Impact of Immunohistochemical Analysis of Sentinel Lymph Node Biopsy on Breast Cancer Management

ELISABETH CHÉREAU[1], CORINNE BEZU[1], JOSEPH GLIGOROV[2], RITA SAKR[1], MARTINE ANTOINE[3], EMILE DARAÏ[1], SERGE UZAN[1] and ROMAN ROUZIER[1]

Departments of [1]Gynecology and Obstetrics, [2]Medical Oncology and [3]Pathology, Tenon Hospital, Paris, France

Abstract. *Background: Little evidence of the impact of immunohistochemical analysis (IHC) on the indications for adjuvant therapies is available. This study determined the modification rate of adjuvant chemotherapy and lymph node area radiotherapy using IHC and its impact on survival. Patients and Methods: Between 2001 and 2005, 416 patients underwent surgery for invasive breast cancer with sentinel lymph node (SLN) biopsy. Results: A total of 112 patients had positive SLNs: 12.5% isolated tumor cells, 35.7% micrometastasis and 51.8% macrometastasis. Only 4% of patients (14 out of 342 patients) had modified indications for chemotherapy and 7% of patients (25 out of 342) for lymph node area radiotherapy due to IHC findings. Conclusion: IHC analysis led to modifications in adjuvant chemotherapy and lymph node area radiotherapy in 4% and 7% of patients, respectively. The prognosis of patients with nodal metastasis discovered by ultrastaging was similar to that for conventional Haematoxylin-Eosin-Safran (HES) staining. Our data support the use of SLN ultrastaging.*

Axillary lymph node status is the most important prognostic factor in breast cancer. Saphir and Amromin (1) reported that the use of a limited number of lymph node sections was insufficient to determine the presence or absence of metastases. These investigators serially sectioned lymph nodes and discovered obscure axillary lymph node metastases. Since these initial reports, many authors have reported the presence of tumor deposits in initially disease-negative axillary lymph nodes on routine histological examination (2-4). However, the frequency of these occult metastases has varied between studies. The prognostic significance of occult metastases has been debated, but its significance was strongly suggested in a previous meta-analysis (4). The examination of all axillary lymph nodes by serial sectioning is not feasible as a routine practice.

The introduction of the sentinel lymph node (SLN) procedure has reduced the number of removed nodes, which increases the feasibility for the routine use of a step-sectioning procedure with or without immunohistochemical (IHC) staining. However, the intensive examination of SLN increases the detection of isolated tumor cells and micrometastases, which has reopened the discussion for the prognostic value of these small metastases.

The detection of isolated tumor cells or micrometastases raises the question regarding the indications for complementary axillary dissection, adjuvant chemotherapy and radiotherapy. Little data on the impact of IHC on the indications for adjuvant therapies are available.

This study determined the impact of IHC on the modification rate of adjuvant chemotherapy and lymph node area radiotherapy and survival.

Patients and Methods

Between 2001 and 2005, 416 women underwent primary surgery for invasive breast cancer with SLN biopsy in Tenon Hospital, Paris, France. Individual patient records were prospectively recorded in the institutional database. Patients' and tumor characteristics (*e.g.* tumor size, number of positives nodes, hormonal receptor status, Scarff-Bloom-Richardson (SBR) grade and Ki67 rate), surgical procedure, adjuvant chemotherapy and outcomes were analyzed. The histological grade was defined according to the modified SBR system, described previously by Contesso *et al.* (5). Patients with strong IHC positivity for HER2 (3+) and patients with HER2 (2+) with amplification detected by fluorescence *in situ* hybridization (FISH) were considered as being HER2-positive.

The SLN biopsies performed between 2001 and 2003 provided important knowledge on the procedure. Our local guidelines determined the indications for SLN biopsy as unifocal T1 tumor with an invasive component and no palpable axillary lymph nodes. Fine-needle aspiration of the lymph node was performed for a clinical suspicion of axillary metastasis. SLN biopsy was performed when fine-needle aspiration did not yield objective tumor cells.

Correspondence to: Elisabeth Chéreau, Service de Gynécologie-Obstétrique, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France. Tel: +33 156017318, Fax: +33 156017317, e-mail: elisabeth.chereau@gmail.com

Keys Words: Breast cancer, sentinel node biopsy, adjuvant therapies, ultrastaging, immunohistochemical analysis.

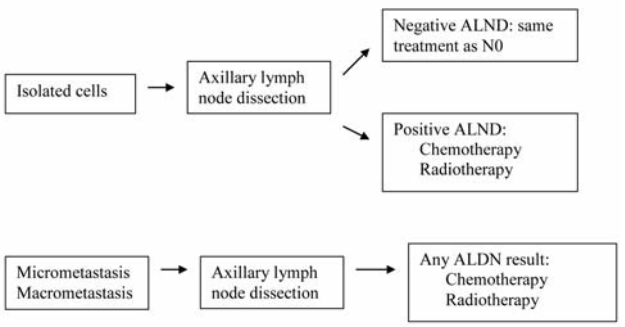

Figure 1. *Local guidelines for breast cancer therapy according to node status. ALND: axillary lymph node dissection.*

Patients with a primary history of breast surgery or neoadjuvant chemotherapy underwent primary axillary lymph node dissection (ALND) without an SLN biopsy.

SLNs were evaluated in frozen sections followed by serial section Hematoxylin-Eosin-Safran (HES) staining. IHC was performed if HES revealed no tumor cells. Lymph node staging followed the accepted pTNM classification: pN0, pN0i+ (0.2 mm, IHC+), pNmi (0.2-2 mm) and pN1a (>2 mm). All positive SLNs underwent complete ALND according to our local protocol.

Complementary ALND was performed when isolated tumor cells were identified according to our local guidelines (Figure 1). Adjuvant radiotherapy and chemotherapy were indicated when complementary ALND revealed positive non-sentinel lymph nodes. No adjuvant chemotherapy or radiotherapy was recommended when SLNs were negative for disease. Complementary ALND was also performed in cases of micro- or macrometastases, but patients underwent adjuvant chemotherapy and radiotherapy regardless of the result. Adjuvant chemotherapy was also recommended for patients with the following tumors: (i) over 20 mm in size, (ii) status of grade 3, (iii) without hormonal receptors, or (iv) present in patients less than 35 years old. Patients with grade 2 tumors (v) received adjuvant chemotherapy if the Ki67 rate was equal to or greater than 25%. Breast radiotherapy was recommended for conservative surgery in all cases of invasive tumors. Lymph node areas (supraclavicular/internal mammary) were irradiated when axillary lymph node micro- or macrometastases were identified. Postmastectomy radiotherapy was indicated for patients with disease positive axillary nodes (micro- or macrometastases) and/or T3-T4 tumors. Isolated tumor cells were considered pN- during this period, except for when micro- or macrometastases in non-sentinel lymph nodes in complementary ALND were detected. Micrometastases were systematically considered pN+.

We determined whether the diagnosis of SLN metastasis was performed using HES and/or IHC/serial sectioning and the changes in the indications for adjuvant chemotherapy and lymph node area radiotherapy according to the final lymph node status. The impact of the type of SLN detection on the prognosis was also determined.

Statistical analysis. Data were analyzed using the Fisher's exact test, the chi-squared test, and the Student's *t*-test. Survival was analyzed using Kaplan–Meier survival curves. Disease-free survival (DFS) was measured from the time of initial treatment to the date of relapse. All analyses were performed using the R package Design, Hmisc, and Survival (http://lib.stat.cmu.edu/R/CRAN/).

Table I. *Patients' and tumor characteristics.*

Number of patients	416
Median age, years (range)	57 (30-91)
Post-menopausal	285 (68%)
Tumor Characteristics	
Median size*, mm (range)	13.5 (1-70)
T1	361 (87%)
T2	52 (12.2%)
T3	3 (0.8%)
ER-positive*	364 (87.5%)
PR-positive*	274 (65.8%)
SBR	
SBR 1	191 (46%)
SBR 2	147 (35%)
SBR 3	59 (14%)
HER2-positive tumors*	31 (7.5%)
IDC	316 (76%)
ILC	48 (11.5%)
Invasive carcinoma associated with DCIS or LCIS	19 (4.5%)
Other histological type	33 (8%)
Triple negative	21 (5%)
Sentinel node (SN) procedure	416
Intraoperative analysis	134 (32%)
Positive SN on preoperative analysis	39 (9%)
Positive SN on definitive histopathology	112 (27%)
By HES	75 (67%)
By IHC	37 (33%)
Positive nodes (%)	
0	301 (72.3%)
1-3	97 (23.3%)
4-9	15 (3.6%)
>9	3 (0.8%)
Type of node metastasis	112
Isolated cells	14 (12.5%)
Micrometastasis	40 (35.7%)
Macrometastasis	58 (51.8%)
Surgery	
Lumpectomy	380 (91.3%)
Mastectomy	36 (8.6%)
Axillary lymph node dissection	170 (40.8%)
Systematic, training	97 (57%)
Because of positive SN	73 (43%)
During first surgery	32 (44%)
Secondary ALND	41 (56%)
Adjuvant therapy	
Chemotherapy	185 (44.4%)
Radiotherapy	378 (90.8%)
Endocrine therapy	365 (87.7%)
Trastuzumab	19 (4.6%)
Recurrence	38 (9%)
Axillary	6
Local recurrence	13
Metastasis	19

*Invasive component. IDC, Invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; ILC, invasive lobular carcinoma; LCIS, lobular carcinoma in situ; ER, estrogen receptor; PR, progesterone receptor; SBR, Scarff-Bloom-Richardson; ALND, axillary lymph node dissection; HES, Haematoxylin-Eosin-Safran; IHC, immunohistochemical.

Table II. *Modifications of adjuvant therapies according to node status.*

	Isolated cells	Micrometastasis	Macrometastasis
Number of patients	14 (12.5%)	40 (35.7%)	58 (51.8%)
Per operative diagnosis	1 (7%)	3 (7.5%)	34 (58.6%)
Diagnosis by HES	2 (14%)	16 (40%)	57 (98.2%)
Diagnosis by IHC	12 (86%)	24 (60%)	1 (1.8%)
ALND	11 (78%)	37 (92.5%)	55 (94.8%)
Systematic, training	3 (27%)	15 (40.5%)	12 (21.8%)
Because of positive SN	8 (73%)	22 (59.5%)	43 (78.2%)
During first surgery	1 (12%)	2 (10%)	29 (67.5%)
Secondary ALND	7 (88%)	20 (90%)	14 (32.5%)
Positive nodes on ALND	0	8 (21.6%)	24 (43.6%)
Adjuvant chemotherapy	7 (50%)	37 (92.5%)	56 (96.5%)
Indication on IHC only	0	13 (32.5%)	1 (1.8%)
Lymph node area radiotherapy	0	40 (100%)	56 (96.5%)
Indication on IHC only	0	24 (60%)	1 (1.8%)
Modification of indication of chemotherapy because of IHC		14/342 (4%)	
Modification of indication of radiotherapy because of IHC		25/342 (7%)	

SN, Sentinel node; ALND, axillary lymph node dissection; HES, Haematoxylin-Eosin-Safran; IHC, immunohistochemical.

All patients provided informed written consent for the therapeutic procedures and data analysis related to their malignancy, in accordance with institutional guidelines and the Declaration of Helsinki.

Results

A total of 416 patients were included in the present study. The population characteristics are described in Table I. The median age of the patients was 57 years, and 68% were post-menopausal. The median tumor size (invasive component) was 13.5 mm (1-70 mm). A total of 87% of tumors were T1, 12.2% of tumors were T2, and 0.8% of tumors were T3, according to the TNM classification. The most frequent SBR grade were, SBR 1 (46%) and SBR 2 (35%). The *Cerb-B2* oncogene was overexpressed in 7.5% of these tumors. The most frequent histological tumor type was invasive ductal carcinoma (76%). Only 5% of patients had hormone receptor triple-negative status. Lymph nodes were positive in 27% of patients.

Most of the patients underwent lumphectomy (91.4%) and 40.8% of patients had ALND. ALND was performed during the learning curves in 57% of cases prior to the preoperative results of the SLN examination.

A total of 112 patients had positive SLNs. SLN metastases were diagnosed using HES in 75 patients (67%) and using IHC in 37 patients (33%).

Finally, 44.4% of patients received adjuvant chemotherapy. The recurrence rate in this cohort was 9% with a median follow-up of 5.6 years for all patients.

Table II details the modifications in the indications of adjuvant chemotherapy and/or radiotherapy, according to metastasis size.

Among the patients with macrometastases, 57 of the 58 patients were diagnosed using HES. Finally, 1 of these patients received adjuvant therapies based on IHC.

Among the 40 patients with micrometastases in SLN biopsies, 40% were diagnosed using HES with serial sectioning, and 60% of these patients were diagnosed using IHC. Thirty-seven (92.5%) patients underwent ALND. Positive non-sentinel nodes were discovered in 40 (21.6%) patients. Among these 40 patients, 92.5% underwent adjuvant chemotherapy. This indication was based solely on the results of IHC in 32.5% of patients (13 of 37 patients). All patients underwent lymph node area radiotherapy, and 60% of these patients had this indication based on IHC results (*i.e.* micrometastases discovered by ultrastaging).

Among the 14 patients with isolated tumor cells, 14% were diagnosed using HES with serial sectioning, and 86% were diagnosed using IHC. Seventy-eight percent of these patients underwent ALND. None of these patients had positive non-sentinel nodes. Fifty percent of these patients underwent chemotherapy, but none of these patients underwent lymph node area radiotherapy. No indications for adjuvant therapy were based solely on IHC, because these patients were considered to be of node-negative status.

In the entire population, based on IHC, 4% of patients (14 of 342 patients with negative SLN biopsies, using HES) had a modification of chemotherapy indication, and 7% (25 of 342 patients) had a modification of lymph node area radiotherapy.

No significant difference between patients with positive SLN biopsies diagnosed using HES or IHC, were observed in DFS in patients with micrometastases (five-year DFS was

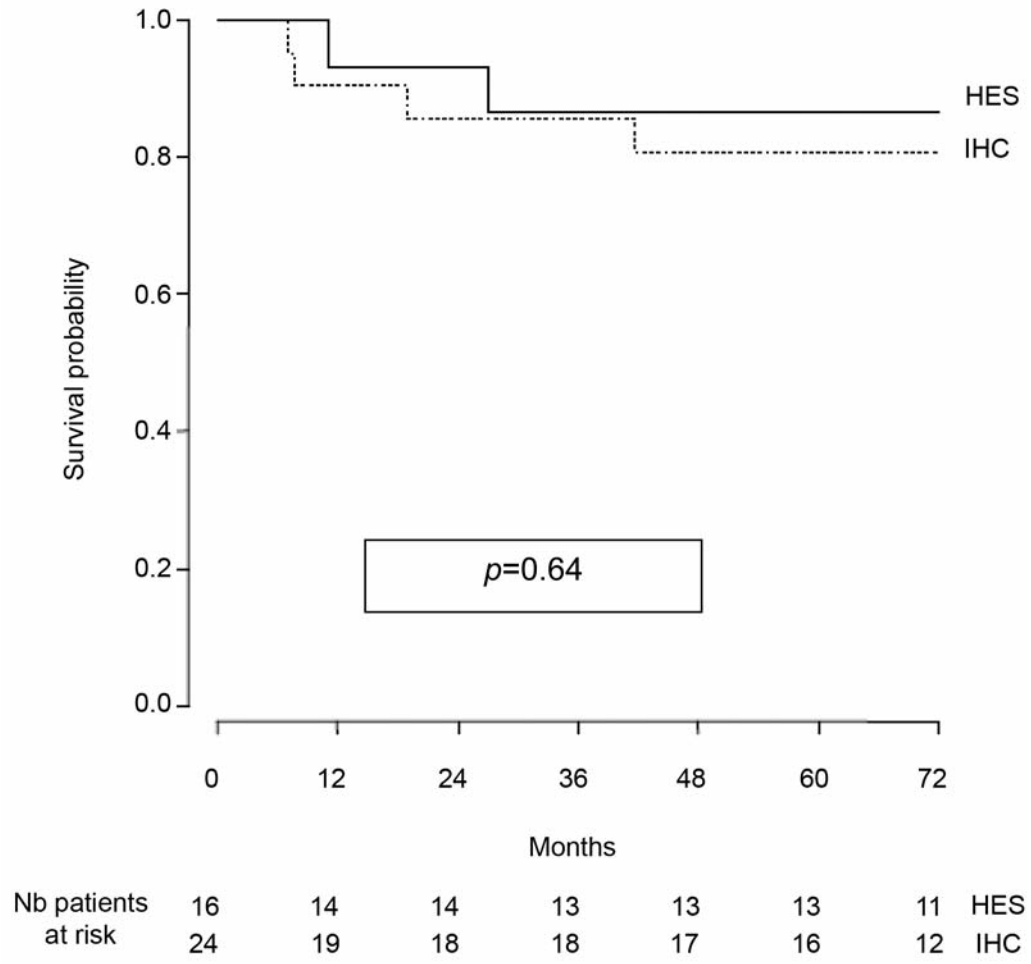

Figure 2. *Disease-free survival for patients with micrometastasis diagnosed according to Haematoxylin-Eosin-Safran (HES) and immunohistochemical analysis (IHC).*

86% for HES *vs*. 81% for IHC, p=0.64) (Figure 2). Additionally, no difference in disease-free survival in patients with nodal metastasis was observed between patients diagnosed using IHC or conventional HES (five-year DFS was 78% for conventional HES *vs*. 85% for IHC, p=0.37). A significant difference was observed between patients without nodal involvement and patients with nodal metastasis diagnosed using IHC and using HES (p=0.003) (Figure 3).

Discussion

In this study, the rate of metastases that were detected using IHC were 1.8%, 60% and 86% for macrometastases, micrometastases and isolated tumor cells, respectively. In this cohort, 4% of the patients (14 out of 342 patients) with negative SLN biopsies using HES, required a modification of their chemotherapy indication following IHC, and 7% of the patients (25 out of 342 patients) required a modification for lymph node area radiotherapy. The impact of the type of SLN detection on prognosis was determined. No significant difference in the DFS of patients with micrometastasis was observed between patients with positive SLN biopsies diagnosed using HES and those using IHC (p=0.64), nor in patients with nodal metastasis diagnosed using IHC and those using conventional HES (p=0.37).

The role of IHC in the definitive assessment of SLNs. SLN metastases were diagnosed using HES and IHC in 67% and 33% of cases, respectively. The detection rate of micrometastases varies among centers due to the use of different protocols and histopathological techniques, including single level analysis, step sectioning and serial sectioning (6). Available data suggest that 15%-48% of all SLN metastases are micrometastases, and these findings have led to an upstaging from 9% to 25% in initially node-negative patients (2, 4, 7).

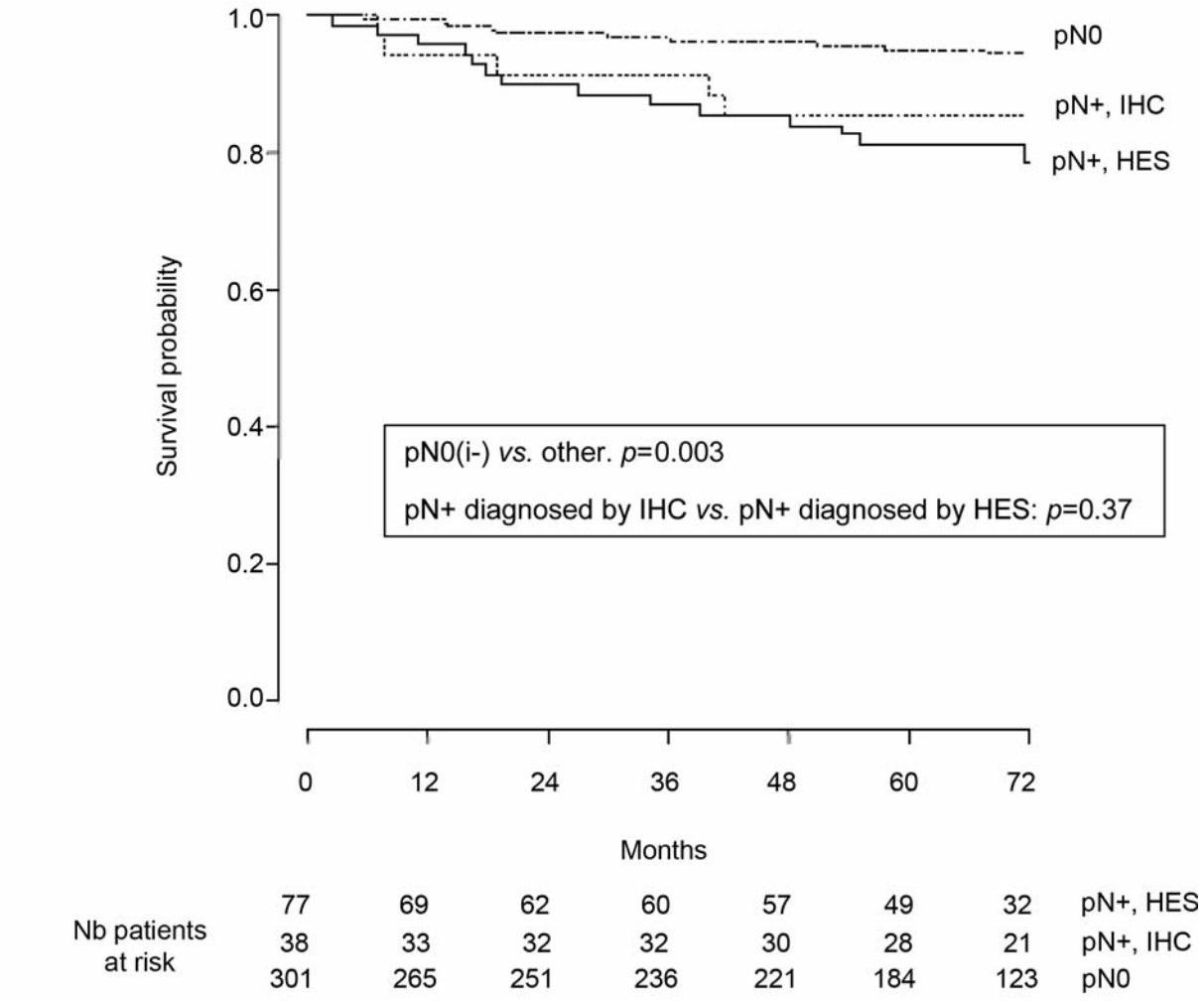

Figure 3. *Disease-free survival for patients without nodal involvement or with nodal involvement diagnosed according to Haematoxylin-Eosin-Safran (HES) and immunohistochemical analysis (IHC).*

The introduction of IHC techniques that target epithelial cytokeratins has modified the definitive assessment of SLN, particularly SLNs that appear negative using conventional HES. The IHC approaches have enabled the detection of otherwise occult metastases and additional extremely small lesions that would otherwise have been missed. The Philadelphia Consensus Meeting (8) recommended serial sections of less than 2 mm for the detection of macrometastases. The addition of IHC was not routinely recommended. This recommendation is consistent with the guidance from the American Society of Clinical Oncology because isolated cancer cells that are detected by the pathological examination of SLNs using specialized techniques were of unknown clinical significance in 2005 (9). The European recommendations in 2003 suggest screening for micrometastases, and they advise against routine IHC analysis for the same reasons (6). In a recent retrospective study, Weaver *et al*. confirmed that occult metastases were an independent prognostic variable in patients with SLNs that were negative on initial examination; the difference in outcome at five years, was small but significant (1.2 percentage points). However, these data do not indicate the clinical benefit of additional evaluation, including IHC analysis, of initially negative sentinel nodes in patients with breast cancer (10), if no treatment modification is proposed, or if the treatment modification is inefficient.

Impact of diagnosis of micrometastases and isolated tumor cells in SLNs. Although several studies on the association between isolated tumor cells and micrometastases in lymph nodes and survival have been published, none of these studies

have provided a complete and systematic overview of the existing evidence (11-21). Similarly, de Boer *et al*. (4) evaluated the association between occult metastases, isolated tumor cells, and micrometastases in axillary lymph nodes of patients with invasive breast cancer and DFS and overall survival (OS). This study revealed that the presence (*vs*. the absence) of occult metastases was associated with a poorer 5-year DFS [pooled(RR)=1.55, 95%(CI)=1.32 to 1.82] and OS [pooled(RR)=1.45, 95%(CI)=1.11 to 1.88], although these endpoints were not consistently assessed in multivariable analyses. The authors concluded that the presence (*vs*. the absence) of metastases of 2 mm or less in diameter in axillary lymph nodes that were detected on single-section examination was associated with lower DFS and OS rates, but the independent prognostic value of occult metastases, including isolated tumor cells, micrometastases, and macrometastases, after an intensive pathological assessment of all axillary lymph nodes, remained undetermined. In our study, the prognosis of patients with metastases that were detected by ultrastaging and HES was similar but poorer than that of pN0(i-) patients. This is consistent with the analyses of Weaver *et al*. and de Boer *et al*.

Our data quantified the therapeutic changes in surgery, chemotherapy, and radiation therapy. Changes in chemotherapy and radiation therapy for micrometastases, but not isolated tumor cells, were examined because isolated tumor cells were considered to confer pN– status and micrometastases pN+. However, this decision was based on the fact that the pejorative value of isolated tumor cells is less (or lacking) compared to micrometastases or macrometastases, a fact that is debatable. This type of division was not performed by de Boer *et al*. (4) or Weaver *et al*. (10). However, the presence of isolated tumor cells is an independent prognostic factor in the NSABP32 analysis (22). Therefore, the prognostic value of isolated tumor cells should be reconsidered based on these latest publications.

Some authors suggest that complementary ALND is not worthwhile and does not improve prognosis. Complementary ALND may increase survival by reducing residual disease in non-SLNs with metastasis. The number of such patients is estimated at 15% (23), but this value was null in our series. However, non-SLNs were only subject to conventional histological assessment. Our current adjuvant treatment strategy includes complementary ALND only for patients with isolated tumor cells from a tumor with no indication of chemotherapy, based on biological factors (*e.g*. grade and hormone receptor status) and tumor size. Of note, only seven (1.7%) patients in our previous series were of this case type. The impact of adjuvant chemotherapy on prognosis modification is probably limited because these patients have limited disease that is unlikely to be chemosensitive (low proliferation). Our results suggest that a demonstration of the survival impact of complementary ALND using a randomized controlled trial is not feasible. Therefore, the decision to perform complementary ALND may be empirically based on the adjuvant chemotherapy indication. However, this latter point requires reconsideration in the light of the prognostic value of isolated cell highlighted in recent publications.

In our study, the most important therapeutic change occurred in the radiation field (7%), which had not been evaluated previously. In contrast to chemotherapy, radiation is administered based on tumor burden factors but not biological factors. Unfortunately, our series was too limited to determine the impact of radiotherapy modification on survival. Therefore, the most important treatment for occult metastases, including complementary ALND, chemotherapy or radiation therapy, was not determined; additional studies are required in order to perform this determination.

Funding Sources

None.

Conflict of Interests

None.

References

1 Saphir O and Amromin GD: Obscure axillary lymph-node metastasis in carcinoma of the breast. Cancer *1*: 238-241, 1948.

2 Cote RJ, Peterson HF, Chaiwun B, Gelber RD, Goldhirsch A, Castiglione-Gertsch M *et al*: Role of immunohistochemical detection of lymph-node metastases in management of breast cancer. International Breast Cancer Study Group. Lancet. England p. 896-900, 1999.

3 Cummings MC, Walsh MD, Hohn BG, Bennett IC, Wright RG and McGuckin MA: Occult axillary lymph node metastases in breast cancer do matter: results of 10-year survival analysis. Am J Surg Pathol *26*: 1286-1295, 2002.

4 de Boer M, van Dijck JA, Bult P, Borm GF and Tjan-Heijnen VC: Breast cancer prognosis and occult lymph node metastases, isolated tumor cells, and micrometastases. J Natl Cancer Inst. United States p. 410-425, 2010.

5 Contesso G, Mouriesse H, Friedman S, Genin J, Sarrazin D and Rouesse J: The importance of histologic grade in long-term prognosis of breast cancer: a study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. J Clin Oncol 5: 1378-1386, 1987.

6 Cserni G, Amendoeira I, Apostolikas N, Bellocq JP, Bianchi S, Bussolati G *et al*: Pathological work-up of sentinel lymph nodes in breast cancer. Review of current data to be considered for the formulation of guidelines. Eur J Cancer England p. 1654-1667, 2003.

7 Weaver DL, Krag DN, Ashikaga T, Harlow SP and O'Connell M: Pathologic analysis of sentinel and nonsentinel lymph nodes in breast carcinoma: a multicenter study. Cancer. United States: 2000 American Cancer Society p. 1099-1107, 2000.

8 Schwartz GF, Giuliano AE and Veronesi U: Proceedings of the consensus conference on the role of sentinel lymph node biopsy in carcinoma of the breast, April 19-22, 2001, Philadelphia, Pennsylvania. Cancer *94*: 2542-2551, 2002.

9 Lyman GH, Giuliano AE, Somerfield MR, Benson AB, 3rd, Bodurka DC, Burstein HJ *et al*: American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol United States p. 7703-7720, 2005.
10 Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, Harlow SP *et al*: Effect of occult metastases on survival in node-negative breast cancer. N Engl J Med *364*: 412-421, 2011.
11 Dowlatshahi K, Fan M, Snider HC and Habib FA: Lymph node micrometastases from breast carcinoma: reviewing the dilemma. Cancer. United States p. 1188-1197, 1997.
12 Gray RJ, Cox CE and Reintgen DS: Importance of missed axillary micrometastases in breast cancer patients. Breast J. United States p. 303-307, 2001.
13 Mittendorf EA and Hunt KK: Significance and management of micrometastases in patients with breast cancer. Expert Rev Anticancer Ther *7*: 1451-1461, 2007.
14 Noguchi M: Therapeutic relevance of breast cancer micrometastases in sentinel lymph nodes. Br J Surg *89*: 1505-1515, 2002.
15 Quan ML and Cody HS 3rd: Missed micrometastatic disease in breast cancer. Semin Oncol. United States p. 311-317, 2004.
16 Rutgers EJ: Sentinel node biopsy: interpretation and management of patients with immunohistochemistry-positive sentinel nodes and those with micrometastases. J Clin Oncol. United States p. 698-702, 2008.
17 Sakorafas GH, Geraghty J, Pavlakis G: The clinical significance of axillary lymph node micrometastases in breast cancer. Eur J Surg Oncol. England p. 807-816, 2004.
18 Steinhoff MM: Axillary Node Micrometastases: Detection and Biologic Significance. Breast J p. 325-329, 1999.
19 Tjan-Heijnen VC, Buit P, de Widt-Evert LM, Ruers TJ and Beex LV: Micro-metastases in axillary lymph nodes: an increasing classification and treatment dilemma in breast cancer due to the introduction of the sentinel lymph node procedure. Breast Cancer Res Treat *70*: 81-88, 2001.
20 Wada N and Imoto S: Clinical evidence of breast cancer micrometastasis in the era of sentinel node biopsy. Int J Clin Oncol *13*: 24-32, 2008.
21 Weaver DL: Sentinel lymph nodes and breast carcinoma: which micrometastases are clinically significant? Am J Surg Pathol. 27: 842-845, 2003.
22 Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP *et al*: Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol. England: 2010 Elsevier Ltd p. 927-933, 2010.
23 Viale G, Maiorano E, Mazzarol G, Zurrida S, Galimberti V, Luini A *et al*: Histologic detection and clinical implications of micrometastases in axillary sentinel lymph nodes for patients with breast carcinoma. Cancer. United States: 2001 American Cancer Society p. 1378-1384, 2001.

Received March 19, 2012
Revised April 18, 2012
Accepted April 20, 2012