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OPTIMAL NUMBER OF RADIOACTIVE SENTINEL LYMPH NODES TO REMOVE FOR ACCURATE AXILLARY STAGING OF BREAST CANCER¹

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Abstract

Background—Although sentinel lymph node (SLN) biopsy is the standard technique for staging the axilla of clinically node negative breast cancer, the optimal number of radioactive SLN to remove to ensure accuracy and minimize morbidity is still actively debated. The purpose of this study was to determine the minimum number of SLNs to excise to ensure accurate axillary staging of SLN positive patients.

Methods—One hundred twenty six patients with invasive breast cancer underwent SLN biopsy by periareolar injection of radiolabeled technetium sulfur colloid on the day of surgery. The sequence in which SLNs were removed and the corresponding ex vivo radioactive counts were recorded. SLNs were removed until radioactive counts in the axilla were <10% the ex vivo counts of the hottest SLN.

Results—A radioactive SLN was identified in every patient. The mean number of SLNs identified was 2.86 (range: 1–8). Clinicopathologic features associated with a positive SLN included a palpable tumor ($p=0.0035$), increasing tumor size ($p=0.0039$), increasing histologic grade ($p=0.0234$), and angiolymphatic invasion ($p<0.001$). The highest radioactive counts were found in the first node in 100 patients (79.4%), the second node in 15 (11.9%) and the third or later node in 11 patients (8.7%). Among the 38 patients with a positive SLN (30.2%), the hottest node was the first positive SLN in 27 patients (71.1%). The first positive SLN was the first node removed in 31 patients (81.6%), following the second node in 37 patients (97.4%) and was removed in all patients by the third SLN.

Conclusion—These data support the trend of limiting SLN biopsy to 3 lymph nodes. Removing all SLN with radioactive counts > 10% of the ex vivo counts of the hottest SLN did not increase accuracy.

Background

Sentinel lymph node biopsy has become the standard method for the accurate surgical staging of the axilla for breast cancer. This technique has been demonstrated to be accurate and reliable in comparison to complete axillary dissection, with a false negative rate of 8%, fewer associated morbidities including pain, decreased arm mobility and lymphedema, and no differences in local recurrence or survival at follow up (1). Early results from the NSABP-32 trial, the largest

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multicenter trial to date for the evaluation of SLN biopsy, confirm these findings (2). Specifics of the sentinel lymph node technique have been evaluated in several large trials (reviewed in 3). The use of both vital blue dye and radioactive colloid as lymphatic tracers has been shown to increase the success rate and the accuracy of the SLN technique, but has also been shown to increase the number of lymph nodes removed (4–7).

Controversy exists regarding the optimal number of axillary lymph nodes to remove for accurate SLN directed staging of breast cancer. Current practice is guided by the convention that all lymph nodes with radioactive counts > 10% of the ex vivo counts measured in the hottest SLN are removed. While it has been established that the SLN with the most radioactive counts is not always the SLN most likely to harbor metastatic disease (6,7), the use of relative radioactive counts to define the extent of SLN dissection has not been directly investigated. Establishing the criteria for the optimal extent of SLN biopsy technique will require an evaluation of the optimal number of nodes to remove and the determination of which nodes are “sentinel”- based upon radioactive counts, order of removal, uptake of vital blue dye, or a combination of these factors. This study evaluated the relationship between the order in which SLN were removed, their relative radioactive counts and the identification of the first positive SLN in a series of SLN procedures performed by a single surgeon using a standardized technique. The goal of this study was to determine the optimal number of sentinel lymph nodes required to accurately stage the axilla.

Patients and Methods

Patients

A retrospective chart review was performed with Institutional Review Board approval using records for 126 patients with clinically node-negative invasive breast cancer who underwent lymphatic mapping and sentinel lymph node biopsy between July 2003 and June 2006. All patients were diagnosed with invasive breast cancer by core needle biopsy or by image guided excisional biopsy prior to the sentinel node procedure.

Lymphatic Mapping and Sentinel Lymph Node Biopsy Technique

All procedures were performed by one surgeon (RL) using a standard technique. Patients underwent injection of 400–1000 μ Curie of filtered technetium sulfur colloid (CIS-US, Bedford, MA) using a subareolar technique on the day of surgery (8). A handheld gamma probe (Navigator GPS, US Surgical; Neoprobe, Neoprobe Corporation) was used to identify transcutaneous hotspots and to guide the dissection of the axilla. Vital blue dye was not used for identification of SLN in this series. The sequence in which SLNs were removed and the corresponding ex vivo radioactive counts were recorded. SLNs were removed until radioactive counts in the axilla were <10% the ex vivo counts of the hottest SLN. The SLNs were identified in the order in which they were excised and not based upon the radioactive counts. This method ensured that the order of lymph node excision and radioactive counts would be recorded independently. The number of SLN removed by the surgeon were recorded and used for this analysis. Additional SLNs and nonsentinel nodes identified in the pathology specimens were not included in the final analysis. All SLNs were evaluated intraoperatively by touch preparation cytology (9). If metastases were identified in the SLN, a completion axillary dissection was performed at the time of initial surgery. Completion axillary dissections were not performed for patients with a negative SLN.

Pathologic Examination of Sentinel Lymph Nodes

All SLNs were bivalved and sectioned as close to 2 mm as possible and entirely submitted for histologic evaluation, regardless of node size, unless the lymph node was grossly positive. For grossly positive lymph nodes, one section was submitted for histology. All nodes were

submitted for permanent sectioning, hematoxylin and eosin staining, and immunohistochemistry using cytokeratin antibodies. Micrometastatic disease was defined as metastatic disease identified by hematoxylin and eosin staining, measuring between 0.2 and 2mm in size. Isolated tumor cells, measuring <0.2mm and found on cytokeratin immunohistochemistry only, were classified as N0 disease.

Data Analysis

Data was entered into an Excel (Microsoft, Redmond, WA) spreadsheet format for evaluation. All statistical analysis was performed using SAS 9.0 software (SAS Institute, Cary, North Carolina). Continuous variables were evaluated using a Student's t-test. Categorical variables were evaluated using a chi-squared test or Kruskal-Wallis ANOVA. Differences between groups were considered significant when the *p*-value was <0.05.

Results

Demographics and Clinicopathologic Characteristics

Between June 2003 and July 2006, 126 patients underwent sentinel lymph node biopsy at the time of definitive surgery for biopsy proven breast cancer. Sentinel lymph nodes were successfully identified in every patient. The false negative rate could not be determined as no patient underwent completion axillary dissection if the SLN was negative for metastasis. The mean age of all patients was 56.2 years. Tumor location, mastectomy vs. partial mastectomy, expression of estrogen receptor and progesterone receptor, and amplification of HER-2 were not significantly different among SLN positive and SLN negative patients. However, a palpable tumor (*p*=0.0035), increasing tumor size (*p*=0.0039), increasing histologic grade (*p*=0.0234), and angiolymphatic invasion (<0.001) were significantly associated with a positive SLN on univariate analysis. The demographic and clinicopathologic characteristics are summarized in Table 1.

Sentinel Lymph Nodes

The mean number of SLN removed was 2.86 (range 1–8) and the median was 2. More than 3 SLN were removed in 33 (26.2%) of the patients. The number of SLN removed did not differ significantly between SLN positive and SLN negative patients. The first SLN was found to have the highest radioactive counts in 100 (79.4%) patients. The highest counts were found in the second SLN in 15 (11.9%), the third SLN in 8 (6.3%) and in later nodes in 3 (2.4%) patients. The SLN data for the entire cohort is summarized in Table 2 and Table 3. Completion axillary dissections were not performed for patients with a negative SLN; thus, we could not evaluate the false negative rate in our series.

Thirty-eight patients (30.4%) were found to have a positive SLN. Eleven patients (28.9%) were found to have H&E confirmed micrometastases (0.2mm to 2 mm) and were included in the SLN positive group. A total of 50 positive SLN were identified in 38 patients, with one positive SLN identified in 27 patients (71.1%), two positive SLNs identified in 10 patients (26.3%) and 3 positive SLNs identified in 1 patient (2.6%).

Among patients with a positive SLN, the first positive SLN was the first SLN removed in 31 (81.6%) patients, the second node removed in 6 (15.8%), and the third node removed in 1 (2.6%) patient. In no patient was the first positive SLN discovered after the third SLN was removed. With regards to radioactive counts, the first positive SLN had the highest counts in 30 (78.9%) patients and the second highest counts in 6 patients (15.8%). Counts ranged from 22% to 68% of the hottest SLN in cases where the SLN with the highest counts was not positive for metastasis. Evaluating both the order of SLNs removed and the radioactive counts, the first SLN removed had the highest or second highest counts in 30 patients (79.0%). The first or

second SLN removed accounted for the highest or second highest counts and was the first positive SLN in 37 patients (97.4%) . These data are summarized in Table 4.

Discussion

Although sentinel lymph node (SLN) biopsy is the standard technique for staging the axilla of clinically node negative breast cancer, the optimal number of radioactive SLN to remove to ensure accuracy and minimize morbidity is still actively debated. In this retrospective review of a single surgeon case series, the mean number of SLN identified was 2.86, range 1–8. Among several case series and clinical trials of SLN technique previously published, the reported mean number of SLN excised ranges from 1.8 to 4.4, with 4 of the 10 reports cited reporting a mean less than 2 (10–18). These series are summarized in Table 5. More than 3 SLN were removed in 26.3% of patients in our series, with prior trials and series reporting rates of 7.2% to 46.1% (10–18). The large multicenter trial from the University of Louisville reported more than 3 SLNs removed in 22.1% of their patients (10). This wide variation of total number of SLN excised in published case series and clinical trials is compelling, as it suggests that variations of technique and surgeon experience may contribute to the differences in number of SLN required for accurate staging of the axilla.

In evaluating the association of clinical and pathologic factors to the identification of a positive sentinel lymph node and the number of sentinel lymph nodes excised, we found that a palpable tumor, increasing tumor size, increasing histologic grade and angiolymphatic invasion were all significantly associated with a positive sentinel lymph node. None of these factors however, was significantly associated with the identification of more than 3 SLN in SLN positive or SLN negative patients. For comparison, the University of Louisville trial found that a palpable tumor, intradermal injection of radiotracer and surgeon inexperience were the three most significant factors associated with the identification of 4 or more SLN, but not tumor size, tumor location, or histologic subtype (19). The University of Louisville study suggests that a palpable tumor is the only pathologic determinant of the identification of multiple sentinel nodes, while technical factors and surgeon inexperience account for the remaining number of cases of multiple SLNs. The lack of a significant association of these clinical and pathologic factors with multiplicity of SLNs in our dataset may be due to the small number of cases in our series, but may also be due to surgeon experience and to technique.

Our series is quite comparable to the majority of published series evaluating the optimal number of SLN to harvest to ensure accuracy of the technique. When all series reporting order and number of SLN excised are compared, a positive SLN was identified in the first three SLN excised in all series with >96% accuracy. In our small series, conducted by a single experienced surgeon using a standard technique, 100% accuracy was achieved excising 3 SLN. This high rate of accuracy is reflected in the other smaller series conducted by few experienced surgeons (15–18). The data regarding radioactive counts and identification of the first positive sentinel lymph node is unique to our series and to the University of South Florida and the Ohio State University reports, as other series do not report the radioactive counts as absolute or relative values. An accuracy of >96% was achieved with removal of the three nodes with highest counts across all three series.

The identification of micrometastases using SLN technique is an important area of active investigation. Micrometastases, defined as lymph node tumor deposits 0.2mm to 2.0mm in size, are associated with a significant decrease in disease free survival and overall survival (reviewed in 20). Their detection requires serial sectioning, which is labor intensive, but is feasible when using SLN technique where only a few lymph nodes are analyzed. In our series, 11 of 38 SLN positive patients were found to have micrometastatic disease. The clinicopathologic characteristics and SLN profile of these patients did not differ significantly

from the remaining SLN positive patients. In this subgroup, the first SLN was the first positive SLN in 10 patients (90.9%) and was the hottest SLN in all patients. Three of the SLN positive patients with micrometastatic disease had two positive SLN, and no patient in this subgroup had more than three SLN excised.

The data regarding number of SLN excised becomes more interesting when surgical complications and resource utilization associated with SLN biopsy are considered. Data from the ACOSOG Z0010 prospective trial comparing SLN dissection only with SLN dissection followed by complete axillary lymph node dissection found an increase in rates of axillary seroma formation and wound infections in patients who had more than four SLN removed (21). Cost-effectiveness and cost-utilization studies have found that excision of increasing numbers of SLN is associated with increased OR time, increased pathology costs, and increased procedure costs (17). Thus, limiting the number of sentinel lymph nodes excised based upon number of nodes and other objective criteria is an important strategy for optimizing accuracy of the procedure while limiting morbidity and procedure costs.

These data demonstrate that in the hands of an experienced surgeon, using a standard technique, the total number of sentinel lymph nodes excised can be limited to no more than 3 without compromising the accuracy of the technique. Furthermore, micrometastatic disease is detectable using these same criteria. Current practice, guided by the convention of excising all SLN with >10% of the counts of the hottest SLN, results in oversampling. While no clear data exists to limit axillary dissection based upon relative radioactive counts, limiting SLN dissection to no more than 3 SLN is well supported by several case series including this report. Other clinical and pathologic factors, including the presence of a palpable tumor or neoadjuvant chemotherapy prior to SLN biopsy, may influence a surgeon's choice of appropriate technique.

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Table 1**Patient Demographics and Clinicopathologic Features Associated with SLN Positivity**

Clinicopathologic Feature	Total (%)	SLN Positive (%)	SLN Negative (%)	P Value
Patient age				
Mean	56.2	55.5	56.5	0.65
<50	36 (23.7)	9 (23.7)	27 (30.7)	0.42
>50	90 (76.3)	29 (76.3)	61 (69.3)	
Palpable Tumor				
Yes	58 (46.0)	25 (65.8)	33 (37.5)	0.0035*
No	68 (54.0)	13 (34.2)	55 (62.5)	
Tumor Location				
UOQ	81 (64.3)	24 (63.2)	57 (64.8)	0.710
LOQ	11 (8.7)	3 (7.9)	8 (9.1)	
UIQ	20 (15.9)	5 (13.2)	15 (17.0)	
LIQ	14 (11.1)	6 (15.8)	8 (9.1)	
Procedure Performed				
Mastectomy	22 (17.5)	7 (18.4%)	15 (17.0%)	0.852
Partial Mastectomy	104 (82.5%)	31 (81.6%)	73 (83.0%)	
Tumor Size				
T1	77(61.1)	17 (44.7)	60 (68.2)	0.0039*
T2	46 (36.5)	18 (47.4)	28 (31.8)	
T3	3 (2.4)	3 (7.9)	0 (0)	
Histologic Grade				
1	28 (22.2)	3 (7.9)	25 (28.4)	0.0234*
2	58 (46.0)	23 (60.5)	35 (39.8)	
3	40 (31.8)	12 (31.6)	28 (31.8)	
Angiolymphatic Invasion				
Yes	34 (27.0)	20 (52.6)	14 (15.9)	<0.001*
No	92 (73.0)	18 (47.4)	74 (84.1)	
Receptor Status				
ER Positive	106 (84.1)	33 (86.8)	73 (83.0)	0.5837
PR Positive	97 (77.0)	31 (81.6)	66 (75.0)	0.4207
HER-2 Amplified	14 (11.1)	6 (15.8)	8 (9.1)	0.4501
HER-2 Subtype	8 (6.3)	3 (7.9)	5 (5.7)	0.4065
Triple Negative	11 (8.7)	2 (5.3)	11 (10.2)	0.1298

* statistically significant difference between SLN positive and SLN negative patients, p<0.05

Table 2

Total Number of Sentinel Lymph Nodes Removed Per Patient

Number SLNs Removed	All Patients (%)	SLN Positive Patients	SLN Negative Patients
1	20 (15.8%)	4 (10.5%)	16 (18.2%)
2	52 (41.2%)	15 (39.5%)	37 (42.0%)
3	21 (16.7%)	7 (18.4%)	14 (15.9%)
4	13 (10.3%)	3 (7.9%)	10 (11.4%)
5	11 (8.7%)	6 (15.8%)	5 (5.7%)
6	8 (6.3%)	2 (5.3%)	6 (6.8%)
>6	1 (0.8%)	1 (2.6%)	0 (0%)

Table 3

Sentinel Lymph Nodes Identified by Order and Radioactive Counts (126 patients)

Order of SLN Removed	Highest Radioactive Counts
1 st	100 (79.4)
2 nd	15 (11.9)
3 rd	8 (6.3)
>3 rd	3 (2.4)

Table 4

First Positive Sentinel Lymph Node Identified by Order and Radioactive Counts (38 SLN Positive Patients)

Order of SLN Removed	Radioactive Counts	Positive SLN (%)	Cumulative Number (%)
1 st	Highest	27 (71.1)	27 (71.1)
	2 nd Highest	3 (7.9)	30 (79.0)
	>2 nd Highest	1 (2.6)	31 (81.6)
2 nd	Highest	3 (7.9)	34 (89.5)
	2 nd Highest	3 (7.9)	37 (97.4)
	>2 nd Highest	0 (0)	37 (97.4)
3 rd	Highest	1 (2.6)	38 (100)
	2 nd Highest	0 (0)	
	>2 nd Highest	0 (0)	

Table 5

Institution	Key Clinical Trials and Case Series Evaluating Number of Sentinel Lymph Nodes Removed			Number SLN Required to Identify 1 st Positive SLN (%)	
	Total Patients	SLN Positive Patients (%)	Mean # SLN Removed	More than 3 SLN removed (%)	
University of Louisville (10)	3882	1358 (35.0%)	Median 2	300 (22.1%)*	1- 1011 (74.4%) 2- 151 (11.1%) 3- 56 (4.1%) >3- 35 (2.6%)
Memorial Sloan Kettering (11)	1561	449 (28.8%)	2.3	241 (15.4%)	1- 338 (75.3%) 2- 79 (17.6%) 3- 23 (5.1%) >3- 8 (1.8%)
William Beaumont (12)	720	167 (23.2%)	4.4	77 (46.1%)*	1- 128 (76.6%) 2- 24 (14.4%) 3- 10 (6.0%) >3- 5 (3.0%)
University of Michigan (13)	725	132 (18.0%)	2.5	133 (18.3%)	1- 87 (65.9%) 2- 27 (20.5%) 3- 16 (12.1%) >3- 2 (1.5%)
Virginia Mason (14)	541	129 (23.8%)	1.95	57 (10.5%)	1- 127 (98.4%) 2- NR 3- 2 (1.6%) >3- NR
University of South Florida (15)	465	128 (27.5%)	Median 2	56 (11.0%)	1- 114 (89.1%) 2- 12 (9.4%) 3- 2 (1.6%) >3- 0 (0%)
Ludwig Boltzman (16)	263	105 (39.9%)	1.8	19 (7.2%)	1- 96 (91.4%) 2- 8 (7.6%) 3- 1 (1.0%) >3- 0 (0%)
Ohio State University (17)	353	104 (29.5%)	2.5	66 (18.7%)	1- 86 (82.7%) 2- 15 (14.4%) 3- 1 (1.0%) >3- 1 (1.0%)
Mayo Clinic (18)	472	103 (22%)	2.5	85 (18.0%)	1- 90 (87.4%) 2- 8 (7.8%) 3- 3 (2.9%) >3- 2 (1.9%)
Wagga Wagga (19)	113	33 (29.2%)	1.9	NR	1- 29 (87.9%) 2- 3 (9.1%) 3- 1 (3.0%) >3- 1 (3.0%)
Case Western Reserve	126	38 (30.2%)	2.8	33 (26.2%)	1- 31 (81.6%) 2- 6 (15.8%) 3- 1 (2.6%) >3- 0 (0%)

* Represents only sentinel lymph node positive patients