Pergamon

European Journal of Cancer Vol. 31A, No. 5, pp. 661–664, 1995

0959–8049/95 $9.50+0.00

0959-8049(94)00504-4

Prognostic Value of Total Cathepsin B in Invasive Ductal Carcinoma of the Breast

M. Budihna, J. Škrk, B. Zakotnik, D. Gabrijelčič and J. Lindtner

Total cathepsin B (catB) was determined by ELISA in 62 specimens of invasive ductal carcinoma of the breast. It was measured in μg/g of tumour protein (μg/gtp). The median catB was 91 μg/gtp, not varying significantly with T-stage or with age. It was higher in lymph-node negative (143 μg/gtp) than in lymph-node positive patients (49μg/gtp) ($P = 0.0005$), in grade 3 (132 μg/gtp) than in grade 1 and 2 tumours (72 μg/gtp) ($P = 0.07$) and in hormone receptor-negative (155 μg/gtp) than in hormone receptor-positive tumours (72 μg/gtp) ($P = 0.025$). The recurrence-free survival (RFS) at 54 months for patients with tumours with catB ≤23 μg/gtp was 22% and for catB >23 μg/gtp, 68% ($P = 0.0004$). CatB >23 μg/gtp did not significantly influence the RFS. Multivariate analysis showed that lymph nodes involvement ($P = 0.003$) and catB ($P = 0.007$) were independent prognostic factors.

Key words: breast carcinoma, disease-free survival, cathepsin B
Eur J Cancer, Vol. 31A, No. 5, pp. 661–664, 1995

INTRODUCTION

CATHEPSIN B is one of the proteolytic enzymes involved in the process of metastatic spread. It is found in the lysosomes [1], is able to cleave basement membranes [2] and degrade extracellular matrix, and thus could promote the seeding of tumour cells [3]. Little is known about recurrence-free (RFS) survival of patients with breast carcinoma in relation to the tumour concentration of cathepsin B. The aim of this study was to determine the RFS of patients with invasive ductal carcinoma of the breast in relation to the tumour concentration of cathepsin B.

PATIENTS AND METHODS

Patients

Between 1 January 1988 and 31 December 1989, 62 non-selected patients were treated for operable invasive ductal carcinoma of the breast, graded according to the criteria described by Bloom and Richardson [4]. All patients were female, aged 32–78 years (median 56 years). Table 1 shows the distribution of patients according to their tumour stage and number of positive lymph nodes. The primary treatment was modified radical mastectomy or breast-conserving surgery with axillary dissection (59 patients) or without (3 patients). All patients with conserving surgery had postoperative radiotherapy with 50 Gy to the whole breast, and a boost to the tumour bed of 10–20 Gy. Postoperative radiotherapy after total mastectomy was given if the tumours were in advanced stage. Lymph node-positive premenopausal patients and postmenopausal patients below 70 years with negative hormone receptors or high-grade tumours received adjuvant chemotherapy. Postmenopausal patients with positive lymph nodes and with positive hormone receptors received adjuvant endocrine therapy.

Table 1. Tumour stage and number of positive lymph nodes

Tumour stage	No. of positive lymph nodes 0–3	>3	Unknown	All
T1	11	1	1	13
T2	27	5	0	32
T3	6	5	0	11
T4	2	2	2	6
All	46	13	3	62

The median follow-up of patients was 44 months (4–54).

Cathepsin B assay

Samples of tumours were obtained immediately after surgical removal and immersed in liquid nitrogen. Possible necrotic parts were carefully removed and this was controlled histologically. The tissue homogenates (also called cytosolic fraction) were prepared from tissue samples by homogenisation and centrifugation (40 000 *g*), as reported by McGuire and associates [5]. Some of the homogenate was used for steroid hormonal receptor determination and the rest for biochemical studies. Total cathepsin B levels (active and inactive) were determined in those samples by ELISA [6]. Cathepsin B ELISA and other tests are commercially available from BioASS (D-86911 Diessen, F.R.G.). Microtitre plate wells (Nunc, Denmark) were coated overnight at 4°C with 2.5 mg/l of sheep anti-cathepsin B IgG in

Correspondence to M. Budihna.
M. Budihna, J. Škrk, B. Zakotnik and J. Lindtner are at the Institute of Oncology, Zaloška 2–4, 61105 Ljubljana, Slovenia; and D. Gabrijelčič is at BioAss, Fritz-Winter Str. 32, D-86911 Diessen, F.R.G.
Revised 6 Oct. 1994, accepted 24 Nov. 1994.

15 mmol/l carbonate buffer, pH 9.6. Thereafter the wells were washed with phosphate-buffered saline (pH 7.1, containing 0.5 g/l Tween 20). Aliquots (0.1 ml) of the standard solutions or samples (tumour cytosols diluted with phosphate-buffered saline-Tween containing 20 g/l bovine serum albumin) were applied to each well, incubated for 2 h at 37°C and washed again for incubation. Then, 0.1 ml of rabbit anti-cathepsin B IgG solution (0.5 mg/l in washing buffer containing 20 g/l bovine serum albumin) was added to each well and incubated again for 2 h at 37°C. After washing, the bound rabbit antibodies were determined with goat anti-rabbit IgG peroxidase conjugate (dilution 1: 100000) and incubated for 2 h at 37°C. After washing with phosphatase-buffered saline, 0.1 ml solution of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline sulphonic acid)] (0.1 g/l) and hydrogen peroxide (0.12 g/l) in 0.01 mol/l citrate phosphate buffer, pH 4.1 was added to the wells and absorbance measured at 410 nm (SLT-ELISA reader) after incubation for 30 min at 37°C. Blanks, containing washing buffer instead of samples, were performed simultaneously. The concentration of cathepsin B was measured in μg/g of tumour protein (μg/gtp). The specimen of cytosol was used for cathepsin B determination only once.

Since macrophages also contain cathepsin B [7], histological slides were examined, retrospectively, for possible infiltration of the tumour with these cells, but no notable infiltration was found.

Statistical methods

The RFS was calculated using the Kaplan–Meier product limit method [8]. The association of different tumour concentrations of cathepsin B with RFS was studied. The statistical difference in RFS was calculated with the log rank test [9]. The difference in distribution of patients in various groups was calculated by Fisher's exact test and the difference between the medians of tumour concentration of cathepsin B in different groups was calculated by the Mann–Whitney test. Survival analysis with covariates was performed based on the Cox proportional hazards model [10].

RESULTS

The concentration of cathepsin B in tumours ranged from 14 to 716 μg/gtp (median 91 μg/gtp). Table 2 shows the range and median tumour concentration of cathepsin B by tumour stage, number of positive lymph nodes, histological grade, age and hormone receptors.

Patients with a tumour concentration of cathepsin B ≤23 μg/gtp had the shortest RFS. Patients with cathepsin B tumour levels above 23 μg/gtp had longer RFS, although there was no significant difference with regard to how much above that value it was (Figure 1). Table 3 shows the distribution of patients,

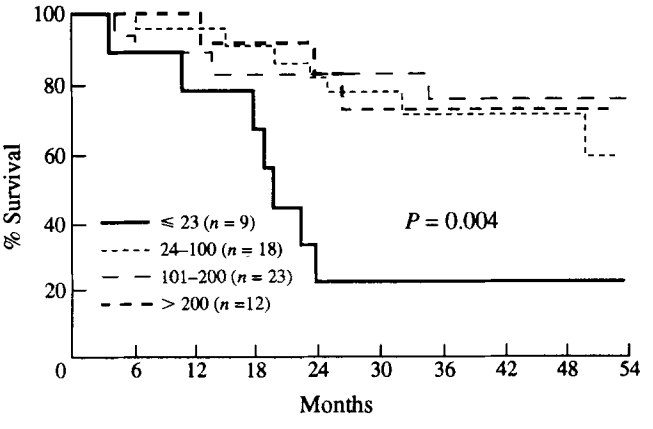

Figure 1. Recurrence-free survival according to different cathepsin B tumour concentration in μg/gtp. *n*, number of patients.

Table 2. Tumour concentration of cathepsin B in different prognostic groups

Prognostic group	No. of patients	Tumour concentration of cathepsin B (μg/gtp) Range	Median	*P* value
Tumour stage				
T 1 and 2	45	14–716	77	
T 3 and 4	17	16–455	81	0.66
No. of positive lymph nodes*				
0	29	15–455	143	
1–3	17	14–240	50	
>3	13	14–182	41	0.0005
Grade				
1 and 2	39	15–716	72	
3	23	14–455	132	0.07
Age (years)				
≥50	39	16–716	107	
<50	23	14–455	72	0.56
HR+	47	15–401	72	
HR−	15	14–716	155	0.025
Total	62	14–716	91	

* *n* = 59, missing data for 3 patients. HR, hormonal receptor.

Table 3. Distribution of patients with low (≤23 μg/gtp) and high (>23 μg/gtp) tumour concentration of cathepsin B according to known prognostic groups

Prognostic group	Cathepsin B ≤23 μg/gtp	Cathepsin B >23 μg/gtp	*P* value
Tumour stage			
T 1 and 2	6	39	
T 3 and 4	3	14	0.47
No. of positive lymph nodes*			
0–3	4	42	
>3	4	9	0.06
Grade			
1 and 2	8	31	
3	1	22	0.08
Age (years)			
≥50	6	20	
<50	3	33	0.56
HR+	8	39	
HR−	1	14	0.30

*Missing data for 3 patients. HR, hormonal receptor.

using a cut-off value for cathepsin B concentration in the tumour of 23 μg/gtp, according to T-stage, number of positive lymph nodes, histological grade and hormone receptor status. The univariate analysis of RFS according to standard prognostic factors and cathepsin B concentration with a cut-off value of 23 μg/gtp is shown in Table 4. Patients with a tumour concentration of cathepsin B ≤23 μg/gtp had a shorter RFS (22%) than patients with a tumour concentration of cathepsin B >23 μg/gtp (68%) (Figure 2). When standard prognostic factors and cathepsin B were entered in the multivariate model, lymph nodes ($P = 0.003$) and cathepsin B ($P = 0.007$) remained as independent prognostic factors.

Table 4. Recurrence-free survival at 54 months according to known prognostic groups and tumour concentration of cathepsin B

	No. of patients	Recurrence-free survival (%)	*P* value
Tumour stage			
T 1 and 2	45	64	
T 3 and 4	17	53	NS
No. of positive lymph nodes*			
0–3	46	71	
>3	13	36	0.0003
Grade			
1 and 2	39	73	
3	23	46	NS
Age (years)			
≥50	39	64	
<50	23	59	NS
HR+	47	63	
HR−	15	56	NS
Cathepsin-B			
≤23 μg/gtp	9	22	0.0004
>23 μg/gtp	53	68	
Total	62	62	

* Missing data for 3 patients. HR, hormonal receptor.

DISCUSSION

As cathepsin B cleaves basement membranes and degrades extracellular matrix, it could promote the seeding of tumours [3]. We expected that patients with higher tumour concentration of cathepsin B would have a higher recurrence rate than patients with lower concentration of cathepsin B. This could also be expected considering the cathepsin B concentration in normal tissue and in the tumour. For example, Lah and associates

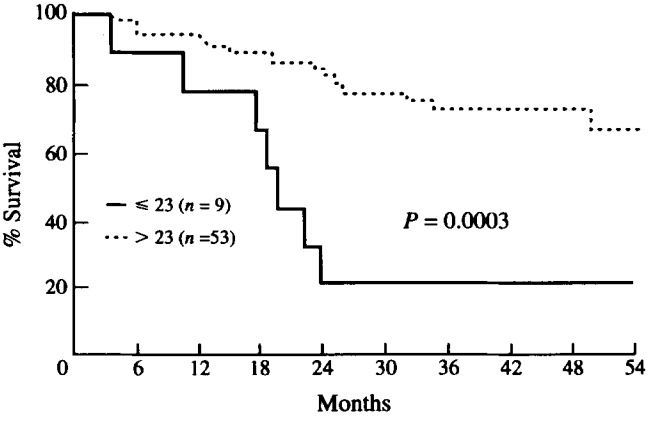

Figure 2. Recurrence-free survival according to the cut-off value for cathepsin B tumour concentration of 23 μg/gtp. *n*, number of patients.

[11] reported an 18.5-fold increase in cathepsin B activity in carcinoma of the breast compared to the activity of cathepsin B in the normal glandular tissue of the breast. At the same time, they observed a lower activity of cysteine proteinase inhibitors in two-thirds of breast carcinomas. Similar results on the activity of cathepsin B in breast carcinoma and normal glandular tissue of the breast were reported by Gabrijelčič and associates [6] and Vashista and associates [12]. Elevated cathepsin B activity was also observed in colorectal carcinoma compared with its activity in normal colon mucosa [13], and in lung carcinoma compared with normal lung parenchyma [14]. Increased activity of cathepsin B in tumours has been correlated with increased metastatic capability of animal tumours and malignancy of human tumours [2]. Hirano and associates [15] found higher levels of cathepsin B in serum and urine in cancer patients with remote metastases.

In our study, the median tumour concentration of cathepsin B was independent of T-stage, but it was significantly higher in node-negative tumours than in node-positive tumours. It was marginally higher in histological grade 3 than in grades 1 and 2 tumours. The tumour concentration of cathepsin B was independent of age, but it was significantly ($P = 0.025$) higher in hormone receptor-negative than in hormone receptor-positive tumours (Table 2). The lowest tumour concentrations of cathepsin B ($\leq$23 μg/gtp) were rather uniformly distributed among early and advanced tumours, tumours in younger and older patients, and among hormone receptor-negative and -positive tumours. Cathepsin B in the tumour $\leq$23 μg/gtp, however, occurred relatively more often in > three lymph node-positive group than in the group with $\leq$ three positive lymph nodes ($P = 0.06$) and appeared more often in the tumours with histological grades 1 and 2 than in grade 3 tumours ($P = 0.08$).

There is no general correlation between different levels of proteinases and length of RFS of patients. Lah and associates found no difference in 2-year disease-free survival in patients with breast carcinoma in relation to cathepsin B activity [11]. Yamashita and colleagues [16] found, unexpectedly, that the prognosis of breast cancer was poor when the total plasminogen activator (PA) activity (tissue-type and urokinase-type) was low and not high. It is interesting that they found shorter RFS and overall survival in patients where the total PA activity in the tumour was $\leq$60 U/mg of protein. Patients with total PA activity in the tumour above this cut-off level had longer RFS and overall survival, with no relationship to how much higher the total activity of PA in the tumour was. This is analogous to the findings in our study with cathepsin B, where only low tumour concentrations of total (active and inactive) cathepsin B ($\leq$23 μg/gtp) were associated with aggressive behaviour of the tumour, whereas the concentration of cathepsin B above 23 μg/gtp was associated with significantly better prognosis independent of the cathepsin B concentration in the tumour (Figure 1). We have no explanation for this phenomenon. Further detailed studies on more patients are needed to resolve these conflicting results.

Even though the observed group was small and the follow-up time was relatively short, it appears that the total cathepsin B concentration in the tumour might be a powerful independent prognostic factor for RFS in invasive ductal carcinoma of the breast.

1. Howie AJ, Burnett D, Crocker J. The distribution of cathepsin B in human tissues. *J Pathol* 1985, **145**, 307–314.
2. Sloane BF, Moin K, Krepela E, Rozhin J. Cathepsin B and its endogenous inhibitors: the role in tumour malignancy. *Cancer and Metastasis Rev* 1990, **9**, 333–352.
3. Buck MR, Karustis DG, Day NA, Honn KV, Sloane BF. Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumour tissues. *Biochem J* 1992, **282**, 273–278.
4. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. A study of 1049 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957, **11**, 359–377.
5. McGuire WL. Estrogen receptors in human breast cancer. *J Clin Invest* 1973, **52**, 73–77.
6. Gabrijelčič D, Svetič B, Spajič D, *et al.* Cathepsins B, H and L in human breast carcinoma. *Eur J Clin Chem Clin Biochem* 1992, **30**, 69–74.
7. Machleidt W, Asfalg-Machleidt I, Jochum M, Jänicke F, Schmitt M. Lysosomal cystein proteinases as mediators in inflammation and tumour spread: control of the extracellular activity. *Fibrinolysis* 1992, **6**(suppl. 4), 125–129.
8. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
9. Peto R, Pike MC, Armitage P, Breslow NE, *et al.* Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 1975, **35**, 1–39.
10. Cox DR. Regression models and life tables. *J Royal Stat Soc* 1972, **34(B)**, 187–202.
11. Lah TT, Kunovar-Kokalj M, Strukelj B, *et al.* Stefins and lysosomal cathepsins B, L and D in human breast carcinoma. *Int J Cancer* 1992, **50**, 36–44.
12. Vashista A, Baker PR, Preece PE, Wood RAB, Cuschieri A. Inhibition of proteinase-like peptidase activities in serum and tissue from breast cancer patients. *Anticancer Res* 1988, **8**, 765–790.
13. Sheahan K, Shuja S, Murnane MJ. Cystein protease activities and tumour development in human colorectal carcinoma. *Cancer Res* 1989, **49**, 3809–3814.
14. Krepela E, Kasafirek E, Novak K, Viklicky J. Increased cathepsin B activity in lung tumours. *Neoplasma* 1990, **37**, 61–70.
15. Hirano T, Manabe T, Takeuchi S. Serum cathepsin B levels and urinary excretion of cathepsin B in the cancer patients with remote metastasis. *Cancer Letters* 1993, **70**, 41–44.
16. Yamashita J, Ogawa M, Inada K, *et al.* Breast cancer prognosis is poor when the total plasminogen activator activity is low. *Br J Cancer* 1993, **67**, 374–378.