ORIGINAL ARTICLE
UDC: 618.19-006:616.428-037:57.083.3

Arch Oncol 2006;14(1-2):19-22.
DOI: 10.2298/AOO0602019I

Factors correlating with lymph node metastases in patients with T1 ductal invasive breast cancer

Tatjana Ivković-Kapicl[1], Milana Panjković[2], Dejan Ninčić[1], Slavica Knežević-Ušaj[1]

ABSTRACT

BACKGROUND: *Identification of reliable predictors of axillary lymph node metastases (ALNM) may be useful in selecting appropriate management for patients with T1-size breast cancer. This study was undertaken to determine the association between ALNM and several variables, including age, tumor size, grade, estrogen receptor status, progesterone receptor status, p53 and c-erbB2 protein expression, and Ki-67 proliferative index.*

METHODS: *In a retrospective study, 74 patients with pT1b and pT1c ductal invasive breast carcinoma and with known nodal status were analyzed. The size of the infiltrating tumor was microscopically evaluated. The histological grading was performed using the modified criteria of Bloom and Richardson, as described by Elston and Ellis. The immunophenotype of the tumor was determined as: the expression of estrogen (ER) and progesterone (PR) receptors, p53, c-erbB2 and Ki-67. The patients were grouped by age as follows: <50, 50-70, and >70 years old.*

RESULTS: *Twenty six patients (35%) were node positive. Tumor size was related directly to nodal positivity. Nodal positivity was significantly related to negative PR status, p53 protein overexpression and high Ki-67 index ($p<0.05$). No significant association was found between nodal positivity and patient age, tumor grade, ER status, and c-erbB2 expression.*

CONCLUSION: *These data suggest that PR status, Ki-67 proliferation index, and p53 protein expression might provide additional information to the lymph node status in T1 ductal breast carcinomas.*

KEY WORDS: *Breast Neoplasms; Carcinoma, Infiltrating Duct; Lymph Nodes; Neoplasm Metastasis; Prognosis; Immunohistochemistry; Neoplasm Staging*

[1]Institute of Oncology Sremska Kamenica, Sremska Kamenica, [2]Institute of Pulmonary Diseases, Sremska Kamenica, Serbia; Address correspondence to: Asist. Dr. Tatjana Ivković-Kapicl, Institute of Oncology Sremska Kamenica, Institutski put 4, 21204 Sremska Kamenica, Serbia, E-mail: kapicl@Eunet.yu; The manuscript was received: 20.01.2006, Provisionally accepted: 06.02.2006, Accepted for publication: 28.02.2006

INTRODUCTION

The number of tumor-related features available to predict the prognosis of patients with breast cancer has grown impressively in recent years. Lymph-node status, tumor size and histologic grade are now supplemented with measurements of steroid hormone receptors, proliferation index, ploidy, tumor suppressor genes, growth factors, oncogenes, and oncogenes products (1-4). One of the best-established genes for tumorigenesis that is found to be amplified in patients with breast cancer is c-erbB2. The function of this gene is to act as a growth factor receptor. It is a well known prognostic and predictive factor in breast cancer (4-6). The p53 tumor suppressor gene, located on the short (p) arm of chromosome 17, is another established breast cancer progression gene that regulates the cell cycle and DNA repair. The mutation of p53 is associated with genetic instability (4,7,8). Apart from progression and suppressor genes, some of which have clearly been shown to possess prognostic value, a number of other molecules, are also biologically important in initiation and progression of breast cancer. Among them are an estrogen regulated protein Factor VIII, and Ki-67.

The presence of lymph node metastases in breast carcinoma is directly proportional to tumor size. The axillary lymph nodes are affected in less than 10% of all cases of pT1a and non-palpable pT1b early breast carcinoma (9). Mammographic screening, which allowed the early detection of breast carcinomas with a low risk of lymph node metastases, has promoted a series of studies that debated the usefulness of axillary node dissection.

However, the frequency of lymph node metastases associated with small tumors is too variable to rule out axillary dissection altogether (10-12). There are other clinical and pathological factors that might influence the nodal status in pT1 breast cancer (10,13).

For many years, axillary dissection has been a routine method for determining the status of axillary lymph nodes. The recent introduction of the sentinel lymph node procedure has already reduced the number of unnecessary lymphadenectomies and the number of axillary dissection associated complications. However, some patients will require chemotherapy even thought the sentinel lymph node is negative (14,15).

The evaluation of the specific tumor phenotype and the clinical features of each single patient need to be considered for the provision of individualized treatment. To provide this information, we evaluated the correlation between tumor size and lymph node status in a series of 74 pT1ductal breast carcinomas. In addition, we evaluated which clinicopathological factors correlating with lymph node metastases in patients with T1 ductal invasive breast cancer.

MATERIAL AND METHODS

We studied 74 patients with pT1 ductal invasive breast carcinoma who underwent total axillary dissection between 1998 and 2001, in the Institute of Oncology, Sremska Kamenica. None of the patients was submitted to sentinel node procedure.

Palpable tumors were cut along their major diameter and measured. Because all the lesions examined were <2 cm, it was possible to confirmed microscopically the gross evaluation of tumor size. Non-palpable lesions were identified by a hook-wire. In non-palpable lesions, tumor size was microscopically measured and the largest diameter of the invasive component was reported. When the invasive tumor was associated with *in situ* carcinoma, only the invasive carcinoma was considered for tumor staging. Tumors were categorized using the TNM system (16). Invasive carcinomas were classified as pT1b (6-10 mm) and pT1c (11-20 mm).

Surgical specimens were 10% buffered formalin fixed and paraffin wax embedded; 4 μm sections were cut and stained with hematoxylin and eosin (H&E). Two to five sections for each block were examined after H&E staining. The histological grading was performed using the modified criteria of Bloom and Richardson, as described by Elston and Ellis (17). The streptavidin-biotin peroxidase complex method was used for immunohistochemistry. Briefly, 4 μm sections were cut from paraffin wax blocks, dewaxed, and hydrated through graded alcohols to water. Estrogen (ER) and progesterone (PR) receptor (Dako, Glostrup, Denmark), p53 (Dako, Glostrup, Denmark), and c-erbB2 (Dako, Glostrup, Denmark) immunostainings were performed, preceded by antigen retrieval with incubation in 10 mM citrate buffer for 5 minutes at 100 W and 15 minutes at 800 W (domestic microwave), in thermo resistant container. Distilled water and buffer were added every 5 minutes to the container to prevent drying during the incubation process. Immunostaining for Ki-67 (Dako, Glostrup, Denmark) was carried out with no previous antigen retrieval step. Following endogenous peroxide and protein blocking step, the slides were incubated with primary antibodies. After brief washes, incubation in a cocktail of biotinylated rabbit anti-mouse IgG/IgM for 30 minutes was performed. The sections were then washed and incubated with streptavidin-biotinylated horseradish peroxidase complex for 30 minutes reacted with 3-amino-9-ethylcarbazole (AEC, DAKO) and hydrogen peroxide to visualize the end product. Hematoxylene was used as counter stain.

Nuclear staining for hormone receptors, Ki-67 and p53, and membranous staining for c-erbB2 were evaluated. Cut off values for positivity were established as follows: ER, PR, p53 and Ki-67: >10% marked and very strong nuclear staining; c-erbB2: >10% weak to moderate and strong complete membrane staining (18).

Negative controls were carried out by omission of the primary antibody. As positive controls, sections from previously studied cases of breast cancer know to express ER, PR, p53, c-erbB2 and Ki-67 were used.

Statistical differences between lymph node status and tumor size, histological grade, ER and PR content, p53 expression, c-erbB2 expression and Ki-67 expression were calculated using the χ^2 test and Students t test. A value of $p<0.05$ was considered significant.

RESULTS

Of 74 patients with T1 ductal invasive breast carcinoma, 24 (32.4%) had pT1b, and 50 (67.6%) had pT1c tumors (Table 1). There were not cases of pTa tumors in our study.

The mean age of all patients was 58±12.1 years. Axillary metastatic lymph nodes were found in 26 of 74 patients (35%). The number of lymph nodes recovered from all specimens ranged from 10 to 24.

Tumor size was related directly to nodal positivity. Cases of IC pT1b were less frequently node positive, than pT1c tumors (21% v 42%; $p<0.1$). In lymph node positive patients the mean size of tumors was significantly larger than in group of lymph node negative patients (1.59±0.42 cm v 1.36±0.46 cm; $p<0.05$).

Table 1. Relation between node positivity and different tumor clinicopathological parameters in the 74 patients

Clinicopathological features	No. of cases	Lymph node status Positive	Negative	p Value
Tumor size				
pT1b	24	5	19	NS
pT1c	50	21	29	
Histological grade				
I	24	6	18	NS
II	43	15	28	
III	7	5	2	
Age in years				
<50	20	11	9	
50-70	41	13	28	NS
>70	13	2	11	
Estrogen receptor content				
Positive	57	18	39	NS
Negative	17	8	9	
Progesterone receptor content				
Positive	55	15	40	**<0.05**
Negative	19	11	8	
c-erbB2 expression				
Positive	10	6	4	NS
Negative	64	20	44	
p53 expression				
Positive	14	9	5	**<0.05**
Negative	60	17	43	
Ki-67 index				
High	53	23	30	**<0.05**
Low	21	3	18	

Of 74 patients, 57 (77%) had ER positive tumors, and 55 (74%) had PR positive tumors (Figure 1). In progesterone receptor positive tumors, the percentage of node positivity was significantly lower than in progesterone negative tumors (27% v 58%; $p<0.01$).

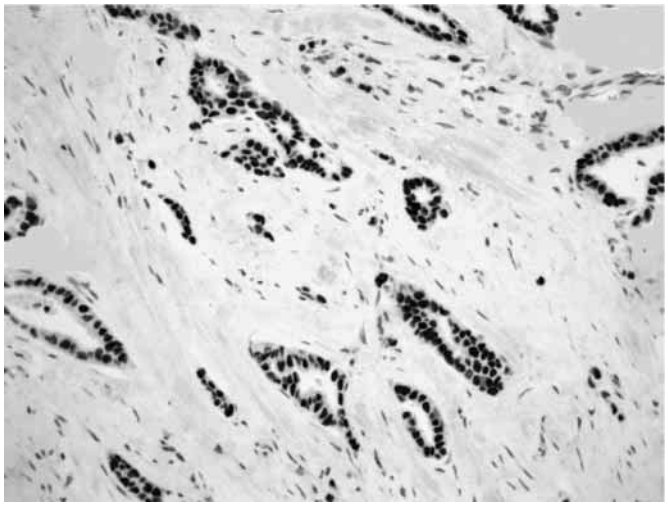

Figure 1. Intense nuclear localization of estrogen receptors was detected in tumor cells, B-SA, x100

In our group of patients, there were 14 (19%) patients with positive tumors p53 protein expression (Figure 2).

In tumors with positive p53 protein expression, the percentage of node positivity was significantly higher than in p53 protein negative tumors (64% v 28%; $p<0.05$).

Ki-67 proliferative index was significantly related to nodal positivity. In fact, 43% (23 of 53) of cases with high proliferative index were node positive compared with 14% (3 of 21) of tumors with low Ki-67 proliferative index. In our group of patients, c-erbB2 overexpression was determinated in 10 (13.5%) cases (Figure 3).

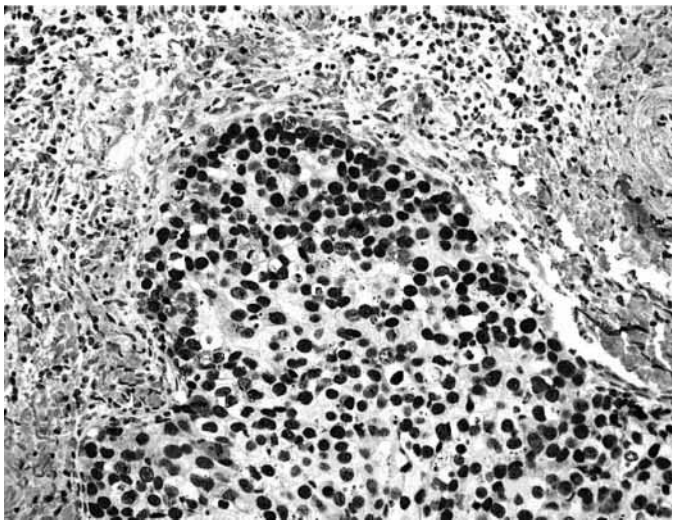

Figure 2. Intense nuclear localization of p53 protein was detected in tumor cells, suggesting accumulation of abnormal mutant p53 protein, B-SA, x200

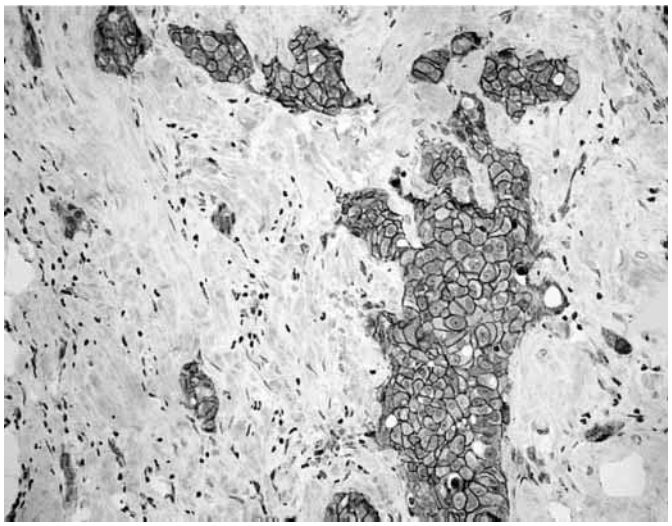

Figure 3. A strong membrane staining for c-erbB2 (HER2) was observed in tumor cells, B-SA, x200

No significant relation was found between nodal status and patient age at diagnosis, histological grade, ER status, and c-erbB2 expression.

DISCUSSION

Axillary lymph node status and tumor size have long been considered to be the most significant prognostic factors for breast cancer patients (1-4).

This study reports an overall rate of axillary lymph node metastases of 35% among 74 women with ductal invasive breast carcinomas measured ≤2 cm. In our study, we confirmed that tumor size remains an important predictor of axillary node metastases in breast carcinomas. However, we showed that other factors as p53 overexpression, PR status and Ki-67 proliferative index correlate with ALNM.

Contrasting data have been published previously on the incidence of axillary lymph node metastases according to tumor size (10-13,19). Overall, the rate of ALNM in patients with pT1 breast cancers has been reported to be from 18 to 38.5%. Axillary metastasis rates for pT1a (≤0.5 cm) tumors have varied even more widely, from 0% to 28%. This variability might have several causes, such as the need to examine a sufficient number of lymph nodes to obtain reliable results, and the poor reproducibility of the measurement of small invasive cancers. The definition of tumor size in the literature varies significantly, possibly affecting the incidence of positive lymph nodes too. In our work tumor diameter was determinated microscopically (10). This allowed us to limit our measurement to the invasive component of each tumor, and to exclude the associated *in situ* component. Seidman and co-workers demonstrated that this kind of tumor size measurement is a better predictor of lymph node status than the total tumor size (20). Abner at al. demonstrated that the rate of axillary lymph node metastases associated with macroscopic tumor size did not differ significantly from that associated with microscopic tumor size (21). The relatively large tumor size of our sample without pT1a cases may partly explain the high rate of nodal involvement in this series.

Among patients affected with small breast cancer, younger patients have a higher incidence of axillary nodal metastases than older patients. Mustafa et al. demonstrated that young age is a strong predictor of nodal metastases in univariate and multivariate analyses of 2185 patients with invasive breast carcinomas measuring ≤1 cm (22). Additionally, histological grade and nuclear grade are also significantly associated with ALNM. The discrepancy between our findings and those from other studies may be caused by the relatively small number of cases of our sample, and differences in the grading methods used to evaluate the histological malignancy grade. Beside, the malignancy grade of the invasive breast carcinoma is a powerful prognostic factor in tumors larger than 10-15 mm.

The presence of steroid hormone receptors (ER and PR) represents a relatively weak prognostic factor for patients with breast cancer, but these receptors are the strongest predictive factors for response to hormonal therapy. Most of the tumors are obviously receptor-positive (1-3). Our series demonstrated that PR-negative status was significantly associated with axillary node metastases. Wenger et al. demonstrated that steroid receptor status, S-phase fraction and DNA ploidy were associated with axillary nodal positivity (23). Ravdin et al. found tumor size, patient age and progesterone receptor status to be independent predictors of axillary nodal status in breast cancer patients with tumors of ≤5 cm (24).

In our study, we found p53 protein expression and high Ki-67 score are associated with ALNM. Gasparini et al. reported that high Ki-67 scores are associated with poor histologic differentiation and with lymph node metastasis (25).

In our study, 10 (13.5%) patients with pT1 tumors had c-erbB2 protein overexpression. Generally, the HER2/neu gene has been found to be amplified and/or overexpressed in approximately 20 to 30% of invasive breast cancers, most commonly in invasive ductal carcinomas (5). C-erbB2 expression showed no significant differences between the node negative and node positive patients in our groups. Arisio et al. found no significant correlation between nodal positivity and ER, PR,53 and c-erbB2 status (10). There are many studies that have shown that HER2 overexpression is associated with other adverse prognostic factors such as positive lymph nodes, larger tumor size, high proliferative index, high histologic grade, p53 mutation, intratumoural necrosis, and lack of expression of estrogen and progesterone receptors (26,27).

CONCLUSION

Our study has demonstrated association of large tumor size, lack of expression of progesterone receptors, p53 protein overexpression, and Ki-67 high proliferative index with axillary lymph node involvement in pT1 ductal invasive breast cancer. However, it is difficult to characterize a subgroup that has an acceptably low risk of nodal involvement, since the impact of each factor on the reduction of lymph node positivity is relatively small. The indications provided by the pathological parameters of the tumor, together with the clinical data, might provide additional information to the histology of lymph nodes, and might be useful for individualized treatments.

REFERENCES

1. Tot T. Assessment of the most important prognostic factors. In: Tot T, Tabar L, Dean P, editors. Practical Breast Pathology. New York: Thieme Medical Publishers; 2002. p.125-35.
2. Mansour EG, Ravdin PM, Dressler L. Prognostic factors in early breast carcinoma. Cancer 1994;74 Suppl:381-400.
3. Donegan WL. Tumor-related prognostic factors for breast cancer. Ca Cancer J Clin 1997;47:28-51.
4. Schnitt SJ. Traditional and newer pathologic factors. J Natl Cancer Inst Monographs 2001;30:22-6.
5. Schnitt S. Breast cancer in the 21st century: New opportunites and new challenges. Mod Pathol 2001;14(3):213-8.
6. Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, and target for therapy. The Oncologist 1998;3:237-52.
7. Hall PA. P53:The challenge of linking basic science and patient management. The Oncologist 1998;3:218-24.
8. Harris C, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 1993; 329:1318-1327.
9. Silverstein MJ. Recent advances: diagnosis and treatment of early breast cancer. Br Med J 1997;314:1736.
10. Arisio R, Sapino A, Gassoni P, Accinelli G, Cuccoresae MC, Mano MP, et al. What modifies the relation between tumour size and lymph node metastases in T1 breast carcinomas? J Clin Pathol 2000;53:846-50.
11. Iwasaki Y, Fukutomi T, Akashi-Tanaka S, Nanasawa T, Tsuda H. Axillary node metastasis from T1N0M0 breast cancer: possible avoidance of dissection in a subgroup. Jpn J Clin Oncol 1998;28:601-3.
12. Brenin D R, Manasseh D, El-Tamer M, Troxel A, Schnabel F, Ditkoff B A, et al. Factors correlating with lymph node metastases in patients with T1 breast cancer. Ann Surg Oncol 2001;8(5):432-7.
13. Cabioglu N, Yazici MS, Arun B, Broglio KR, Hortobagyi GN, Price JE, et al. CCR7 and CXCR4 as novel biomarkers predicting axillary lymph node metastasis in T1 breast cancer (abstr.). Clin Cancer Res 2005;11:5686-93.
14. Lane KT, Esserman LJ. Sentinel lymph node dissection in early-stage breast cancer. Oncol Exchange 2004;3:6-13.
15. Fleming FJ, Kavanagh D, Crotty TB, Quinn CM, McDermotr EW, O'Higgins N, et al. Factors affecting metastases to non-sentinel lymph nodes in breast cancer. J Clin Pathol 2004;57:73-6.
16. TNM classification of malignant tumors (UICC). 6th ed. In: Sobin LH, Wittekind CH, editors. Wiley-Liss; 2002.
17. Symmers W, Elston C, Ellis I. Systemic Pathology: The Breast. 2nd ed. New York: Churchill Livingstone Company; 1997.
18. Ivkovic-Kapicl T. Značaj neovaskularizacije kod infiltrativnog duktalnog carcinoma (Magistarska teza). Novi Sad: Medicinski fakultet Univerziteta u Novom Sadu; 2004.
19. Chao T, Chen M, Wang C, Jan Y, Hwang T, Chen S. Small invasive breast carcinomas in taiwanese women. Ann Surg Oncol 2003;10(7):740-7.
20. Seidman J, Grabau DA, Sorensen DB. Relationship of the size of the invasive component of the primary breast carcinoma to axillary lymph node metastasis. Cancer 1995;75:65-71.
21. Abner AL, Collins L, Peiro G, Recht A, Come S, Shulman LN, et al. Correlation of tumour size and axillary lymph node involvement with prognosis in patients with T1 breast carcinoma. Cancer 1998;83:2502-8.
22. Mustafa IA, Cole B, Wanebo HJ, Bland KI, Chang HR. The impact of histopathology on nodal metastases in minimal breast cancer. Arch Surg 1997;132:384-91.
23. Wenger CR, Beardslee S, Owens MA, Pounds G, Oldtaker T, Vendely P, et al. DNA ploidy, S-phase, and steroid receptors in more than 127,000 breast cancer patients. Breast Cancer Res Treat 1993;28:9-20.
24. Ravdin PM, De Laurentiis M, Vendely T, Clark GM. Prediction of axillary lymph node status in breast cancer patients by use of prognostic indicators. J Natl Cancer Inst 1994;86:1771-5.
25. Gasparini G, Pozza F, Meli S, Reitano M, Santini G, Bevilacqua P. Breast cancer cell kinetics: Immunohistochemical determination of growth fractions by monoclonal antibody Ki-67 and correlation with flow cytometric S-phase and with some features of tumor aggressiveness. Anticancer Res 1991;11:2015-21.
26. Bhatavdekar JM, Patel DD, Shah NG, Vora HH, Suthar TP, Chikhlikar PR, et al. Prognostic significance of immunohistochemically localized biomarkers in stage II and stage III breast cancer: a multivariate analysis. Ann Surg Oncol 2000;7(4):305-11.
27. Piccart MJ, Mano M, Lohrisch C, Di Leo A. Herceptin for the treatment of breast cancer: what we know-and what we have yet to learn. Cancer Futures 2002;1:73-9.