Sentinel Lymph Node Biopsy for Breast Cancer: Impact of the Number of Sentinel Nodes Removed on the False-Negative Rate

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BACKGROUND: Numerous studies have demonstrated that sentinel lymph node (SLN) biopsy can accurately determine axillary nodal status for breast cancer, but unacceptably high false negative rates have also been reported. Attention has been focused on factors associated with improved accuracy. We have previously shown that injection of blue dye in combination with radioactive colloid reduces the false negative rate compared with injection of blue dye alone. We hypothesized that this may be from the increased ability to identify multiple sentinel nodes. The purpose of this analysis was to determine whether removal of multiple SLNs results in a lower false negative rate.

STUDY DESIGN: The University of Louisville Breast Cancer Sentinel Lymph Node Study is a prospective multiinstitutional study. Patients with clinical stage T1-2, N0 breast cancer were eligible for enrollment. All patients underwent SLN biopsy using blue dye alone, radioactive colloid alone, or both agents in combination, followed by completion level I and II axillary dissection.

RESULTS: A total of 1,436 patients were enrolled in the study from August 1997 to February 2000. SLNs were identified in 1,287 patients (90%), with an overall false negative rate of 8.3%. A single SLN was removed in 537 patients. Multiple SLNs were removed in 750 patients. The false negative rates were 14.3% and 4.3% for patients with a single sentinel node versus multiple sentinel nodes removed, respectively ($p = 0.0004$, chi-square). Logistic regression analysis revealed that use of blue dye injection alone was the only factor independently associated with identification of a single SLN ($p < 0.0001$), and patient age, tumor size, tumor location, surgeon's previous experience, and type of operation were not significant.

CONCLUSIONS: The ability to identify multiple sentinel nodes, when they exist, improves the diagnostic accuracy of SLN biopsy. Injection of radioactive colloid in combination with blue dye improves the ability to identify multiple sentinel nodes compared with the use of blue dye alone. (J Am Coll Surg 2001;192:684–691. © 2001 by the American College of Surgeons)

Sentinel lymph node (SLN) biopsy is a minimally invasive alternative to axillary lymph node dissection for breast cancer. SLN biopsy is performed by injecting a vital blue dye, radioactive colloid, or both around the site of the primary breast cancer. These agents travel through afferent lymphatic channels to lodge in the first draining, or "sentinel" nodes.

Two key factors are used to assess the success of 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 successful localization and removal of a sentinel node. More important is the false negative rate, because it defines the frequency with which the sentinel node is pathologically negative when other axillary nodes harbor metastases. Before consideration can be given to abandoning the gold-standard level I and II axillary dissec-

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tion, SLN biopsy must be established as an accurate and reproducible alternative with an acceptably low false negative rate.

Although numerous studies have demonstrated that SLN biopsy can accurately determine axillary nodal status for breast cancer, variable and, in some cases, unacceptably high false negative rates have been reported in a number of studies.[1-8] So attention has been focused on technical factors associated with improved accuracy. We have previously shown that the use of blue dye in combination with radioactive colloid injection reduces the false negative rate compared with injection of blue dye alone.[8] Our experience with the use of blue dye as a single agent has suggested that it is often straightforward to identify a single blue node by following the afferent lymphatic channel, but that identification of additional blue nodes is more difficult after removal of the first. We also have shown previously that when radioactive colloid is used, the most radioactive, or "hottest," SLN is not infrequently negative for tumor when a less radioactive SLN is positive.[9] So we hypothesized that the use of radioactive colloid in combination with blue dye reduces the false negative rate by improving the ability to identify sentinel nodes beyond the first node.

METHODS

The University of Louisville Breast Cancer Sentinel Lymph Node Study is a prospective multiinstitutional study involving 148 surgeons. Investigators represent a wide variety of practice environments from across the country, most from private general surgery practices. The study was approved by the Institutional Review Board of each participating institution, and informed consent was obtained from all patients after discussion of risks and benefits with the operating surgeon. Patients with biopsy-proved clinical stage T1-2, N0 breast cancer were eligible for participation. Some patients with T2 tumors clinically were found to have T3 tumors pathologically after resection of the primary tumor; these patients were included in this study.[8-10]

All patients underwent SLN biopsy using blue dye alone, radioactive colloid alone, or both blue dye and radioactive colloid at the discretion of the operating surgeon. All patients had a completion level I and II axillary dissection after the sentinel node procedure. Recommended guidelines for performance of SLN biopsy were provided in the protocol. 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 sentinel node. Although many participants participated in formal SLN biopsy training courses, this was not mandatory for participation in this study. Biopsy of nonaxillary (eg, internal mammary) nodes was not required in this study. Only two patients underwent internal mammary SLN biopsy; both were negative for tumor.

Sentinel nodes were evaluated histologically by serial sections with hematoxylin and eosin staining at intervals no greater than 2 mm. Use of immunohistochemistry was at the discretion of the participating institution; antibodies for cytokeratin were used in 47% of patients. Nonsentinel nodes were evaluated by routine histology.

Comparison between the clinicopathologic characteristics of patients with single lymph nodes and multiple lymph nodes harvested was performed using Student's *t*-test and chi-square analysis. To assess the relationship of other factors on the identification rate and number of SLNs removed, univariate chi-square testing was performed; multivariate logistic regression analysis was then used to determine independent factors predicting outcomes. Fisher's exact test was used for false negative rate comparisons. Significance was determined at $p < 0.05$.

RESULTS

A total of 1,436 patients were enrolled in the study from August 1997 to February 2000. The mean number of SLN cases performed per surgeon was 9.7 (range 1 to 94), and consecutive cases were reported for all patients enrolled in the study. Blue dye alone, radioactive colloid alone, and the combination of blue dye plus radioactive colloid were used in 16%, 5%, and 79% of patients, respectively. When radioactive colloid was used, peritumoral injection was performed in 74% of cases. SLNs were identified in 1,287 patients (90%) with an overall false negative rate of 8.3%.

The mean number of SLNs removed per patient was 2.2. A single SLN was removed in 537 patients (41.7%); multiple nodes (mean 3.02 nodes per patient) were removed in 750 patients (58.3%). Patients in each group (single SLN versus multiple SLN) were well balanced in terms of age, tumor stage, tumor location, pathology of primary tumor, type of surgical procedure performed for treatment of the primary tumor, and percentage with axillary metastases (Table 1). Of these clinicopathologic characteristics, only age (≥ 50 years) was significantly

Table 1. Clinicopathologic Characteristics of Patients Undergoing Sentinel Lymph Node Biopsy

Characteristic	Single sentinel lymph node removed	Multiple sentinel lymph nodes removed
Age (mean) (y)	59.7	58.2
Tumor stage		
T1a	6.6%	7.3%
T1b	24.8%	18.4%
T1c	41.5%	39.9%
T2	24.6%	26.8%
T3	2.5%	2.1%
Tumor location		
Upper outer quadrant	50.7%	49.1%
Upper inner quadrant	15.5%	13.5%
Lower outer quadrant	13.8%	12.4%
Lower inner quadrant	5.4%	7.3%
Central	11.5%	14.3%
Other	3.2%	3.5%
Pathology		
Ductal	79.7%	79.1%
Lobular	7.6%	8.5%
Other	12.7%	12.4%
Type of operation		
Total mastectomy	25.5%	30.1%
Partial mastectomy	71.7%	67.2%
Other	2.8%	2.7%
Axillary nodal metastases	28.7%	31.1%

associated with a lower overall SLN identification rate in a stepwise logistical regression analysis ($p < 0.005$), consistent with our previous analysis.[8]

The false negative rate in patients who had one sentinel node harvested was 14.3%, compared with a false negative rate of 4.3% in patients who had multiple (two or more) sentinel nodes removed ($p = 0.0004$, chi-square) (Table 2). False negative rates associated with the use of blue dye alone, radioactive colloid alone, or blue dye plus radioactive colloid were 11.9%, 11.8%, and 7.3%, respectively. In a previous analysis, we showed that the use of dual agent injection (blue dye plus radioactive colloid injection) improved the false negative rate compared with the use of either agent alone.[8] The present updated analysis supports that finding, although the false negative rates are now marginally statistically significant (dual agent versus single agent injection, false negative rates 7.3% versus 11.9%, $p = 0.058$, Fisher's exact test).

We subsequently performed several analyses to evaluate related covariants associated with identification of multiple SLNs. The mean numbers of SLNs removed were 1.59 and 2.28 for patients undergoing blue dye injection alone and radioactive colloid injection (either alone or in combination with blue dye), respectively ($p < 0.0001$, Student's *t*-test). Use of blue dye injection alone or radioactive colloid alone compared with radioactive colloid in combination with blue dye, was more

Table 2. Impact of Number of Sentinel Nodes Removed on the False Negative Rate

SLN removed (n)	Patients with SLN identified (n)	Patients with true positive SLN (n)	Patients with false negative SLN (n)	False negative rate (%)
One	537	132	22	14.3
Two or more	750	223	10	4.3*

*$p = 0.0004$, chi-square.
SLN, sentinel lymph node.

often associated with the identification of a single SLN (Table 3; $p<0.0001$). The injection technique used does not appear to predict a significantly lower false negative rate (Table 3). A multivariate logistic regression model confirmed that use of blue dye injection alone, when compared with other techniques, was the only factor independently associated with identification of a single sentinel node ($p<0.0001$). Patient age, tumor size, tumor location, surgeon's stated experience before enrolling in this study, and type of operation were not significant independent factors predictive of the number of SLNs identified (Table 4). Logistic regression analysis also revealed no significant association between the type of radioactive colloid used (filtered or unfiltered), the location of radioactive colloid injection (peritumoral versus subdermal or dermal), or the time lapse (<2, 2–4, 4–8, >8 hours) between injection and operation and the number of SLNs identified (Table 5).

The effect of the sequence in which the sentinel lymph nodes were harvested was also examined. If only the first SLN had been harvested from all patients, there would have been an additional 57 false negative results; the false negative rate would have been as high as 28.8%. Subsequent removal of additional sentinel nodes decreases the false negative rate to 4.3%. But only 1.4% of positive sentinel nodes were found beyond the fourth sentinel node removed.

DISCUSSION

SLN biopsy has become increasingly accepted as an alternative to axillary lymph node dissection for nodal staging of breast cancer. Considerable controversy exists as to the optimal technique that should be used. Our results indicate that removal of more than one sentinel node is strongly associated with a decreased false negative rate, and that radioactive colloid injection facilitates identification of multiple sentinel nodes. That is not to say that more than one sentinel node must be removed in every patient. Rather, the data suggest that more than one sentinel node is present in the majority of patients, and that identification of all sentinel nodes is important for reducing the false negative rate.

Subtle differences in technique may lead to significant differences in the accuracy of nodal staging. Lymphatic drainage patterns can be complex, with many lymphatic channels leading to or emanating from various lymph nodes. One or more lymphatic channels can carry embolizing tumor cells, resulting in the possibility of more than one "true" sentinel node. It is believed that vital blue dye concentrates in the first draining "true" sentinel nodes, and that it is uncommon for enough blue dye to pass through the first node into second-echelon nodes to cause blue staining of the secondary nodes. So a blue-stained lymph node is thought to represent a true sentinel node, because the blue dye indicates a direct lymphatic drainage pathway from the tumor. Although blue dye staining is helpful for identifying sentinel nodes, it is not 100% reliable, at least in multiinstitutional practice. It has been demonstrated that even when a combination of blue dye and radioactive colloid is used, not all positive sentinel nodes are blue.[11] In fact, in a previous anal-

Table 3. Factors Associated with Identification of Single Versus Multiple Sentinel Lymph Nodes

	Single sentinel lymph node removed		Multiple sentinel lymph nodes removed	
Injection technique	**n**	**%**	**n**	**%**
Blue dye alone	124	62.0*	76	38.0*
Use of radioactive colloid	413	38.0	674	62.0
Radioactive colloid alone	41	60.3	27	39.7
Radioactive colloid + blue dye	372	36.5	647	63.5

*$p<0.0001$ versus use of radioactive colloid, chi-square.

Table 4. Multivariate Logistic Regression Analysis of Factors Predicting the Removal of Multiple Sentinel Lymph Nodes

Characteristic	Odds ratio	95% Confidence interval	p-Value
Patient age (<50 vs. ≥50 y)	0.798	0.60–1.06	0.13
Tumor size (T1, T2, or T3)	1.074	0.84–1.37	0.57
Tumor location (upper outer quadrant vs. others)	0.959	0.78–1.08	0.74
Surgeon experience (<10, 10–20, >20 cases performed before the study)	0.917	0.78–1.08	0.29
Type of operation (lumpectomy vs. mastectomy)	1.211	0.92–1.60	0.18
Injection technique (blue dye vs. radioactive colloid alone or in combination with blue dye)	0.360	0.056–0.50	<0.0001

Table 5. Number of Sentinel Lymph Nodes Removed by Characteristics of Radioactive Colloid Used

Radioactive colloid characteristics	Odds ratio	95% Confidence interval	p-Value
Location of radioactive colloid injection (peritumoral vs. dermal/subdermal)	0.726	0.52–1.01	0.053
Type of radioactive colloid used (filtered vs. unfiltered)	0.924	0.62–1.37	0.70
Time lapse between radioactive colloid injection and sentinel lymph node procedure (<2 h, 2–4 h, 4–8 h, >8 h)	0.970	0.91–1.04	0.04

ysis, we found that only two-thirds of all positive sentinel nodes contained blue dye staining. Furthermore, we showed that all blue nodes and all nodes with 10% or more of the ex vivo count of the hottest SLN should be harvested to minimize the false negative rate.[9] This so-called "10% rule" provides a very practical definition of a sentinel node and appears equally applicable to breast cancer and melanoma.[12] We believe that the blue dye and radioactive colloid techniques provide overlapping and complementary techniques for SLN identification.

Some valid concerns have been raised that the use of radioactive colloid results in removal of an excessive number of radioactive nodes that may be second-echelon nodes and not true sentinel nodes.[13,14] But in this analysis, when radioactive colloid was used and the "10% rule" was used, the mean number of SLNs harvested was 2.28. So there is little evidence that radioactive colloid injection results in indiscriminate removal of an inordinate number of axillary nodes. Some might argue that the use of radioactive colloid may decrease the false negative rate simply by allowing removal of a greater number of nodes, and that more positive nodes would be identified by chance alone. Mathematically, it is implausible that an increase in the number of sentinel nodes removed from 1.59 (blue dye alone) to 2.28 (radioactive colloid) would explain the significant reduction in false negative rate purely by chance. It appears clear that use of radioactive colloid in combination with blue dye results in more consistent identification of the potentially very important second or third sentinel node.

There also has been concern that prolonged time lapse after radioactive colloid injection leads to identification of more radioactive nodes that may not be true sentinel nodes. In this analysis, an increasing length of time from injection of the radioactive colloid to SLN biopsy was not associated with an increased number of nodes removed, indicating that the radioactive tracer accumulates and, for the most part, is retained in the first draining nodes. The type of radioactive technetium sulfur colloid used (unfiltered versus 0.2 micron filtered) appears to have little effect on the number of sentinel nodes identified, as shown previously.[9]

Although the relationship between location of radioactive colloid injection (peritumoral versus dermal or subdermal) and the number of sentinel nodes identified did not reach statistical significance ($p = 0.053$), this may yet prove important. Dermal[15] and subdermal[16] injection techniques have been tested in some studies, but further study is necessary to demonstrate convincingly that these injection techniques result in an acceptably low false negative rate, especially in a multiinstitutional setting.

The issue of the best injection technique continues to be hotly debated. Although the present study is not a randomized study, it does encompass a wide range of surgical practices and hospital environments and is reflective of what is happening in the "real world," where SLN biopsy may be broadly used. Our results support the use of dual agent (blue dye plus radioactive colloid) injection to optimize the accuracy of SLN biopsy and probably to shorten the learning curve to allow surgeons to master this technique. Certainly, advocates of various injection techniques have reported good results in single institution studies. Our results do not detract from the excellent results obtained in some centers using other techniques, but rather suggest that, in widespread multiinstitutional practice, dual agent injection is the preferred technique to reduce false negative results.

Most surgeons in our study had little previous experience with SLN biopsy, and these results represent their initial experiences with the technique. We found no difference in number of SLNs removed between surgeons new to the technique and those who had performed more than 10 sentinel node cases before participating in this study. It is important to note that the surgeon experience reported here represents the surgeon's stated experience before entering the study, as filled out in a prestudy surgeon registration form. But these results regarding surgeon experience and the number of SLNs removed should not be misconstrued to indicate that surgeon experience is not important for assuring reproducible and accurate results. In fact, we have found a significant correlation between surgeon experience and SLN identification and false negative rates that will be presented in an upcoming article.

In conclusion, we have demonstrated that the ability to identify multiple sentinel nodes, when they exist, reduces the false negative rate. Use of radioactive colloid and blue dye injection together improves the ability to identify all sentinel nodes and is associated with improved accuracy. These data provide useful guidelines for SLN identification to minimize false negative results.

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Invited Commentary

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The sentinel node concept in breast cancer has been validated in large numbers of women, and there is no doubt that this is a valuable technique for reducing the morbidity of axillary staging. Attention is now focused on improving our understanding of why a sentinel node cannot be identified in all patients, and determining factors responsible for false negative results after lymphatic mapping. A variety of factors such as tumor location, patient age, earlier breast biopsy, and body mass index have been shown to correlate with failure to identify a sentinel node, although none of these has been found to be significant in a majority of studies. In contrast, there is agreement that surgeon experience impacts on both the ability to identify a sentinel node and its predictive value.

In the study presented here, Wong and coauthors suggest that the number of sentinel nodes removed significantly alters the false negative rate of the procedure, and that lymphatic mapping with blue dye alone makes it much more likely that only a single sentinel node will be identified.

A review of the literature does not clearly substantiate this observation. Studies of mapping with blue dye alone report a mean of 1.8 to 2.1 sentinel nodes identified, and studies with blue dye plus radioactivity report means of