Predictors for nonsentinel node involvement in breast cancer patients with micrometastases in the sentinel lymph node

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Sentinel lymph node (SLN) biopsy in breast cancer allows for a more thorough pathologic assessment with serial sectioning and cytokeratin staining. This has resulted in increased detection of micrometastatic disease (tumor size <2 mm) in the SLN. Unfortunately, the value of completion axillary dissection after finding micrometastatic disease in the SLN remains poorly defined. Over a 2-year period, a prospective database of 305 patients who underwent SLN biopsy for breast cancer at Baylor University Medical Center was reviewed. Eighty-four (27.5%) of the patients had evidence of metastatic disease in the SLN. Twenty-four of the 41 patients identified as having micrometastatic disease in the SLN underwent completion axillary lymph node dissection. In these patients, all nonsentinel nodes were further studied by serial sectioning and immunohistochemistry. The median age of these 24 patients was 52 years (range, 34–83). Their primary tumor stages were T₁a and T₁b (n = 5), T₁c

(n = 15), and T₂ (n = 4). A total of 328 nonsentinel lymph nodes were examined, including 225 from patients with infiltrating ductal carcinoma (n = 17) and 103 from patients with infiltrating lobular carcinoma (n = 7). In the patients with infiltrating ductal carcinoma, no additional nodal metastases were identified, while in those with infiltrating lobular carcinoma, additional nodal disease was found in 5 lymph nodes (2 of 12 patients, 17%). Primary tumor characteristics were not predictive of additional nodal disease. These data suggest that patients with micrometastasis in the SLN from infiltrating lobular carcinoma have a significant risk of harboring additional nodal disease and should undergo completion axillary dissection. However, those with micrometastatic disease from infiltrating ductal carcinoma have a very low incidence of additional metastasis and may not need completion axillary dissection.

Regional lymph node metastasis has long been considered the most important predictor of survival in patients with invasive breast cancer. As the number of lymph node metastases increases, patient survival decreases (1–3). The number of lymph nodes containing tumor also determines the need for and type of adjuvant therapy. As breast cancer surgery becomes more conservative, the best method of obtaining this information is evolving. Traditionally, an axillary dissection comprising level I and II lymph nodes was done to provide staging and prognostic information (4). With the advent of sentinel lymph node (SLN) biopsy, this same information can be obtained by removal of only a few nodes, thus avoiding much of the morbidity associated with a standard axillary dissection.

SLN biopsy findings have been shown to be a very sensitive predictor of regional metastasis, with accuracies of 90% to 100% (5–15). Recent reports suggest that the SLN is often the only node containing metastatic disease (6, 7, 10, 11, 16–18). Thus, removing additional lymph nodes may not provide additional useful information. It may be valuable to identify the subset of patients in whom tumor cells are confined only to the sentinel node. Some studies have suggested that small primary tumors with micrometastases (tumor size <2 mm) in the SLN harbor a very low incidence of nonsentinel lymph node (non-SLN) metastasis (3, 19). Turner et al suggested that the size of the primary tumor and peritumoral lymphovascular invasion correlate with non-SLN metastasis (20). Unfortunately, most prior studies have not included extensive pathologic evaluation of the non-SLNs. The purpose of this study was to correlate the size of nodal metastasis and tumor histology as predictors of involvement of non-

SLNs based on serial sectioning and immunohistochemistry of all non-SLNs.

PATIENTS AND METHODS

Between October 1997 and December 1999, 305 patients who were enrolled in an institutional review board–approved prospective study underwent SLN biopsy at Baylor University Medical Center in Dallas, Texas. The SLN was identified by preoperative injection of unfiltered technetium 99 sulfur colloid (99 Tc; CIS, Bedford, Mass) and intraoperative injection of blue dye. A total of 1 mCi of 99 Tc in an 8-cc volume was injected around the tumor the morning of surgery in the nuclear medicine department. Preoperative lymphoscintigraphy was done in all patients, and a hand-held gamma probe (C-trak; Carewise Medical, Palo Alto, Calif) was used intraoperatively to identify the hot nodes. Approximately 5 minutes before incision, 3 to 5 cc of isosulfan blue dye (Lymphazurin; USSC, Norwalk, Conn) was injected peritumorally. Nodes were considered sentinel if they were hot, blue, or both hot and blue. The nodes were then sent for pathological review.

SLNs suggestive of disease were evaluated by frozen section. Tissue sections were fixed in 10% formalin and embedded in paraffin. All SLNs were serially sectioned along their long axis

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Table 1. Sentinel lymph nodes positive for disease according to stage of primary tumor

Tumor stage	n	No. positive SLN	%
T _{1a}	22	2	9
T _{1b}	86	17	20
T _{1c}	142	42	30
T ₂	48	22	46
T ₃	4	1	25

at 1- to 2-mm intervals. Three sections were taken from each node, at the 30-, 60-, and 90- μ levels, and were stained with routine hematoxylin and eosin. An additional section of each SLN was taken for immunohistochemical studies, which were performed on a Ventana Basic DAB 320 Automated Immunostainer (Tucson, Ariz) using the standard avidin-biotin-peroxidase technique. Pancytokeratin primary antibody (AE1–AE3, dilution 1/2000; Boehringer Mannheim, Indianapolis, Ind) was used for these studies. All patients with positive results on the SLN biopsies were advised to undergo completion axillary dissection.

All non-SLNs obtained from the axillary dissection were recut at the 30-, 60-, and 90- μ levels and stained with hematoxylin and eosin. An additional section was obtained for immunohistochemical staining.

RESULTS

Of the 305 patients who underwent SLN biopsy, 84 (28%) had positive findings for metastatic disease in the SLN. These patients ranged in age from 32 to 83 years, with a median age of 53 years. Tumor pathology included infiltrating ductal carcinoma in 67 patients and infiltrating lobular carcinoma in 17 patients. As tumor size increased, so did the percentage of SLNs that were positive for disease (Table 1). One half of all patients with disease in the SLN had a T_{1c} tumor. Micrometastases were identified in the SLNs of 41 patients (49%).

Of the 17 patients with infiltrating lobular carcinoma in the SLN, 5 had macrometastasis and 12 had micrometastasis. Four of the 5 patients with macrometastasis had completion axillary dissection, and 3 (75%) of these patients had positive findings in node(s) other than the SLN by hematoxylin-eosin staining. Of the 12 patients with infiltrating lobular carcinoma who had micrometastatic disease, only 7 agreed to undergo completion

Table 3. Cytokeratin staining of axillary lymph nodes in 24 patients with sentinel lymph node micrometastases who underwent completion axillary lymph node dissection

	Infiltrating breast carcinoma	
	Lobular	Ductal
Cytokeratin staining of all axillary nodes	7	17
Total number of nodes studied	103	225
Median number of nodes per patient	12	13
Additional nodes positive by cytokeratin	5	0
Number of patients with additional positive nodes	2 (29%)	0

axillary dissection, and 2 (29%) of these patients were found to have additional positive node(s) on hematoxylin-eosin staining (Table 2).

In the group of 67 patients with infiltrating ductal carcinoma, 38 had macrometastasis and 29 had micrometastasis. Most of the patients with macrometastasis (35/38) underwent completion axillary dissection, and 11 (31%) of these patients were found to have other positive nodes. Of the 29 patients with micrometastatic disease, 17 underwent completion axillary dissection. None of these patients were found to have additional nodal disease (Table 2), a significant difference from patients with macrometastasis in the SLN ($P = 0.025$).

The 24 patients with micrometastatic disease who underwent completion axillary dissection were further evaluated. These patients ranged in age from 34 to 83 years, with a median age of 52 years. Five of these patients had a primary tumor size ≤ 1 cm, 12 had a primary tumor between 1 and 2 cm, and 7 had a primary tumor > 2 cm. Eleven (46%) of these patients had lymphovascular invasion. Three patients had grade 1 tumors, 11 had grade 2 tumors, 6 had grade 3 tumors, and in 4 patients the grade was unknown. The tumors were negative for estrogen receptor in 4 patients and for progesterone receptor in 5 patients.

Fifty of the 84 patients with metastatic disease underwent frozen section analysis. This analysis successfully identified metastasis in the sentinel node only about half of the time. All patients whose sentinel node was positive on frozen section analysis had an immediate axillary dissection. In all, 63% of the positive nodes were found by hematoxylin-eosin staining alone; 30%, by frozen section and hematoxylin-eosin staining; and 8%, by cytokeratin staining alone.

Table 2. Patients with sentinel and axillary lymph nodes positive for disease among 305 breast cancer patients evaluated with sentinel lymph node biopsy

	Infiltrating ductal carcinoma		Infiltrating lobular carcinoma	
	Macrometastases	Micrometastases	Macrometastases	Micrometastases*
Number of patients	38	29	5	12
Number who underwent completion axillary dissection	35	17	4	7
Number with additional nodes positive on hematoxylin-eosin staining	11 (31%)	0	3 (75%)	2 (29%)

*Macrometastases are defined as > 2 mm; micrometastases, as < 2 mm.

All of the lymph nodes obtained through completion axillary dissections of patients with micrometastatic disease were serially sectioned and stained by immunohistochemical techniques in a blinded fashion. A total of 328 non-SLNs were examined, including 225 from the 17 patients with infiltrating ductal carcinoma and 103 from the 7 patients with infiltrating lobular carcinoma. Among the 103 lymph nodes associated with infiltrating lobular carcinoma, 5 nodes from 2 patients were positive for disease by cytokeratin staining. Of the 225 nodes associated with infiltrating ductal carcinoma, no additional nodal metastases were seen, even with immunohistochemical stains ($P = 0.117$) (Table 3).

The 17 patients with micrometastases who refused axillary dissection have now been followed for a mean of 30 months, and there have been no axillary recurrences.

DISCUSSION

The best predictor of survival among patients with breast cancer has long been considered to be axillary lymph node status. As breast cancers are being detected and treated at very early stages, the incidence of axillary nodal involvement has decreased. Previously, an axillary dissection comprising level I and II nodes was done to provide staging and prognostic information (4). However, most researchers point to the NSABP B04 trial, which showed no survival benefit when comparing mastectomy with axillary dissection, mastectomy alone, or mastectomy plus radiation for clinically negative axilla (21). Axillary node dissection can be associated with a wide range of complications, including paresthesia due to intercostobrachial nerve injury, wound infection, seroma, drain complications, and lymphedema (22), which develops in 15% to 20% of patients after breast cancer treatment (23). SLN biopsy can provide the prognostic information that is needed while avoiding the morbidity of an axillary dissection in patients with a low risk of axillary metastasis.

With an SLN biopsy, only a few nodes are biopsied, and the examination of these nodes can be more thorough than that with axillary dissection. Serial sectioning and immunohistochemical staining can identify small deposits of tumor. Retrospective and prospective studies have shown that these intensive pathologic studies in patients with clinically negative nodes can detect occult metastatic disease in as many as 33% of patients with breast cancer (24). This study found that approximately 14% of the total group of patients who underwent axillary dissection for breast cancer had micrometastasis, which was largely detected by serial sectioning and immunohistochemical staining. Trojani et al (25) found a higher incidence of micrometastasis in patients with lobular carcinoma, which correlates well with the results of this study.

The significance of these micrometastases has remained controversial. Earlier investigations showed micrometastases to have no significance in survival (26, 27). More recent studies, however, suggest that micrometastases can have a negative impact on disease-free and overall survival (25, 28–32). However, in all of these prior studies on micrometastases, a completion axillary dissection had been done. Therefore, these micrometastatic deposits had been removed.

When the SLN is positive for disease, the current standard management includes completion axillary dissection based on

the belief that axillary dissection may have therapeutic benefit in a patient with known metastasis. Protocol Z0011 of the American College of Surgeons Oncology Group (ACOSOG) randomizes patients with SLN positive for metastatic disease to either completion axillary dissection plus adjuvant therapy or adjuvant therapy alone. The rationale for the ethical randomization into this study is based on several concepts. It is possible that after accurate staging with SLN biopsy, no residual tumor is left in the remainder of the axilla. Giuliano et al (6, 9) have shown that in approximately 70% of cases in which the SLN is positive, it is the only involved node. However, it is also possible that some women with SLN metastases will have micro- or macrometastases in non-SLNs that will progress and develop into regional recurrences that may or may not be associated with a decrease in overall survival. On the other hand, metastases in the non-SLNs may be destroyed or inhibited by the adjuvant systemic chemotherapy that is given to most women with involved lymph nodes. Opposing tangential fields of radiation therapy may also ablate some metastases.

Historically, the need for systemic adjuvant chemotherapy or hormonal therapy has been based upon the presence or absence of axillary metastasis. Over the past few years, this focus has changed. The characteristics of the primary tumor, including size and biologic characteristics, determine the need for these systemic agents. This means that many women with negative axilla receive adjuvant chemotherapy. Since dissection quite possibly won't change therapy recommendations, the continued need for immediate axillary dissection comes into question.

In this study, a thorough evaluation of the non-SLNs in patients with micrometastases showed that the incidence of additional metastasis was markedly lower in the invasive ductal group compared with the infiltrating lobular group. The results of the prospective ACOSOG protocol may give further guidance for the management of patients with micrometastases. These data suggest that patients with micrometastasis in the SLN from infiltrating lobular carcinoma have a significant risk of harboring additional nodal disease and should undergo completion axillary dissection. However, those with micrometastatic disease from infiltrating ductal carcinoma have a very low incidence of additional metastasis and may not need completion axillary dissection.

1. Moore MP, Kinne DW. Axillary lymphadenectomy: a diagnostic and therapeutic procedure. *J Surg Oncol* 1997;66:2–6.
2. Wilking N, Rutqvist LE, Carstensen J, Mattsson A, Skoog L. Prognostic significance of axillary nodal status in primary breast cancer in relation to the number of resected nodes. Stockholm Breast Cancer Study Group. *Acta Oncol* 1992;31:29–35.
3. Chu KU, Turner RR, Hansen NM, Brennan MB, Bilchik A, Giuliano AE. Do all patients with sentinel node metastasis from breast carcinoma need complete axillary node dissection? *Ann Surg* 1999;229:536–541.
4. Dees EC, Shulman LN, Souba WW, Smith BL. Does information from axillary dissection change treatment in clinically node-negative patients with breast cancer? An algorithm for assessment of impact of axillary dissection. *Ann Surg* 1997;226:279–286; discussion 286–287.
5. McMasters KM, Giuliano AE, Ross MI, Reintgen DS, Hunt KK, Byrd DR, Klimberg VS, Whitworth PW, Tafra LC, Edwards MJ. Sentinel-lymph-node biopsy for breast cancer—not yet the standard of care. *N Engl J Med* 1998; 339:990–995.
6. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping

- and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–398; discussion 398–401.
7. Alex JC, Krag DN. The gamma-probe-guided resection of radiolabeled primary lymph nodes. *Surg Oncol Clin N Am* 1996;5:33–41.
 8. Albertini JJ, Lyman GH, Cox C, Yeatman T, Balducci L, Ku N, Shivers S, Berman C, Wells K, Rapaport D, Shons A, Horton J, Greenberg H, Nicosia S, Clark R, Cantor A, Reintgen DS. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. *JAMA* 1996;276:1818–1822.
 9. Giuliano AE, Jones RC, Brennan M, Statman R. Sentinel lymphadenectomy in breast cancer. *J Clin Oncol* 1997;15:2345–2350.
 10. Barnwell JM, Arredondo MA, Kollmorgen D, Gibbs JF, Lamonica D, Carson W, Zhang P, Winston J, Edge SB. Sentinel node biopsy in breast cancer. *Ann Surg Oncol* 1998;5:126–130.
 11. Veronesi U, Paganelli G, Galimberti V, Viale G, Zurrida S, Bedoni M, Costa A, de Cicco C, Geraghty JG, Luini A, Sacchini V, Veronesi P. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. *Lancet* 1997;349:1864–1867.
 12. Guenther JM, Krishnamoorthy M, Tan LR. Sentinel lymphadenectomy for breast cancer in a community managed care setting. *Cancer J Sci Am* 1997;3:336–340.
 13. Borgstein PJ, Pijpers R, Comans EF, van Diest PJ, Boom RP, Meijer S. Sentinel lymph node biopsy in breast cancer: guidelines and pitfalls of lymphoscintigraphy and gamma probe detection. *J Am Coll Surg* 1998;186:275–283.
 14. Crossin JA, Johnson AC, Stewart PB, Turner WW Jr. Gamma-probe-guided resection of the sentinel lymph node in breast cancer. *Am Surg* 1998;64:666–668; discussion 669.
 15. Krag D, Weaver D, Ashikaga T, Moffat F, Klimberg VS, Shriver C, Feldman S, Kusminsky R, Gadd M, Kuhn J, Harlow S, Beitsch P. The sentinel node in breast cancer—a multicenter validation study. *N Engl J Med* 1998;339:941–946.
 16. Cody HS III. Sentinel lymph node mapping in breast cancer. *Oncology (Huntingt)* 1999;13:25–34; discussion 35–36, 39, 43.
 17. Czerniecki BJ, Scheff AM, Callans LS, Spitz FR, Bedrosian I, Conant EF, Orel SG, Berlin J, Helsabeck C, Fraker DL, Reynolds C. Immunohistochemistry with pancytokeratins improves the sensitivity of sentinel lymph node biopsy in patients with breast carcinoma. *Cancer* 1999;85:1098–1103.
 18. Giuliano AE. Sentinel lymphadenectomy in primary breast carcinoma: an alternative to routine axillary dissection. *J Surg Oncol* 1996;62:75–77.
 19. Reynolds C, Mick R, Donohue JH, Grant CS, Farley DR, Callans LS, Orel SG, Keeney GL, Lawton TJ, Czerniecki BJ. Sentinel lymph node biopsy with metastasis: can axillary dissection be avoided in some patients with breast cancer? *J Clin Oncol* 1999;17:1720–1726.
 20. Turner RR, Chu KU, Qi K, Botnick LE, Hansen NM, Glass EC, Giuliano AE. Pathologic features associated with nonsentinel lymph node metastases in patients with metastatic breast carcinoma in a sentinel lymph node. *Cancer* 2000;89:574–581.
 21. Fisher B, Redmond C, Fisher ER, Bauer M, Wolmark N, Wickerham DL, Deutsch M, Montague E, Margolese R, Foster R. Ten-year results of a randomized clinical trial comparing radical mastectomy and total mastectomy with or without radiation. *N Engl J Med* 1985;312:674–681.
 22. Cox CE, Haddad F, Bass S, Cox JM, Ku NN, Berman C, Shons AR, Yeatman T, Pendas S, Reintgen DS. Lymphatic mapping in the treatment of breast cancer. *Oncology (Huntingt)* 1998;12:1283–1292; discussion 1293–1294, 1297–1298.
 23. Petrek J, Lerner R. *Diseases of the Breast*. Philadelphia: Lippincott-Raven, 2000.
 24. Leong AS. The prognostic dilemma of nodal micrometastases in breast carcinoma. *Gan To Kagaku Ryoho [Japanese Journal of Cancer and Chemotherapy]* 2000;27 Suppl 2:315–320.
 25. Trojani M, de Mascarel I, Bonichon F, Coindre JM, Delsol G. Micrometastases to axillary lymph nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. *Br J Cancer* 1987;55:303–306.
 26. Cady B. Case against axillary lymphadenectomy for most patients with infiltrating breast cancer. *J Surg Oncol* 1997;66:7–10.
 27. Fisher ER, Palekar A, Rockette H, Redmond C, Fisher B. Pathologic findings from the National Surgical Adjuvant Breast Project (Protocol No. 4). V. Significance of axillary nodal micro- and macrometastases. *Cancer* 1978;42:2032–2038.
 28. Rosen PP, Saigo PE, Braun DW, Weathers E, Fracchia AA, Kinne DW. Axillary micro- and macrometastases in breast cancer: prognostic significance of tumor size. *Ann Surg* 1981;194:585–591.
 29. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. *Lancet* 1990;335:1565–1568.
 30. Hainsworth PJ, Tjandra JJ, Stillwell RG, Machet D, Henderson MA, Rennie GC, McKenzie IF, Bennett RC. Detection and significance of occult metastases in node-negative breast cancer. *Br J Surg* 1993;80:459–463.
 31. de Mascarel I, Bonichon F, Coindre JM, Trojani M. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. *Br J Cancer* 1992;66:523–527.
 32. Gerber B, Krause A, Reimer T. Immunohistochemically detected lymph-node micrometastases in breast cancer and their correlation with prognostic factors. *The Breast Journal* 1997;3:106–111.