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Primary Breast Cancer Features Can Predict Additional Lymph Node Involvement in Patients with Sentinel Node Micrometastases

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Abstract

Objective: The aim of this retrospective study was to identify biological features of primary breast cancer from which to predict the presence of further axillary involvement in patients bearing micrometastases in the sentinel lymph node (SLN).

Methods: From a starting group of 690 patients, we isolated patients with micrometastases in the SLN. Those patients were classified according to the presence/absence of further metastases in nonsentinel lymph nodes (NSLNs). We examined primary tumor features to identify any relevant difference. Analysis of primary tumors evaluated histology, tumor size, lymphovascular invasion, mitotic index (Mib-1), estrogen and progesterone receptor status (ER/PR status), C-erb B-2 (HER-2/neu) expression and amplification, and p53 expression. Chi square analysis for statistical significance was applied.

Results: Of the original 690 patients, 296 showed some kind of metastases in the SLN; 238 patients had gross metastases in the SLN. After axillary lymph node dissection (ALND), 102 patients (43%) had NSLNs with metastases, and 136 (57%) had negative axillary non-sentinel nodes. Another 58 patients harbored solitary micrometastases in the SLN. After ALND, 8 (14%) patients had further NSLN involvement, and 50 (86%) had negative axillary nodes.

Conclusions: Analysis of the primary breast lesion in patients with micrometastatic SLN and metastatic NSLNs revealed the presence of lymphovascular invasion, Mib-1 index > 10%, and tumor size > 2 cm. Patients without lymphovascular invasion, Mib-1 < 10% and T size < 2 cm could avoid further ALND.

Axillary lymph node status is the single most important prognostic factor in patients with early breast cancer; however, about 25% of lymph node-negative patients still develop lymph node and distant metastatic disease.[1,2] Detection of micrometastases in sentinel lymph node (SLN) has been incorporated into the staging system and resulted in upstaging of many breast tumors. However, the significance of micrometastases remains controversial. On the one hand, several investigators have found that the presence of micrometastases in SLN correlates with the absence of metastases in nonsentinel lymph nodes (NSLN),[2,3] or at least the risk of macrometastases in the NSLN in patients is minimal.[4] A recent investigation suggests that the presence of SLN micrometastases does not lead to axillary recurrence or distant disease and therefore supports the theory that

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formal axillary lymph node dissection (ALND) may be omitted in these patients.[5]

On the other hand, other investigations show[6–9] that up to 26% of patients with micrometastases in the SLN were found to have disease in the corresponding NSLN, whereas a recent study indicates that the risk of non-SN metastases with micrometastasis in the SN is around 10% or 15%, depending on the method of detection of SN involvement.[10] Again, the clinical significance of micrometastases is unclear in itself: some authors were not able to find a correlation or effect on recurrence or survival rates,[11–13] but other studies suggest that micrometastases can be considered as gross metastases. Sedmak and colleagues[14] studied the disease-free and overall survival of patients with micrometastases and found that their survival curves were significantly worse than those for patients without micrometastases, and recent reviews and papers confirm this.[15,16]

Axillary lymph node dissection is a surgical procedure that can lead to significant morbidity. Therefore, it would be of great value if we had a guide for estimating the risk of NSLN involvement: the low-risk subgroup could safely avoid any further ALND. About NSLNs, Cox and colleagues[17] emphasized the role of NSLNs in the prognosis of patients with breast cancer, demonstrating in their study that the total number of NSLNs was predictive of survival. Turner and colleagues[6] demonstrated that the size of the primary tumor and the size of the SLN metastasis are associated with the presence of NSLN metastases. They also found that extranodal hilar tissue invasion and lymphovascular invasion were significantly associated with NSLN metastases. It has also been reported that the vascularity of the primary tumor, used as an index of angiogenesis and vascular invasion, correlates with the presence of bone marrow micrometastases.[1]

In this retrospective study of ours, all the SLNs and NSLNs (where ALND was performed) of 690 patients were reviewed and reclassified as bearing micrometastases or gross metastases. We identified a subgroup of patients with micrometastases in the SLN and metastastic disease in the NSLN, and we examined the primary tumor-related characteristics of these patients in order to identify any biological marker able to predict the presence of metastatic disease in NSLNs.

MATERIALS AND METHODS

Histopathological features of 690 patients with early breast cancer treated between November 1997 and December 2004 were reviewed. Patient mean age was 63 years (range: 28–83 years); all patients were women. All tumors were 3 cm in diameter or less and had clinically negative axillary lymph nodes, and all patients underwent operation in our department. Of the 690 patients, 523 (76%) underwent breast conservation surgery (lumpectomy or quadrantectomy, with radio-guided localization if required); another 167 (24%) patients underwent mastectomy.

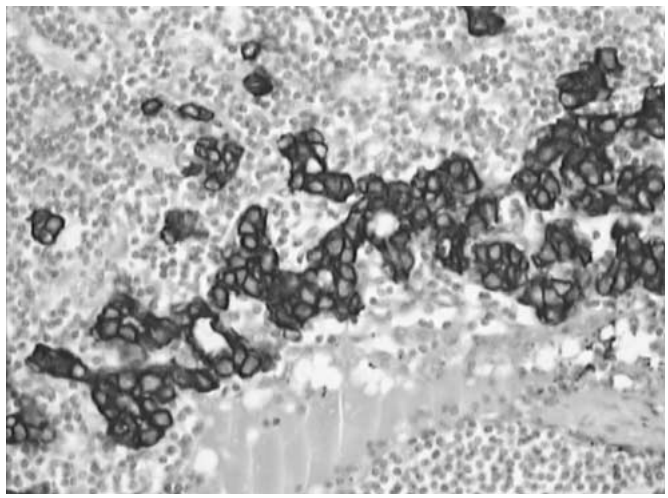

Figure 1. Lymph node micrometastasis (sampled with IHC).

All patients underwent SLN biopsy. Ethical permission was asked and granted by the Local Ethics Committee. To localize SLN, we injected 0.4 cc of Nanocoll (99m-Tc-labeled human albumin, Amersham Health, Italy) under sonographic or stereotactic guidance the morning of surgery (average dose, 130 MBq, range: 110–155 MBq). ScintiProbe (GIO, Italy) was used to localize the SLN. In the first 50 cases, during surgery, we injected subdermally 1.0 cc of lymphazurin 1% (Hirsch Industries, Inc, Richmond, VA); then SLN localization was radioguided only. Each SLN was subjected to multiple sectioning and gross histopathologic analysis using hematoxylin and eosin (H&E) staining. Immunohistochemistry (IHC) was performed on 20 sections to detect cytokeratin (MAK-6, Ciba-Corning, CA).

All patients with SLN micrometastases or gross metastases underwent ALND. Micrometastases were defined as breast cancer deposits (negative by H&E, if that was the case, but at least positive by IHC; see Fig. 1) between 0.2 and 2 mm (major diameter). Isolated tumor cells were not considered as micrometastases, and the SLN was deemed to be free of disease.

In the starting group of 690 patients, we isolated patients with micrometastases in the SLN. Again, patients with SLN micrometastases were classified according to the presence/absence of metastases in NSLN. We

examined primary tumor features in both patients with and without metastases in the NSLNs, in order to identify any relevant difference. Analysis of primary tumors evaluated histology, tumor size, lymphovascular invasion (LVI), mitotic index (Mib-1), estrogen and progesterone receptor status (ER/PR status), C-erb B-2 (HER-2/neu) expression (staining) and amplification (fluorescence in-situ hybridization, FISH), and p53 expression.

Chi square analysis for statistical significance was applied for comparisons between the two groups.

RESULTS

The features of the starting cohort (690 patients) are summarized in Table 1. The starting cohort consisted of 8% patients with T1a breast cancer, 47% with T1b, 29% with T1c, and 16% with T2 (always with T < 3 cm) breast cancer. Mean dimension of the primary tumor was 1.3 cm (range: 0.5–3 cm). Tumors were localized to the upper outer quadrant in 42% of cases, lower outer quadrant in 16%, upper inner quadrant in 13%, lower inner quadrant in 12%. In this series, 17% of the patients presented with sub-areolar or peri-areolar tumors. The histopathologic diagnoses of the primary tumors were as follows: invasive ductal carcinoma (65% of cases), invasive lobular carcinoma (24%), invasive tubular (4%), invasive mucinous carcinoma (2%), papillary carcinoma (1%), and other diagnoses (in situ carcinoma—both ductal and lobular, medullary carcinoma, malignant lymphoma, mixed lesions) in 25 cases (4%).

Sentinel lymph node localization and excision was successful in 682 patients of the 690 (99%); 296 patients of 682 (43%) showed metastatic disease in the SLN; 260 SLNs with metastases were located in the axilla (88%), 21 in the internal mammary chain (7%), and 15 both in the axilla and the internal mammary chain (5%). About primary breast cancer, invasive ductal carcinoma was the most frequent histology (491 cases, 72%); 238 patients were found to have gross metastases in the SLN. After ALND, 102 patients (43%) had NSLNs with metastases, and 136 (57%) had negative axillary nonsentinel nodes.

Fifty-eight patients harbored micrometastases in the SLN, and these constituted our subset of interest (see Fig. 2). Micrometastases were always solitary. After ALND, 8 (14%) patients had further NSLN involvement, and 50 (86%) had negative axillary nodes.

Statistical analysis of the primary breast lesion in patients with micrometastatic SLNs and metastatic NSLNs revealed a strong association with the presence of LVI ($P < 0.05$), Mib-1 index > 10% ($P < 0.05$), and tumor size > 2 cm ($P < 0.03$). In fact, as regards patients with additional NSLN involvement, 6 patients out of 8 presented with a primary breast lesion between 2 and 3 cm in size, whereas only 5 patients out of 50 had tumors greater than 2 cm in diameter in the group without any further nodal involvement. Again, only 2 patients with additional involvement had no LVI or Mib-1 < 10%, and only 3 patients without NSLN involvement had LVI or Mib-1 > 10%. These distributions are summarized in Table 2.

As a consequence, patients with micrometastatic SLN, but with no metastases in the NSLNs, showed smaller

Table 1.
Features of the starting cohort (690 patients, mean age 63 years)

	Number	Percentage
Dimension		
T1a	55	8
T1b	324	47
T1c	198	29
T2 (< 3 cm)	113	16
Localization		
UOQ	289	42
LOQ	111	16
UIQ	92	13
LIQ	81	12
PA	117	17
Surgery		
Conservative	523	76
Mastectomy	167	24
Histology		
I. duct.	448	65
I. lob.	166	24
I. tub.	28	4
Other	48	7

UOQ: upper outer quadrant; LOQ: LOQ: lower outer quadrant; UIQ: upper inner quadrant; LIQ: lower inner quadrant; PA: sub-areolar or peri-areolar. I. duct.: invasive ductal carcinoma; I. lob.: invasive lobular carcinoma, I. Tub.: invasive tubular carcinoma, other. in-situ carcinoma, medullary carcinoma, malignant lymphoma, mixed lesions.

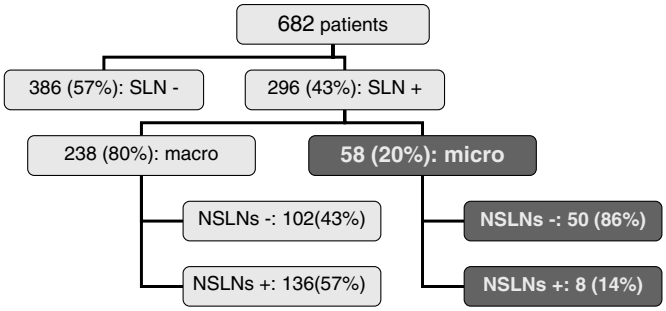

Figure 2. Selection of patients. –: free of metastases; + : metastatic node(s); macro: gross metastases; micro: micrometastases.

Table 2.
Distribution of larger tumor lesions, lymphovascular invasion (LVI), and high mitotic activity between patients with micrometastatic sentinel lymph node and with or without additional nonsentinel lymph node (NSLN) involvement

	T > 2 cm	LVI	Mib-1 > 10%
Patients with further NSLN +	6 / 8	6 / 8	6 / 8
Patients NSLN –	5 / 50	3 / 50	3 / 50

size breast lesions (T < 2 cm), no LVI, and less mitotic activity with Mib-1 index > 10%. Four of these patients (less than 10%) were found to have positive signal for C-erb B-2 (HER-2/neu) and p53.

Analysis of histology, hormonal receptor status, C-erb B-2 amplification, and p53 expression did not show any statistically significant difference between the two groups.

DISCUSSION

The aim of this study was to identify those patients with micrometastatic SLN that are likely to have NSLN metastases. To do that, we evaluated some biological features of primary breast lesions: histology, tumor size, lymphovascular invasion, mitotic index (Mib-1), estrogen and progesterone receptor status (ER/PR status), C-erb B-2 (HER-2/neu) expression, and p53 expression. The choice of these features was made for two reasons: first, in literature several papers emphasize the role of some aspects such as tumor size and lymphovascular invasion as predictive factors for the presence of axillary metastases.[6,18,19] Second, those parameters could be detected quite easily in any academic pathology department without any special instrumentation or skills.

In early breast cancer, micrometastases in SLN carry just a low risk of NSLN involvement, whereas gross SLN metastases do not. In this series, only 8 of 58 patients with micrometastases in the SLN had further NSLN metastases (14%), whereas 43% of patients with gross SLN metastases presented with further axillary involvement. This simple study identifies a subset of patients with micrometastatic SLN disease who are likely to have additional metastatic disease in the NSLNs. This ''high-risk'' subset was found to present invasive carcinoma with LVI, Mib-1 index > 10%, and tumor size > 2 cm. In contrast, in this study patients with micrometastases in the SN and without NSLN metastases had smaller breast lesions and no peritumoral LVI.

Two consequences can be drawn from these findings:

- First, we can separate a subset of patients with SLN micrometastases and the most favorable combination of predictive factors that could avoid ALND in clinical trials. In fact, those patients are very unlikely to have NSLN metastatic disease, although further studies are required to confirm the predictive power of these biological characteristics. Sentinel lymph node micrometastases are quite rare: we started from an initial group of about 700 patients, but from that group we isolated only 58 patients bearing SLN mcrometastases. Then, multivariate analysis could be useful in estimating every single parameter.
- Second, patients with micrometastatic SLNs and carcinoma with LVI, Mib-1 index > 10%, and tumor size > 2 cm are likely to have additional axillary metastases. Therefore, in this subset of patients a more accurate NSLN pathological evaluation could be beneficial, in detecting both gross disease and small metastases. In these selected cases, NSLN evaluation should be similar to what is routinely performed for SLNs, with multisectioning and immunohistochemical staining for cytokeratin.

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