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Sentinel Node Metastasis in Patients with Breast Carcinoma Accurately Predicts Immunohistochemically Detectable Nonsentinel Node Metastasis

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Background: Sentinel lymphadenectomy is highly accurate for identifying axillary metastasis from a primary breast carcinoma. Nonsentinel axillary lymph nodes (NSNs) are unlikely to contain tumor cells if the axillary sentinel node (SN) is tumor free. We previously showed that the size of the primary tumor and the size of its SN metastasis predict the risk of NSN tumor involvement detected by hematoxylin and eosin staining. This study used immunohistochemical staining (IHC) to determine the likelihood of NSN axillary metastasis in the presence of SN metastasis.

Methods: Between 1991 and 1997, axillary lymphadenectomy was performed in 156 women (157 axillary basins) who had primary breast carcinoma with SN metastasis. By hematoxylin and eosin staining, we identified NSN metastasis in 55 axillae (35%). IHC was then used to re-examine all NSNs (1827 lymph nodes) from the remaining 102 axillae. The incidence of IHC-detected NSN involvement was analyzed with respect to clinical and tumor characteristics.

Results: By using IHC, we identified NSN metastasis in 15 (14.7%) of the 102 axillae. By multivariate analysis, the size of the SN metastasis ($P = .0001$) and the size of the primary tumor ($P = .038$) were the only independent variables predicting NSN metastasis determined by using either hematoxylin and eosin staining or IHC. Only the number of SN metastases (1 vs. >1) was a significant ($P = .04$) predictor of IHC-detected NSN metastasis.

Conclusions: Use of IHC increases the likelihood of detection of NSN metastasis, and the risk of IHC-detected metastasis increases with the size of the SN metastasis and the size of the primary tumor. If SN involvement is micrometastatic (≤2 mm) or detected by using IHC, tumor cells are unlikely to be found in other axillary lymph nodes in patients with a small primary tumor. The clinical significance of micrometastatic disease in lymph nodes is controversial, and a prospective randomized study is necessary to resolve this important issue.

Key Words: Breast carcinoma—Sentinel node—Axillary lymphadenectomy—Immunohistochemistry.

The increased use of screening mammography has resulted in the earlier detection and smaller size of invasive breast cancers, thereby decreasing the incidence of axillary metastases.[1] This decrease challenges the efficacy of routine standard level I and II axillary lymph node dissection (ALND) for all patients with invasive breast cancer. However, although the incidence of axillary metastasis is low in patients with T1a lesions,[2–5] it is still high enough to require accurate staging of the axillary basin.[6,7] The recent emergence of sentinel lymphadenectomy (SLND) may eliminate routine ALND in patients with invasive breast cancer, especially those with small tumors and noninvolved sentinel nodes (SNs).

SLND is a highly accurate technique for identifying axillary metastases from invasive breast carcinoma.[8–11] The SN is the first lymph node draining a specific breast cancer. It may be identified by using a vital blue dye alone or in combination with a radioactive colloid.[8,10] If

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histopathological examination shows that the SN is free of metastasis, then other nodes in the same axillary drainage basin are highly unlikely to contain tumor cells,[9] and the patient is unlikely to benefit from ALND.

We recently demonstrated that even if the SN contains tumor cells, the status of the remaining nonsentinel nodes (NSNs) in the axillary basin can be predicted by the size of the SN metastasis and the size of the primary tumor.[12] In that study, if the SN contained micrometastatic tumor foci ($\leq$2 mm), the rate of NSN metastasis detected by hematoxylin and eosin (H&E) staining was 0 in patients with T1a and T1b primary tumors and less than 10% in patients with T1c and T2 primary tumors. In the present study, we examined NSNs by using immunohistochemical (IHC) staining and compared the rate of IHC-detected NSN metastasis with various clinicopathological prognostic factors.

PATIENTS AND METHODS

Patients and Operative Procedure

Intraoperative lymphatic mapping and SLND for patients with potentially curable breast cancer was initiated at our institution in 1991. From September 1991 through September 1995, SLND was undertaken in all patients scheduled for ALND, including those with locally advanced disease, large primary tumors, and/or palpable axillary lymph nodes. SLND was followed immediately by a completion ALND that removed all nodes in levels I and II and occasionally some nodes from level III. From October 1995 through July 1997, SLND was undertaken only in patients with no clinical evidence of axillary involvement and was not followed immediately by completion ALND unless frozen-section examination of the SN revealed tumor cells.

Of the 421 patients (422 axillae) in whom an SN was identified, 162 (38.5%) had a tumor-involved SN. Five patients refused completion ALND; and, therefore, our study group consisted of 157 patients, including one case of bilateral synchronous breast cancer. All 158 operations were performed by the same senior surgeon (A.E.G.) after informed consent had been obtained.

Our technique of dye-directed SLND for patients with invasive breast cancer has been previously described.[13,14] In brief, 3 to 5 ml of isosulfan blue dye (Lymphazurin 1%; Hirsch Industries, Inc., Richmond, VA) is injected into the breast parenchyma surrounding the primary tumor or biopsy cavity. Care is used to not inject the dye into the biopsy cavity itself. After 5 to 7 minutes, an axillary incision is made and a blue lymphatic tract is identified. This tract is dissected both proximally and distally until a blue-stained lymph node (SN) is identified. The SN is excised and sent for frozen section analysis.

During the assessment period (1991–1994) of the SLND technique, some patients underwent SN localization using radiocolloid in addition to dye. Before surgery, 0.25 to 1.0 mC of a technetium-labeled radiopharmaceutical (Tc-99m albumin colloid, DuPont de Nemours, Billerica, MA; or Tc-99m sulfur colloid, CisUS, Bedford, MA) was injected into the breast parenchyma surrounding the primary tumor or into the wall of the cavity created by the previous biopsy. For nonpalpable lesions, a needle left in the breast parenchyma after either mammographic or ultrasonographic localization was used as a guide to instill radioisotope in the area of the primary tumor. Care was used to not contaminate the skin with the radiopharmaceutical. After the induction of general anesthesia or heavy intravenous sedation, blue dye was also injected as described above. A handheld γ-counter (C-Trak, Carewise Medical, Palo Alto, CA; or Neoprobe 1000, Neoprobe Corp., Dublin, OH; or Navigator, USSC, Norwalk, CT) covered with a sterile plastic sheath was used to localize the SN. The lymphatic drainage pattern was mapped in the operating room, and a transverse axillary incision was made in the skin overlying the area with the greatest radioactivity. Blunt dissection was then carefully performed with the tips of a curved hemostat until the signal intensified; the lymph node with the greatest radioactivity and/or a blue stain was identified as the SN. This node was excised and sent to pathology. The residual radioactivity in the axilla was then measured; if the basin remained hot, an attempt was made to find a second SN by tracking blue-stained lymphatics.

When completion ALND was performed immediately after SLND, the ALND specimen was submitted separately for histopathological examination. After September 1995, completion ALND was performed as a second procedure if examination of permanent sections of the SN identified tumor cells not found during frozen-section examination. All patients underwent either segmental mastectomy or mastectomy after SLND.

Patients were prospectively followed, and both clinical and tumor characteristics were entered into the data base. Clinical characteristics included age, mode of tumor detection, and lymph node status; tumor characteristics included histological type, grade, size, and angiolymphatic invasion. The size of the primary tumor was determined from histopathological sections, by measuring the invasive component, and was used to classify the tumor according to guidelines of the American Joint Committee on Cancer.[15] The size (maximum diameter) of SN metastases, the number of tumor-positive SNs, and

the mode of tumor detection (H&E or IHC) were also recorded prospectively. SN tumor foci were defined as micrometastases (≤2 mm, using H&E staining) or macrometastases (>2 mm, using H&E staining). An SN metastasis detected only by IHC was identified as an IHC metastasis. Estrogen receptor status, progesterone receptor status, *HER-2/neu* expression, DNA ploidy, and S-phase were evaluated as tumor-associated indicators of prognosis.

Histological Examination of Axillary Lymph Nodes

All SNs and NSNs were examined initially by multiple pathologists at our institution and reviewed again by one pathologist (R.R.T.). SNs were evaluated independently of NSNs. The size and method of detection of each SN metastasis was reviewed. Each SN was bisected and a frozen section was obtained. The SN then was blocked individually, with preparation of permanent section levels from each paraffin block. If H&E was negative for tumor cells, the SN was examined with IHC, using an antibody cocktail (MAK-6; Ciba-Corning, Alameda, CA) directed against low and intermediate molecular weight cytokeratin. Six to eight histological sections (including the frozen section) of each SN were examined.

The ALND specimens (NSNs) were examined by using standard histopathological techniques. Lymph nodes were identified visually or with manual palpation; no lymph node clearing solution was used. Lymph nodes more than 3 to 4 mm in diameter were grossly sectioned and all nodal tissue was embedded in paraffin. One or two H&E-stained sections were evaluated. Cytokeratin IHC stains were examined by one pathologist (R.R.T.) on NSNs that were tumor free by H&E. Among 158 axillary specimens, one was not available for examination. Thus, we studied 157 axillary specimens from 156 patients who had a tumor-involved SN.

Statistical Analysis

All data were reviewed and analyzed by the biostatistical unit at our institution. The Pearson χ^2 test was used to assess the relationship between NSN metastasis and each of the following potential predictors: age, tumor grade, hormone receptors, S-phase fraction, DNA ploidy, HER-2/neu expression, angiolymphatic invasion, tumor size, palpable primary tumor, palpable axillary nodal disease, size of SN metastasis, and the number of tumor-involved SNs. Multivariate analysis was performed by using logistic regression, and stepwise procedure was used for covariate selection.

RESULTS

The median age of the 156 patients was 52.5 years (range, 28–91 years). The median size of primary tumors was 2.0 cm (range, 0.1–11 cm). The mean number of SNs identified was 1.8 (range, 1–7), and the mean number of tumor-involved SNs was 1.3 (range, 1–5). The mean number of NSNs identified was 17.8 (range, 5–60). Most patients (79%) underwent segmental mastectomy and ALND; 33 patients (21%) underwent modified radical mastectomy.

By using H&E, we identified at least one tumor-involved NSN in 55 axillae (35%) (Table 1). Of 1827 NSNs removed from the remaining 102 axillae (65%) with no H&E evidence of NSN involvement, 29 nodes (1.59%) from 15 axillae (14.7%) contained metastases identified by using IHC.

The four following clinicopathological factors were significant predictors of NSN metastasis by univariate analysis: size of SN metastasis, size of primary tumor, number of tumor-positive SNs, and angiolymphatic invasion. Among the other risk factors studied, only the clinical status of the axilla and tumor grade approached statistical significance (Table 2). Multivariate analysis, using logistic regression and stepwise procedure, defined only two covariates, i.e., size of primary tumor and size of SN metastasis (Table 2). The number of tumor-positive SNs and angiolymphatic invasion dropped out because of their significant correlation with primary tumor size and size of SN metastasis.

There was a clear correlation between the size of the SN metastasis and the incidence of IHC-detected NSN metastasis, and between the size of the primary tumor and the incidence of IHC-detected NSN metastasis: as the diameter of the primary tumor increased above 5 cm and as the diameter of its SN metastasis increased above 2 mm, the incidence of NSN metastasis reached 78.6% and 62.4%, respectively. Conversely, the incidence of NSN metastasis was 20% with primary tumors 1 cm or smaller in diameter, and 12.1% with IHC-detected SN involvement (Table 1).

To determine whether IHC-detected NSN metastases could be predicted by clinicopathological factors, we analyzed the frequency of NSN metastases in the 102 axillae with no H&E evidence of NSN involvement. Only the number of tumor-positive SNs was significant (Table 3).

DISCUSSION

Standard H&E examination shows that two-thirds of patients whose primary breast tumor has spread to axil-

TABLE 1. *Rate of NSN metastasis according to clinicopathological factors and staining technique*

Clinicopathological factors	H&E(+) NSN (%)	H&E or IHC(+) NSN (%)
Total number of axillae	55/157 (35)	70/157 (44.6)
Size of SN metastasis		
Macrometastasis (>2 mm)	48/85 (56.5)	53/85 (62.4)
Micrometastasis (≤2 mm)	7/39 (17.9)	13/39 (33.3)
Immunohistochemistry	0/33	4/33 (12.1)
Size of primary tumor		
T3 (>5 cm)	11/14 (78.6)	11/14 (78.6)
T2 (2–5 cm)	23/57 (40.4)	26/57 (45.6)
T1c (1–2 cm)	19/66 (28.8)	29/66 (43.9)
T1b (0.5–1 cm)	2/15 (13.3)	3/15 (20)
T1a (<0.5 cm)	0/5	1/5 (20)
Number of tumor-positive SNs		
1	37/122 (30.3)	47/122 (38.5)
>1	18/35 (51.4)	23/35 (65.7)
Angiolymphatic invasion		
Yes	21/46 (45.7)	27/46 (58.7)
No	34/111 (30.6)	43/111 (38.7)
Palpable axillary node		
Yes	13/22 (59.1)	14/22 (63.6)
No	42/135 (31.1)	56/135 (41.5)
Tumor grade		
Poor	25/52 (48.1)	29/52 (55.8)
Moderate	27/90 (30)	37/90 (41.1)
Well	3/15 (20)	4/15 (26.7)
Age		
<50 y	16/59 (27.1)	22/59 (37.3)
≥50 y	39/98 (39.8)	48/98 (49)
Palpability		
Yes	43/117 (36.8)	56/117 (47.9)
No	12/40 (30)	14/40 (35)
HER-2/neu expression		
High	5/22 (22.7)	7/22 (31.8)
Low	30/88 (34.1)	41/88 (46.6)
Estrogen receptor		
Positive	48/131 (36.6)	60/131 (45.8)
Negative	7/26 (26.9)	10/26 (38.5)
S-phase fraction		
High	24/54 (44.4)	28/54 (51.9)
Low	26/78 (33.3)	36/78 (46.2)
Progesterone receptor		
Positive	36/106 (34)	47/106 (44.3)
Negative	19/50 (38)	23/50 (46)
DNA ploidy		
Yes	19/63 (30.2)	29/63 (46)
No	34/82 (41.5)	39/82 (47.6)

NSN, nonsentinel lymph node; H&E, hematoxylin and eosin staining; IHC, immunohistochemical staining; SN, sentinel node.

TABLE 2. *Predictive value of clinicopathological factors with respect to NSN metastasis*

Clinicopathological factors	*P*	
	Univariate	Multivariate
Size of SN metastasis	<.001	.0001
Number of tumor-positive SNs	.004	–
Size of primary tumor	.005	.038
Angiolymphatic invasion	.022	–
Palpable axillary node	.053	–
Tumor grade	.081	–
Patient age	.153	–
Palpability	.158	–
HER-2/neu expression	.211	–
Estrogen receptor	.492	–
S-phase fraction	.520	–
Progesterone receptor	.846	–
DNA ploidy	.855	–

NSN, nonsentinel lymph node; SN, sentinel node.

lary SNs will have no evidence of tumor in the NSNs.[8] In our previous study, we found that this group of patients could be identified by examining the size of the primary tumor and the size of its SN metastasis.[12] The size of the SN metastasis was particularly important; i.e., the rate of NSN involvement was less than 10% when the SN had a micrometastasis and more than 55% when the SN had a macrometastasis. If the primary tumor was T1 (≤2 cm), the rate of NSN involvement decreased to less than 5% in patients with SN micrometastasis. In these patients, completion ALND may increase the risk of operative morbidity without yielding a clinical benefit.

Although H&E examination of axillary lymph nodes is the standard of care for patients with invasive breast carcinoma, it is not as sensitive as IHC for identifying metastases. In the current study, we therefore examined NSNs by using IHC in addition to H&E. Use of IHC increased the detection of metastasis in NSNs by 14.7% compared with use of H&E. This increase was very similar to the 14.3% increase in metastases that we previously reported for SNs examined by using IHC.[9] In the current study, IHC increased the overall rate of NSN metastasis from 35% to 44.6%.

Examination of NSNs by using IHC confirmed our previous observation that the likelihood of NSN metastases depends on the size of the SN metastasis and the size of the primary tumor.[12] It also showed that only the number of tumor-involved SNs affected the likelihood of NSN metastasis missed by H&E but detected by IHC; i.e., more than one tumor-involved SN increased the likelihood of IHC-detected NSN metastases. The significance of the number of tumor-involved SNs can be illustrated in two patients, each of whom had a primary tumor smaller than 1 cm (T1a or T1b) and SN micrometastases. IHC identified NSN metastases in both patients. The patient with a T1a lesion had extensive ductal carcinoma in situ with focal invasive carcinoma and three SNs that contained IHC-detected metastases. By IHC examination, we detected multiple NSN metastases. The patient with a T1b lesion also had extensive ductal carcinoma in situ with multifocal invasive carcinoma. Five SNs were identified in this patient, and all had metastases detected only by using IHC; four NSNs had micrometastases detected by using IHC. Thus, the number of

TABLE 3. *Increase in detection of NSN metastasis by immunohistochemistry*

Clinicopathological factors	NSN converted by IHC (%)	*P*
Total number of axillae	15/102 (14.7)	
Size of SN metastasis		.779
Macrometastasis (>2 mm)	5/37 (13.5)	
Micrometastasis (≤2 mm)	6/32 (18.8)	
Immunohistochemistry	4/33 (12.1)	
Size of primary tumor		.519
T3 (>5 cm)	0/3	
T2 (2–5 cm)	3/34 (8.8)	
T1c (1–2 cm)	10/47 (21.3)	
T1b (0.5–1 cm)	1/13 (7.7)	
T1a (<0.5 cm)	1/5 (20)	
Number of tumor-positive SNs		.043
1	10/85 (11.8)	
>1	5/17 (29.4)	
Angiolymphatic invasion		.190
Yes	6/25 (24)	
No	9/77 (11.7)	
Palpable axillary node		1.000
Yes	1/9 (11.1)	
No	14/93 (15.1)	
Tumor grade		.921
Poor	4/27 (14.8)	
Moderate	10/63 (15.9)	
Well	1/12 (8.3)	
Age		.855
<50 y	6/43 (14)	
≥50 y	9/59 (15.3)	
Palpability		.227
Yes	13/74 (17.6)	
No	2/28 (7.1)	
HER-2/neu expression		.490
High	2/17 (11.8)	
Low	11/58 (19)	
Estrogen receptor		1.000
Positive	12/83 (14.5)	
Negative	3/19 (15.8)	
S-phase fraction		.494
High	4/30 (13.3)	
Low	10/52 (19.2)	
Progesterone receptor		1.000
Positive	11/70 (15.7)	
Negative	4/31 (12.9)	
DNA ploidy		.110
Yes	10/44 (22.7)	
No	5/48 (10.4)	

NSN, nonsentinel lymph node; SN, sentinel node.

tumor-involved SNs was directly related to the likelihood of IHC-detected NSN metastases, regardless of the size of the primary tumor or the size of SN metastases. This reinforces the importance of focused examination of the SN.

Although the chance of finding tumor cells in an NSN clearly decreased with decreasing size of the primary tumor and its SN metastasis, the overall frequency of NSN metastasis was higher by using IHC. The rate of NSN involvement was 20% in patients with T1a and T1b lesions, 12.1% in patients with IHC-detected SN metastasis, and 33.3% in patients with H&E-detected SN micrometastasis (Table 1). The clinical significance of IHC-detected metastases in the axillary lymph nodes is not known, and the clinical significance of H&E-detected micrometastases remains controversial. The reported studies are retrospective and generally conflicting,[16–22] in part because their results are based on different methods of detecting and defining micrometastasis. Some used multiple sections with H&E, whereas others used IHC but with different antibodies.[17,21] At the present time, the surgical management and staging of invasive breast carcinoma relies on conventional H&E for detection of metastases in excised lymph nodes. IHC is more sensitive but also more expensive and time consuming, making it impractical for routine analysis of NSN specimens. In addition, small clusters of cytokeratin-positive cells or isolated cytokeratin cells are often seen in SNs by using IHC and may be of no clinical significance.

More accurate surgical sampling based on SLND and more focused pathological examination of the SLND specimen by using IHC herald a new era in the management of patients with breast carcinoma. However, the uncertain significance of axillary micrometastases, especially those detected by using IHC, has created a dilemma regarding appropriate surgical or medical management. To take full advantage of technological advances, we must determine the clinical significance of axillary micrometastases in patients with invasive breast cancer. A prospective randomized study is needed to determine the clinical significance of micrometastases detected by either H&E or IHC.

In summary, SLND with focused histopathological examination of the SN is predictive of metastases in other axillary nodes (NSNs). Both the size of the SN metastasis and the number of tumor-involved SNs appear to be useful in determining the likelihood of NSN metastases. A prospective randomized trial is indicated to determine whether completion ALND after SLND has a significant impact on the clinical outcome of patients with invasive breast carcinoma, and to determine the clinical significance of axillary micrometastases, particularly those detected by using IHC. The American College of Surgeons Oncology group recently started a trial of intraoperative lymphatic mapping and SLND for breast cancer, which addresses these questions. This trial should provide information to further examine the usefulness of focused examination of the SN and its accuracy as a predictor of NSN tumor status.

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REFERENCES

1. Cady B. Case against axillary lymphadenectomy for most patients with infiltrating breast cancer. *J Surg Oncol* 1997;66:7–10.
2. White RE, Vezeridis MP, Konstadoulakis M, Cole BF, Wanebo HJ, Bland KI. Therapeutic options and results for the management of minimally invasive carcinoma of the breast: influence of axillary dissection for treatment of T1a and T1b lesions. *J Am Coll Surg* 1996;183:575–82.
3. Chontos AJ, Maher DP, Ratzer ER, Fenoglio ME. Axillary lymph node dissection: is it required in T1a breast cancer? *J Am Coll Surg* 1997;184:493–8.
4. Fein DA, Fowble BL, Hanlon AL, et al. Identification of women with T1–T2 breast cancer at low risk of positive axillary nodes. *J Surg Oncol* 1997;65:34–9.
5. Whitten TM, Fraser HR, Christensen WN, Turk PS. Axillary lymph node metastasis in stage T1a breast cancer: a pathologic review of 82 patients. *Am Surg* 1997;63:144–9.
6. Giuliano AE, Barth AM, Spivack B, Beitsch PD, Evans SW. Incidence and predictors of axillary metastasis in T1 carcinoma of the breast. *J Am Coll Surg* 1996;183:185–9.
7. Shetty MR. Axillary lymph node metastasis in carcinoma of the breast. *J Am Coll Surg* 1997;184:671–3.
8. Giuliano AE, Jones RC, Brennan M, Statman R. Sentinel lymphadenectomy in breast cancer. *J Clin Oncol* 1997;15:2345–50.
9. Turner RR, Ollila DW, Krasne DL, Giuliano AE. Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. *Ann Surg* 1997;226:271–8.
10. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. *Lancet* 1997;349:1864–7.
11. Krag D, Weaver D, Ashikaga T, et al. The sentinel node in breast cancer: a multicenter validation study. *N Engl J Med* 1998;339:941–6.
12. Chu KU, Turner RR, Hansen N, Brennan M, Bilchik A, Giuliano AE. Do all patients with sentinel node metastasis from breast carcinoma need complete axillary node dissection? *Ann Surg* 1999; 229:536–41.
13. Giuliano AE, Kirgan DM, Guenther M, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–401.
14. Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. *Ann Surg* 1995;222:394–401.
15. Fleming ID, Cooper JS, Henson DE, et al. *AJCC Cancer Staging Manual*. 5th ed. Philadelphia: Lippincott-Raven, 1997:171–80.
16. Clare SE, Sener SF, Wilkens W, Goldschmidt R, Merkel D, Winchester DJ. Prognostic significance of occult lymph node metastases in node-negative breast cancer. *Ann Surg Oncol* 1997; 4:447–51.
17. Hainsworth PJ, Tjandra, Stillwell RG, et al. Detection and significance of occult metastases in node-negative breast cancer. *Br J Surg* 1993;80:459–63.
18. Ludwig Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. *Lancet* 1990;335:1565–8.
19. Dowlatshahi K, Fan M, Snider HC, Habib FA. Lymph node micrometastasis from breast carcinoma. *Cancer* 1997;80:1188–97.
20. Wells CA, Heryet A, Brochier J, et al. The immunohistochemical detection of axillary micrometastases in breast cancer. *Br J Cancer* 1984;50:193–7.
21. Trojani M, de Mascarel I, Bonichon F, et al. Micrometastases to axillary lymph nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. *Br J Cancer* 1987;55:303–6.
22. 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;66:523–7.