Predictive Factors for Metastatic Involvement of Nonsentinel Nodes in Patients With Breast Cancer

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Background: The potential morbidity of an axillary lymph node dissection in patients with breast cancer can be avoided in patients with a negative sentinel node (SN).

Hypothesis: It may be possible to identify a subset of patients with a positive SN and without metastases in the remaining axillary lymph nodes.

Design: Case-control study.

Setting: Both primary and referral hospital care.

Patients: Data were studied for 255 consecutive patients with stage T1 or T2 breast cancer who had a successful identification of the SN.

Interventions: In patients with a positive SN, histological examination of all non-SNs that were negative by routine examination was the same as that for SNs (multiple sectioning and immunohistochemical analysis).

Main Outcome Measures: The incidence of non-SN metastases was correlated with the surface area and number of SN metastases and primary tumor characteristics. A micrometastasis was defined as less than 1 mm².

Results: Of 255 patients, the SN appeared to be positive in 93 (36%). Subsequent axillary lymph node dissection revealed positive non-SNs in 46 patients (49%). Patients with a single positive SN and patients with metastases less than 1 mm² in the SN had significantly less non-SN involvement than patients with more than 1 positive SN (40% vs 78%) and patients with macrometastases (27% vs 49%).

Conclusions: The incidence of non-SN metastases seemed to be related to the number of positive SNs and the size of SN metastases. However, in the group of patients with a positive SN, it was not possible to identify a subset of patients without non-SN metastases.


M ost patients who are eligible for a curative resection of breast cancer will not have axillary lymph node metastases. The sentinel node (SN) biopsy can now accurately identify this group of patients1-4 and an axillary lymph node dissection (ALND) can be limited to patients with a positive SN. Moreover, the SN biopsy has the potential of becoming a routine procedure in centers with surgeons who have acquired adequate experience.5,7 Several studies have shown that the SN seems to be the only positive axillary node in more than 50% of patients who have undergone a completion ALND.1,3,5,9,10 It has been suggested that primary tumor size and SN tumor load both correlate with the incidence of additional metastases in the remaining axillary lymph nodes.9,10 The chances of finding non-SN metastases are particularly low in patients with micrometastases in the SN, and the question is whether all patients with breast cancer with a positive SN will actually require an ALND.

This question must be seen in view of the fact that multiple sectioning of SNs and the use of immunohistochemical analysis may result in a 5-fold increase in the detection of lymph node metastases compared with routine histological examination.11 This has resulted in the controversial situation that occurs when an SN biopsy is performed to avoid an ALND in patients with a negative axilla, while more axillary metastases are detected than before. Most of these will be micrometastases.12

The purpose of this study was to assess the incidence of non-SN axillary nodal metastases in patients with positive SN biopsy findings and to examine whether a subset of patients can be identified in whom a completion ALND can be safely
PATIENTS AND METHODS

PATIENTS

The data from 255 consecutive patients who underwent a successful SN procedure for stage T1 and T2 breast cancer (according to the AJCC TNM staging system) at our institution were studied. Each patient in whom the SN was positive underwent an ALND. A successful procedure was defined as the ability to identify the SN according to the criteria described in detail elsewhere.7 Sentinel nodes were defined as all radioactive nodes that were excised; non-SNs were defined as all nonradioactive nodes removed either during the SN procedure (ie, palpable nodes) or the completion ALND. In patients with a single positive SN, a number of factors, including age, primary tumor characteristics, size of the SN metastases, and mode of detection, were evaluated for their predictive value for the incidence of non-SN axillary metastases.

SN PROCEDURE

The technical aspects and criteria used for the SN procedure are described in detail elsewhere.1,7,13 In brief, surgery was performed within 24 hours after injection of the radioactive tracer. Before the incision, 0.5 mL of 2.5% Patent Blue V solution (Guerbet, Aulnay-sous-Bois, France) was injected. Prior to surgery the SN was localized with a handheld gamma probe (Navigator; Radiation Monitoring Devices, Watertown, Mass). Dissection in the axillary fat was guided by the gamma probe, and the SN was identified by focal tracer activity and blue staining. The number of radioactive nodes that were resected was determined by the residual radioactive count in the axilla, which should be less than 10% of radioactivity of the most radioactive node (ex vivo count with the gamma probe). Palpation of the open axilla was performed to detect enlarged non-SNs. These were excised and underwent histological examination separately.

RESULTS

Of 255 patients who underwent a successful SN procedure, 162 (64%) had no metastases to the SN, 70 (27%) had a single positive SN, and 23 (9%) had more than 1 positive SN. The average number of radioactive nodes resected was 1.7 per patient (range, 1-4 nodes). In patients with a single positive SN, more than 1 radioactive node was resected in 30 cases (43%).

All 93 patients with 1 or more positive SN underwent ALND. Routine histological examination revealed non-SN metastases in 34 patients. More extensive sectioning and the use of immunohistochemical analysis was performed in the remaining 59 patients and revealed non-SN metastases in an additional 12 patients. Therefore, the overall incidence of non-SN metastases in patients with a positive SN was 49% (46 of 93). All 12 patients who were found to have non-SN metastases after more extensive examination of the axillary lymph nodes had small metastatic deposits in only 1 or 2 nodes.

With increasing primary tumor size (T1a, T1b, T1c, and T2 tumors), the SN was found to be positive in 17%, 24%, 33%, and 53% of patients (P = .002). However, the incidence of non-SN metastases was the same (50%) regardless of primary tumor size (Table 1).

Non-SN metastases were found in 18 (78%) of 23 patients with multiple positive SNs and in only 28 (40%) of 70 patients with a single positive SN (P = .002, Table 2).

HISTOPATHOLOGIC EXAMINATION

Sentinel Nodes

Lymph nodes smaller than 1 cm in diameter were halved, and lymph nodes that were 1 cm were lamellated into pieces approximately 0.5 cm in size. If the initial paraffin sections were negative, 4-step ribbons were cut from each block at 250-µm intervals. From each ribbon, one section was stained with hematoxylin-eosin, and one was used for standard immunohistochemical analysis with CAM5.2 (Beckton Dickinson, San Jose, Calif) to facilitate detection of micrometastases. In this way, 10 slides were systematically prepared from each block when the original hematoxylin-eosin section was negative. Surface area of the metastases was measured with an interactive video overlay system (QPRODIT; Leica, Cambridge, England) by tracing the metastases with a mouse on the computer screen at appropriate magnifications. Micrometastases were defined as those with an area less than 1 mm². For the purpose of this study, all SNs with tumor metastatic deposits smaller than 3 mm² were submitted to standard step sectioning, also if metastases were already apparent on the routine first section with hematoxylin-eosin. In this fashion we could be sure that the largest metastatic surface area was measured for each microscopic SN metastasis.

Nonsentinel Nodes

All non-SNs were initially examined by 1-level hematoxylin-eosin staining. In patients with a positive SN, all non-SNs that were negative on initial examination underwent multiple-step sectioning and immunohistochemical analysis as for the SN.

Primary Tumor

Mitoses were counted as described previously14 in 10 fields of vision at original magnification ×400 (total area, 1.59 mm²) in the most poorly differentiated area in the periphery of the tumor, taking the total as the mitotic activity index.15 The estrogen receptor status was determined by standard immunohistochemical analysis (Dako, Glostrup, Denmark) and evaluated according to the Histo-score.16 Histologic tumor grading was performed according to Elston and Ellis.17
In the 70 patients with a single SN positive for metastatic involvement, micrometastases were found in 30 patients (43%). Intraoperative frozen section analysis of the SN in 43 of these 70 patients detected only 15% of micrometastases but 91% of macrometastases (Table 3). The micrometastases were seen with hematoxylin-eosin and multiple sectioning in 18 patients and with immunohistochemical analysis only in the remaining 12 patients. Non-SNs harbored metastases in 27% of patients with SN micrometastases and in 50% of patients with SN metastases larger than 1 mm² (Table 4). In the patients with micrometastases, non-SNs were positive in 8 patients (6 of 18 metastases detected with hematoxylin-eosin and 2 of 12 metastases detected with immunohistochemical analysis). Of these 8 patients, 3 were found to have palpable grossly involved metastatic non-SNs during exploration of the axilla. In the remaining 5 patients, the positive non-SNs were single nodes with micrometastases, of which 4 were seen only after multiple sectioning and immunohistochemical analysis.

Of all tumor-related factors, only a positive estrogen receptor status was related to a lower incidence of non-SN metastases, but this was not statistically significant (P = .21). Small tumor size or nonpalpability and a low mitotic activity index did not decrease the chances of non-SN metastases being present (Table 4).

**COMMENT**

In patients with breast cancer, ALND is performed for staging and locoregional control, and the presence of axillary metastases has important prognostic implications. The SN biopsy procedure now seems to be a reliable, minimally invasive technique that allows for an accurate determination of axillary nodal involvement. An ALND can be avoided in most patients who are proven to have a negative SN.1-3,6,8,18,19 Accurate axillary staging by means of the SN biopsy is not only dependent on the reliability of the procedure but also on the extent of histological examination of the SN. To which extent this should be performed is still a matter of debate.20 Hartveit21 proposed that metastatic tumor cells are not distributed throughout the node in random fashion, but along the
hilar axial plane first, thereby suggesting that if the proper technique of lymph node sectioning was observed, additional sectioning would not be necessary. This was also suggested by Turner et al in a more recent study in relation to the sectioning of SNs. However, in practice it may not always be possible to accurately identify the hilar orientation of the lymph node, and the advantage of the SN procedure is that more elaborate sectioning and immunohistochemical analysis can be limited to only a few nodes. Moreover, it has been shown that both the number of sections obtained and the use of immunohistochemical analysis will increase the number of metastases that will be detected. This in turn will result in an increase in the proportion of patients with micrometastases in the SN. Both the definition and the clinical significance of a micrometastasis has been a matter of extensive debate. The cutoff point for a micrometastasis has varied between 0.2 mm and 2.0 mm in diameter in published studies. Huvos et al are the first to arbitrarily define a micrometastasis as being less than 2 mm in diameter, and this cutoff point has been used by most investigators since then. Fisher et al used an additional cutoff point of 1.3 mm because this represented the largest metastasis that was not detected by routine histological examination. In this study, most metastases smaller than 1 mm² required multiple sectioning before being detected. This is clearly illustrated in this study by the fact that in an intraoperative frozen (single) section, only 15% of metastases less than 1 mm² were detected compared with 91% of metastases larger than 1 mm².

It was found in this study that one third of patients with a positive SN had no more than micrometastatic tumor deposits (<1 mm²) in the SN. The increased detection rate of micrometastases in the SN is expected to result in an increase of the proportion of patients in whom the axillary metastases will be limited to the SN. Indeed, we found that metastases were limited to the SN in 73% of patients (22/30) with micrometastases, whereas this was the case in 51% of patients (20/40) with single-node macrometastatic involvement and in only 22% of patients (5/23) with more than 1 SN involved.

Therefore, in patients with single-node metastatic involvement, most will not receive any benefit from a completion ALND, and the question has arisen whether all patients with a positive SN may need a completion ALND. Although the SN is reported to be the only positive axillary node in more than 50% of patients in most studies, there seems to be a considerable range from 38% to 67%. The extent of examination of axillary lymph nodes may play a role in this respect. It is known from earlier studies that 9% to 31% of patients may be converted to a diagnosis of node positive with repeated sectioning of lymph nodes that were negative on routine histological examination. In this study 37% of patients (34/93) with a positive SN had non-SN metastases after routine examination, which increased to 49% (46/93) after more extensive sectioning and with the use of immunohistochemical analysis. This is quite similar to the findings of Chu et al who also showed an increase in non-SN metastases (35% to 46%) after the use of immunohistochemical analysis (but without additional sectioning).

Nevertheless, the extent of examination of non-SNs cannot explain the wide variety in non-SN involvement found in different studies. It may be that tumor size and other primary tumor characteristics ultimately determine the incidence of non-SN involvement. However, unlike the reports by other investigators, we did not find that primary tumor characteristics were significantly predictive of non-SN involvement in patients with a positive SN.

The number of nodes involved and the size of the SN metastases did clearly correlate with non-SN involvement. Our finding that 27% of patients (8/30) with micrometastases (<1 mm²) in a single SN had non-SN involvement is similar to the findings by Chu et al in which 24% of patients with metastases less than 2 mm in diameter had non-SN involvement. Like them, we saw the incidence decrease when these SN metastases are detected with immunohistochemical analysis only. We choose to measure the surface area instead of the diameter of metastatic deposits because this allows for a more objective distinction between micrometastases and macrometastases.

Three patients with SN micrometastases had grossly involved palpable axillary nodes. This finding can be explained by the SN concept. Metastatic tumor cells are thought to progress in a sequential fashion from first- to second-echelon nodes, as it has been shown for both the radioactive tracer and the blue dye. With increasing tumor load of the first echelon node(s), metastatic cells will advance to second-echelon nodes by alternative routing. The same may be true for the radioactive tracer and blue dye when grossly involved metastatic first-echelon nodes may no longer be able to contain any of the injected radioactive tracer or blue dye. Therefore, a reactive node with a micrometastasis may occasionally be a second-echelon node. This is also in accordance with the finding in several studies in which SNs were occasionally found to be negative for metastatic tumor cells, while nodes that were neither radioactive nor blue were grossly involved with tumor metastases. Palpation of the open axilla during the SN biopsy is therefore a simple and necessary requirement.

Finding grossly involved palpable axillary nodes in the axilla obviates the need for an SN biopsy, and these patients clearly require an ALND for locoregional control. That leaves us with a group of patients with microscopic involvement of the SN of which a few (5 of 27 in this study) may have microscopic involvement limited to a single additional non-SN.

Although it has not been possible to identify a subset of patients in whom the non-SNs will be negative, the question of whether or not we can identify these patients may not be relevant. Greco et al showed in a recent study that axillary relapses in untreated axillas occurred in 5.3% of patients with T1 tumors and in 18.4% of patients with T2 tumors during a median follow up of 5.2 years. No systemic chemotherapy or axillary radiotherapy was administered to any of the patients. The occurrence of clinically involved axillary nodes was much lower than the expected 25% to 30%, and 50% incidence of positive nodes with axillary dissection. An SN biopsy will completely remove the axillary metasta-
ses in 75% of patients with micrometastases in the SN, while leaving only minimal microscopic disease in the remaining 25% of patients. When the axillary relapse in the untreated axilla is much lower than expected, the risk may be particularly low for patients left with micrometa-
static axillary disease. Since axillary staging has been ade-
quately performed and since patients will conse-
quently receive adjuvant systemic treatment, this may be reason enough to omit an ALND in patients with micro-
metastases in the SN. Randomized trials comparing dif-
ferent modes of axillary therapy in SN-positive patients
should also include a no-treatment arm for this group of
patients.

In conclusion, although patients with only 1 posi-
tive SN and a low tumor load in the SN have a signifi-
cantly lower chance of having second-echelon meta-
stases, it was not possible to identify a subset of patients
in whom non-SN metastases will be absent.

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