Characteristics of the Sentinel Lymph Node in Breast Cancer Predict Further Involvement of Higher-Echelon Nodes in the Axilla

A Study to Evaluate the Need for Complete Axillary Lymph Node Dissection

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**Background:** Sentinel lymph node (SLN) biopsy techniques provide accurate nodal staging for breast cancer. In the past, complete lymph node dissection (CLND) (levels 1 and 2) was performed for breast cancer staging, although the therapeutic benefit of this more extensive procedure has remained controversial.

**Hypothesis:** It has been demonstrated that if the axillary SLN has no evidence of micrometastases, the non-sentinel lymph nodes (NSLNs) are unlikely to have metastases.

**Objective:** To determine which variables predict the probability of NSLN involvement in patients with primary breast carcinoma and SLN metastases.

**Methods:** An analysis of 101 women with SLN metastases and subsequent CLND was performed. Variables included size of the primary tumor, tumor volume in the SLN, staining techniques used to initially identify the micrometastases (cytokeratin immunohistochemical vs hematoxylin-eosin), number of SLNs harvested, and number of NSLNs involved with the metastases. Tumor size was determined by the invasive component of the primary tumor. Patients with ductal carcinoma in situ who were upstaged with cytokeratin staining were considered to have stage T1a tumors.

**Results:** Sentinel lymph node micrometastases (<2 mm) detected initially by cytokeratin staining were associated with a 7.6% (2/26) incidence of positive CLND compared with a 25% (5/20) incidence when micrometastases were detected initially by routine hematoxylin-eosin staining. Sentinel lymph node micrometastases, regardless of identification technique, inferred a risk of 15.2% (7/46) for NSLN involvement. As the volume of tumor in the SLN increased (ie, <2 mm, ≥2 mm, grossly visible tumor), so did the risk of NSLN metastases (P<.001).

**Conclusions:** Our study demonstrated that patients with micrometastases detected initially by cytokeratin staining had low-volume disease in the SLN with a small chance of having metastases in higher-echelon nodes in the regional basin other than the SLN. Characteristics of the SLN can provide information to determine the need for a complete axillary CLND. Complete lymph node dissection may not be necessary in patients with micrometastases detected initially by cytokeratin staining since the disease is confined to the SLN 92.4% of the time. However, the therapeutic value of CLND in breast cancer remains to be determined by further investigation.


**PGNOSTIC FACTORS** have been used to help identify high-risk patients with invasive breast carcinoma, such as tumor size, tumor grade, S-phase fraction, DNA index, tumor ploidy, and estrogen and progesterone receptor status; however, nodal status still remains the most significant predictor of recurrence and survival. The status of the regional lymph node basin is a reflection of the biological aggressiveness of the primary tumor and may influence therapeutic intervention in patients with invasive breast carcinoma. Therefore, it is of paramount importance to identify patients with metastatic disease to the axillary lymph nodes for prognostic and therapeutic purposes.

**See Invited Critique at end of article**

Surgical intervention in the diagnosis and treatment of breast carcinoma has undergone an extensive transformation from radical mastectomy to lumpectomy, lymph node dissection, and irradiation in appropriately selected patients. The trend
PATIENTS AND METHODS

At H. Lee Moffitt Cancer Center (Tampa, Fla), from 1996 to 1997, 111 patients with breast carcinoma had intraoperative lymphatic mapping with SLN biopsy and subsequent CLND if the SLN was positive. Ten patients who had fewer than 10 lymph nodes in the axillary CLND were eliminated from the study since it could not be determined if this low number was secondary to missed lymph nodes in the specimen by the pathologist or to lack of removal by the surgeon. Two patients with ductal carcinoma in situ who were upstaged using cytokeratin staining were considered to have T1a tumors. These 2 patients had microinvasive disease apparently missed when their primary tumor was examined and were included in the series of patients with invasive cancers who had a positive SLN and underwent CLND.

NUCLEAR MEDICINE

Patients were initially seen in the nuclear medicine department for injection of 450 µCi of technetium Tc 99m (0.45-µCi filter) sulfur colloid. The radiocolloid was injected into the breast parenchyma around the periphery of the tumor. Volumes of 6 mL were administered and the injections were diffuse enough around the tumor or biopsy cavity to allow the radiocolloid to be taken up by the breast lymphatic system. If the tumor was detected mammographically, a localization wire was placed and the radiocolloid injected around the tumor. If the tumor was palpable, the injections were done tightly around the circumference of the tumor. If an excisional biopsy was performed, injection was done under ultrasound guidance, taking care not to inject the biopsy cavity.

INTRAOPERATIVE LYMPHATIC MAPPING

Patients were taken to the operating room 2 to 24 hours after being injected with the filtered technetium Tc 99m. Because of particle size and flow characteristics, an intraoperative injection of 5 mL of 1% Lymphazurin (isosulfan) blue dye was given in a similar fashion. Following injection, manual compression of the breast and massage for 5 minutes was performed to increase interstitial pressure and to ensure proper migration of the blue dye into the lymphatic channels. Prior to making the skin incision, a handheld gamma probe was used to identify the most radioactive area (the "hot spot") in the axilla. Once the location of the SLN was identified, an incision was made (2-4 cm) overlying the area of highest activity. Careful dissection was undertaken and lymphatic channels were clamped or tied.

Localization ratios were used to eliminate uncontrollable variables that might affect identification of an SLN. A node was considered to be the SLN if it met 1 of the following 3 criteria: (1) the node was blue, (2) the node had a blue-stained afferent lymphatic vessel leading to it, or (3) the node had an in vivo activity ratio of 3:1 (SLN vs background) or an ex vivo ratio of 10:1 (activity in the SLN vs neighboring nonsentinel lymph node [NSLN]).

HISTOLOGIC EXAMINATION

The SLN was bivalved and the interior examined. Sentinel lymph nodes larger than 3 mm were submitted for serial sectioning (2-3 mm intervals) for touch imprint cytology and immunohistochemistry. Touch imprint cytology is a technique developed for intraoperative examination of lumpectomy margins and SLNs of specimens injected with radiocolloid mapping agents. It avoids cutting the hot specimens on the cryostat and involves a simple touch or the scrape of a glass slide to the bivalved SLN or margin. If there are any cancer cells present, they come off on the slide, undergo cytologic preparation and stain, and are read. This technique can identify metastatic disease in the SLN 70% of the time if it exists. If no gross disease exists, the entire SLN is processed by sectioning at 3- to 5-mm intervals and making 1 to 10 blocks of each node depending on the size of the SLN. These blocks are then cut and stained with hematoxylin-eosin. Cytokeratin stains are performed on each block if there are no gross signs of disease and the findings from intraoperative touch preparation are negative. The section on which the cytokeratin stain is performed is at the same level as the hematoxylin-eosin so that if metastatic breast cancer is identified with the cytokeratin stain, the hematoxylin-eosin–stained block can be reexamined to find the abnormal cells. The cytologic examination of the cells for malignancy is much more meaningful with the hematoxylin-eosin stain, since the cytokeratin obscures the cytologic detail of the cell.

Patients with a positive intraoperative diagnosis underwent a CLND. If the diagnosis of metastatic disease in the SLN could not be made intraoperatively, the patients underwent a CLND on another day. The specimens were quarantined for 48 hours to allow for decay of the 99Tc and were then processed for routine hematoxylin-eosin staining. Any specimens that were negative on gross examination and hematoxylin-eosin staining were stained with a monoclonal antibody against low-molecular-weight cytokeratin (CAM5.2) using the avidin-biotin complex technique with diaminobenzidine chromogen. If the lymph node was positive by cytokeratin staining, then it was resectioned and stained again with hematoxylin-eosin in an attempt to confirm the micrometastases.

STATISTICAL ANALYSIS

Associations between ordinal variables (eg, tumor size and number of positive nonsentinel lymph nodes [NSLNs]) were assessed using the Spearman correlation coefficient. The Wilcoxon rank sum test was used to compare groups with respect to ordinal variables. All analyses used a 2-tailed significance level of .05 and were performed using SAS (SAS Institute, Cary, NC) Procs Freq (version 6.12), Corr, and NParlway. Ninety-five percent confidence intervals for proportions were computed using the method of Clopper and Pearson.

toward conservative surgical intervention without compromising care is also being applied to the evaluation of the axillary lymph nodes. Traditionally, the status of the axillary lymph nodes was determined by complete lymph node dissection (CLND); however, sentinel lymph node (SLN) mapping has been demonstrated to...
be effective in determining regional lymph node involvement and to have lower morbidity. \(^1\) The SLN is the first node or nodes to receive lymphatic drainage from the primary tumor and therefore is at highest risk for containing metastatic disease. \(^3\) The average number of axillary SLNs harvested per patient is 2. \(^4\) In contrast, axillary CLND yields a range of 15 to 30 lymph nodes, a number that does not permit the pathologist to perform a detailed examination, owing to time and financial restraints. \(^7\) A more detailed examination of the SLN with multilevel serial sectioning and cytokeratin staining can be performed on an SLN that is at highest risk of metastases. New techniques using cytokeratin staining are detecting micrometastatic disease, which had previously gone undetected by standard histologic techniques.

Because the SLN is at highest risk for metastases, the other regional lymph nodes should be evaluated against the SLN. If the SLN is negative for tumor, the remaining lymph nodes in the basin have a low probability (1%-2%) of containing metastatic disease. \(^1\) Likewise, when the SLN contains tumor, the patients are recommended to undergo a CLND. This analysis may identify patients with a positive SLN who do not need to be exposed to the morbidity and cost associated with a CLND.

### RESULTS

#### PRIMARY TUMOR SIZE AND INCIDENCE OF NSLN METASTASES

As the tumor size increased, the incidence of NSLN metastases increased \((P = .005)\). T1a, T1b, T1c, T2, and T3 tumors had a 25%, 30%, 40%, 46.4%, and 80% incidence, respectively, of metastatic disease in higher-echelon nodes (Table 1).

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Primary tumor size</td>
<td>No. of positive SLNs</td>
<td>.005</td>
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<tr>
<td>Primary tumor size</td>
<td>No. of SLNs harvested</td>
<td>.73</td>
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<tr>
<td>HE-positive SLN</td>
<td>No. of positive NSLNs</td>
<td>.024</td>
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<tr>
<td>CK-positive SLN</td>
<td>No. of positive NSLNs</td>
<td>.042</td>
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<td>Volume of disease</td>
<td>No. of positive NSLNs</td>
<td>.001</td>
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* SLN indicates sentinel lymph node; NSLN, nonsentinel lymph node; HE, hematoxylin-eosin; and CK, cytokeratin.

### PATHOLOGY RESULTS

Metastatic disease was confined to the SLN 40.6% (41/101) of the time. Twenty-six patients had low-volume metastases that could only be initially detected by cytokeratin and 2 of these patients had NSLN involvement. Routine initial hematoxylin-eosin detected metastatic disease in 75 patients, and 52% (39/75) had NSLN metastases. A larger tumor burden \((P \leq .02)\) was associated with a positive hematoxylin-eosin–positive SLN compared with smaller tumor volumes in the SLN when the SLN metastatic deposits were detected initially by cytokeratin \((P \leq .04)\).

Evaluation of the 101 patients with positive SLNs demonstrated that tumor size, the volume of tumor in the SLN, and tumors detected by detailed examination and cytokeratin staining techniques were significant variables in predicting positive higher-echelon nodes.

### COMMENT

Given the importance of regional nodal status in breast carcinoma, axillary staging continues to be vital. Many institutions worldwide have demonstrated that lymphatic mapping techniques can be used in breast carcinoma, providing accurate axillary nodal staging with reduction in morbidity. \(^6\) Several investigators have demonstrated that the SLN was the only site of disease in 40 patients (60%). \(^3\) Identifying specific characteristics of the SLN that can predict patients who may benefit from a CLND is a significant goal. Variables such as lymphovascular invasion, tumor size, nuclear grade, patient age, mitotic count, and estrogen and progesterone receptors have all been evaluated as predictors of axillary nodal involvement. \(^12\) It is well known that tumor size is significant in determining the risk for axillary node metastases; however, the volume of disease in the SLN or the initial method of histologic analysis used to identify the metastases are currently factors being considered by investigators. \(^15\)

Whether a pathological examination identifies disease in the SLN is determined by the intensity of the examination. The standard of care across the country for the histologic examination of the regional basin is to make 1 section of each node and stain that section with hematoxylin-eosin. This method studies only 1% of the submitted material. However, if there are just 1 to 2 SLNs that are the nodes most likely to contain metastases, a

| Table 1. Predictors of Axillary Metastases in the SLN and NSLN* |
|-------------------|-------------------|---------|
| Variable 1          | Variable 2          | \( P \)  |
| Primary tumor size  | No. of positive SLNs| .005    |
| Primary tumor size  | No. of SLNs harvested| .73    |
| HE-positive SLN     | No. of positive NSLNs| .024   |
| CK-positive SLN     | No. of positive NSLNs| .042   |
| Volume of disease   | No. of positive NSLNs| .001   |

* SLN indicates sentinel lymph node; NSLN, nonsentinel lymph node.

| Table 2. Tumor Volume in SLN vs NSLN Involvement* |
|-------------------|-------------------|---------|
| Volume of Disease in SLN | Incidence of NSLN Involvement, % |
| <2 mm              | 15.2               |
| >2 mm              | 58.3               |
| Gross volume       | 64.5               |

* SLN indicates sentinel lymph node; NSLN, nonsentinel lymph node.
more detailed examination can be performed, including more sections and cytokeratin staining.

Our study shows that 26% of the time, if metastatic disease exists in the SLN, it is of such low volume that it can only initially be identified with cytokeratin stains. Thus, in 26% of patients with metastatic disease in the regional basin, the standard level 1 and 2 axillary CLND and superficial examination of all 15 to 20 nodes in the specimen probably do not detect disease.

Primary tumor size and volume of disease in the SLN were significant indicators of the incidence of NSLN metastases in the regional basin. As the tumor size and the volume of disease in the SLN increased, the incidence of NSLN metastases also increased. Furthermore, a detailed examination of the SLN by serial sectioning and cytokeratin staining (after routine hematoxylin-eosin staining produced negative results) upstaged patients and provided more accurate staging. The clinical relevance of this upstaging has yet to be determined. When the SLN is negative for tumor it is unlikely that the NSLNs contain disease. Moreover, if the SLN contains micrometastases detected initially by cytokeratin only, the likelihood of having NSLN metastases is small. Metastatic disease was confined to the SLN in 92.4% of the patients in this study when the SLN was positive for micrometastases detected initially by cytokeratin staining.

The clinical relevance of positive micrometastases by cytokeratin is unknown. Some studies have demonstrated no prognostic difference in patients with low-volume axillary nodal micrometastases vs other investigators who have reported a higher recurrence rate and lower survival in these patients. Metastatic disease detected initially by hematoxylin-eosin staining typically indicates a larger volume of disease in the SLN. Thus, the method of initial detection of metastases is a reflection of the volume of disease in the SLN and points to the likelihood that higher-echelon nodes are involved.

In conclusion, variables such as the size of the primary tumor, the volume of disease in the SLN, and the method of detection of micrometastases, can predict further involvement of neighboring NSLN. This study confirms the result of a study from the John Wayne Cancer Center (Santa Monica, Calif) that showed that tumor volume in the SLN predicted NSLN involvement. Sentinel lymph node biopsy is an accurate method for nodal staging in patients with breast cancer, and when combined with detailed histologic examination of the SLN, a subgroup of patients who may not require CLND can be identified. The clinical relevance of micrometastases detected by cytokeratin and the role of CLND in patients with micrometastases is under investigation through clinical trials and requires further investigation in prospective studies with an emphasis on recurrence and survival. This issue is being addressed in an ongoing American College of Surgeons Oncology Trials Group, patients with a negative SLN are not receiving further surgery nor adjuvant therapy. A blinded cytokeratin analysis of the SLN will attempt to address the relevance of upstaging with cytokeratin staining. Patients with a positive SLN will be randomized to CLND and adjuvant therapy vs adjuvant therapy alone. This arm of the study will examine the role of CLND in treating women with invasive breast cancer.

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REFERENCES

22. De Mascarel I, Bonichon F, Coindre J, et al. Prognostic significance of breast...
Kamath and associates determined that characteristics of the SLN confirm the need to complete axillary nodal staging procedures. This is the first paper that provides objective data showing that examination of the SLN with serial sectioning and cytokeratin staining will identify patients with low tumor volume. Further, the authors confirm that metastatic tumor deposits are confined to 92% of SLN evaluations.

Traditional approaches suggest that the status of the axillary lymphatic system is best determined by complete axillary LND, which has evolved from a comprehensive (level I-III; Patey) LND to the Auchincloss-Madden (levels I-II) nodal sampling technique. The advent of SLN mapping, championed by Giuliano et al, has more recently been confirmed to be a highly efficacious method for determining pathological regional lymph node status; the technique ensures morbidity much lower than that achieved with partial or complete axillary LND. The significance of this study by Kamath et al suggests that primary tumor size and tumor volume in the SLN are predictors of the incidence of non-SLN metastases in the nodal basin. Further, the method for detecting micrometastases is increasingly evident as a principle for detecting involvement of contiguous non-SLN. Thus, the present study confirms the findings of the John Wayne Cancer Center, that tumor volume in the SLN is predictive of non-SLN involvement.

As indicated by the authors, the American College of Surgeons Oncology Trials Group initiated studies for patients with SLN sampling to determine overall value. Patients harboring positive SLN will be randomized to CLND plus adjuvant therapy vs adjuvant therapy alone. The aim of this arm of the study is to verify the importance of axillary LND as definitive therapy for individuals with positive SLN and to determine whether therapeutic LND will enhance disease-free and overall survival. Moreover, it is essential that this trial be completed to answer the query of the therapeutic value for completion of LND for the clinically node-negative axilla with histologically confirmed SLN.

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