact that few of the participating surgeons had significant experience with SLN biopsy before entering the study. This suggests that dermal injection of radioactive colloid permits reliable and accurate SLN biopsy despite relative surgeon inexperience. Thus, the dermal injection technique may shorten the learning curve associated with SLN biopsy. We believe that dermal injection is the single technical breakthrough that will allow SLN biopsy to become adopted more broadly as a replacement for routine axillary dissection. Further examination of the effect of evolving techniques in SLN biopsy on learning curves is underway and may help resolve this issue.

Another advantage of dermal radioactive colloid injection is that the transit time from the injection site to the axillary nodes is rapid. It is possible to perform SLN biopsy within 30 to 60 minutes after dermal injection. With peritumoral injection, it has been proposed that a 2- to 3-hour delay improves the ability to identify SLNs.[5] We have shown previously that obtaining a preoperative lymphoscintigram (nuclear medicine scan) does not improve the axillary SLN identification rate or false-negative rate. It does, however, add time, cost, and patient inconvenience to the procedure.[36] Dermal injection, therefore, allows more efficient use of time.

It might be argued that we should not rush to accept this new SLN technique based on a single study. However, the results of this study represent nearly 40% of the published literature. We report on a total of 511 patients with dermal injection from a large multiinstitutional experience. This number of patients exceeds the previously reported collective experience with blue dye as a single agent, from a few single-institution studies. It also exceeds the sample size of the only two other large multiinstitutional studies that have been reported, with superior SLN identification and false-negative rates. Therefore, we believe that our results provide substantial and credible evidence that dermal radioactive colloid injection is superior to other techniques when applied in a multiinstitutional setting.

One concern regarding dermal injection of radioactive colloid is that internal mammary nodes may not be identified using this technique. We take the position that SLN biopsy is performed to stage the axillary lymph nodes, because these are the nodes used to make clinical decisions regarding adjuvant therapy. In this view, SLN biopsy is a less morbid replacement for routine axillary dissection.[37] Therefore, we have not sought to perform biopsies on internal mammary nodes. In a few patients the axillary nodes are negative for tumor but an internal mammary node is positive, but the number of patients in whom this makes a difference in treatment and outcome is vanishingly small. The proof that dermal injection accurately reflects the lymphatic drainage of the breast cancer to the axilla rests in the low false-negative rate. This is the same standard that has been applied to other techniques.

Another frequent concern is the ability to ascertain the exact location of the skin overlying the tumor to inject. The breast is a three-dimensional structure, and the site for dermal injection may not always be obvious, especially for tumors located deep within the breast parenchyma. In practice, however, we have found that this concern does not frequently arise. For palpable tumors, it is a simple matter; for nonpalpable tumors, wire localization using standard mammographic or ultrasound techniques (even for patients undergoing mastectomy) permits accurate identification of the tumor site. It is helpful for the radiologist to mark the skin overlying the tumor with an indelible marker to facilitate dermal injection in the proper place. If this is not done, it is usually a simple matter to judge the location of the tumor based on the location, direction, and depth of the embedded wire. If it is still not possible to determine the proper skin site to inject because the tumor is very deep within the breast tissue, it may be reasonable to perform peritumoral injection.

It is reasonable to ask whether the use of blue dye adds to the ability to identify the SLN. In previous analyses,[8,38] we found that the use of dual agents (radioactive colloid plus blue dye) improves the accuracy of SLN biopsy compared with single-agent injection (blue dye alone or radioactive colloid alone). Peritumoral injection of blue dye, first studied by Giuliano et al,[2,6,15] is considered the gold standard for SLN identification, because the identification of a blue lymphatic channel leading to a blue-stained SLN indicates a direct lymphatic pathway from the site of the tumor to the node. We have shown previously that only two thirds of positive SLNs contain blue dye staining.[39] However, occasionally a blue-stained SLN with only minimal radioactivity is the only node to contain metastasis. Blue dye injection adds a visual signal that complements the use of the hand-held gamma probe for SLN identification. Therefore, we believe that peritumoral injection of blue dye in conjunction with dermal injection of radioactive colloid provides overlapping methods to identify the SLN and optimize the procedure.

Results from our previous analysis[8] and from other investigators[4,7] indicate that tumors in the upper outer quadrant are associated with an increased false-negative rate. The likely reason is related to the difficulty in discriminating a radioactive signal from background when peritumoral injection is performed near the axilla. In other words, the closer the zone of diffusion is to the axilla, the more difficult it is to identify an SLN. This is evidenced by the significant increase in the false-negative rate for upper outer quadrant tumors when peritumoral radioactive colloid injection was used (see Table 3). Interestingly, however, the SLN identification rates were not affected by upper outer quadrant tumor location: SLNs were still identified, albeit the wrong nodes were removed more commonly for upper outer quadrant tumors. We hypothesized that dermal injection of radioactive colloid would reduce this problem. Although it did not reach statistical significance, the false-negative rate was still greater for upper outer quadrant tumors when dermal injection of radioactive colloid was used.

Theoretically, there is relatively little uptake of tracer from breast tissue with peritumoral compared with intradermal injection because of the richness of the cutaneous lymphatics from the breast to the axilla. The results of the current study show that dermal injection is associated with SLNs that are at least fivefold more radioactive than with peritumoral injection (see Table 4). The higher radioactive counts simplify the procedure because there is greater ease in identifying the "hot spot" in the axilla and ultimately the SLN. This may also allow use of a smaller dose of radioactive colloid, administered in a simplified fashion.

There is concern that the increased radioactivity seen with dermal injection could lead to the harvest of an inordinate number of SLNs. We previously validated the so-called 10% rule for SLN biopsy, which was used in the current study.[39] This states that all nodes with radioactive counts of 10% or more of the ex vivo count of the most radioactive ("hottest") node should be removed to reduce the false-negative rate. Dermal injection of radioactive colloid resulted in a mean number of SLNs removed that was not different from peritumoral injection (2.29 vs. 2.57, respectively). Therefore, there is no evidence that dermal radioactive colloid injection leads to the indiscriminate removal of more nodes. Further, the proportionally hotter node may be easier to localize during surgery and may play a part in identifying the "correct" SLN.

There is a recent study on the use of the subareolar injection technique for radioactive colloid.[40] Although the initial data appear promising, there are no available data on false-negative rates because a completion axillary dissection was not performed. We have only limited data for either the subareolar or periareolar technique, but it will be interesting to see what the SLN identification and false-negative rates are as more experience is gained. One heralded advantage of subareolar injection is the ease of the technique; we agree that simplicity of injection is important and have found the dermal technique easy to use. Perhaps periareolar or subareolar injection techniques may improve the false-negative rate for upper outer quadrant tumors, although this remains to be determined.

CONCLUSIONS

We report what is to our knowledge the largest experience with dermal injection of radioactive colloid injection for SLN biopsy for breast cancer. Dermal injection improves the SLN identification rate and minimizes the false-negative rate compared with peritumoral injection. Dermal injection is associated with SLNs that are five- to sevenfold more radioactive than with peritumoral injection, which simplifies SLN localization and may shorten the learning curve.

Acknowledgments

The authors thank the members of the University of Louisville Breast Cancer Study Group for their dedicated and ongoing participation. They also thank Sherri Matthews for expert assistance with the manuscript and data management and Stephen Maniscalco, MD, for database design and maintenance.

References

1. McMasters KM, Giuliano AE, Ross MI, et al. Sentinel lymph node biopsy for breast cancer—not yet the standard of care. N Engl J Med 1998; 339:990–995.
2. Turner RR, Ollila DW, Krasne DL, et al. Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. Ann Surg 1997; 226:271–278.
3. Veronesi U, Paganelli G, Viale G, et al. Sentinel lymph node biopsy and axillary dissection in breast cancer: results in a large series. J Natl Cancer Inst 1999; 91:368–373.
4. Krag D, Weaver D, Ashikaga T, et al. The sentinel lymph node in breast cancer: a multicenter validation study. N Engl J Med 1998; 339:941–946.
5. Bass SS, Cox CE, Ku NN, et al. The role of sentinel lymph node biopsy in breast cancer. J Am Coll Surg 1999; 189:184–194.

6. Giuliano AE, Jones RC, Brennan MB, et al. Sentinel lymphadenectomy in breast cancer. J Clin Oncol 1997; 15:2345–2350.
7. O'Hea BJ, Hill ADK, El-Shirbiny AM, et al. Sentinel lymph node biopsy in breast cancer: initial experience at Memorial Sloan-Kettering Cancer Center. J Am Coll Surg 1998; 186:423–427.
8. McMasters KM, Tuttle TM, Carlson DJ, et al. Sentinel lymph node biopsy for breast cancer: A suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. J Clin Oncol 2000; 18:2560–2566.
9. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph nodes. Lancet 1997; 349:1864–1867.
10. Linehan DC, Hill ADK, Akhurst T, et al. Intradermal radiocolloid and intraparenchymal blue dye injection optimize sentinel node identification in breast cancer patients. Ann Surg Oncol 1999; 6:450–454.
11. Roumen RM, Geuskens LM, Valkenburg JG. In search of the true sentinel node by different injection techniques in breast cancer patients. Eur J Surg Oncol 1999; 25:347–351.
12. Bland KI. Refining the optimal technical approach for sentinel node localization in breast cancer patients. Ann Surg Oncol 1999; 6:418–419.
13. Cady B, Chung M. Invited commentary: Sentinel node biopsy—comment on techniques. Surgery 2000; 128:13–15.
14. Hill ADK, Mann GB, Borgen PI, et al. Sentinel lymphatic mapping in breast cancer. J Am Coll Surg 1999; 188:545–549.
15. Giuliano AE, Kirgan DM, Guenther JM, et al. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994; 220:391–401.
16. Guenther JM, Krishnamoorthy M, Tan LR. Sentinel lymphadenectomy for breast cancer in a community managed care setting. Cancer J Sci Am 1997; 3:336–340.
17. Flett MM, Going JJ, Stanton PD, et al. Sentinel node localization in patients with breast cancer. Br J Surg 1998; 85:991–992.
18. Krag DN, Weaver DL, Alex JC, Fairbanks JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol 1993; 2:335–340.
19. Pijpers R, Meijer S, Hoekstra OS, et al. Impact of lymphoscintigraphy on sentinel node identification with technetium-99m colloidal albumin in breast cancer. J Nucl Med 1997; 38:366–368.
20. Roumen RMH, Valkenburg JGM, Geuskens LM. Lymphoscintigraphy and feasibility of sentinel node biopsy in 83 patients with primary breast cancer. Eur J Surg Oncol 1997; 23:495–502.
21. Borgstein PJ, Pijpers R, Comans EF, et al. Sentinel lymph node biopsy in breast cancer: guidelines and pitfalls of lymphoscintigraphy and gamma probe detection. J Am Coll Surg 1998; 186:275–283.
22. Miner TJ, Shriver CD, Jaques DP, et al. Ultrasonographically guided injection improves localization of the radiolabeled sentinel lymph node in breast cancer. Ann Surg Oncol 1998; 5:315–321.
23. Krag DN, Ashikaga T, Harlow SP, et al. Development of sentinel node targeting technique in breast cancer patients. Breast J 1998; 4:67–74.
24. Offodile R, Hoh C, Barsky SH, et al. Minimally invasive breast carcinoma staging using lymphatic mapping with radiolabelled dextran. Cancer 1998; 82:1704–1708.
25. Snider H, Kowlatshahi K, Fan M, et al. Sentinel node biopsy in the staging of breast cancer. Am J Surg 1998; 176:305–310.
26. Crossin JA, Johnson AC, Stewart PB, et al. Gamma probe-guided resection of the sentinel lymph node in breast cancer. Am Surg 1998; 64:666–669.
27. Winchester DJ, Sener SF, Winchester DP, et al. Sentinel lymphadenectomy for breast cancer: experience with 180 consecutive patients: efficacy of filtered technetium-99m sulphur colloid with overnight migration time. J Am Coll Surg 1999; 188:597–603.
28. Rubio IT, Korourian S, Cowan C, et al. Sentinel lymph node biopsy for staging breast cancer. Am J Surg 1998; 176:532–537.
29. Albertini JJ, Lyman GH, Cox C, et al. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. JAMA 1996; 276:1818–1822.
30. Barnwell JM, Arredondo MA, Kollmorgen D, et al. Sentinel node biopsy in breast cancer. Ann Surg Oncol 1998; 5:126–130.
31. Nwariaku FE, Euhus DM, Beitsch PD, et al. Sentinel lymph node biopsy, an alternative to elective axillary dissection for breast cancer. Am J Surg 1998; 176:529–531.
32. Tafra L, Lannin DR, Swanson MS, et al. Multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue dye. Ann Surg 2001; 233:51–59.
33. Borgstein PJ, Meijer S, Pijpers R. Intradermal blue dye to identify sentinel lymph node in breast cancer [research letter]. Lancet 1997; 349:1668–1669.
34. Canavese G, Gipponi M, Catturich A, et al. Sentinel lymph node mapping opens a new perspective in the surgical management of early-stage breast cancer: a combined approach with vital blue dye lymphatic mapping and radioguided surgery. Semin Surg Oncol 1998; 15:272–277.
35. Morrow M, Rademaker AW, Bethke KP, et al. Learning sentinel node biopsy: results of a prospective randomized trial of two techniques. Surgery 1999; 126:714–722.
36. McMasters KM, Wong SL, Tuttle TM, et al. Preoperative lymphoscintigraphy for breast cancer does not improve the ability to identify axillary sentinel lymph nodes. Ann Surg 2000; 231:724–731.
37. Schrenk P, Rieger R, Shamiyeh A, et al. Morbidity following sentinel lymph node biopsy versus axillary lymph node dissection for patients with breast carcinoma. Cancer 2000; 88:608–614.
38. Wong SL, Edwards MJ, Tuttle TM, et al. Sentinel lymph node biopsy for breast cancer: impact of the number of sentinel nodes removed on the false negative rate. Presented at the American College of Surgeons 86th Annual Clinical Congress, Chicago, IL, October 2000.
39. Martin RCG, Edwards MJ, Wong SL, et al. Practical guidelines for optimal gamma probe detection of sentinel lymph nodes in breast cancer: results of a multi-institutional study. Surgery 2000; 128:139–144.
40. Klimberg VS, Rubio IT, Henry R, et al. Subareolar versus peritumoral injection for location of the sentinel node. Ann Surg 1999; 229:860–865.

Discussion

DR. KIRBY I. BLAND (Birmingham, Alabama): I congratulate Dr. McMasters and Associates of the University of Louisville Breast Cancer study group for bringing to our attention this multi-institutional prospective nonrandomized study involving 229 surgeons for the treatment of clinical stage T1-3 No, as this course will initiate inappropriate adjuvant therapy decisions and the risk of persistent or recurrent disease in the axilla.

I therefore agree with the authors that the confirmation of the correct pathological state of the sentinel node is crucial, as the false-negative rate is the essential factor to evaluate with sentinel lymph node biopsy in axillary staging. This false negative rate of less than 5% has been an achievable goal, as is apparent in the current study quoted by Dr. McMasters and associates. You confirm that dermal injection enhances sentinel lymph node identification and the relative degree of sentinel lymph node radioactivity.

From a personal standpoint my experience at Brown University and currently at the University of Alabama in Birmingham is that use of the blue dye technique alone is technically challenging and has a steep learning curve. However, the false negative sentinel lymph node identification rate can be significantly decreased when blue dye is combined with radioactive sulphur colloid. Since the seminal report by Pat Borgen and his group at Memorial Sloan-Kettering presented before the Society of Surgical Oncology in '99, I have utilized exclusively the dermal injection technique together with the subareolar blue dye injection technique advo-

cated by Klimberg and associates at Arkansas. This combination has achieved a greater than 95% true positive sentinel node detection rate in my personal series of over 60 patients. I have a few questions.

Please comment on the dermal injection guidelines of your group as compared with the Memorial group. I currently use 900 to 950 microcuries of unfiltered technetium -99m sulfur colloid in small volumes with a single injection on the proximate areolar margin towards the tumor bed or axilla. This is injected some 12 to 18 hours before the sentinel lymph node sampling the following morning. Does this represent the most efficient use of resources in sentinel lymph node staging?

Secondly, can you give advice regarding management of tumors that have had previous biopsies and surgical scars in the upper outer quadrant? Do you feel that sentinel lymph node staging for this presentation should include dermal injection on the proximate side of the surgical defect? Might this interfere in sentinel node detection with dermal injection in the upper outer quadrant near the axilla?

Many of us continue to use 'belt and suspenders,' utilizing both techniques, dye and radioactive tracer, in each patient. Should one technique fail, the other usually does not. I have observed technical failure of both methods in two patients who had extra-capsular nodal invasion with tumor replacement. If you analyze your cohort of patients, are there any caveats in which you detect differences for histological presentations of the index tumor? Or for metastases?

Are you continuing to use both techniques, dye plus radiosulphur colloid, now that you have convinced us that a dermal injection of radioactive sulphur colloid alone provides a detection rate of 98%?

I enjoyed the advance copy of this paper, and I congratulate the authors.

DR. FREDERICK MOFFAT (Miami, Florida): This is a wonderful paper. For those of you who don't do sentinel node biopsy, it is impossible to overstate the problem that is posed by the radioactive diffusion zone with intraparenchymal injection. Both from the standpoint of injecting and getting either injection into the underlying muscle or into the axillary fat with diffusion of radioactivity or just in terms of the shine-through, which can't be retracted away, particularly without upper outer quadrant tumors.

I have a couple of brief questions for Dr. McMasters.

First of all, in 94% of cases with the technetium sulphur colloid you injected blue dye. What was the degree of concordance or the reciprocal discordance between the blue dye and radio-labeling of the sentinel nodes?

Secondly, there is a concern with the use of dermal or intradermal dye that in fact, the migration rates or migration patterns may not exactly duplicate that of the breast parenchyma. In series where peritumoral injection has been done, the rate of migration to at least one sentinel node outside the axilla range is between 5 and 10%, and most of these, of course, are internal mammary nodes.

You didn't break down the areas where your sentinel nodes were found, and I would appreciate getting some insight into high axillary nodes and internal mammary nodes. Does the presumption of embryological similarity to lymphatic drainage really hold a hundred percent?

Thirdly, just a very minor question. There is a tempest in a teapot debate about whether the colloid should be filtered or unfiltered. Which one did you use?

Thank you very much. I enjoyed the paper.

DR. DON M. MORRIS (Albuquerque, New Mexico): I, too, want to address the last issue. It's the critical issue, at least for me.

What studies have been done to show that if you inject the skin you get the same sentinel node as if you inject peritumoral?

I have done a smaller number of cases where I have used a different tracer in the skin and a different tracer in the tumor, and I don't get the same sentinel node. And I would really like that issue clarified.

If injecting the skin gives you the same sentinel node, it clearly is better. But if it does not, then we've got a problem.

Thank you.

DR. SUSAN KLIMBERG (Little Rock, Arkansas): I would like to thank the Association for the privilege of the floor and Dr. McMasters and the Louisville group for the opportunity to discuss their paper and see it well in advance.

I confess my ignorance in understanding the difference between subdermal and dermal. Can you explain? Nonetheless, I believe you are right in your results that show faster and more concentrated localization with the dermal injection. When one injects intraparenchymal with blue dye it almost always appears on the skin; the deeper you inject the longer it takes. The importance of this work can be seen in the fact that the NSABP B32 trial, or the sentinel lymph node trial, which started with peritumoral injection of both technetium and blue dye will now add a dermal injection to the protocol.

However, it is our belief that it is the drainage of the breast and not the specific location of the tumor that defines the sentinel lymph node. Based on the central embryological origin of the lymphatic plexus, we initially reported at this meeting three years ago the injection of technetium in the subareolar plexus in 70 breasts in comparison to peritumoral blue dye as the standard. All blue nodes were hot, confirming its accuracy.

Earlier this year Borgstein validated those results using peritumoral technetium and subareolar blue dye in 220 patients, again confirming its accuracy. In two small series both Kern et al., with subareolar blue dye and no technetium, and our institution with subareolar technetium and peritumoral blue dye followed by axillary node dissection, revealed no false positive with subareolar injection. In the multicenter trial by Krag, all the false negatives were in the upper outer quadrant.

In your study you state that tumors in the upper outer quadrant had a higher false negative rate but what percent of the total of false negatives were in the upper outer quadrant compared to the other quadrants of the breast?

It may be not so much the shine-through, or not all of it is shine-through, but Krag also postulated it may be the particular confluence of the lymphatics in the upper outer quadrant.

I know you have a smaller subset of patients injected subareolar. Can you tell us those results? Any false negatives?

You used filtered technetium. Krag has shown that unfiltered technetium sulfur colloid lessens the occurrence of multiple sentinel lymph nodes. Would you comment on the average number of sentinel lymph nodes identified by each of these techniques and your philosophy in using filtered versus unfiltered sulfur colloid for this study?

Another important point in your data that needs to be brought out is that 50 percent of individuals are using immunohistochemistry. And the importance of the work they are doing at Louisville is that you can get a pulse on what is going on with sentinel lymph node in the nation. This shows a pattern of utilization that has been

ferreted out by the American Pathology Association as not standard of care in assessing lymph nodes. What is your philosophy and practice at your institution?

Thank you very much.

DR. ROGER FOSTER (Atlanta, Georgia): I enjoyed this paper. I have a very brief comment and question. The goal is clearly to identify a positive node in the axilla, whether it is done by a sentinel node procedure or by palpation with the finger. And my question is how. . . I think the rate of false negatives is relatively high here. How is that defined?

I think it is important to remember that the surgeon must palpate the axilla and remove a clinically involved node, which, because it is choked with tumor, may not take up either of the markers.

And my second question is how often were nonaxillary sentinel nodes identified in this group and biopsied? I always felt that was part of the tradeoff, was to identify a positive a positive nonaxillary node. And if you are using the sentinel node technique, that compensates for an occasional false negative test.

Thank you.

DR. KELLY M. MCMASTERS (Louisville, Kentucky): I'd like to thank the discussants for their thoughtful comments and questions. I will try to take them in order.

Dr. Bland asked about the specifics of our dermal injection guidelines in which we use filtered technetium sulphur colloid, 0.5 millicuries in several locations into the skin overlying the tumor. The original Memorial Sloan-Kettering experience used a slightly different injection technique with more colloid that was unfiltered in a single injection site performed many hours, 12 to 18 hours, ahead of time. We find it is very convenient to do this injection on the morning of surgery within 30 minutes of the time of the operation. We don't do lymphocintigrams, which we have not found to be helpful in identifying the sentinel lymph nodes, so we take the patient straight from the nuclear medicine area to the operating room to perform the procedure.

I think that the difference between using unfiltered and filtered colloid, which was brought up by several of the discussants – we have looked at our data and, in fact, some of the surgeons used unfiltered technetium sulphur colloid. We find absolutely no difference in the mean number of sentinel lymph nodes removed in the false negative rate and in the sentinel lymph node identification rate, whether you use unfiltered colloid or filtered colloid. And I know there is a lot of controversy about this in the literature, but when it is used in actual patients in a large study in a multi-institutional experience, there is absolutely no difference you can find. So I think you can use whatever you like.

Dr. Bland asked about previous excisional biopsy; where should we inject, and is it still accurate. We can find no impact of the biopsy type, that is, excisional biopsy, we thought might make the procedure less accurate. We cannot prove that is true in the analysis of this large database. We do inject around the scar from the biopsy site like we would do for melanoma, not just on the axillary side.

Is blue dye necessary in conjunction with the radioactive colloid injected dermally? We still think that the blue dye peritumorally is an important component of this technique. It provides some overlapping and complementary technique to identify sentinel lymph nodes, and I think the blue dye technique is certainly considered the gold standard because a blue afferent lymphatic channel leading from the site of the tumor to a lymph node does indicate a direct lymphatic drainage pattern.

We have seen in the previous analysis of these data some lymph nodes are blue and not very radioactive. Some nodes are radioactive and not blue at all. In fact, in answer to several questions, only two-thirds of the sentinel nodes that are positive in our series are actually stained blue. So you cannot rely – at least in this large multi-institutional experience – you can't rely on the blue dye to be there every time.

Dr. Moffat asked about the concordance with blue dye of radioactive colloid. Again, only two-thirds of our sentinel nodes overall were blue stained, and we think that the combination of the techniques does give the best results.

Our philosophy of sentinel lymph node biopsy is a little bit different than some other people. We believe that sentinel lymph node biopsy is a less invasive, less morbid alternative to axillary lymph node dissection. As such, our goal is to stage the axillary lymph nodes, not the regional lymph nodes. So we did not set out in this protocol to biopsy internal mammary lymph nodes, which we have not used for many years, to make treatment decisions in breast cancer. Our goal is to stage the axillary lymph nodes.

There is some question, however, about whether or not if you inject the skin it will always drain to the same lymph nodes, or would it identify internal mammary nodes if they exist. There is some evidence that perhaps that is not true, that you will not identify internal mammary nodes with a dermal injection. For us it does not make much difference because we are staging the axillary lymph nodes and we have shown the gold standard, which is a large series of patients in which you examine the false negative rate with backup axillary dissection. That is the same standard that has been applied to every other technique. We have a larger sample size with dermal injection in this large multi-institutional experience with a lower false negative rate and a higher sentinel lymph node identification rate than the composite experience using blue dye alone or the prior multiinstitutional experience using radioactive colloid in a peritumoral location. So we think that it is an accurate way of finding the SLN, or the optimal injection technique for sentinel lymph node biopsy.

Dr. Klimberg confessed that she didn't know the difference between subdermal and dermal injection and I confess that I didn't know at first either. But my understanding is that with dermal injection it is as I show, where you raise a wheal, like injection of local anesthesia that we are all used to. Subdermal injection is beneath the skin into the subcutaneous tissue just beneath the skin and leads to fairly similar results and, I believe, can also be performed accurately.

I'm glad to see that the NSABP B32 study, Suzanne, is now including dermal injection, as I feel that it gives superior results.

We are also intrigued with the subareolar and periareolar injection techniques and, as you know, we had 85 patients in our study that had sub or periareolar injection regardless of the tumor location in the breast. Of those patients, there was one false negative result for a 5.9 percent false negative rate so far. Too few numbers yet to be able to validate that as an experience. But as you know, the way to validate that is the way that we are going to continue to do this, which is get a larger series of patients and look at the false negative rate when we have more patients. But we think that is very exciting and may be the way to reduce the false negative rate for these upper outer quadrant tumors. That's a hypothesis that can be tested.

Dr. Foster asked us about the palpation of the axilla. We include that as part of the technique. Part of the detail of the procedure is after you have used the gamma probe and the blue dye to look for

sentinel lymph nodes, absolutely, you must palpate. If you find a palpably suspicious node, that should obviously be removed.

There are sometimes, as Dr. Bland brought up, nodes that are completely replaced with tumor that will not take up the blue dye or the radioactive colloid and they can be removed by careful clinical judgment.

Because of the recommendations of a recent consensus panel from the American College of Surgeons and the Pathology Society, we have ceased to perform immunohistochemistry for routine evaluation of sentinel nodes.

I'd like to thank the Association once again for the privilege of the floor and the opportunity to present this study.