Occurence of Stromal Myofibroblasts in the Invasive Ductal Breast Cancer Tissue is an Unfavourable Prognostic Factor

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Abstract. *Background: Numerous experimental studies have described the capacity of myofibroblasts to stimulate mammary cancer cells in a paracrine manner. Until now, the prognostic significance of myofibroblasts present in breast cancer has not been examined. Patients and Methods: In paraffin sections, originating from 45 patients with primary invasive breast cancer, immunohistochemical reactions were performed using antibodies directed against smooth muscle actin, Ki-67, VEGF, bFGF and UPA. Results: The cases with higher content of myofibroblasts in the tumour tissue manifested higher grade, more pronounced expression of Ki-67, VEGF and bFGF and shorter overall survival and relapse-free survival. Conclusion: The present study for the first time documents the unfavourable prognostic significance of myofibroblasts in tissues of invasive ductal mammary carcinomas.*

Throughout the world, breast cancer represents the most frequent malignant tumour in females, being responsible for about 32% of the estimated new female cancer cases. The incidence of breast cancer remains high and the clinical course is highly variable (1). The detection of new prognostic and predictive factors in breast cancer in order to provide a target for new therapeutic techniques as well as to define both prognosis and sensitivity to various therapies is being sought worldwide. In line with such classical prognostic indices as stage, grade, or histological type, only the estimation of oestrogen alpha receptors, progesterone receptors and of HER-2 have been included in routine pathological practice (2, 3).

Apart from neoplastic cells, tumours are also composed of connective tissue (fibroblasts, myofibroblasts and extracellular matrix) blood vessels and frequently vast inflammatory infiltrates and necrotic areas. In recent years increasing attention has been devoted to the effects of the non-neoplastic tissues of the tumour on the dynamic nature of neoplastic disease. However, to date few reports have appeared (4), which have reflected among other factors the difficulty of reliable confirmation of clinical data in *in vitro* and *in vivo* experiments.

Invasive breast carcinomas frequently include myofibroblasts with expression of α-smooth muscle actin (α-SMA) (5). Myofibroblasts previously termed 'activated fibroblasts' are also present in areas of wound healing and chronic inflammation and function to promote angiogenesis, stimulate proliferation of epithelial cells and produce extracellular matrix, growth factors and cytokines (4).

In cases of breast cancer a few reports have suggested that myofibroblasts may secrete growth factors (6), and their presence in cancer tissue correlates with higher grade, more intense proliferation (7, 8), and higher expression of HER-2 (8). The prognostic significance of myofibroblast presence in cancer tissue has not previously been examined and is very important not only for the detection of a novel prognostic index but any unfavourable effect of myofibroblasts on survival of breast cancer patients would open new avenues for therapy of the tumours through the potential to inhibit the formation of myofibroblasts or their activity.

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In the present study the immunohistochemical determination of α-SMA expression in breast cancer myofibroblasts was examined in relation to overall and relapse free survival. In order to more accurately clarify the potential role of myofibroblasts, the expressions of Ki-67, VEGF, bFGF and UPA in cancer cells were also estimated.

Patients and Methods

Patients. Immunohistochemical analysis was performed retrospectively on tissue samples which had been taken for routine diagnostic purposes. The cases were selected based on availability of tissue and were not stratified for known preoperative or pathological prognostic factors. The study was approved by an Institutional Review Board and the patients gave their informed consent before their inclusion into the study. Forty-five post-menopausal patients with primary invasive breast cancer who were diagnosed in the years 1997 to 1998 in the Wielkopolskie Cancer Centre in Poznan, Poland, were included in the studies. The data on post-surgical treatment of the patients are listed in Table I. The patients were monitored by periodic medical check-ups, ultrasonographic and radiological examinations. The tumour samples from studied tumours were fixed in 10% buffered formalin and then embedded in paraffin. In each case, haematoxylin and eosin stained preparations were subjected to histopathological evaluation by two pathologists. Tumour histology was determined according to WHO criteria. The stage of the tumours was assessed according to the Unio Internationalis Contra Cancrum (UICC) (9). The tumour grade was estimated according to the method of Bloom-Richardson as modified by Elston and Ellis (10).

Table I. *Patient and tumour characteristics.*

Characteristics	No. (%)
All patients	45
Age (mean 61.47)	
≤50	6 (13.33)
50-60	12 (26.66)
>60	
27 (60.00)	
Histology	
Invasive ductal breast cancer	45
Grade	
1	5 (11.11)
2	19 (42.22)
3	21 (46.66)
pT	
1	10 (22.22)
2	35 (77.78)
pN	
1	19 (42.22)
2	25 (55.56)
3	1 (2.22)
pM	
0	45
Therapy	
Tamoxifen	36 (80.00)
CMF (cyclophosphamide + methotrexate + 5-Fluorouracil	12 (26.67)
AC (doxorubicin + cyclophosphamide)	5 (11.11)
Radiotherapy	12 (26.67)
Other (paclitaxel, docetaxel, letrozol, clodronate disodium, anastrozol, aminoglutethimid)	11 (24.44)

Immunohistochemistry. Sections of the freshly cut (4 μm) formalin-fixed paraffin embedded tissue were mounted on Superfrost slides (Menzel Gläser, Braunschweig, Germany), dewaxed with xylene, and gradually rehydrated. The activity of endogenous peroxidase was blocked by 5 min incubation in 3% H_2O_2. The sections were boiled for 15 minutes in Target Retrieval Solution (DakoCytomation, Warszawa, Poland) in a microwave oven at 250 W. Then, immunohistochemical reactions were performed using monoclonal antibodies detecting SMA (clone 1A4) (DakoCytomation) at a dilution of 1:200, Ki-67 (clone MIB-1) (DakoCytomation) at a dilution 1:100, mouse monoclonal antibodies against VEGF (clone G153-694) (BD Pharmingen, Franklin Lakes, NJ, USA) at a dilution of 1:400, mouse monoclonal antibodies against UPA (clone 10D.7.2) (Chemicon, Temecula, CA, USA) at a dilution of 1:250 and polyclonal rabbit antibodies against bFGF (Chemicon) at a dilution of 1:400. The antibodies were diluted in the background reducing Antibody Diluent (DakoCytomation). The sections were incubated with antibodies for one hour at room temperature. Subsequently, incubations were performed with biotinylated antibodies (20 min, room temperature) and with streptavidin-biotinylated peroxidase complex (20 min, room temperature) (LSAB+, HRP, DakoCytomation). NovaRed (Vector Laboratories, Peterborough, UK) was used as a chromogen (5 min at room temperature). All the sections were counterstained with Meyer's haematoxylin. In each case controls were included in which the specific antibody was substituted by the Primary Negative Control (DakoCytomation).

Scoring of immunostaining results. The intensity of immunohistochemical reactions was estimated independently by two pathologists. In doubtful cases a re-evaluation was performed using a double-headed microscope and staining was discussed until a consensus was achieved.

When the intensity of myofibroblast manifestation was evaluated by SMA expression, a simplified scale was employed, which involved only the percentage of myofibroblasts in the tumour stroma (0 – no reaction, 1 – <10% of myofibroblasts in tumour stroma, 2 – 10-30% of myofibroblasts in tumour stroma, 3 – >30% of myofibroblasts in tumour stroma) as previously described (8). The expression of Ki-67 was quantified by counting the cells with a positive reaction.

In order to evaluate the expression of VEGF, bFGF and UPA a semi-quantitative scale the immunoreactive score (IRS) was applied, in which the intensity of the colour reaction and the percentage of positive cells were taken into account. The score represented a product of points given for the evaluated characters and it ranged from 0 to 12 (Table II) (11).

Statistical analysis. The statistical analysis of the results took advantage of Statistica 98 PL software (Statsoft, Krakow, Poland). The Kaplan-Meier statistics and log-rank tests were performed using SPSS software (release 10.0; SPSS Inc., Chicago, IL, USA) to estimate the significance of differences in survival times. The length of relapse-free survival was defined as the time between the primary surgical treatment and diagnosis of a relapse or death.

Table II. *Evaluation criteria of VEGF, bFGF and UPA expression using the immunoreactive score (11).*

Positive cells (%)	Points	Intensity of reaction	Points
No positive cells	0	No reaction	0
<10% of positive cells	1	Weak reaction	1
10-50% of positive cells	2	Moderate reaction	2
51-80% of positive cells	3	Intense reaction	3
>80 of positive cells	4		

Results

Patients. During the follow-up period of 96 months, 31 patients (68.88%) had recurrent disease and 32 patients (71.11%) died of the disease. The mean progression-free survival time was 57.36 months (range 6 to 96 months), while the mean overall survival time was 61.64 months (range 12 to 96 months).

Immunostaining. The detection of SMA revealed a cytoplasmic localization and of variable intensity in individual cases. SMA was present in myofibroblasts (Figure 1), myoepithelial cells and in muscles of blood vessels. According to the applied scale, the mean manifestation of myofibroblasts amounted to 1.93±1.07 with a range of 0 to 3 (Figure 2).

The immunohistochemical reactions of antibodies to Ki-67 showed nuclear localisation with variable colour intensity in individual cases. The reactions developed in the tumour cells. The mean percentage of Ki-67 positive breast cancer cells was 48.22±18.83 (Figure 2).

In the cases of VEGF, bFGF and UPA cytoplasmic reactions were obtained, of various intensity in individual cases and localised in the tumour cells. Mean reaction intensity in IRS score was 3.78±3.6 for VEGF, 4.96±4.14 for bFGF and 4.27±3.22 for UPA (Figure 2).

Relationships between presence of myofibroblasts and histopathological parameters. The analysis of the relationship between the presence of myofibroblasts in tumour tissue and clinical/pathological data demonstrated higher proportions of myofibroblasts in tumours of higher grade or in cases in which a relapse of the disease was noted or the patient died (Table III). No signifant relationship could be detected between the presence of myofibroblasts and pT, pN or age of the patient (Table III).

Relationships between presence of myofibroblasts and expression of studied proteins. The analysis of the relationship between the presence of myofibroblasts in tumour tissues and the expression of Ki-67, VEGF, bFGF and UPA in cancer cells showed that in cases with myofibroblasts present in higher numbers a higher proportion of the cancer cells manifested expression of Ki-67 (Figure 3A) and the expression of VEGF (Figure 3B) and bFGF (Figure 3C) in the tumour cells was elevated (Table III).

Table III. *Correlations between presence of myofibroblasts in tumour stroma and various clinicopathologic parameters and expression intensity of studied antigens (p-values, Chi-square test).*

Characteristics	Myofibroblasts
Grade	0.0348
pT	0.7512
pN	0.4179
Age	0.1872
Relapse	0.0066
Death	0.0067
Ki-67	0.0070
VEGF	0.0017
bFGF	0.0039
UPA	0.0699

Table IV. *Relationships between overall survival time and relapse-free survival and expression of Ki-67, VEGF, bFGF and UPA.*

Studied antigen	Overall survival	Relapse-free survival
Ki-67	<50% of positive cells, n=28 ≥50% of positive cells, n=17 p=0.0005	<50% of positive cells, n=28 ≥50% of positive cells, n=17 p=0.0016
VEGF	IRS score 0-3, n=23 IRS score 4-12, n=22 p=0.0002	IRS score 0-3, n=23 IRS score 4-12, n=22 p=0.0012
bFGF	IRS score 0-3, n=17 IRS score 4-12, n=28 p=0.0012	IRS score 0-3, n=17 IRS score 4-12, n=28 p=0.0056
UPA	IRS score 0-3, n=20 IRS score 4-12, n=25 p=0.0017	IRS score 0-3, n=20 IRS score 4-12, n=25 p=0.0013

Relationships between presence of myofibroblasts and patients survival. The Kaplan-Meier calculations demonstrated that the cases with a higher proportion of myofibroblasts in the cancer tissue (score 2-3) manifested a significantly abbreviated overall survival (Figure 4A) and a progression-free survival (Figure 4B).

Relationships between expression of Ki-67, VEGF, bFGF and UPA and patients survival. The cases with higher proportions of Ki-67 positive cells and higher expression of VEGF, bFGF and UPA exhibited a significantly shorter overall survival and relapse-free survival (Table IV).

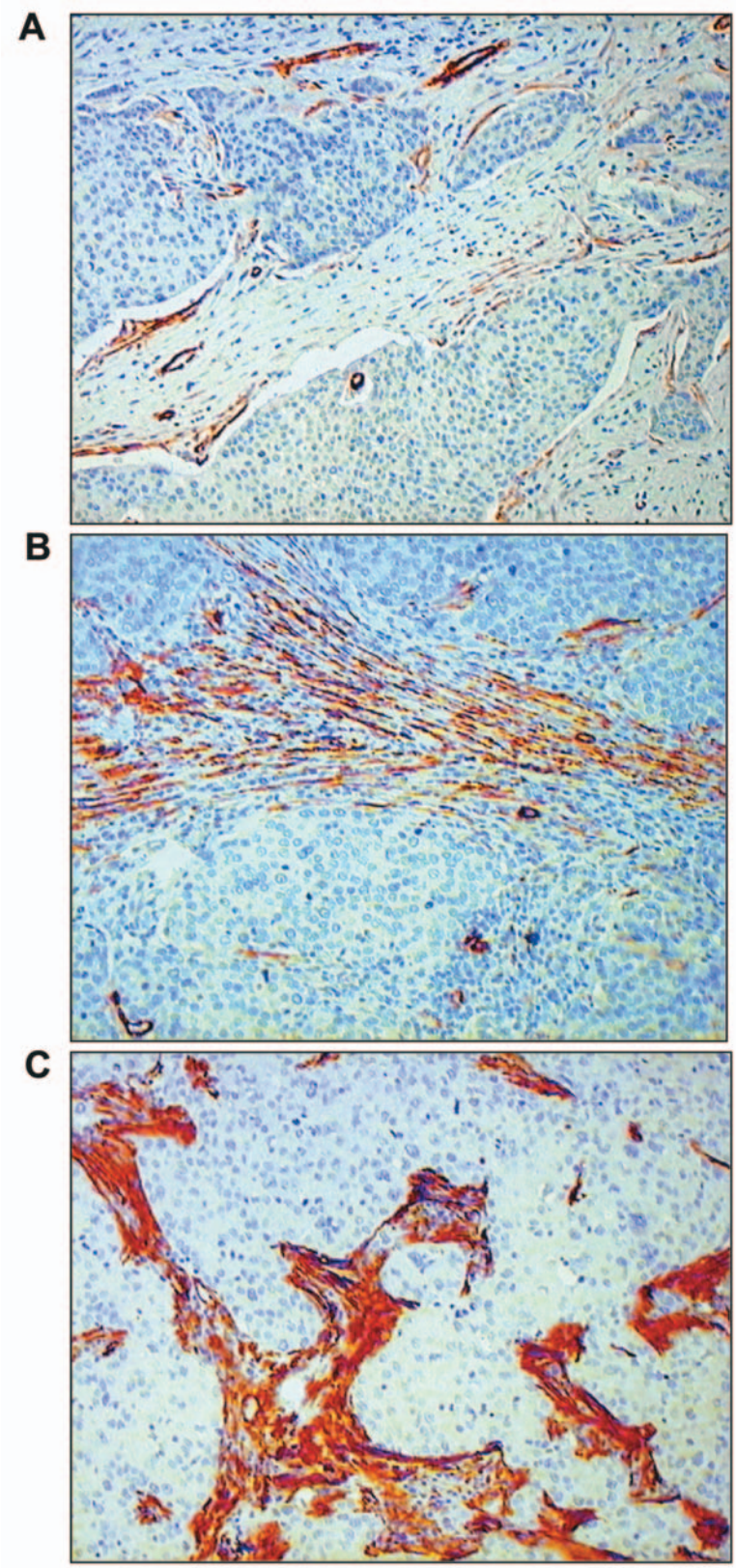
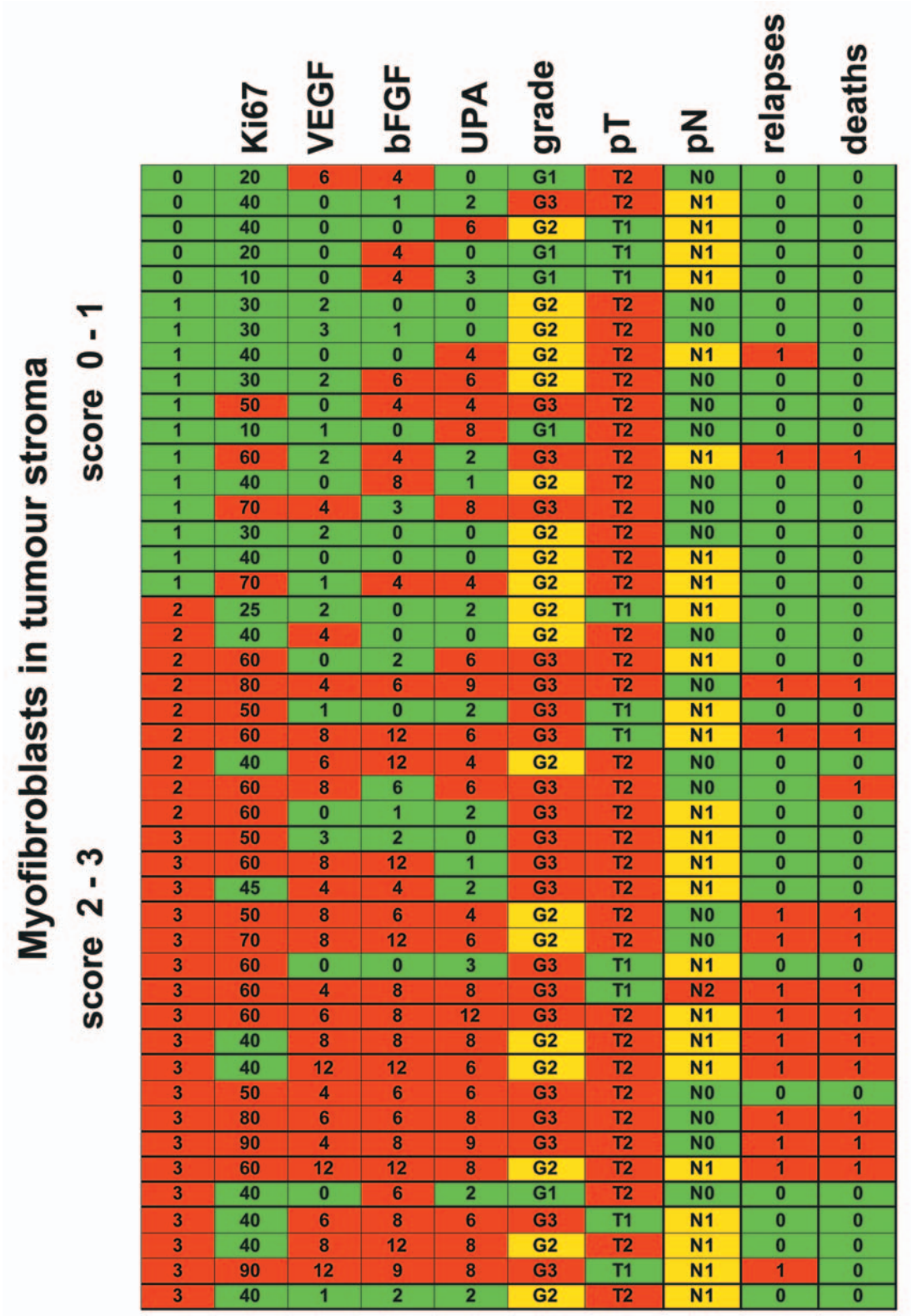


Figure 1. *Immunohistochemical localization of smooth muscle actin (SMA) expression in breast cancer tumours: (A) Reaction in individual myofibroblasts in tumour stroma and in blood vessels (score 1) (hematoxylin, x200); (B) Reaction in numerous myofibroblasts in tumour stroma and in blood vessels (score 2) (hematoxylin, x200), (C) Reaction in numerous myofibroblasts in tumour stroma (score 3) (hematoxylin, x400).*



Myofibroblasts in tumour stroma		Ki67	VEGF	bFGF	UPA	grade	pT	pN	relapses	deaths
score 0 - 1	0	20	6	4	0	G1	T2	N0	0	0
	0	40	0	1	2	G3	T2	N1	0	0
	0	40	0	0	6	G2	T1	N1	0	0
	0	20	0	4	0	G1	T1	N1	0	0
	0	10	0	4	3	G1	T1	N1	0	0
	1	30	2	0	0	G2	T2	N0	0	0
	1	30	3	1	0	G2	T2	N0	0	0
	1	40	0	0	4	G2	T2	N1	1	0
	1	30	2	6	6	G2	T2	N0	0	0
	1	50	0	4	4	G3	T2	N0	0	0
	1	10	1	0	8	G1	T2	N0	0	0
	1	60	2	4	2	G3	T2	N1	1	1
	1	40	0	8	1	G2	T2	N0	0	0
	1	70	4	3	8	G3	T2	N0	0	0
	1	30	2	0	0	G2	T2	N0	0	0
	1	40	0	0	0	G2	T2	N1	0	0
	1	70	1	4	4	G2	T2	N1	0	0
score 2 - 3	2	25	2	0	2	G2	T1	N1	0	0
	2	40	4	0	0	G2	T2	N0	0	0
	2	60	0	2	6	G3	T2	N1	0	0
	2	80	4	6	9	G3	T2	N0	1	1
	2	50	1	0	2	G3	T1	N1	0	0
	2	60	8	12	6	G3	T1	N1	1	1
	2	40	6	12	4	G2	T2	N0	0	0
	2	60	8	6	6	G3	T2	N0	0	1
	2	60	0	1	2	G3	T2	N1	0	0
	3	50	3	2	0	G3	T2	N1	0	0
	3	60	8	12	1	G3	T2	N1	0	0
	3	45	4	4	2	G3	T2	N1	0	0
	3	50	8	6	4	G2	T2	N0	1	1
	3	70	8	12	6	G2	T2	N0	1	1
	3	60	0	0	3	G3	T1	N1	0	0
	3	60	4	8	8	G3	T1	N2	1	1
	3	60	6	8	12	G3	T2	N1	1	1
	3	40	8	8	8	G2	T2	N1	1	1
	3	40	12	12	6	G2	T2	N1	1	1
	3	50	4	6	6	G3	T2	N0	0	0
	3	80	6	6	8	G3	T2	N0	1	1
	3	90	4	8	9	G3	T2	N0	1	1
	3	60	12	12	8	G2	T2	N1	1	1
	3	40	0	6	2	G1	T2	N0	0	0
	3	40	6	8	6	G3	T1	N1	0	0
	3	40	8	12	8	G2	T2	N1	0	0
	3	90	12	9	8	G3	T1	N1	1	0
	3	40	1	2	2	G2	T2	N1	0	0

Figure 2. *Comparison of myofibroblast presence in tumour stroma in studied patients as related to clinical and pathological variables and to expression of Ki-67, VEGF, bFGF and UPA in cancer cells. Red, myofibroblasts score: 2-3, Ki-67 in ≥50% of the cells, VEGF, bFGF and UPA IRS score ≥4, G3, T2, N2, relapses and deaths; green, myofibroblasts score: 0-1, Ki-67 in <50% of the cells, VEGF, bFGF and UPA IRS score <4, G1, T1, N1, no relapses and alive; yellow, G2, N1.*

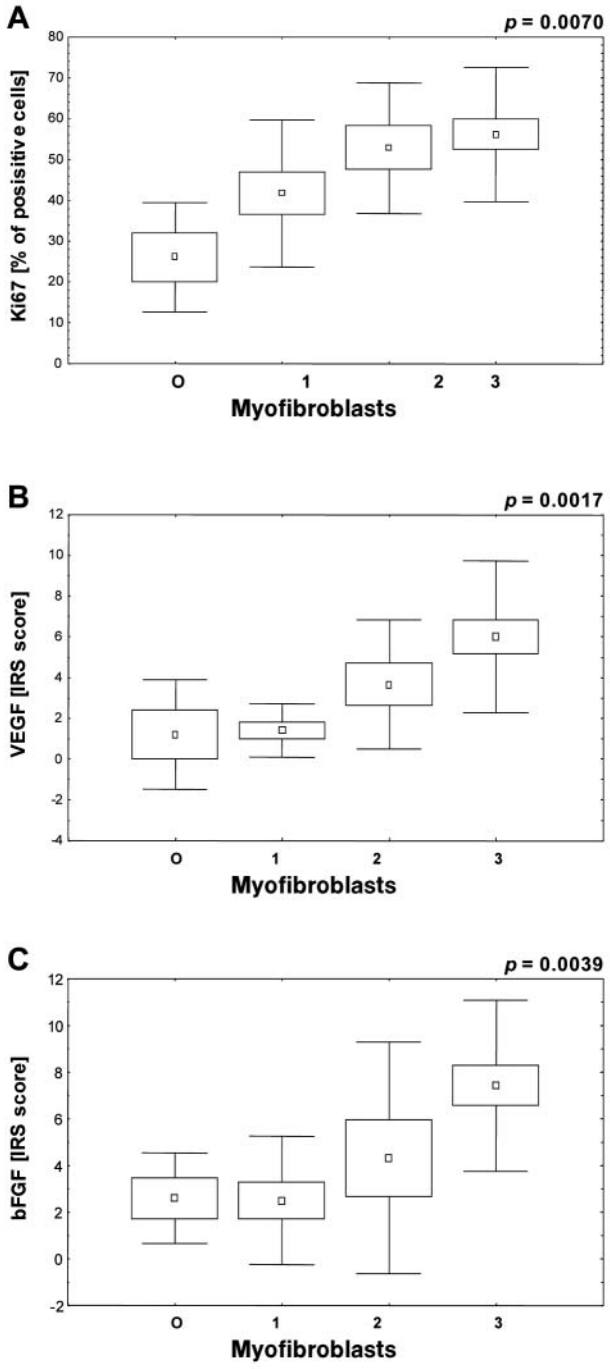


Figure 3. *Positive correlation between myofibroblast presence in tumour stroma in studied patients as related to: (A) Ki-67, (B) VEGF and (C) bFGF expression in breast cancer cells.*

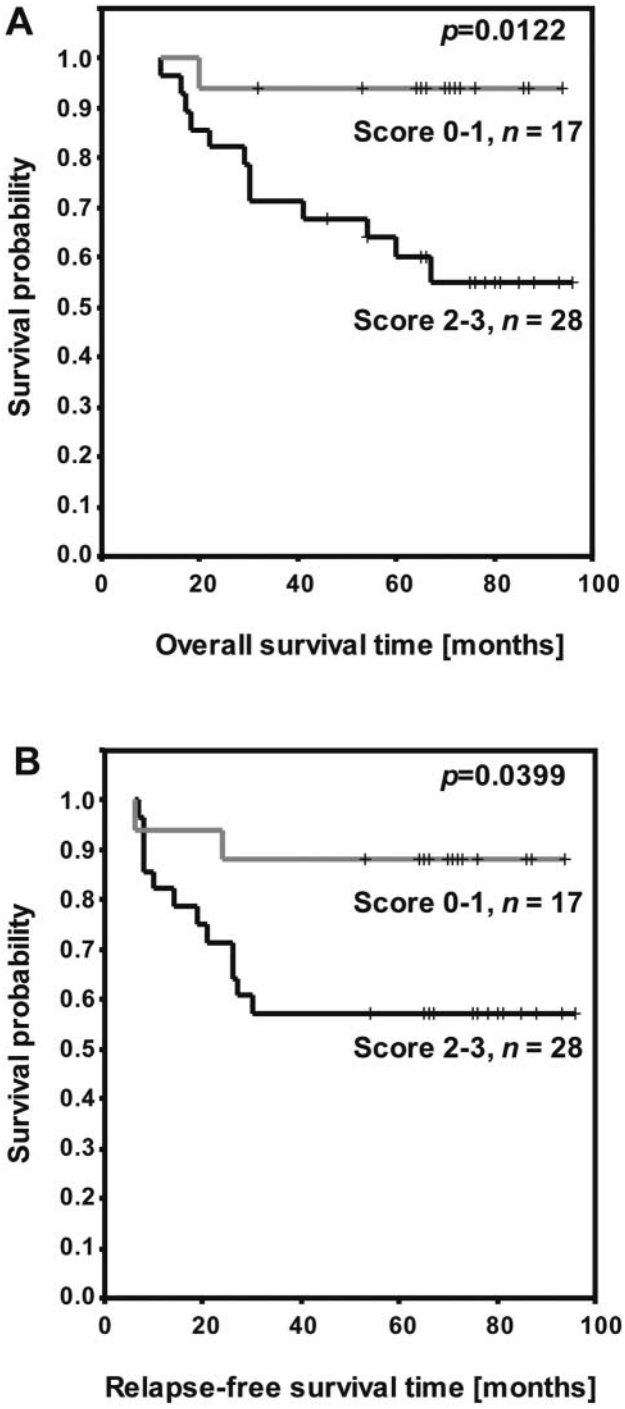


Figure 4. *Kaplan-Meier curves for survival and myofibroblast presence in tumour stroma in the studied group of 45 invasive breast cancer patients: (A) overall survival and (B) relapse-free survival.*

Discussion

In the present study the significance of the presence of myofibroblasts in tumour stroma, as detected by smooth muscle actin expression has been illustrated.The prognostic value of Ki-67, VEGF, bFGF and UPA expression in breast cancer cells was also examined and our studies confirmed that the proteins represented unfavourable prognostic indices in breast cancer (3, 12-18).

Myofibroblasts were present in the tumour stroma in a high proportion of breast cancer tissues, confirming the results of previous study (8) and those of Yazhou *et al.* (7). Similarly the relationsship between a higher proportion of myofibroblasts and higher tumour grade but no relationship between presence of myofibroblasts and stage orage of patient have also been confirmed (7, 8). Reproducibility of the results points to their high reliability.

The positive correlation between the presence of myofibroblasts and the intensity of tumour cell proliferation, measured by expression of Ki-67 suggested that myofibroblasts present in tumour structures may support tumour cell proliferation.

An elevated expression of VEGF and bFGF, the variables thought to promote invasiveness and angiogenesis has been demonstrated in the present study in tumours with higher proportions of myofibroblasts in their structure. Myofibroblasts have been shown to secrete insulin-like growth factor-2 (IGF-2) and hepatocyte growth factor (HGF) (6). Orimo *et al.* (19) have shown that in athymic mice grafted with breast cancer cells or with myofibroblasts isolated either from breast cancer or from healthy tissues the myofibroblasts accelerated tumour growth and intensified angiogenesis in the tumour. This effect has been related to secretion by the myofibroblasts of stroma cell-derived factor 1 (SDF-1/CXCL12) which stimulates breast cancer cell proliferation through the CXCR4 receptor and may induce recruitment of endothelial progenitor cells (19, 20). The present results suggest that this mechanism is probably also important also in human breast cancer as well as in the animal model.

This study has shown for the first time the prognostic value of myofibroblast presence in tissues of invasive ductal breast cancer. The cases with a higher proportion of myofibroblasts in the tumour tissue manifested a significantly shorter overall survival and relapse-free survival time.

In the present study for the first time cells distinct from neoplastic ones have been associated with the survival of patients with invasive ductal breast cancers. The phenomenon should be confirmed on higher numbers of patients.

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References

1 Jemal A, Thomas A, Murray T and Thun M: Cancer statistics. CA Cancer J Clin *52*: 23-47, 2002.

2 Mori I, Yang Q and Kakudo K: Predictive and prognostic markers for invasive breast cancer. Pathol Inst *52*: 186-194, 2002.

3 Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connoly JL, Hayes DF, Edge SB, Lichter A and Schnitt SJ: Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med *124*: 966-978, 2000.

4 De Wever O and Mareel M: Role of tissue stroma in cancer cell invasion. J Pathol *200*: 429-447, 2003.

5 Chaponnier C and Gabbiani G: Pathological situations characterized by altered actin isoform expression. J Pathol *204*: 386-395, 2004.

6 Offersen BV, Nielsen BS, Hoyer-Hansen G, Rank F, Hamilton-Dutoit S, Overgaard J and Andreasen PA: The myofibroblast is the predominant plasminogen activator inhibitor-1-expressing cell type in human breast carcinomas. Am J Pathol *163*: 1887-1899, 2003.

7 Yazhou C, Wenlv S, Weidong Z and Licun W: Clinicopathological significance of stromal myofibroblasts in invasive ductal carcinoma of the breast. Tumor Biol *25*: 290-296, 2004.

8 Surowiak P, Suchocki S, Györffy B, Gansukh T, Wojnar A, Maciejczyk A, Pudelko M and Zabel M: Stromal myofibroblasts in breast cancer: relations between their occurrence, tumor grade and expression of some tumor markers. Fol Histochem Cytobiol *44*: 105-110, 2006.

9 Elston CW and Ellis IO: Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology *5*: 403-410, 1991.

10 Sobin LH and Wittekind C: TNM classification of malignant tumours. 5th edition. Wiley-Liss Inc., New York, 2002.

11 Remmele W and Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe *8*: 138-140, 1987.

12 van Diest PJ, van der Wall E and Baak JP: Prognostic value of proliferation in invasive breast cancer: a review. J Clin Pathol *57*: 675-681, 2004.

13 Bando H, Weich HA, Horiguchi S, Funata N, Ogawa T and Toi M: The association between vascular endothelial growth factor-C, its corresponding receptor, VEGFR-3, and prognosis in primary breast cancer: a study with 193 cases. Oncol Rep *15*: 653-659, 2006.

14 Ryan BM, Konecny GE, Kahlert S, Wang HJ, Untch M, Meng G, Pegram MD, Podratz KC, Crown J, Slamon DJ and Duffy MJ: Survivin expression in breast cancer predicts clinical outcome and is associated with HER2, VEGF, urokinase plasminogen activator and PAI-1. Ann Oncol *17*: 597-604, 2006.

15 Jin Q, Hemminki K, Enquist K, Lenner P, Grzybowska E, Klaes R, Henriksson R, Chen B, Pamula J, Pekala W, Zientek H, Rogozinska-Szczepka J, Utracka-Hutka B, Hallmans G and Forsti A: Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis. Clin Cancer Res *11*: 3647-3653, 2005.

16 Linderholm BK, Lindh B, Beckman L, Erlanson M, Edin K, Travelin B, Bergh J, Grankvist K and Henriksson R: Prognostic correlation of basic fibroblast growth factor and vascular endothelial growth factor in 1307 primary breast cancers. Clin Breast Cancer *4*: 340-347, 2003.

17 Faridi A, Rudlowski C, Biesterfeld S, Schuh S, Rath W and Schroder W: Long-term follow-up and prognostic significance of angiogenic basic fibroblast growth factor (bFGF) expression in patients with breast cancer. Pathol Res Pract *198*: 1-5, 2002.

18 Urban P, Vuaroqueaux V, Labuhn M, Delorenzi M, Wirapati P, Wight E, Senn HJ, Benz C, Eppenberger U and Eppenberger-Castori S: Increased expression of urokinase-type plasminogen activator mRNA determines adverse prognosis in ErbB2-positive primary breast cancer. J Clin Oncol *24*: 4245-4253, 2006.

19 Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA: Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell *121*: 335-348, 2005.

20 Burger JA and Kipps TJ: CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood *107*: 1761-1767, 2006.

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