Annals of Surgical Oncology, **8**(2):133–137
Published by Lippincott Williams & Wilkins

A New Radiocolloid for Sentinel Node Detection in Breast Cancer

N. Hodgson, MD, P. Zabel, MSc, A. G. Mattar, MD, C. J. Engel, MD, D. Girvan, MD, and R. Holliday, MD

Background: The optimal radioactive tracer and technique for sentinel lymph node localization in breast cancer is yet to be determined. The dilemma of small particle size with dispersion to second echelon nodes versus failure of migration of larger radiocolloids needs to be resolved. A new radiocolloid preparation with particle size under 0.1 micron was developed with excellent primary/post lymphatic entrapment ratio.

Objective: To assess the feasibility of a new ^{99m}Tc radiocolloid cysteine-rhenium colloid in sentinel lymph node (SLN) localization for breast cancer.

Methods: Forty-seven patients with newly diagnosed T1 or T2 breast cancer underwent injection of ^{99m}Tc-labeled cysteine-rhenium colloid followed by lymphoscintigraphy. Same day SLN biopsy with patent blue dye and intraoperative gamma probe to identify SLNs were performed.

Results: SLN mapping and intraoperative localization were successful in 46/47 (98%) of patients. The blue dye radioactive tracer concordance was 94%. There was one false-negative in a patient with a nonpalpable tumor that underwent ultrasound-guided peritumoral radiocolloid injection.

Conclusions: ^{99m}Tc-cysteine-rhenium colloid is highly effective in identifying SLNs. It has the advantage of smaller particle size than sulfur colloid with easier lymphatic migration. It has a more neutral pH with less pain on injection and does not require filtration, thereby minimizing radiation exposure to technologists.

Key Words: Sentinel lymph node—Radiocolloid—Breast cancer.

Sentinel lymph node (SLN) dissection has been advocated as a potential alternative to axillary lymphadenectomy (ALND), and SLN mapping may contribute to more accurate staging, while using less invasive techniques. The effectiveness of SLN localization varies greatly according to radiotracer particle size. One of the most pressing issues in sentinel node surgery is the dilemma caused by small particle-size tracers that label too many secondary nodes and large particle-size tracers that do not adequately label enough sentinel nodes. To date, the ideal radiocolloid for SLN mapping in breast cancer is yet to be determined.

Radiopharmaceutical colloidal preparations were developed shortly after World War II. In 1955, Hultborn and colleagues reported the preoperative use of interstitial colloidal gold injections in breast cancer patients.[1] ^{99m}Tc was introduced as a replacement for radioactive gold because of a better safety profile. ^{99m}Tc has a short half-life (6 hours), low patient dose, no beta emission and good detection energy.[2] Various dyes and radioactive tracers have been used to identify SLNs.[3,4] With widespread application of the sentinel node technique in breast cancer, ^{99m}Tc-labeled sulfur colloid (^{99m}Tc-SC) is presently the most commonly employed for SLN localization. Studies involving melanoma that compare ^{99m}Tc-human serum albumin (HSA) and ^{99m}Tc-SC have reported a higher rate of nonvisualization in SC scans.[5] Lower hot and blue dye concordance has also been reported with SC compared with HSA.[6] However, the optimal radiopharmaceutical is still under investigation.

Received March 18, 2000; accepted September 21, 2000.

From the Departments of Surgery (NH, CJE, RH) and Nuclear Medicine (PZ, AGM), London Health Sciences Center, University of Western Ontario, London, Ontario, Canada.

Presented at the 53rd Annual Meeting of the Society of Surgical Oncology, New Orleans, Louisiana, March 16–19, 2000.

Address correspondence and reprint requests to: R. Holliday, MD, 375 South St, Victoria Campus, London Health Sciences Centre, London, Ontario, Canada N6A 4G5.

For SLN detection, colloids should ideally move to primary (sentinel) lymph node(s) with minimal further migration.

We developed a new ^{99m}Tc-radiocolloid in our center, one that did not require filtration, which displayed optimal properties such as a particle size range of 10–12 nm (scanning electron microscope) and high radiochemical purity and stability.[7] This colloid was formulated by modifying an initial ^{99m}Tc-SC preparation so that it contained perrhenate and a much lower percentage of thiosulfate. The perrhenate is chemically similar to pertechnetate and may serve to decrease particle size in a similar manner shown for carrier technetium.[8] Addition of cysteine also resulted in a smaller particle size. The final preparation is buffered to a neutral pH which contrasts to the typically acidic pH of ^{99m}Tc-SC. It has been termed ^{99m}Tc-cysteine-rhenium colloid to distinguish it from ^{99m}Tc-SC.

This product differs substantially from a European 100 nm ^{99m}Tc-rhenium sulfide, TCK–17[9] because it does not involve tin and or chelation chemistry. Our ^{99m}Tc-cysteine-rhenium colloid displayed very promising results in a rabbit animal model. Primary node entrapment ratio was calculated as the ratio of uptake in primary node/post node lymphatic channel. The formulation of rhenium colloid showed the least leakage past the primary nodal basin compared with sulfur colloid (Reg TSC), filtered sulfur colloid (Eschima TSC), and antimony colloid (Fig. 1).[10]

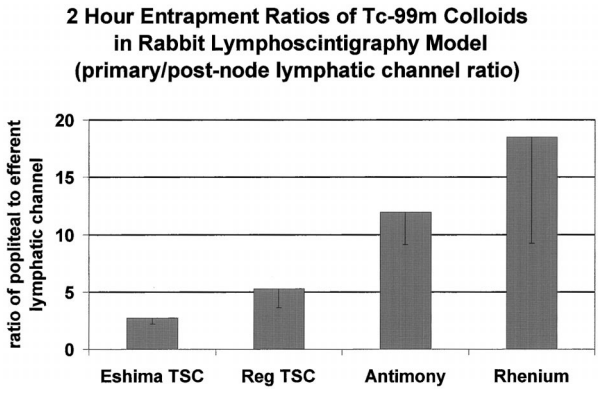

FIG. 1. Anesthetized rabbits received two 500 microCi (18MBq) intradermal foot injections of 99mTc-colloid. Sequential images were performed to 2 hours. Primary node entrapment ratio was calculated as uptake in primary (popliteal) node/post node lymphatic channel. All colloids displayed excellent rapid uptake in popliteal nodes with averages of 5–6% injected dose. Primary node "entrapment ratios" were calculated as the ratio of uptake of in primary node/post node lymphatic channel. Our formulation of 99mTc-rhenium colloid displayed the least leakage past the primary nodal basin resulting in the best entrapment ratio ± SD (Eschima TSC 2.74 ± 0.54, Reg TSC 5.28 ± 1.67, Antimony 11.93 ± 2.78, and Rhenium 18.48 ± 9.26).

The product has not displayed any adverse events when used as either an intravenous diagnostic product (liver, spleen, bone imaging) or a nebulized inhaled product (lung ventilation studies) in over 1000 patients. The ingredients are very similar to our "in house" ^{99m}Tc-SC which has been used clinically without adverse events for 20 years. The newly formulated colloid has a more neutral pH, lower thiosulfate concentration, and the addition of cysteine well below that used in other medicinal products (<0.3 mg/ml). No hypersensitivity reactions have occurred with the ^{99m}Tc-cysteine-rhenium colloid to date.

Our objective was to determine the feasibility of this novel radiocolloid for SLN localization in breast cancer at our center. Endpoints included: (1) SLN localization by lymphoscintigraphy, (2) SLN localization at surgery, and (3) blue dye radioactive tracer concordance.

METHODS

Patients were prospectively enrolled for SLN localization and resection between September 1998 and December 1999, at the London Health Sciences Center (LHSC), University of Western Ontario, Canada. Informed consent from all participants was obtained and internal review board approval was obtained. Participating surgeons and nuclear medicine physicians were trained in the procedure at the H. Lee Moffit Cancer Center (Tampa, Florida). The inclusion criteria were the presence of invasive breast cancer and a clinically negative axilla. Exclusion criteria included: clinically suspicious/abnormal lymph nodes, previous axillary lymphadenectomy, tumor size ≥5 cm or evidence of distant metastases.

Radioactive Tracer

Two to four hours prior to surgery, ^{99m}Tc-cysteine-rhenium colloid in a volume of 4 ml was circumferentially injected intraparenchymally surrounding the tumor. This new "in-house" radiocolloid was developed by our radiopharmacist. It uses cysteine and less thiosulfate and has a more neutral pH. Between 85%–95% of the preparation passes between 0.1 micron low protein binding filters. Four to six peritumoral injection sites were used. Lymphoscintigraphy was then performed and the sentinel nodes marked on the patient's skin by the nuclear medicine physician. Intraoperatively, a hand held gamma probe (Navigator, Autosuture Co,, UK) was used to locate the radioactive sentinel nodes. Patent blue dye in volumes of 2 ml was injected intraparenchymally along the axillary border of the tumor, then the breast was massaged for 10 minutes. A standard axillary incision was made and the sentinel nodes were identified by

locating blue stained afferent lymphatics and dissecting along them to blue nodes. Lymph nodes that did not stain blue were defined as SLNs if in vivo gamma-probe counts were greater than or equal to 4 times background. Background counts were defined as the baseline radioactivity of the nodal basin after removal of SLNs. Ex vivo counts of SLNs were measured and compared to background. Completion axillary node dissection was subsequently performed. Internal mammary node dissection was not performed.

Sentinel nodes were labeled and processed separately for pathological examination. Routine hematoxylin and eosin (H&E) staining was used for sentinel and nonsentinel nodes. For nodes $<.5$ cm whole nodes were examined, half nodes were examined for nodes 0.5 to 1 cm, and for nodes $>$ 1 cm multiple sections of .5 cm were examined. Immunohistochemistry for cytokeratin was performed on all negative sentinel nodes.

RESULTS

A total of 47 patients were prospectively enrolled and underwent SLN localization at LHSC between September 1998 and December 1999. Patient demographics and tumor characteristics are displayed in Table 1. The sentinel node was successfully located in 98% (46/47) of patients. The mean number of sentinel nodes identified was 1.7 ± 0.8 and the mean number of nonsentinel nodes was 12 ± 0.7.

The overall sensitivity of sentinel lymphadenectomy was 90% and specificity was 100%. Positive and negative predictive values were 100% and 96%, respectively.

Concordance between lymphoscintigraphy and intraoperative SLN localization was 98%. The blue dye and radiotracer concordance was 94%, with 44 of 47 SLN (93.6%) both blue and radioactive.

TABLE 1. *Demographic information*

No. of patients	47
Mean age (years)	54 ± 5
Stage, number (%)	
T1a	1 (2)
T1b	3 (4)
T1c	29 (62)
T2	15 (32)
Procedure	
Lumpectomy	89%
Mastectomy	11%
Pathology (%)	
Ductal	44 (94)
Lobular	1 (2)
Other	2 (4)
Neurovascular Invasion Present (%)	11 (23)

Three patients with nonpalpable tumors underwent lymphatic mapping. There was one false-negative result, which occurred in a patient with a nonpalpable tumor. There were no false-negatives in patients with palpable breast tumor.

Internal mammary drainage was identified in 3 of 47 (6%) patients on preoperative lymphoscintigraphy. The remaining 43 patients (94%) demonstrated axillary drainage patterns. Ten patients had positive SLNs; eight were positive on H&E staining and two were positive for micrometastases on immunohistochemical staining for cytokeratin.

DISCUSSION

Currently, the use of SLN mapping is widespread in patients with early stage breast cancer. A number of large randomized clinical trials are underway to assess the morbidity and outcome of SLND versus ALND. The effectiveness of labeling varies considerably according to the tracer particle size. One of the biggest potential difficulties is that small sized tracers may pass through the sentinel nodes and label secondary nodes. Large particle radioactive tracers appear to have little pass-through to secondary nodes, yet their uptake at the injection site is less. SLN localization using our new ^{99m}Tc-cysteine-rhenium colloid is feasible, as demonstrated in this study, where sentinel nodes were successfully detected in 98% of patients. This parallels SLN mapping success rates reported in recent studies.[11–14] The sensitivity of SLN dissection was 90% for all cases ($n = 47$) and 100% for all palpable tumors ($n = 44$).

In our center, only 1 of 47 patients had a false-negative result, and a false-negative rate of 10% (1 of 10 patients with positive nodes) (Table 2). This occurred in a patient with a nonpalpable tumor in which the radiocolloid was injected under ultrasound guidance. Lymphoscintigraphy revealed uptake in the internal mammary chain but no uptake in the axilla. Axillary dissection revealed six positive axillary nodes. False-negatives may occur secondary to failure of migration, diffuse radioactivity of the injection site or residual radioactive nodes distant form the hot spot. Mode of injection, intraparenchymal versus intradermal, may influence radiocolloid migration. In a series of 200 patients, Linehan et al.[15] reported a 98% SLN localization rate with intradermal sulfur colloid injection versus 78% in patients with intraparenchymal injection. Our false-negative may be explained by failure of migration secondary to intraparenchymal injection or our limited experience with the ultrasound-guided technique. Limited volume of radiotracer may also be a potential explanation as this patient only received a 3 ml

TABLE 2.

Mean no. of sentinel nodes	1.7 ± 0.8
Mapping failures	1 (3%)
Sensitivity	90%
Specificity	100%
Positive predictive value	100%
Negative predictive value	96%
False-negative rate	10%

Sensitivity equals the number of positive sentinel lymph nodes divided by the number of patients with axillary lymph node metastases, multiplied by 100. Specificity equals the number of negative SLNs divided by the number of patients without axillary metastases, multiplied by 100. Positive predictive value equals the number of patients with axillary lymph node metastases divided by the number of patients with a positive sentinel node biopsy. Negative predictive value equals the number of patients without lymph node metastases divided by the number of patients with negative lymph node biopsy, multiplied by 100. False-negative rate is the number of negative sentinel nodes divided by the number of patients with positive lymph nodes.

injection of colloid. There were no false-negatives in patients with palpable tumors (44/47). Because more patients will be presenting with nonpalpable tumors, it is important that sentinel node localization in nonpalpable tumors be improved. Only three patients presented with nonpalpable lesions and this is a limitation of the small sample size of this study.

Local failure of internal mammary nodes is rarely recognized yet their prognostic significance may be underestimated. There is no indication for a routine parasternal dissection today based on a current overview of sentinel node dissection in breast cancer.[16]

Presently, sulfur colloid is the predominant radiocolloid used in the United States, including large clinical trials such as the American College of Surgeons Oncologic Group Z00010 and ZN00011 protocols. Krag et al.[17,18] found the highest success rate of SLN resection with unfiltered radiocolloid.

Linehan and colleagues[19] confirmed that unfiltered sulfur colloid was superior to filtered sulfur colloid in SLN localization (88% vs. 73% success rate, respectively). Human serum albumin has a broad diffusion and is rapidly dispersed through to second-echelon nodes and not recommended for SLN mapping.[20] Our institution developed a new "in-house" ^{99m}Tc-cysteine-rhenium colloid. Unlike sulfur colloid, it is smaller with a particle size of 85% < 0.1 microns (10–12 nm) with easier migration into lymphatics (Table 3). It demonstrated an excellent entrapment ratio in the primary nodal basin compared with antimony, sulfur colloid, and filtered sulfur colloid in our rabbit model.[21] ^{99m}Tc-cysteine-rhenium colloid also has the advantage of not requiring filtration and less radiation dose to technologists. Moreover, it also has a neutral pH and causes less pain on injection than sulfur colloid which has an acidic pH.

TABLE 3. *Comparison of particle size of different technetium labeled radiocolloid agents*[21]

Material	Particle Size (nm)
Gold (reference point)	3–5
^{99m}TC Human serum albumin	<3
^{99m}TC antimony colloid	3–50
^{99m}TC nanocolloid	95% < 80
^{99m}TC sulfur colloid (hydrogen sulfide)	<40
^{99m}TC sulfur colloid (thiosulfate method)	100–600
^{99m}TC sulfur colloid (filtered, Eshima method)	<10
^{99m}TC CIS rhenium sulfide	100
^{99m}TC cysteine rhenium (our prep)	10–12
Red blood cell (reference)	8000

In conclusion, randomized clinical studies comparing mode of injection, timing of injection, SLN localization and blue/radioactive concordance rates between rhenium versus sulfur colloid are warranted to determine the optimal radiocolloid and technique.

REFERENCES

1. Hultborn KA, Larsonn L-G, Ragnhult I. The lymph drainage from the breast to the axillary and parasternal lymph nodes studied with the aid of colloidal Au 198. *Acta Radiol* 1955;43:52–63.
2. Weinberg J, Greaney EM. Identification of regional lymph nodes by means of vital staining dye during surgery of gastric cancer. *Surg Gynecol Obstet* 1950;90:561–7.
3. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992;127:392–9.
4. Alex JC, Krag DN. Gamma probe guided localization of lymph nodes. *Surg Oncol* 1993;2:137–43.
5. Moffat FL Jr; Gulec SA, Sittler SY, et al. Unfiltered sulfur colloid and sentinel node biopsy for breast cancer: technical and kinetic considerations *Ann Surg Oncol* 1999;6:746–55.
6. Bedrosion I, Scheff AM, Mick R, et al. 99m Tc-human serum albumin: an effective radiotracer for identifying sentinel lymph nodes. *J Nucl Med* 1999;40:7143–8.
7. Zabel PL, Dunn KE. Development of a smaller "in house" Tc-99m colloid for potential use in sentinel node lymphoscintigraphy, bone marrow scintigraphy and lung aerosol ventilation studies. Proceedings of the Annual Scientific Meeting of the Canadian Society of Nuclear Medicine, Alberta, Canada March 1999.
8. Eschima D, Eschima L, Gotti N, et al. Technetium-99m sulfur colloid for lymphoscintigraphy; effects of preparation parameters. *J Nucl Med* 1996;37:1575–1578.
9. Package Insert Nanocis, CIS Bio International.
10. Zabel PL, Dunn KE. Tc-99m rhenium colloid displays the highest primary lymph node entrapment when compared to 3 other Tc-99m colloids in a rabbit lymphoscintigraphy study. Proceedings of the Annual Scientific Meeting of the Canadian Society of Nuclear Medicine, Quebec April 1–4, 2000.
11. Krag DN, Meijer SJ, Weaver DL, et al. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surg Oncol* 1993;2:335–9.
12. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–8.
13. 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;349:1864–7.
14. Giuliano AE, Jones RC, Brennan M, Statman R. Sentinel lymphadenectomy in breast cancer. *J Clin Oncol* 1997;2345–50.
15. Linehan DC, Hill AD, Akhurst T, Yeung H, et al. Intradermal radiocolloid and intraparenchymal blue dye injection optimize sentinel node identification in breast cancer patients *Ann Surg Oncol* 1999;6:450–4.
16. Bembenek A, Schlag PM. Lymph-node dissection in breast cancer. *Langenbecks Arch Surg* 2000;385:236–45.
17. Krag D, Weaver D, Ashikaga T, et al. The sentinel node in breast cancer: a multicenter validation study. *N Engl J Med* 1998;339: 941–6.
18. .Krag DN, Aschikaga T, Harlow SP, Weaver DL. Development of sentinel node targeting technique in breast cancer patients. *Br Journal* 1998;4:67–74.
19. Nathanson SD, Anaya P, Karvelis KC, Eck L, Havstad S. Sentinel lymph node uptake of two different technetium-labeled radiocolloids. *Ann Surg Oncol* 1997;4:104–10.
20. Linehan DC, Hill AD, Tran KN, Yeung H, Yeh SD, Borgen PI, Cody HS III. Sentinel lymph node biopsy in breast cancer: unfiltered radioisotope is superior to filtered *J Am Coll Surg* 1999;188: 377–81.
21. Strand SE, Berqvist L. Radiolabeled colloids and macromolecules in the lymphatic system. *Crit Rev Ther Drug Carrier Syst* 1989; 6:211–38.