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Histopathologic Validation of the Sentinel Lymph Node Hypothesis for Breast Carcinoma

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Background and Objective

The sentinel node hypothesis assumes that a primary tumor drains to a specific lymph node in the regional lymphatic basin. To determine whether the sentinel node is indeed the node most likely to harbor an axillary metastasis from breast carcinoma, the authors used cytokeratin immunohistochemical staining (IHC) to examine both sentinel and nonsentinel lymph nodes.

Methods

From February 1994 through October 1995, patients with breast cancer were staged with sentinel lymphadenectomy followed by completion level I and II axillary dissection. If the sentinel node was free of metastasis by hematoxylin and eosin staining (H&E), then sentinel and nonsentinel nodes were examined with IHC.

Results

The 103 patients had a median age of 55 years and a median tumor size of 1.8 cm (58.3% T1, 39.8% T2, and 1.9% T3). A mean of 2 sentinel (range, 1–8) and 18.9 nonsentinel (range, 7–37) nodes were excised per patient. The H&E identified 33 patients (32%) with a sentinel lymph node metastasis and 70 patients (68%) with tumor-free sentinel nodes. Applying IHC to the 157 tumor-free sentinel nodes in these 70 patients showed an additional 10 tumor-involved nodes, each in a different patient. Thus, 10 (14.3%) of 70 patients who were tumor-free by H&E actually were sentinel node-positive, and the IHC lymph node conversion rate from sentinel node-negative to sentinel node-positive was 6.4% (10/157). Overall, sentinel node metastases were detected in 43 (41.8%) of 103 patients. In the 60 patients whose sentinel nodes were metastasis-free by H&E and IHC, 1087 nonsentinel nodes were examined at 2 levels by IHC and only 1 additional tumor-positive lymph node was identified. Therefore, one H&E sentinel node-negative patient (1.7%) was actually node-positive ($p < 0.0001$), and the nonsentinel IHC lymph node conversion rate was 0.09% (1/1087; $p < 0.0001$).

Conclusions

If the sentinel node is tumor-free by both H&E and IHC, then the probability of nonsentinel node involvement is <0.1%. The true false-negative rate of this technique using multiple sections and IHC to examine all nonsentinel nodes for metastasis is 0.97% (1/103) in the authors' hands. The sentinel lymph node is indeed the most likely axillary node to harbor metastatic breast carcinoma.

Tumor size and axillary lymph node status still are the most important prognostic factors in potentially curable carcinoma of the breast. Physical examination, radiologic imaging of the axilla, or prognostic models based on primary tumor characteristics cannot accurately predict the occurrence of axillary metastases.[1-5] The National Institutes of Health Consensus Conference recommended a level I and II axillary lymph node dissection (ALND) for staging and regional control of breast cancer.[3] To minimize the short- and long-term morbidity associated with ALND, some investigators have proposed a limited axillary sampling.[6] This procedure has less morbidity but misses 24% to 42% of axillary metastases.[7-10] We have shown that our technique of intraoperative lymphatic mapping and sentinel lymphadenectomy (SLND) is likely to have minimal morbidity and is highly accurate for staging the axilla of patients with breast cancer.[11,12] Focused histopathologic examination of the sentinel node using multiple sections, cytokeratin immunohistochemical staining (IHC), and hematoxylin and eosin staining (H&E) increased our detection rate of axillary metastases to 42%, compared with 29% for standard examination of nodes in the ALND specimen using single sections and H&E.[12]

Critics have argued that enhanced detection of metastatic tumor in the SLND specimen may reflect the more intensive histopathologic technique rather than the biologic significance of the sentinel node. Various studies have shown that multiple sections and IHC increase the yield of tumor-involved nodes in 9% to 31% of patients who are node-negative by H&E,[13-22] but such a comprehensive histopathologic evaluation of the entire level I and II dissection specimen is time-consuming and expensive and has not been routinely used, even at major cancer centers. In the current study, we applied cytokeratin IHC staining to *non*sentinel nodes. Our purpose was to determine whether the sentinel node is truly the axillary lymph node most likely to harbor metastatic tumor and to assess the true histologic false-negative rate of SLND at our institution.

METHODS

Patient Selection

Study candidates were patients undergoing operative management of potentially curable invasive breast carcinoma at the John Wayne Cancer Institute from February 1994 to October 1995. The study period represented the mature phase of the surgeon's and pathologist's technical development of the procedure and the resumption of clinical activity after the Northridge earthquake. After informed consent, all patients underwent SLND followed immediately by standard level I and II ALND.

Surgical Techniques

All operations were performed by the senior surgeon (AEG) using previously described techniques for SLND and completion level I and II ALND.[11,12] Briefly, for SLND, 3 to 5 mL of 1% isosulfan blue vital dye (Lymphazurin, Hirsch Industries, Inc, Richmond, VA) was injected into the breast parenchyma surrounding the primary tumor or into the wall of the biopsy cavity. After approximately 3 to 7 minutes, a standard transverse axillary dissection incision was made just inferior to the hair-bearing region of the axilla. Blunt dissection was performed to identify a blue-impregnated lymphatic channel. The blue lymphatic then was followed proximally and distally until the first ("sentinel") node was identified. Sometimes two and rarely more than two blue-stained sentinel nodes were identified along the lymphatic tract. After SLND, a completion level I and II ALND was performed; if there was gross nodal involvement of the axillary nodes, then the dissection was extended to level III. The ALND specimen was submitted as a separate specimen for histopathologic examination.

Pathologic Evaluation

Sentinel Nodes

Sentinel lymph nodes were bisected if they were >4 mm. A frozen section was obtained, and the node was processed in individual blocks for permanent-section histopathologic evaluation with H&E. If no tumor was identified using H&E, then cytokeratin IHC was performed at one level (total of two faces in a bisected node) using an antibody cocktail (MAK-6; Ciba-Corning, Alameda, CA) to low- and intermediate-molecular weight cytokeratins with an automated immunoperoxidase system (Ventana ES; Ventana Medical Systems, Inc, Tucson, AZ). Thus, a tumor-free sentinel node was examined at three sections (six faces): one frozen, one H&E, and one IHC.

Nonsentinel Nodes

Nonsentinel axillary nodes were dissected fresh and processed by routine surgical pathology techniques for isolation of lymph nodes. No lymph node-clearing techniques were used. The nodes were bisected if they were >4 mm, and multiple nodes were embedded per paraffin block and examined with H&E. No frozen section was

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performed. Further examination of nonsentinel specimens depended on sentinel node histology. If the sentinel node had metastatic tumor by H&E, then cytokeratin IHC was not undertaken on corresponding nonsentinel nodes; if the sentinel node was metastasis-free by H&E, all corresponding nonsentinel axillary nodes were examined at two levels (total of four faces in a bisected node) using the cytokeratin antibody cocktail and automated immunoperoxidase system described above. Thus, a nonsentinel node was examined at three sections: one H&E and two IHC. The immunohistochemical stains were reviewed independently by two pathologists (RRT and DLK). A cytokeratin immunostain was considered positive if there were cohesive clusters of malignant-appearing, immunoreactive cells within the lymph node or if there were cytologically atypical individual cells with strong cytokeratin reactivity in subcapsular sinuses.

Statistical Analysis

The statistical analysis was performed by the Statistical Coordinating Unit at the John Wayne Cancer Institute. The Mantel–Haensel test was used to compare the cytokeratin IHC results for sentinel and nonsentinel lymph nodes.

RESULTS

Patient and Primary Tumor Data

Our study population comprised 103 patients in whom a sentinel node was identified and for whom nonsentinel lymph node paraffin blocks were available. The median age of the 103 patients eligible for study was 55 years (range, 28–88 years). Forty-two patients were premenopausal, 60 were postmenopausal, and 1 was male. Sixty-three patients (61.2%) had a palpable tumor by physical examination and 40 (38.8%) had a nonpalpable tumor detected by mammography or ultrasonography. Eighty-three patients (80.6%) underwent breast-conserving surgery and 20 (19.4%), including the male patient, underwent a modified radical mastectomy.

Median tumor size was 1.8 cm (range, 0.1–7.5 cm) (Table 1). Using American Joint Committee on Cancer guidelines,[23] there were 60 T1, 41 T2, and 2 T3 lesions. Estrogen receptor assays were performed on the primary tumor in 101 patients: estrogen receptors were present in 82 patients (81.2%). Progesterone receptor assays were performed in 100 patients: progesterone receptors were present in 64 patients (64%). Flow cytometry was performed on the primary tumor in 90 patients: 40 (44.4%) had diploid tumors and 56 (62.2%) had a low proliferative index.

Table 1. PRIMARY TUMOR CHARACTERISTICS

Median size (cm)	1.8
Histologic type	
Invasive ductal	91
Invasive lobular	10
Special subtypes	2
Estrogen receptor status	
Positive	82
Negative	19
Insufficient material	2
Progesterone receptor status	
Positive	64
Negative	36
Insufficient material	3
DNA content	
Diploid	40
Aneuploid	50
Insufficient material	13
S-Phase	
Low	56
High	27
Insufficient material	20

Sentinel and Nonsentinel Node Data

The 103 successful SLND procedures yielded a mean of 2 sentinel nodes (range, 1–8). The completion level I and II ALND specimens provided a mean of 18.9 additional nonsentinel axillary lymph nodes (range, 7–37). Gross examination and routine histologic examination results of the sentinel lymph nodes with H&E showed metastatic carcinoma in 33 patients (32%). The remaining 70 patients (68%) had tumor-negative SLND specimens by H&E; sentinel and nonsentinel nodes from these patients were therefore the focus of further histopathologic evaluation.

Of the 157 sentinel nodes removed from the 70 patients whose sentinel node was tumor-free by H&E, cytokeratin IHC showed 10 lymph nodes with cytokeratin-immunoreactive metastases. Each node was from a different patient and each was identified independently by both pathologists. Thus, IHC detected occult metastatic tumor in 10 (6.4%) of 157 sentinel nodes that were metastasis-free by H&E, and it upstaged 10 (14.3%) of 70 patients who were metastasis-free by H&E (Table 2).

The crux of this study was histopathologic examination of the 1087 nonsentinel nodes removed from the 60 patients whose SLND specimen was metastasis-free by both H&E and IHC. The H&E of these nonsentinel nodes showed no metastases and cytokeratin IHC showed only one lymph node with metastasis (Table 3). This node was found in a postmenopausal woman whose primary tumor had good prognostic features (T1b, ER+, PR+, diploid, low grade, low proliferative index). Thus, whereas IHC found metastases in 10 of 157 sentinel nodes judged tu-

Table 2. RESULTS OF CYTOKERATIN IMMUNOHISTOCHEMISTRY IN NODES THAT WERE METASTASIS-FREE BY H&E EXAMINATION

	H&E	IHC	IHC Conversion Rate (%)	
Lymph node evaluation				
Tumor in sentinel nodes	0/157	10/157	6.4	($p < 0.0001$)
Tumor in nonsentinel nodes only	0/1087	1/1087	0.1	
Patient evaluation				
Patients with sentinel node metastasis	0/70	10/70	14.3	($p < 0.0001$)
Patients with nonsentinel node metastasis only	0/60	1/60	1.7	

H&E = hematoxylin & eosin; IHC = immunohistochemistry.

mor-free by H&E, IHC identified only 1 tumor-involved lymph node among 1087 nonsentinel nodes judged tumor-free by H&E ($p < 0.0001$ by Mantel–Haensel test). Therefore, only 1 (1.7%) of 60 patients whose sentinel node was metastasis-free by IHC had tumor in a nonsentinel node by IHC (Table 2). By contrast, 43 (41.8%) of 103 patients had sentinel node metastasis using H&E and IHC ($p < 0.0001$ by Mantel–Haensel test). Thus, the true histologic false-negative rate of SLND using multiple sections and IHC examination of all nonsentinel lymph nodes for metastasis is 1 (0.97%) of 103 patients in our hands.

DISCUSSION

The size of the primary tumor and the status of the axillary lymph nodes are the two most important prognostic factors in patients with breast cancer. In general, patients without metastatic tumor in the regional nodes fare significantly better than those with regional node metastases. However, recurrent locoregional or distant metastatic disease ultimately develops in 20% to 25% of patients without evidence of axillary metastases by routine H&E. These patients presumably had subclinical distant metastases at the time of definitive breast cancer operation, subclinical axillary node involvement, and/or internal mammary node involvement.

Table 3. FINAL TUMOR STATUS OF SENTINEL AND NONSENTINEL AXILLARY LYMPH NODES

Tumor Status	Number (%) of Patients
All nodes metastasis-free	59 (57.3)
Nodal metastases detected	44 (42.7)
Sentinel node only	25 (24.3)
Sentinel and nonsentinel nodes	18 (17.5)
Nonsentinel nodes only	1 (1.0)
Total	103 (100.0)

The ability to detect metastatic tumor in the axillary lymph nodes is directly related to the extent of axillary dissection and the methods used for histopathologic examination (Table 4). Several studies have shown that H&E of multiple levels rather than a single level increases detection of metastatic tumor in the axillary nodes; the largest trials report a 9% to 17% rate of conversion from node-negative to node-positive.[13,14,24] In a report from the Ludwig Breast Cancer Study Group,[13] lymph nodes from 921 patients were sectioned at 6 levels for repeat H&E examination after initial H&E of a single section was negative. Examination of these multiple sections identified metastatic tumor in 83 patients (9%). In this study, these micrometastases were associated with diminished survival.

In addition to the number of sections examined, the ability to detect metastatic tumor also is related to the type of stain used. In several studies, application of epithelial membrane antigen,[20] mucin,[16] or cytokeratin[20] antibodies to H&E metastasis-free lymph nodes showed a 12% to 15% incidence of occult axillary metastases. These studies in reality examined multiple sections, because blocks of H&E-negative nodes were recut and stained with IHC. To avoid examining multiple sections and to determine the impact of the type of stain on the detection of metastasis, Trojani et al.[17] took the original metastasis-free H&E slides of axillary lymph nodes from patients with breast cancer and removed the H&E cytoplasmic stain. They then performed immunostaining using five monoclonal antibodies directed toward epithelial cell antigens and detected metastases in 14% of lymph nodes previously found to be metastasis-free by H&E. More recent studies,[18–22] combining improved immunohistochemical techniques with antibodies against cytokeratin filaments and multiple sections of lymph nodes, have shown a negative-to-positive conversion rate of 23% to 31%.

Immunohistochemical staining is an extremely sensitive method for the detection of micrometastases. However, pathologists must be aware that benign cytokeratin-immunoreactive findings do exist.[25–28] Lymph nodes may

Table 4. DETECTION OF OCCULT NODAL MICROMETASTASES IN BREAST CANCER PATIENTS WITH TUMOR-FREE LYMPH NODES BY ROUTINE H&E

Reference (year)	Serial Sections	Immunohistochemistry	Occult Micrometastasis Detected [number (%)]
Friedman et al.[14] (1988)	X		43/456 (9)
Bettelheim et al.[13] (1990)	X		83/921 (9)
Wells et al.[20] (1984)		X	7/45 (15)
Trojani et al.[17] (1987)		X	21/150 (14)
Hainsworth et al.[16] (1993)		X	41/343 (12)
Bussolati et al.[19] (1986)	X	X	12/50 (24)
Chen et al.[21] (1991)	X	X	23/80 (29)
de Mascarel et al.[18] (1992)	X	X	50/218 (23)
Nasser et al.[22] (1993)	X	X	50/159 (31)

H&E = hematoxylin and eosin.

contain benign epithelial inclusions or cytokeratin-immunoreactive mesenchymal cells. Benign epithelial inclusions are rare in lymph nodes, but the pathologist must be able to recognize them. During the study period, we encountered two examples of benign inclusions and set strict criteria that combined immunostaining intensity with cytologic–morphologic features for lymph node IHC diagnosis. An immunohistochemical stain was considered positive if it showed a cohesive cluster of malignant-appearing immunoreactive cells or cytologically atypical cells with strong cytokeratin reactivity in subcapsular sinuses.

Although the value of detecting a nodal micrometastasis (≤2 mm) is controversial,[29–31] there is a growing body of evidence that these patients have a poorer survival than do node-negative patients.[13,14,16,17,21] With the trend toward early mammographic diagnosis of smaller tumors, a method to optimize detection of micrometastasis may become even more important in the future. Cytokeratin immunohistochemistry is a highly sensitive method, converting 12% to 31% of node-negative patients to node-positive status.[16,17,20–22] However, multiple sections and immunohistochemical stains of every node in an axillary dissection specimen are costly, time-consuming, and rarely performed. By focusing the pathologist's efforts on one or two sentinel nodes, we can enhance the detection of occult metastases without the extraordinary labor and expense associated with multiple sections and immunostains of the entire axillary dissection specimen.[11,12]

In our previous studies, nonsentinel nodes were examined only with standard H&E. Therefore, we could not determine whether detection of a metastasis in the sentinel node was due to the biologic significance of that node or the histopathologic technique of lymph node examination. In the current study, we applied cytokeratin IHC at two levels of nonsentinel nodes to mimic the evaluation of sentinel nodes examined with frozen section, permanent H&E section, and cytokeratin IHC at one level. Thus, the nonsentinel node was scrutinized more intensely than was the sentinel node, because we examined a second IHC-stained section rather than a frozen section. Of the 70 patients whose sentinel specimen was metastasis-free by H&E, 10 had sentinel node metastasis by IHC, an upstaging rate of 14.3%. Of the 60 patients whose sentinel specimen was metastasis-free by both H&E and IHC, only 1 had a nonsentinel node metastasis—an upstaging of 1.7% (0.97% false-negative rate for the entire study population)—despite the more intensive examination of nonsentinel nodes. To find this 1 nonsentinel node metastasis, we examined more than 2000 levels in 1087 nonsentinel nodes with IHC. This confirms histopathologically that the sentinel node identified by meticulous intraoperative lymphatic mapping, excised by selective lymphadenectomy, and examined by focused histopathologic review including immunostains is indeed the most likely axillary lymph node to harbor metastatic tumor in patients with breast cancer.

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Discussion

DR. WILLIAM C. WOOD (Atlanta, Georgia): Dr. Giuliano, I enjoyed your presentation very much, and I appreciated the privilege of reading the manuscript. I would congratulate you first for testing the hypothesis that the sentinel node does accurately reflect what is happening in the remainder of the axilla, and more than that, for developing the techniques of doing this in an easy way with the blue dye and then teaching that to so many of the rest of us.

Even more impressive to me is that you have taken the criticism offered at this meeting and others when you have presented these data and gone back to your clinical laboratory and have responded by now quantifying the meaning of the false-negative rate and the criticism that you were only adequately examining the sentinel nodes. To quote a historical figure, "I believe, gentlemen, this is no humbug." I am convinced absolutely that your work is changing forever the way we will approach patients with negative axillae in breast cancer.

Because we are all following your clinical experience and are not yet at the place, at least speaking for myself, where I can approach the 1% or less false-negative rate, I have two specific questions about technique. If a blue lymphatic is identified and traced to a node that is not blue, do you sample that and consider that a sentinel node? What if in examining the axilla looking for a blue lymphatic or a sentinel node, you identify a clearly suspicious hard node that you had not palpated? Do you excise this and consider this one of the sentinel nodes even though it was not located with the aid of the blue dye?

DR. BLAKE CADY (Boston, Massachusetts): I appreciate being able to read the complete manuscript of this excellent study of a timely and important subject. Dr. Giuliano has again confirmed the anatomic truth behind his concept of a sentinel node or nodes as the entrance to the regional axillary lymph node basin and the accuracy of the histologic examination of the sentinel node.

However, more important than the mere discovery of still more micrometastases is the question, what do they mean? Do a few cytokeratin-positive cells in a subcapsular sinus of a single lymph node make a difference in outcome? The discovery may accomplish no more than stage shifting, the Will Rogers effect. Stage shifting by itself never cured a single patient.

If a node-negative patient with an excellent prognosis is made node positive and receives adjuvant therapy to accomplish a marginal gain in outcome, is this a good thing? Our mad rush to enlist patients into adjuvant therapy programs for a 2%, 3%,