Cysteine Rhenium Colloid: A Novel Radiocolloid for Identifying Sentinel Lymph Nodes in Breast Cancer Surgery

Biniam Kidane,1 Pamela L. Zabel,2,3,4 Vaibhav Gupta,5 Caroline Whiston,1,2,4 Frances Wright,6,7,8 Muriel Brackstone1,4,9

Abstract

Cysteine rhenium colloid (CRC) requires less medical isotope than standard sulfur colloid (SC) for sentinel lymph node biopsy (SNB). Our retrospective cohort study on 1205 consecutive early, clinically node-negative breast cancer patients who underwent SNB with either isotope suggests CRC is an alternative to SC in detecting sentinel lymph nodes, and uses less medical isotope.

Background: Medical isotopes are required for sentinel node lymphoscintigraphy in breast cancer, but are in critical shortage. Our center uses a modification of the standard SC, called CRC, that has been shown to require less medical isotope for the same procedure. Our objective was to determine if there was a significant difference between SC and CRC in successful lymph node identification in breast cancer patients. Patients and Methods: This was a retrospective cohort study using prospectively-collected data on 1205 consecutive early, clinically node-negative breast cancer patients who underwent a SNB between 2002 and 2008 at 2 tertiary hospitals in Canada. Results: There was no difference in successful lymph node identification rate (P = .50) or in the mean number of positive nodes identified between the 2 colloids (P = .88). The CRC group had a significantly lower rate of delayed adverse events (4.91% vs. 0.59%, P < .0001) even after adjusting for whether axillary dissection occurred on the same day as the biopsy (adjusted odds ratio, 0.12; 95% confidence interval, 0.04-0.40; P = .001). Conclusion: Our findings suggest that there is no significant difference between CRC and SC in detecting sentinel nodes; however, CRC uses less medical isotopes. In the current climate of critical shortages of medical radioisotopes, radiocolloids should be selected for use based on amount of radioisotope required.

Clinical Breast Cancer, Vol. 15, No. 1, e41-5 © 2015 The Authors. Published by Elsevier Inc. All rights reserved.

Keywords: Clinical trial, Lymphoscintigraphy, Nuclear medicine, Sentinel node biopsy, Sulfur colloid

Introduction

Breast cancer is the most common noncutaneous cancer in women, and the most powerful prognostic factor of survival is regional lymph node status.1 Thus, it is important to identify patients with nodal involvement to differentiate those that will clearly benefit from further treatments and staging. Sentinel lymph node (SLN) biopsy (SNB) has largely supplanted axillary dissection as a staging procedure because it reduces surgical morbidity and directs patient management based on nodal status. Retrieval of sentinel nodes during SNB is guided by preoperative sentinel node lymphoscintigraphy (SL) and application of blue dye.2 SL uses scintigraphic visualization of lymphatic drainage of a specific tumor site after injection of a radiolabeled colloid. The current standard in Canada for this procedure is technetium-99m (Tc-99m) sulfur colloid (SC). Typically, the SC used for SL requires a filtration step that wastes 70% to 90% of the isotope because most of the particles...
Cysteine Rhenium Colloid in Sentinel Node Biopsy

are greater than the 220-nm size limit required for SL.1 This filtration step can also increase cost, time, and radiation exposure to health care professionals. SL is listed as a top priority study in the dissemination of medical isotopes during times of critical shortages.

A member of our group (PLZ) developed a modification of SC, called cysteine rhenium colloid (CRC), which has the optimal size range for breast cancer SL such that the filtration is avoided and waste is reduced. Thus, SL using Tc-99m CRC uses the same clinical methodology and requires 70% to 90% less technetium waste is reduced. Thus, SL using Tc-99m CRC uses the same range for breast cancer SL such that the called cysteine rhenium colloid (CRC), which has the optimal size dissemination of medical isotopes during times of critical shortages.

Health care professionals. SL is listed as a top priority study in the final steps were included in the study. Patients were excluded if they had T1 or T2 tumors who were clinically node-negative were included in the study. Patients were excluded if they had neoadjuvant therapy. The lymphoscintigraphy report, operative note, postoperative note, and pathology report were obtained. Using the individual-level data from these deidentified reports, each patient was evaluated for: successful lymph node identification (primary outcome) and a number of secondary outcomes including any adverse events (either intraoperative from the operative report or delayed as described in the postoperative/clinic notes), the mean number of positive nodes in the 602 patients in the SC group, 600 (99.5%) had successful identification of SLNs and 597 (99.17%) of the 603 of the CRC group had successful identification; there was no significant difference in successful identification rate (P = .50). There was a significantly greater mean number of SLNs identified in the SC group (2.71 vs. 2.06; P < .0001); the median number of nodes identified was 2 for both with interquartile ranges of 1 to 3 and 2 to 4 nodes for the CRC and SC groups, respectively. In contrast, the total number of positive nodes identified (defined by histology) was significantly greater in the CRC group (170 (28%) vs. 138 (23%); P < .0001). Furthermore, the CRC group had a significantly greater proportion of positive nodes in the first 2 SLNs (P = .004

Related to SNB that required medical assessment/intervention and were reported within 30 days after the procedure. Data abstraction was completed by the same trained abstractor with regular consistency checks. Continuous data were assessed using independent samples t tests with Wilcoxon rank sum test being applied in cases of nonnormal distribution. Categorical data were assessed using Fisher exact test. Multivariable logistic regression was used for adjusted analyses. Results from this study were analyzed using SPSS/PASW v20 statistical software (SAS).

Table 1 Comparison of Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cysteine Rhenium Colloid</th>
<th>Sulfur Colloid</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.48 (SD, 12.05)</td>
<td>59.56 (SD, 12.40)</td>
<td>.003</td>
</tr>
<tr>
<td>Successful Identification of Sentinel Nodes</td>
<td>597 (99.17%)</td>
<td>600 (99.50%)</td>
<td>.50</td>
</tr>
<tr>
<td>Mean Sentinel Nodes Identified, n</td>
<td>2.06 (SD, 1.41)</td>
<td>2.71 (SD, 1.57)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total Positive Nodes, n</td>
<td>170 (13.20%)</td>
<td>138 (8.41%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Blue Dye Used</td>
<td>581 (99.15%)</td>
<td>594 (99.66%)</td>
<td>.28</td>
</tr>
<tr>
<td>Intraoperative Adverse Event</td>
<td>0 (0.00%)</td>
<td>1 (0.17%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Delayed Adverse Event</td>
<td>3 (0.59%)</td>
<td>26 (4.91%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sentinel Nodes Identified Using Tc-99</td>
<td>585 (97.34%)</td>
<td>574 (96.31%)</td>
<td>.31</td>
</tr>
<tr>
<td>Sentinel Nodes Identified Using Blue Dye</td>
<td>522 (86.86%)</td>
<td>513 (86.07%)</td>
<td>.69</td>
</tr>
<tr>
<td>Axillary Dissection</td>
<td>124 (20.56%)</td>
<td>115 (19.10%)</td>
<td>.52</td>
</tr>
<tr>
<td>Same Day Axillary Dissection</td>
<td>32 (25.81%)a</td>
<td>93 (80.87%)a</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*Percentage of all who had axillary dissection.

Technical Aspects of CRC

The Tc-99m CRC was developed and compared with many other colloidal products for size range, radiochemical purity, stability, optimal formulation, intravenous biokinetics in mice, and for lymphatic migration from intradermal foot injection in rabbits.4-6 CRC has a mean size of 10 nm to 12 nm based on electron microscopy,4-6 Using filter retention testing, > 90% of the radioactive colloid is retained on a 0.22 µm filter.4-6 The smaller size of Tc-99m CRC optimizes it for clinical use in SL because it concentrates in the sentinel node and shows minimal leakage to nonsentinel nodes.6 Two pilot human clinical trials were completed in melanoma and breast cancer lymphatic staging.7,8 The sterile compounding methods and extensive quality control methods were those previously used with our own SC preparation licensed with Health Canada.

Results

The SC group was older than the CRC group with a statistically significant mean age difference of 2 years (P = .003) (Table 1). Of the 602 patients in the SC group, 600 (99.5%) had successful identification of SLNs and 597 (99.17%) of the 603 of the CRC group had successful identification; there was no significant difference in successful identification rate (P = .50). There was a significantly greater mean number of SLNs identified in the SC group (2.71 vs. 2.06; P < .0001); the median number of nodes identified was 2 for both with interquartile ranges of 1 to 3 and 2 to 4 nodes for the CRC and SC groups, respectively. In contrast, the total number of positive nodes identified (defined by histology) was significantly greater in the CRC group (170 (28%) vs. 138 (23%); P < .0001). Furthermore, the CRC group had a significantly greater proportion of positive nodes in the first 2 SLNs (P = .004

Deidentified data were obtained from the charts for 602 (SHSC) and 603 (LHSC) consecutive early, clinically node-negative breast cancer patients who underwent SNB between 2002 and 2008. Patients with T1 or T2 tumors who were clinically node-negative were included in the study. Patients were excluded if they had neoadjuvant therapy. The lymphoscintigraphy report, operative note, postoperative note, and pathology report were obtained. Using the individual-level data from these deidentified reports, each patient was evaluated for: successful lymph node identification (primary outcome) and a number of secondary outcomes including any adverse events (either intraoperative from the operative report or delayed as described in the postoperative/clinic notes), the mean number of lymph nodes identified, and the metastatic status of these nodes based on the pathology reports. Delayed adverse events were defined as complications (eg, wound infection, hematoma, seroma) related to SNB that required medical assessment/intervention and were reported within 30 days after the procedure. Data abstraction was completed by the same trained abstractor with regular consistency checks. Continuous data were assessed using independent samples t tests with Wilcoxon rank sum test being applied in cases of nonnormal distribution. Categorical data were assessed using Fisher exact test. Multivariable logistic regression was used for adjusted analyses. Results from this study were analyzed using SPSS/PASW v20 statistical software (SAS).
and $P = .046$) (Figure 1, Table 2). There was no significant difference in proportion of positive nodes after the second SLN (Figure 1, Table 2). There was no difference in mean number of positive nodes between the 2 groups ($P = .88$). There was no difference between groups in frequency of blue dye use ($P = .28$) or in detection rate of SLNs using either the blue dye method ($P = .31$) or scintigraphy ($P = .69$). There was no difference in progression to axillary lymph node dissection (ALND) rates ($P = .52$). However, the SC group had a significantly greater rate of same-day ALND. Although there was no difference in rates of intraoperative adverse events, the CRC group had a significantly lower rate of delayed adverse events (0.59% vs. 4.91%; $P < .0001$). The CRC group had a significantly lower rate of delayed adverse events even after adjusting for same-day ALND (adjusted odds ratio, 0.12; 95% confidence interval [CI], 0.04-0.40; $P = .001$).

### Discussion

Overall, our findings suggest that CRC is not significantly different from SC in successful identification of SLNs. Although there was a significantly greater mean number of SLNs identified in the SC group, the total number of positive nodes was significantly greater in the CRC group, supporting earlier preclinical data that showed less migration of CRC to nonsentinel nodes compared with SC. Moreover, histological examination showed that the CRC group had a significantly greater proportion of positive nodes in the first 2 SLNs but not in subsequent SLNs. A potential explanation for this discrepancy might be that there might have been differences in the distribution of tumor size/stage between groups or differences in pathological examination techniques, but this information was not captured in our data extraction. In the end, there was no significant difference between the 2 groups in mean number of positive nodes identified. Thus, CRC seems equivalent and perhaps better able to identify sentinel nodes without requiring the resection of additional nodes (ie, which did not contribute to clinical nodal staging), as seen with SC.

There was a significantly lower rate of delayed adverse events and of same-day ALND in the CRC group. However, the latter finding was related to the routine practice at the hospital using SC, where

### Table 2 Sentinel Lymph Node Pathology

<table>
<thead>
<tr>
<th>Sentinel Lymph Node</th>
<th>Cysteine Rhenium Colloid</th>
<th>Sulfur Colloid</th>
<th>Odds Ratio (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>118</td>
<td>483</td>
<td>80</td>
<td>516</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>322</td>
<td>30</td>
<td>434</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>156</td>
<td>15</td>
<td>276</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>68</td>
<td>8</td>
<td>153</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>43</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>26</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

For each identified sentinel node, the number of patients in whom pathological analysis revealed positive (presence of disease) and negative (no disease) disease is shown. The odds ratio describes the odds of identifying positive nodes with cysteine rhenium colloid compared with the odds of identifying positive nodes with sulfur colloid for each sentinel node.
Cysteine Rhenium Colloid in Sentinel Node Biopsy

surgeons perform intraoperative frozen sections and proceed to same-day ALND if positive. The higher incidence of delayed adverse events in the SC group was specifically related to the SNB and not infiltrated because of adverse events related to same-day ALND: the CRC group had a significantly lower rate of delayed adverse events even after adjusting for same-day ALND (adjusted odds ratio, 0.12; 95% CI, 0.04-0.40; \( P = .001 \)). It is recognized that these data are collected retrospectively and as such, differences in delayed adverse events might be differentially captured and recorded by the 2 institutions, which could account for this difference.

Previous studies have reported the radiopharmaceutical quality and stability of CRC, and its efficacy in detection of SLNs.\(^6\)\(^7\) It has not previously been compared clinically with the standard SC in the setting of breast cancer. Because of the infrastructure barriers of carrying out a fully randomized single-center prospective comparison of CRC and SC, a comparison of 2 contemporaneous cohorts at 2 different centers was undertaken. Because the 2 cohorts being compared were at different sites, variation in established methods beyond the difference in colloid used were unavoidable. Furthermore, breast cancer care requires a coordinated multidisciplinary team approach (including, but not limited to, surgeons, radiologist, oncologists, and pathologists); it is possible that any differences detected in SLN identification rates and adverse events are more related to the differences between the 2 teams at the 2 different hospitals rather than the 2 different colloids used. In a review of SLN biopsy methodology in breast cancer, Bonnema and van de Velde concluded that SLN biopsy is a generally robust procedure despite large variations in isotope technique (ie, choice and dosage of isotope, carrier, particle size, timing of injection, and definition of a successful result).\(^2\) That being said, we discuss these differences and the potential effect on our conclusions below.

The first difference between the 2 cohorts in this study was in radiocolloid injection technique. Evidence shows that periareolar injection yields the best detection rates and is thus the technique advocated by Canadian guidelines and others.\(^10\)\(^11\) Periareolar injection was used in CRC and SC. However, in the CRC group, intradermal periareolar injection was used and in the SC group subdermal (subcutaneous) periareolar injection at 4 quadrants was used. Both injection sites target the subdermal lymphatic plexus and thus should have the same drainage pattern, explaining why most studies have grouped both injection methods together.\(^9\) More recently, a study demonstrated no difference in SLN identification rate using either injection method.\(^12\)

In general, there is variability among centers that use SC in the practice of filtration, which is where up to 90% of Tc-99m is lost on the filter.\(^6\) Some centers use filtered SC (usually through a 0.22-μm filter to obtain the ideal colloid particle size to be trapped within the SLN) and others use unfiltered SC, presumably in an effort to avoid loss of significant amounts of radiocolloid, and instead increase the dose injected to compensate for particle size limitations. The literature is somewhat divided on which technique is better. Some studies suggest that filtered SC results in higher yield of SLNs and secondary nodes.\(^9\)\(^12\) Conversely, other studies found that unfiltered SC is superior and suggest that the filtration allows the smaller SC particles to spread extensively, essentially causing signal washout and making it more difficult to localize true SLNs.\(^11\) In the end, even studies supportive of filtration point out that it is unclear whether the higher detection rates in those studies is due to increased detection of true SLNs or whether the detection rate is inflated by increased detection of nonsentinel secondary/tertiary nodes caused by increased migration of smaller particles to non-SLNs because of limited trapping. In the SC group in our study unfiltered SC was used. Thus, higher dose and volume of Tc-99m were required compared with the CRC group. The present study did not compare CRC and filtered SC; filtration might result in an equivalent use of volume, but would waste significant amounts of isotope and increase cost, time, and radiation exposure to the health care professional. For these reasons, CRC might be more advantageous than filtered SC.

The literature suggests that 85% to 99% of all positive nodes are found in the first 2 SLNs.\(^14\) Furthermore, several studies have found increased postoperative complication rates, operating room time/ cost, and pathology costs when more than 3 or 4 SLNs are removed.\(^14\)\(^15\) Our findings are consistent with the literature that if positive nodes existed, they were found within the first 2 SLNs. Furthermore, our finding that the SC group had significantly greater rates of delayed adverse events might have been a consequence of the greater mean number of SLNs removed, as suggested by the literature.\(^14\)\(^15\)

Technetium-99 SC is the only commercially available product available in Canada. Nanocollod human serum albumin (Nanocoll) and antimony trisulphide colloid are mainly used in Europe.\(^15\) Tc-99m Lymphoseek, a mannose-based colloid, was recently approved in the United States, after our study was completed.\(^11\) Although promising, the studies supporting the use of this new radiocolloid were criticized because they only compared detection rates between Lymphoseek and blue dye rather than another radiocolloid. Comparison of Tc-99m filtered SC, Tc-99m unfiltered SC, Tc-99m antimony trisulphide colloid and Tc-99m CRC in a rabbit model, showed that all colloids migrated to the first draining lymph node but that CRC had the highest entrapment in the first lymph node, so that there was less relative leakage past the sentinel node.\(^13\)\(^16\) Nanocoll is not licensed in North America and was not available for comparison during this initial development of the Tc-99m CRC. There is a paucity of clinical human studies comparing the different radiocolloids head-to-head, thus, evidence on the comparative efficacy of each radiocolloid is limited. Cost comparisons are also challenging to make because of the extra factors that contribute to the final cost for a sentinel lymphoscintigraphy procedure. The Tc-99m CRC is not yet commercially available but would likely have a pricing similar to the only other commercially available product in North America, Tc-99m SC. At our center, current daily reagent costs to produce Tc-99m SC average approximately $100 USD, with cost per patient varying depending on the number of patients booked per day and the timing between injection and procedure, to correct for the 6-hour half-life. Additional costs of manpower and filters would increase the costs by approximately $25 USD per vial with a slightly increased radiation exposure for the technologist. Thus, using these figures, one could estimate a potential cost savings of up to $25 USD per vial with use of CRC compared with SC. Ultimately, however, costs are likely similar between different colloids and pricing differences can be
influenced more by regional contracts than by true cost differences. In the end, we believe that the true decision about which colloids to use should be driven instead by those that use the least amount of technetium because of its scarce availability worldwide.

Retrospective analyses, carry with them an inherent risk of bias. Because we did not capture exact tumor size and histologic tumor phenotype in the data abstraction, there may be differences in the distribution of these factors between groups that might have biased our findings. This study was not randomized and the 2 groups being compared were at 2 different sites with 2 different sets of protocols. Thus, there is a risk that our findings were due to or influenced by unidentified confounding variables. That being said, there was no significant difference between groups in use of blue dye, successful identification of SLNs using blue dye, intraoperative adverse events, or ALND rates. Future studies should include a prospective, randomized and blinded comparison between the 2 colloids within the same center. A cost effectiveness analysis could then be undertaken.

Conclusion

Our findings suggest that CRC is not significantly different from SC in demonstrating very high rates of successful identification of SLNs. CRC seems better able to identify positive lymph nodes within the first 2 to 3 SLNs compared with SC. Our findings show that CRC requires lower volumes and radiation doses to effect similar detection rates in SLN procedures done for breast cancer staging. Preliminary clinical trials have shown CRC to be just as effective as filtered SC and requires 70% to 90% less technetium. Although the control group in this study used unfiltered SC and thus did not waste 70% to 90% of the technetium through filtration, they did use much higher radioisotope volumes and radiation doses. Finding ways to minimize use of medical isotopes is a critical step in overcoming the recurrent medical isotope shortages on a global basis. Our findings suggest that CRC appears to be a viable alternative to SC in detecting SLNs. Prospectively, randomized controlled studies are required to validate the present study.

Clinical Practice Points

- Sentinel node lymphoscintigraphy with Tc-99m SC is commonly used. Medical isotopes such as this are repeatedly in critical shortage globally, and sustainable use is prudent to ensure their availability.
- Cysteine rhenium colloid appears to be as effective as SC in detecting SLNs. Furthermore, it seems better able to identify positive lymph nodes within the first 2 to 3 SLNs and has significantly lower rates of delayed adverse events compared with SC.

- These findings contribute to evidence that CRC might be a viable alternative to SC and has the added benefit of using less medical isotope. The present study might lead to further randomized studies comparing the 2 isotopes head-to-head, which might eventually lead to a change in clinical practice.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research (grant number 200909MIS-MIS-211892) (Alternate Radiopharmaceuticals for Medical Imaging: MIS 100933). The dedicated clinical care provided by the Departments of Nuclear Medicine and Surgery at LHSC and SHSC is gratefully acknowledged.

Disclosure

The authors have stated that they have no conflicts of interest.

References