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ORIGINAL ARTICLE

Distant metastasis after radical treatment of breast cancer: risk factors and their prognostic relevance in 378 consecutive patients

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Summary

Purpose: *To evaluate the prognostic significance of 16 clinical, pathomorphological and immunohistochemical features for predicting distant metastasis (DM) and 5-year overall survival (OS) in breast cancer (BC) patients.*

Patients and methods: *A retrospective study of 378 patients with invasive BC ($T_{1-3}N_{0-3}M_0$), who were operated between 2000 and 2003 at our Institution, was carried out. Almost 80% had undergone modified radical mastectomy (MRM). Tumor size (T), axillary lymph nodes status (N), age, menstrual status, histological type, grade (G), lymphovascular invasion (LVI), in situ component, estrogen receptor (ER), progesterone receptor (PgR) content, HER-2, Ki-67, p53, bcl-2, cathepsin D and E-cadherin were evaluated. Mean follow-up time was 56 months (range 1-88).*

Results: *During the follow-up period 66 (17.4%) patients developed DM and 76 (20.1%) patients died. Univariate analysis showed that T (p=0.0001), N (p=0.0001), presence of comedo type in situ component (p=0.0001), LVI (p=0.016), Ki-67 (+) (p=0.007) and cathepsin D (+) (p=0.013) were independent prognostic indicators for increased risk for DM. After multivariate analysis only N (+) status (odds ratio/OR 8.8; 95% confidence interval/ CI 3.5-21.77; p=0.0001) and presence of comedo type in situ component (OR 2.4; 95% CI 1.19-4.74; p=0.015) retained their significant association with DM development. The same 2 factors also influenced 5-year OS: N(+), OR 3.8; 95% CI: 1.36-10.56; p=0.011; and comedo type in situ component, OR 3.3; 95% CI: 1.61-6.56; p=0.001.*

Conclusion: *N (+) status and presence of comedo type in situ component are the most reliable predictors of unfavorable events in BC patients. Our study is among the first ones to find a relationship between the presence of in situ component and risk for DM in patients after MRM. The results also show that comedo type intraductal component, no matter how extensive it is, bears high risk for DM equal to N1 axillary status and patients with presence of such intraductal component should be treated as N(+). The evaluation of optimal number of risk markers is substantial for making an individualized decision regarding adjuvant therapy, especially in N0 group.*

Key words: breast cancer, comedo type, distant metastasis, in situ component, N status, prognostic factors

Introduction

Postoperative treatment of BC patients depends on their individual risk for unfavorable events. The tumor features that give information for potential risk for local recurrence (LR), DM and overall survival of the patients when no adjuvant treatment is administered are called prognostic factors [1].

According to St. Gallen consensus meeting in 2005, basic prognostic factors for BC patients are T, N, G, ER and PgR status, age, HER-2 and LVI [2]. LVI is considered as prognostic factor only in N(–) patients. Besides these already established factors, in the last decades many other tumor features are widely examined in order to evaluate their prognostic significance [1,3-8].

Development of DM indicates a more aggressive disease behavior and shorter overall survival. That's why finding reliable markers for higher risk of such an

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unfavorable event is extremely important for more proper treatment and more strict follow-up in shorter intervals.

The aim of present study was to evaluate the prognostic significance of 16 clinical, pathomorphological and immunohistochemical features regarding the development of DM and OS in BC patients.

Patients and methods

Study group

378 patients with invasive T1-3N0-3M0 BC without DM at entry were studied. The study group consisted of all consecutive patients with newly diagnosed invasive BC who had been radically operated in our institution between 2000 and 2003.

Criteria for exclusion were DCIS, LCIS, T4 and M1 at registration, multicentric, multifocal, bilateral tumors and second malignancy (before or after BC). Distribution of cases by stage is shown in Table 1 and patient treatment details are summarized in Table 2.

Mean duration of follow up was 56 months (range 1-88).

Study variables

T, N status, age, menstrual status, histological type, grade, LVI, *in situ* component, ER, PgR, HER-2, Ki-67, p53, bcl-2, cathepsin D and E-cadherin were evaluated.

Tumor size and lymph node status were classified into groups according to TMN 6th edition. Histological grade was classified according to Elston & Ellis modification of Scarff-Bloom-Richardson scoring system. Presence of intraductal component and LVI was investigated in peritumoral parenchyma. ER and PgR status was measured by radioimmunolabeled assay and determined in 92.3% of the cases. Tumors were considered receptor-positive with > 10 fmol/mg protein.

HER-2, Ki-67, p53, bcl-2, cathepsin D and E-cadherin were evaluated retrospectively by immunohistochemical (IHC) assay in 40.7%, 20%, 20%, 19%, 19% and 19% of cases, respectively. Formalin-fixed paraffin-embedded tissues, specific antibodies against the corresponding antigen and standard biotin-streptavidin technique (DAKO, DakoCytomation, Glostrup, Denmark) were used.

The evaluation of HER-2 expression was made by semiquantitative method. HER-2 negative cases were considered those with 0 and 1+ and HER-2 positive with 2+ and 3+. The proliferative activity of the tumor was considered high when nuclear positivity for Ki-67 was seen in more than 20% of the cells. Overexpression of p53 and bcl-2 was reported when the reaction against the corresponding antigen was seen in more than 10% of the tumor cells. The evaluation of cathepsin D was also semiquantitative by Histoscore H score, which included intensity of the cytoplasmic staining and percentage of immunopositive cells. With H score ≥ 3, we considered a tumor as cathepsin D (+). For E-cadherin we evaluated the membrane reaction by semiquantitative method comparing the intensity of staining in tumor cells and in the normal epithelial cells around. The expression was divided in strong, weak and absent.

Table 1. Distribution of cases by stage

Stage	*Number of patients*	*%*
I	123	32.54
II A	126	33.33
II B	37	9.79
III A	60	15.87
III B	32	8.47

Table 2. Treatment modalities

Treatment modality	*Number of patients*	*%*
Surgery		
MRM	324	85.7
BCS	54	14.3
Neoadjuvant chemotherapy	62	16.4
Adjuvant chemotherapy	193	51.2
Radiotherapy	201	54.8
Hormonal therapy	318	85.3

MRM: modified radical mastectomy, BCS: breast-conserving surgery

Statistical methods

Distant metastasis-free survival (DMFS) and 5-year OS were estimated by the Kaplan-Meier method and compared with the log-rank test. To evaluate the independent prognostic significance and relative risk, multivariate analysis of clinical variables was performed by Cox logistic regression method. The model included any variable that achieved a significant difference in univariate analysis and was evaluated in at least 200 patients. Statistical analysis was performed with SPSS, version 11.5. Statistical significance was set at $p<0.05$.

Results

During the follow-up period 66 (17.4%) patients developed DM and 76 (20.1%) patients died. Forty-five (68%) of the patients with DM died.

Evaluated characteristics of all patients and their

significance for predicting DM are summarized in Table 3.

Univariate analysis of data showed that T (p= 0.0001), N (+) (p=0.0001), presence of comedo type *in situ* component (p=0.0001), LVI (p=0.016), Ki-67 (+) (p=0.007) and cathepsin D (+) (p=0.013) were independent prognostic indicators for increased risk for DM.

Factors that showed significance in univariate analysis were included in Cox proportional regression model for multivariate analysis. Grading and age were also included in the model, but Ki-67 and cathepsin D were not because of the small number of evaluated cases (Table 4). In this analysis only presence of metastatic lymph nodes (OR 8.8; 95% CI 3.5-21.77; p=0.0001) and presence of comedo type *in situ* component (OR 2.4; 1.19-4.74; p=0.015) retained their significant association with DM development.

Patients were further compared with regard to the extension of the *in situ* component (extensive vs. not extensive) as far as DM were concerned. Paradoxically, a statistically significant inverse correlation was found (DM in extensive vs. not extensive: 11.4 vs. 21.3%, respectively; x^2= 6.88, p= 0.03).

Also simple presence of any kind of *in situ* component around the invasive tumor was not significant risk marker for DM - 19.4% of patients with and 11.4% without intraductal component experienced DM (x^2=3.61, p=0.058).

Analysis of factors associated with 5-year OS was also performed. Univariate analysis revealed an inverse association between 5-year OS and T size (p=0.0001), N (+) status (p=0.0001), ER(–) status (p=0.031), presence of LVI (p=0.012) and comedo type *in situ* component (p=0.0001). On multivariate analysis again N (+) status (OR 3.8; 95% CI 1.36-10.56; p=0.011) and *in situ* component of comedo type (OR 3.3; 1.61-6.56; p=0.001) were the only factors that retained their relevance with 5-year OS.

Table 3. Univariate analysis of prognostic features and their relevance to DMFS

Feature	*Patients n (%)*	*log-rank*	*DMFS*	*Feature*	*Patients n (%)*	*log-rank*	*DMFS*
Tumor size *	378	18.9	p=0.0001	Histologic type	378	9.47	p=0.39
T1	231 (61.1)			IDC	283 (74.9)		
T2	134 (35.5)			ILC	48 (12.7)		
T3	13 (3.4)			others	47 (12.4)		
N status **	378	77.8	p=0.0001	Grade ***	323	3.9	p=0.140
N0	184 (48.7)			I	94 (29.1)		
N1	104 (27.5)			II	154 (47.7)		
N2	58 (15.3)			III	75 (23.2)		
N3	32 (8.5)			HER-2	154	2.8	p=0.094
Age (years)	378	2.90	p=0.23	(+) 2+ and 3+	35 (22.8)		
≤40	30 (7.9)			(–) 0 and 1+	119 (77.2)		
>40	348 (92.1)			Ki-67	75	7.3	p=0.007
Menstrual status	350	0.09	p=0.76	(+) > 20%	31 (41.3)		
premenopausal	109 (31.1)			(–) < 20%	44 (58.7)		
menopausal	241 (68.9)			p53	75	1.33	p=0.25
ER status	349	0.13	p=0.72	(+)	24 (32)		
(+)	196 (56.2)			(–)	51 (68)		
(–)	153 (43.8)			Bcl-2	72	0.01	p=0.90
PR status	349	3.5	p=0.061	(+)	50 (69.4)		
(+)	180 (51.6)			(–)	22 (30.6)		
(–)	169 (48.4)			Cathepsin D	72	6.2	p=0.013
In situ, comedo type	296	15.8	p=0.0001	(+)	39 (54.2)		
yes	233 (78.7)			(–)	33 (45.8)		
no	63 (21.3)			E-cadherin	72	3.72	p=0.16
LVI	269	5.8	p=0.016	normal	49 (68)		
yes	123 (45.7)			reduced	15 (20.8)		
no	146 (54.3)			absent	8 (11.2)		

DMFS: distant metastasis-free survival, LVI: lymphovascular invasion, IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, ER: estrogen receptors, PR: progesterone receptors, N: lymph nodes

* T_1 vs. T_{2-3}, ** N_0 vs. N_{1-3}, *** G_1 vs. G_{2-3}

Table 4. Multivariate analysis for DMFS by Cox logistic regression

	OR	*95% CI*	*p-value*
Tumor size (cm)			
< 2	1		0.094
2-5	1.7	0.9-3.3	0.100
> 5	3.2	0.9-11.6	0.072
Metastatic axillary lymph nodes			
0	1		0.0001
1-3	2.5	1.07-5.72	0.035
4-10	3.6	1.43-8.94	0.006
>10	8.8	3.5-21.77	0.0001
Grade			
G1	1		0.042
G2	0.98	0.39-2.43	0.963
G3	2.3	0.89-5.91	0.084
Age (years)			0.190
> 40	1.4	0.23-8.2	0.738
≤ 40	2.8	0.76-10.2	0.124
In situ component (comedo type)			
yes	1		
no	2.4	1.19-4.74	0.015
LVI			
no	1		
yes	0.9	0.44-2.05	0.889

DMFS: distant metastasis-free survival, LVI: lymphovascular invasion

Discussion

Our study confirmed the prognostic significance of one of the classic prognostic factors - N status - regarding DM. The axillary lymph node status is known as the most important single prognostic factor [3,4,9-11] and our results also confirmed that observation. As expected, N (+) patients experienced DM significantly more frequently than N(–). The risk for DM in N1 patients was 2.5 times higher than the same risk in N0 group, while in N2 and N3 patients the risk increased to 3.6 and 8.8 times, respectively (p=0.0001).

Presence of *in situ* component around invasive tumor is mainly known as prognostic factor for local recurrence (LR) after breast-conserving surgery [4,12-16]. Although 80% of our patients had undergone MRM, this factor also showed significance as predictor of DM – 30% of the patients with *in situ* component of comedo type experienced DM in comparison with only 11.6% for patients without this specific type of intraductal component (x^2=13.03, p=0.0003).

Our results show that presence of *in situ* component comedo type could be used as prognostic factor also for DM, even after MRM. In the literature we found just one similar article where such a connection with DM risk was detected, but the study group consisted of only 91 BC patients [17].

Our results also show that not all types of *in situ* component bear the same risk for DM and comedo type is the only one which is related with higher risk for unfavorable events, without any difference with regard to its extension.

According to Brower et al. [18] comedo carcinoma is more aggressive than other types of intraductal carcinoma and is associated with poor prognostic factors such as higher ploidy, S-phase fraction, Ki-67(+), ER(–) and PgR(–) status, larger tumor size, HER-2 overexpression and poorer differentiation.

In our study presence of *in situ* component comedo type increased the risk for DM 2.4-fold compared with patients without this feature. This risk is as high adverse prognostic factor as the presence of N1 axillary status (which is 2.5-fold higher that in N0). This fact made us believe that N0 patients with comedo type *in situ* component, even without other unfavorable factors, should be treated in the adjuvant setting as N(+) patients.

Conclusions

This study found that N(+) status and presence of comedo type *in situ* component are the most reliable predictors of unfavorable events in BC patients. Our study is among the first ones to find a relationship between the presence of *in situ* component and the risk for DM in patients after MRM. The results also show that the histologic type of *in situ* component is more important than its extension. Comedo type intraductal component, no matter how extensive it is, bears the greatest risk (equal to N1 axillary status) for DM. We believe that patients with presence of such intraductal component should be treated as N(+).

The evaluation of the optimal number of risk markers is substantial for making an individualized decision regarding adjuvant therapy and follow-up, especially in N0 patients.

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