Practical guidelines for optimal gamma probe detection of sentinel lymph nodes in breast cancer: Results of a multi-institutional study

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Introduction. *Multiple radioactive lymph nodes are often removed during the course of sentinel lymph node (SLN) biopsy for breast cancer when both blue dye and radioactive colloid injection are used. Some of the less radioactive lymph nodes are second echelon nodes, not true SLNs. The purpose of this analysis was to determine whether harvesting these less radioactive nodes, in addition to the "hottest" SLNs, reduces the false-negative rate.*

Methods. *Patients were enrolled in this multicenter (121 surgeons) prospective, institutional review board–approved study after informed consent was obtained. Patients with clinical stage T1-2, N0, M0 invasive breast cancer were eligible. This analysis includes all patients who underwent axillary SLN biopsy with the use of an injection of both isosulfan blue dye and radioactive colloid. The protocol specified that all blue nodes and all nodes with 10% or more of the ex vivo count of the hottest node should be removed and designated SLNs. All patients underwent completion level I/II axillary dissection.*

Results. *SLNs were identified in 672 of 758 patients (89%). Of the patients with SLNs identified, 403 patients (60%) had more than 1 SLN removed (mean, 1.96 SLN/patient) and 207 patients (31%) had nodal metastases. The use of filtered or unfiltered technetium sulfur colloid had no impact on the number of SLNs identified. Overall, 33% of histologically positive SLNs had no evidence of blue dye staining. Of those patients with multiple SLNs removed, histologically positive SLNs were found in 130 patients. In 15 of these 130 patients (11.5%), the hottest SLN was negative when a less radioactive node was positive for tumor. If only the hottest node had been removed, the false-negative rate would have been 13.0% versus 5.8% when all nodes with 10% or more of the ex vivo count of the hottest node were removed (*P *= .01).*

Conclusions. *These data support the policy that all blue nodes and all nodes with 10% or more of the ex vivo count of the hottest SLN should be harvested for optimal nodal staging. (Surgery 2000;128:139-44.)*

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SENTINEL LYMPH NODE (SLN) BIOPSY is a minimally invasive procedure that has been investigated for nodal staging of breast cancer. Increasingly, SLN biopsy has been accepted in many centers as an alternative to axillary lymph node dissection for nodal staging of patients with breast cancer.

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Because the false-negative rate associated with SLN biopsy has been variable in the published literature, there has been concern about the diagnostic accuracy of the technique as it is applied in widespread community surgical practice.[1-8] This concern in amplified by the fact that SLN biopsy has been performed by a variety of different techniques, with the use of an injection of a vital blue dye, radioactive colloid, or a combination of the 2. Therefore, attention has been focused on standardizing and optimizing the technical aspects of the procedure to assure accurate and reproducible nodal staging.

Of critical importance is the definition of a sentinel node. Although most surgeons would agree, in principle, that a sentinel node can be defined as the first node or nodes to receive afferent lymphatic drainage from a tumor, there is actually considerable controversy over the practical definition of a sentinel node.[9-11] Some surgeons believe that the only true way to define a sentinel node is to visualize a blue-stained lymphatic channel as it enters a blue lymph node. However, it is not always a simple matter to identify the sentinel node with the use of blue dye alone,[12] and positive sentinel nodes that contained no blue staining have been reported.[13]

Many surgeons have found that a radioactive colloid injection with intraoperative gamma probe detection greatly facilitates sentinel node identification.[4,5,7-9,13] Multiple radioactive lymph nodes are often identified with the use of the gamma probe. Some of the less radioactive lymph nodes are second-echelon nodes, not true sentinel nodes. However, there is no clear consensus to indicate which radioactive nodes should be removed or which may be true sentinel nodes. Several different definitions of a sentinel node have been proposed. These include definitions based on the absolute number of counts in the node or on the ratio of the in vivo or ex vivo radioactive counts in the node to background radioactivity or to neighboring nonsentinel nodes.[6,9,10,13] All of these definitions are somewhat arbitrary, and none of them is based on empiric data related to the likelihood of false-negative results. For example, the degree of background radioactivity is variable based on the position of the gamma probe within the axilla and the location of the primary tumor, which can account for a significant amount of background radioactivity or "shine through." Furthermore, the absolute number of counts in the node varies depending on factors such as the dose and type of radioactive colloid, the injection site, time interval from injection to operation, the type of gamma probe and its calibration.

The purpose of this analysis was to determine whether harvesting radioactive nodes in addition to the most radioactive ("hottest") node improves the false-negative rate. Moreover, we sought to provide practical guidelines for the removal of sentinel nodes on the basis of the degree of radioactivity of the nodes.

METHODS

Patients were enrolled in the University of Louisville Breast Cancer Sentinel Lymph Node Study, a prospective multicenter (121 surgeons) study, between August 1997 and August 1999. The study was approved by the institutional review board at each institution. Patients with biopsy-proven clinical stage T1-2, N0, M0 invasive breast cancer were eligible. Some patients with T3 tumors were included; the tumors of these patients were clinically staged as T2 N0 tumors, but after the operation the tumor size was found to exceed 5 cm. This analysis includes all patients who underwent axillary SLN biopsy with an injection of both technetium sulfur colloid and isosulfan blue dye. The protocol specified a peritumoral injection of 0.2-μm filtered technetium sulfur colloid (0.5 mCi in a 6-cc volume). Unfiltered technetium sulfur colloid was used in 13% of cases. Peritumoral injection of 5 cc of isosulfan blue dye was also performed in all cases.

After informed consent was obtained, patients underwent SLN biopsy, during which radioactive counts taken during the operation and an evaluation of blue-dye staining were recorded for each SLN removed. Blue-dye staining was graded as follows: 0 = none; 1 = faint blue; 2 = obviously blue. The protocol specified that all blue nodes and all nodes of 10% or more of the ex vivo count of the hottest node should be removed and designated SLNs. These guidelines have been suggested previously.[6,10] All patients then underwent completion level I/II axillary dissection. All SLNs then underwent routine histologic analysis with hematoxylin and eosin staining at a minimum of 2-mm intervals. Immunohistochemistry using antibodies against cytokeratin was performed at some institutions for the analysis of the sentinel nodes. The remaining nonsentinel axillary nodes were examined by standard pathologic measures with hematoxylin and eosin staining. A biopsy of nonaxillary (internal mammary, supraclavicular) nodes was not mandated in the protocol. Only 2 patients in this series underwent internal mammary SLN biopsy; both patients were negative for tumor.

Statistical analysis of the false-negative rates was performed by chi-squared analysis with the use of SAS software (SAS Institute Inc, Cary, NC). Dif-

Table I. Clinicopathologic characteristics of the study population

Variable	No. of patients (%)
Age (y)	
<50	170 (22)
>50	588 (78)
T stage	
T1	541 (71)
T2	196 (26)
T3	21 (3)
Location	
Upper outer quadrant	395 (52)
Upper inner quadrant	120 (16)
Lower outer quadrant	95 (13)
Lower inner quadrant	51 (7)
Central	97 (12)
Pathologic feature	
Infiltrating ductal	649 (85)
Infiltrating lobular	82 (11)
Other	26 (4)
Type of surgery	
Mastectomy	249 (33)
Lumpectomy	509 (67)
Immunohistochemistry performed on SLN	380 (50)

ferences were considered significant at a probability value of less than .05.

RESULTS

The clinicopathologic characteristics of the patients who entered into the study are shown in Table I. A total of 758 patients who underwent injection of both blue dye and radioactive colloid were entered into the study. The median age was 61 years (range, 27-102 years). The median tumor size was 1.86 cm. Most of the patients (67%) underwent breast conservation. SLNs were identified in 672 of 758 patients (89%), and 207 patients (31%) had nodal metastasis (Table II). The false-negative rate was 5.8%. The mean total number of nodes removed (sentinel nodes plus axillary dissection) was 15. The likelihood of a positive SLN correlated with tumor size: the rate of positive SLNs for patients with T1, T2, and T3 was 21.4%, 46.4%, and 61.9%, respectively. Tumor size (T1 vs T2 vs T3) had no significant effect on the SLN identification rate or false-negative rate (data not shown).

Of the 672 patients with SLN identified, 403 patients (60%) had more than 1 SLN removed. The mean number of SLNs removed per patient was 1.96. Patients who underwent filtered (n = 584 patients) versus unfiltered (n = 88 patients) radioactive colloid injection had a mean of 1.97 and 1.97 SLNs removed per patient, respectively.

Table II. SLN identification and positive SLN by tumor size

Variable	N (%)
SLN identified	672/758 (89.0)
False-negative results	12/207 (5.8)
No. of patients with positive lymph node metastasis (%)	
Total	207/672 (31.0)
T1	116/541 (21.4)
T1a	3/58 (5.2)
T1b	31/159 (19.5)
T1c	82/324 (25.3)
T2	91/196 (46.4)
T3	13/21 (61.9)

Table III. Blue dye staining in the sentinel nodes

Blue dye staining	Nodes (no.)	Percentage
SLN negative	606/893	68
SLN positive	285/423	67
Total	891/1316	68

Overall, 68% of the SLNs had evidence of blue-dye staining (Table III). It is noteworthy that 33% of the histologically positive SLNs had no evidence of blue-dye staining.

Of those patients with multiple SLNs removed, histologically positive SLNs were found in 130 patients. In 15 of these 130 patients (11.5%), the hottest SLN was negative when a less radioactive node was positive for tumor (mean ex vivo radioactive count, 46% of the hottest node; range, 12%-81%; Table IV). All of these less radioactive positive SLNs had radioactive counts of 10% or more of the ex vivo count of the hottest node. In 7 of 15 patients, the positive node was the third or fourth sentinel node identified and removed. In 6 of these 15 patients, obvious blue staining was identified in the positive SLN. Faint blue-dye staining was identified in an additional 3 of 15 patients, although no blue dye was evident in 6 of 15 patients.

Table V shows the impact of different criteria for SLN removal on the false-negative rate. If only the hottest node had been removed, the false-negative rate would have been 13.0%. The false-negative rate would have been 11.6% and 8.7%, respectively, if the hottest node plus all obviously blue nodes or the hottest node plus all blue nodes (obvious or faintly stained) had been removed. Of course, it is difficult to know how many of these less radioactive blue nodes would have been identified without the

Table IV. Patients who would have had a false-negative result if only the most radioactive ("hottest") node had been removed

Patient	Ex vivo radioactive count (positive node)	Ex vivo radioactive count (hottest node)	Percent of hottest node	Order positive node was removed	Blue-dye staining
1	359	569	63	2nd of 2	None
2	5391	10,721	50	3rd of 5	Obviously blue
3	29	68	43	3rd of 3	Obviously blue
4	500	800	63	3rd of 3	Obviously blue
5	900	2300	39	2nd of 2	Faint blue
6	24	89	27	4th of 6	Faint blue
7	7000	11,399	61	2nd of 3	Faint blue
8	386	811	48	2nd of 4	None
9	551	1695	33	4th of 4	Obviously blue
10	50	121	42	2nd of 8	None
11	245	745	32	2nd of 2	None
12	155	1323	12	4th of 4	None
13	29	36	81	1st of 3	Obviously blue
14	24	89	27	4th of 6	Obviously blue
15	58	78	74	2nd of 3	None

Table V. Effect of criteria for SLN removal on the false-negative rate

Criteria for removal of SLN	False-negative results/patients with positive nodes in whom SLN was identified (no.)	False-negative rate (%)
Only hottest node removed	27/207	13.0
Hottest node and all obviously blue nodes removed*	24/207	11.6
Hottest node and all blue nodes removed*	18/207	8.7
All blue nodes and all nodes ≥10% or more of the ex vivo count of the hottest node	12/207	5.8†

*This assumes that the faintly blue and/or obviously blue nodes would have been identified without the gamma probe or that blue-dye staining could be established before the node was removed.

†Statistically significant vs only hottest node removed, $P = .01$.

gamma probe and how many of them could have been identified as blue before removal. By harvesting all nodes that were 10% or more of the ex vivo count of the hottest node, the false-negative rate for the study was 5.8%, which is statistically different compared with the 13.0% false-negative rate for the removal of only the hottest node ($P = .01$).

DISCUSSION

Numerous studies have documented that SLN biopsy can accurately determine the nodal status in breast cancer. However, the high false-negative rates in some of the published studies have raised concern about the accuracy of the technique in widespread use. Despite the abundant literature, there has been no clear consensus regarding a practical issue that faces surgeons who perform this procedure: When multiple radioactive nodes are found, which ones should be removed? It is generally accepted that a blue lymphatic channel leading to a blue node is the gold standard for the identification of the SLN. However, there may be 2 or more lymphatic channels leading to separate sentinel nodes. By the use of radioactive colloid injection with intraoperative gamma probe detection, it is usually possible to localize the sentinel nodes without relying completely on visualization of blue dye. As a practical matter, it may be possible to identify the first sentinel node with blue dye alone, but after that first node is dissected, it may be difficult to find additional nodes. It is common, in fact, to localize the sentinel node with the gamma probe, only to find blue-dye staining within the node in retrospect after the node has been removed. Therefore, the gamma probe actually helps to identify blue nodes.

It has been proposed that only nodes with blue-dye staining should be removed and designated SLNs.[9] However, in the present study, one third of the positive SLNs had no blue-dye staining at all. There are several possible reasons that blue dye may not be evident in a true SLN: Timing and site of injection, distance from the tumor to the axilla, efficiency of the lymphatic drainage (which may be less in the postmenopausal breast), and differences in the use of massage to stimulate lymphatic drainage. Although we agree that all blue nodes should be removed regardless of radioactivity because blue-dye staining indicates a direct lymphatic pathway from the tumor to the node, it is clear that some radioactive nodes can be positive when no blue dye is identified.[13] Therefore, when a surgeon is faced with the situation of finding a focal radioactive area in the axilla that is 10% or greater of the ex vivo count of the hottest node after removing the first radioactive SLN, our data indicate that the best course of action would be to continue dissection to identify and remove the additional radioactive node, even if no blue dye is evident.

In our multi-institutional study, we found that the use of blue dye in combination with radioactive colloid resulted in a significant reduction in the false-negative rate compared with use of blue dye alone (unpublished data). The complementary nature of the visual signal provided by the blue dye and the ability to localize the nodes with the gamma probe results in more accurate SLN biopsy. Because multiple radioactive lymph nodes are often identified during dissection, it is essential to determine which of these lesser radioactive lymph nodes should be removed. Although many different sets of guidelines have been proposed, none has been based on an empiric analysis of the probability of false-negative results. The present study therefore provides important practical guidelines for defining which nodes should be removed. The ability to identify a second, third, or fourth SLN, when it exists, reduces the false-negative rate. The gamma probe is extremely helpful in the detection of these additional SLNs.

The false-negative rate for the study was 5.8%. This is only slightly higher than the stated objective of 5% false-negative rate by the American Society of Breast Surgeons[14] and compares quite favorably with the false-negative rates in the published literature.[1-8] This is despite the fact that most of the surgeons in this study are not from large academic medical centers, and most of them had little experience with SLN biopsy before entering the study. With an increase in surgeon experience, we anticipate that the false-negative rate will decline. If only the hottest sentinel nodes had been removed, an additional 15 of the 207 patients with positive axillary lymph nodes would have had false-negative SLN biopsy, resulting in a false-negative rate of 13%. In a similar analysis of the Sunbelt Melanoma Trial of SLN biopsy for melanoma, we found that the false-negative rate would have been 13% if only the hottest node had been removed.[15] Thus, the 10% rule appears useful for reducing the false-negative rate of SLN biopsy for both breast cancer and melanoma.

Removal of all blue nodes and all nodes with 10% or more of the ex vivo count of the hottest node will ensure optimal detection of nodal metastases. Despite the concern that these guidelines will result in the removal of an excessive number of lymph nodes,[9,10] the mean number of SLNs removed in this study was 2 per patient. Another point of controversy in the literature has centered on the merits of the use of filtered versus unfiltered technetium sulfur colloid.[9,10,16,17] It has been proposed that unfiltered colloid is preferred because the overall larger particle size will prevent the colloid from passing through the true SLN to the second echelon nodes. It has also been suggested that filtered colloid results in a greater amount of radioactivity in the non-SLNs. However, in the present study, the mean number of SLNs removed was not affected by the use of filtered versus unfiltered technetium sulfur colloid. Although several radioactive nodes may appear on a preoperative lymphoscintigram, in practice it is rare to find more than 2 to 4 radioactive nodes that fit the 10% rule.[10]

In conclusion, these data support the combined use of both blue dye and radioactive colloid for SLN biopsy in breast cancer. All blue nodes and all nodes with 10% or more of the ex vivo count of the hottest node should be removed for optimal detection of nodal metastases. Although the controversy regarding the definition of the SLN continues, these data provide practical guidelines for SLN identification that should prove helpful in the reduction of the false-negative rate as more surgeons implement SLN biopsy for nodal staging of breast cancer.

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