American Journal of Pathology, Vol. 141, No. 5, November 1992

Short Communication

Cathepsin D in Invasive Ductal NOS Breast Carcinoma as Defined by Immunohistochemistry

No Correlation with Survival at 5 Years

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Cathepsin D expression was assessed by immunohistochemistry in 59 node-negative and 77 node-positive infiltrative ductal not otherwise specified (NOS) breast carcinomas and compared with overall survival at 90 months. Cancer cells in 60% (81/136) of the tumors expressed cathepsin D. In the stroma of 33% (18 of 55) cathepsin D negative tumors, numerous strongly cathepsin D positive, benign macrophage-like cells were found. Multivariate analysis showed no significant correlation of cathepsin D expression and overall survival for all patients for node-negative and node-positive patients and for patients with vimentin-positive and -negative tumors. However, in node-negative but not in node-positive patients, a trend for better survival for patients with cathepsin-positive vimentin-negative tumors and worse survival for those with cathepsin-positive vimentin-positive tumors was noted. Due to the low number of patients in these subgroups, neither trend reached significance. Cathepsin D expression was independent of patient age, size, and histologic grade of tumor, and vimentin expression. However, in the node-positive group, negative correlation of cathepsin D and vimentin expression was found. We suggest that prognostic significance of cathepsin D in infiltrative ductal NOS breast carcinomas may be associated with the pathway of its synthesis rather than with its mere presence in tumor cells. (Am J Pathol 1992, 141:1003–1012)

Cathepsin D is a lysosomal protease that exists in the cell as a precursor form (procathepsin D), intermediate active enzyme, and a mature active enzyme that normally functions in the lysosomes at acidic pH. Synthesis of procathepsin D is regulated by estrogens in estrogen-dependent breast cancer cell lines.[1] In normal mammary cells, most of the procathepsin D is processed to the mature active form in the lysosomes and only small amounts of procathepsin D are secreted. In breast cancer cell lines, processing of the proenzyme is delayed; it accumulates in the cell and at least 45% is secreted.[2] Two activities of secreted procathepsin D have been shown in culture: a proteolytic activity on various substrates including basement membrane and proteoglycans after its autoactivation at acidic pH, and an autocrine mitogenic activity on estrogen-depleted MCF-7 cells.[3] Both suggest a role for cathepsin D in cancer invasion and metastasis, although the exact mechanism is unknown. Further support for such a role comes from transfection experiments. Thus the metastatic activity of a rat tumorigenic cell line that secretes no cathepsin D *in vitro* was significantly higher when the cells were transfected with human procathepsin D gene and injected intravenously into athymic mice.[4]

Immunoenzymatic studies of cathepsin D levels in cy-

Supported in part by a grant from the Mildred Scheel Stiftung of the Deutsche Krebshilfe (Mary Osborn) and by a grant from the National Research Committee (KBN) (Wenancjusz Domagala).

Accepted for publication July 24, 1992.

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tosols of breast carcinomas found that high concentrations of this enzyme were associated with poor prognosis and increased risk of metastases.[5–7] In contrast an immunohistochemical study has shown that positive cathepsin D immunostaining was associated with a significant prognostic advantage in patients with lymph node metastases of breast carcinoma.[8] Significantly prolonged survival was found for the subgroup of patients with estrogen receptor-positive tumors expressing cathepsin D.[8]

Vimentin in breast carcinomas is associated with poor prognostic indicators such as low estrogen receptor level,[9,10] positive epidermal growth factor receptor (EGFR) status,[11] high proliferative activity of the tumor[10,12] and histologic grade of ductal not otherwise specified (NOS) carcinomas.[10,12–14] Recently we have shown a direct relation between vimentin expression and poor prognosis in node negative ductal NOS breast carcinomas.[15]

In view of the apparently conflicting results concerning the prognostic significance of cathepsin D,[5–8] we have used immunocytochemistry to look for a correlation between the expression of vimentin and cathepsin D, as well as for a correlation between overall survival at 5 years and cathepsin D expression in patients with infiltrative ductal NOS breast carcinomas.

Materials and Methods

Patients

Formalin-fixed and paraffin-embedded biopsies from 136 unselected patients who underwent mastectomy for primary infiltrative ductal NOS breast cancer were examined. These were retrieved from the files of the Department of Oncology, Medical Academy of Lodz, Poland, where the patients had been operated on between 1980 and 1985. A computerized database containing the age of the patient, the number of positive lymph nodes, the size of the tumor, histologic type, histologic grade according to Bloom and Richardson,[16] the stage of the disease at diagnosis, the treatment protocol, and the date of the last checkup or death was available on each patient for statistical analysis. Estrogen receptor status was not available. All patients analyzed in this study were used in a previous study on vimentin expression and survival.[15] In that study of 195 cases, 34 were vimentin-positive and 161 were vimentin-negative. Of these, tumor tissues in paraffin blocks were still available for 136 cases (34 vimentin-positive and 102 vimentin-negative), and these were included in the current study. Histologic typing was completed according to published criteria,[17] and histologic grading was according to Bloom and Richardson.[16]

Immunohistochemistry

A rabbit polyclonal cathepsin D antiserum diluted 1:100 (Medac, Hamburg, original supplier Novocastra, Newcastle, England) was used together with a streptavidin-biotin-peroxidase kit (Histostain-SP Kit, Zymed Lab, San Francisco) to determine positivity for cathepsin D. According to the manufacturer, this is a different cathepsin D antibody from that used in the study of Henry et al.[8] For the cathepsin assays, sections were deparaffinized and then trypsinized using 0.1% trypsin for 5 minutes at room temperature. The mouse monoclonal V9 antibody[18] was used in the indirect immunoperoxidase method to determine positivity for vimentin.[14]

Statistical Analysis

Follow-ups were conducted until 1990; 78 patients died of the cancer between 1 and 87 months after initial surgery (average = 36 months) whereas 58 patients were alive at the last follow-up between 42 and 107 months after surgery (average = 84 months). Using the product-limit (PL) estimator P(t),[19] we can calculate 1) the probability of survival for all or any subset of patients at any time in this interval, 2) the mean lifetimes of these patients limited to the end of the interval, and 3) the variances of these statistics. At any point, only the patients still at risk determine the survival probabilities. This permits survival graphs and corresponding statistics for times up to 90 months. To test the significance of the Kaplan-Meier survival plots, χ^2 values were calculated by log-rank statistics (generalized Savage test). Because there were indications that the various parameters were noncumulative, multivariate analysis was performed by calculation of the coefficients of correlation in certain subgroups. The significance of correlation was determined by the *t* statistic.

Results

Cathepsin D Expression in Tumors

Cathepsin D immunostaining was seen as red, coarse, or tiny granules dispersed in the cytoplasm of the cells.

Tumors were regarded as positive for cathepsin D if numerous granules were detected in the majority of tumor cells in a section. Of 136 infiltrating ductal NOS carcinomas in our study, 81 (60%) cases were cathepsin D-positive (Figure 1A, B, C) and 23 (17%) were cathepsin D-negative (Figure 1D). In the other 32 cases, cathepsin D-positive tumor cells were found only focally in a section, in less than 10% of tumor cells.

Initial statistical analysis of the data was completed in

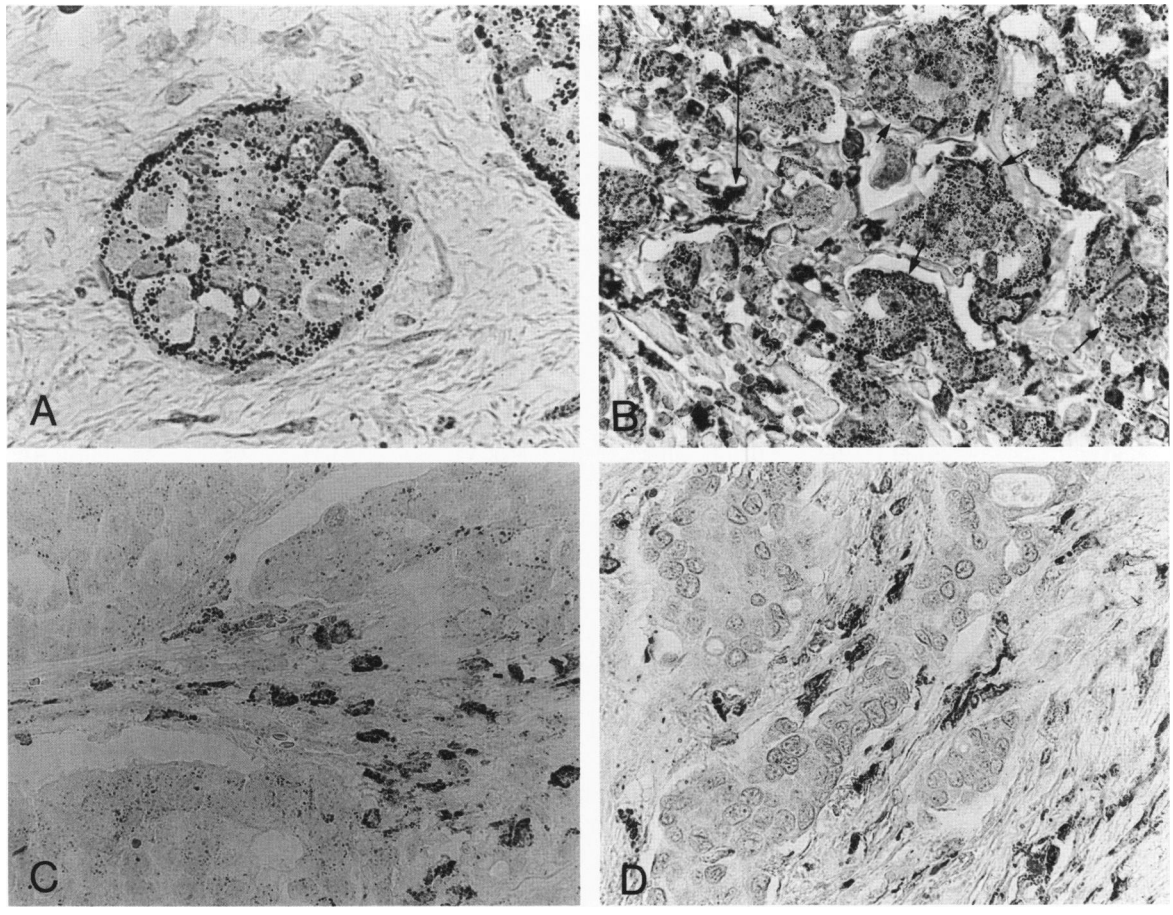


Figure 1. *Invasive ductal NOS breast carcinomas (streptavidin-biotin-peroxidase, light nuclear counterstain).* **A:** *Strong cathepsin D expression. Note numerous coarse cytoplasmic granules positive for cathepsin D (×800).* **B:** *Strong cathepsin D expression in the cytoplasm of tumor cells (short arrows) and in benign stromal cells (long arrow) (×400).* **C:** *Cathepsin D positive tumor cells with tiny cytoplasmic granules and numerous strongly cathepsin D positive stromal macrophage-like cells (×400).* **D:** *Cathepsin D negative tumor. Note strong cathepsin D expression in benign stromal cells (×400).*

two ways. First by including the 32 cases with focal staining in the cathepsin D-negative group (Figure 2A). Thus a total of 81 positive cases plus 55 negative cases (23 negative and 32 focally positive cases) were analyzed. Second by including the 32 focal cases in the positive group (data not shown). In this analysis, a total of 113 positive cases (81 cathepsin D-positive plus 32 focally positive) were analyzed versus 23 negative cases. These analyses showed that there was no significant difference in C+ and C− survival rates regardless of which method was used. In view of this and another report in which cathepsin D concentration was found to be most useful in predicting prognosis when tumors were assigned to only two groups, with low versus high concentrations analyzed[5] we have included the 32 cases with focal staining in the cathepsin D-negative group in the subsequent analyses that are presented in Tables 1 through 3. Table 1 gives an overview of the total material divided according to cathepsin D content, as compared with other criteria. Table 2 and Table 3 use the same display form but show the data subdivided for lymph node-negative and lymph node-positive patients.

The data in these tablets are discussed in greater detail below. Cathepsin D was found in 64% (38 of 59) of the tumors from node-negative patients and in 56% (43 of 77) of tumors from node-positive patients. There was no significant difference in the incidence of cathepsin D with regard to tumor size, histologic grade, or patient age, either in the whole cohort of patients or in node-positive and node-negative subgroups (Tables 1, 2, 3, 4).

Cathepsin D Expression in Benign Cells

Benign stromal cells, some of them presumably macrophages, usually contained numerous coarse, strongly cathepsin D-positive granules. These benign cells could be easily distinguished from cancer cells by their morphology. In the stroma of 33% (18 of 55) cathepsin D-negative tumors, there were numerous benign macro-

Table 1. *Cathepsin D as Compared with Other Prognostic Factors in Infiltrating Ductal NOS Breast Carcinomas**

	Cath D positive/total		% Cath D positive that survive 5 years	% Total that survive 5 years
	No.	%		
All patients	81/136	60	54	50
Vimentin				
Positive	16/34	47	38	38
Negative	65/102	64	58	54
Histologic grade				
I & II†	44/70	63	66	63
III	37/66	56	41	36
Patient's age				
<50 yr	27/39	69	67	62
≥50 yr	54/97	56	48	45
Tumor size				
<5 cm	52/83	63	60	54
≥5 cm	29/53	55	45	43
Axillary lymph nodes				
Positive	43/77	56	44	39
Negative	38/59	64	66	64

* The prognostic value of cathepsin D is nowhere significant ($P < 0.1$).
† Six grade I carcinomas.

phage-like cells that were cathepsin D-positive. These sometimes surrounded and infiltrated clumps of tumor cells. In the remaining cases, a variable number of macrophage-like cathepsin D-positive cells were found. These cells served as a built-in positive control. These benign cells were usually strongly cathepsin D-positive (Figure 1C, D) and could have influenced the results, had tissue cytosols been tested for cathepsin D. For instance, cathepsin D-negative tumors with heavy infiltrates of cathepsin D-positive benign cells plus cathepsin D-positive tumors constitute together 73% (99/136) of breast carcinomas in this series, whereas only 60% of tumors are cathepsin D-positive.

Table 2. *Cathepsin D as Compared with Other Prognostic Factors in Node-negative Infiltrating Ductal NOS Breast Carcinomas**

	Cath D positive/total		% Cath D positive that survive 5 years	% Total that survive 5 years
	No.	%		
All patients	38/59	65	66	64
Vimentin				
Positive	12/20	60	25	35
Negative	26/39	67	85	79
Histologic grade				
I & II	24/34	71	71	71
III	14/25	56	57	56
Patient's age				
<50 yr	16/22	73	75	73
≥50 yr	22/37	59	59	59
Tumor size				
<5 cm	27/41	66	70	66
≥5 cm	11/18	61	55	61

* The prognostic value of cathepsin D is nowhere significant ($P < 0.1$).

Table 3. *Cathepsin D as Compared with Other Prognostic Factors in Node-positive Infiltrating Ductal NOS Breast Carcinomas**

	Cath D positive/total		% Cath D positive that survive 5 years	% Total that survive 5 years
	No.	%		
All patients	43/77	56	44	39
Vimentin†				
Positive	4/14	29	75	43
Negative	39/63	62	41	38
Histologic grade				
I & II	20/36	56	60	56
III	23/41	56	30	24
Patient's age				
<50 yr	11/17	65	55	47
≥50 yr	32/60	53	41	37
Tumor size				
<5 cm	25/42	60	48	43
≥5 cm	18/35	51	39	34

* The prognostic value of cathepsin D is nowhere significant ($P < 0.1$).
† The inverse correlation between cathepsin D and vimentin is significant ($P < 0.05$).

Vimentin Expression in Tumors

Tumors were considered positive for vimentin when there was cytoplasmic staining with V9 antibody in >10% of tumor cells as assessed semiquantitatively. Negative staining of benign epithelial cells and positive staining of fibroblasts, macrophages, lymphocytes, and endothelial cells constituted built-in positive and negative controls. There were 34 vimentin-positive and 102 vimentin-negative cases in this study. Vimentin was found in 34% (20 of 59) of node-negative cases and in 18% (14 of 77) of node-positive ones.

Cathepsin D and Vimentin Expression in Tumors

Cathepsin D positive carcinomas occurred with almost equal frequency in vimentin-positive and -negative subgroups (47% vs. 64%) (Table 1). However, in node-positive patients, a significant reverse association between vimentin and cathepsin D was found ($P < 0.05$). Only 29% of vimentin-positive tumors expressed cathepsin D versus 62% of vimentin-negative cancers (Table 3). This correlation was not seen in node-negative patients in whom 60% of vimentin-positive and 67% of vimentin-

Table 4. *Coefficients of Correlation Between Survival, Lymph Nodes Involved, Tumor Size, Age, Vimentin, and Cathepsin D in Ductal NOS Breast Carcinomas*

	Months	Nodes	Size	Age	Vimentin	Cath-D	Grade
A All patients (n = 136)							
Mean	56.65	3.06	4.40	55.65	0.25	0.60	2.44
Sigma	30.11	4.17	1.72	11.59	0.43	0.49	0.58
Months	1.00						
Nodes	**−0.45**	1.00					
Size	**−0.31**	**0.25**	1.00				
Age	**−0.20**	0.14	**0.20**	1.00			
Vimentin	**−0.20**	−0.02	−0.02	−0.12	1.00		
Cath-D	0.09	−0.04	−0.11	−0.05	−0.15	1.00	
Grade	**−0.28**	**0.18**	0.04	−0.14	0.12	−0.04	1.00
B Node negative (n = 59)							
Mean	65.73	0.00	4.03	54.63	0.34	0.64	2.36
Sigma	27.49	0.00	1.68	13.49	0.47	0.48	0.60
Months	1.00						
Nodes	0.00	0.00					
Size	**−0.26**	0.00	1.00				
Age	−0.13	0.00	0.16	1.00			
Vimentin	**−0.47**	0.00	0.05	−0.15	1.00		
Cath-D	0.03	0.00	−0.09	−0.10	−0.07	1.00	
Grade	−0.19	0.00	0.11	**−0.27**	**0.29**	−0.15	1.00
C Node positive (n = 77)							
Mean	49.70	5.40	4.68	56.43	0.18	0.56	2.51
Sigma	30.17	4.26	1.71	9.82	0.39	0.50	0.55
Months	1.00						
Nodes	**−0.49**	1.00					
Size	**−0.29**	**0.23**	1.00				
Age	**−0.25**	0.18	**0.23**	1.00			
Vimentin	−0.09	0.18	−0.03	−0.07	1.00		
Cath-D	0.09	0.03	−0.09	0.01	**−0.26**	1.00	
Grade	**−0.31**	0.17	−0.06	−0.01	−0.01	0.06	1.00

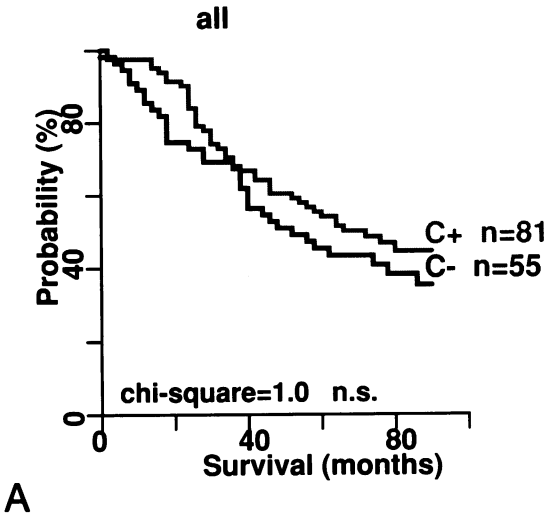
Table 4A–C gives the coefficients of correlation between the prognostic variables, size of tumor (in mm), number of nodes affected, age, grade (1, 2, 3), vimentin (0 = negative, 1 = positive), cathepsin D (0 = negative, 1 = positive), and survival in months after diagnosis (censored to 90 months). The correlations are given in A for all patients, in B for node negative, and in C for node positive patients; n = patients at risk. At 95% significance by *t*-test, correlations of .171, .26, and .23 are required as evidence against the hypothesis of noncorrelation for n = 136, 59, and 77, respectively. Bold face = significant correlations.

negative tumors expressed cathepsin (Table 2). In the group of cathepsin-positive tumors, 20% expressed vimentin (Table 1). When these tumors were divided into node-positive and node-negative subgroups, vimentin was found only in 9% of node-positive cases but in 32% of node-negative ones. In the cathepsin-negative tumor group, vimentin was expressed in 33% (Table 1). When this group was subdivided, it was seen in 29% of node-positive and 38% of node-negative cases.

Cathepsin D and Overall Long-term Survival

In infiltrating ductal NOS breast carcinomas, neither positive nor negative cathepsin D staining was associated with better overall survival at any time between 40 and 90 months. At 20 months, survival for the cathepsin D-positive group was 90% and 74% for the negative group ($P < 0.025$) (Figure 2A). When cathepsin positive tumors were divided into three groups according to intensity of staining, no superior survival was found for patients with strongly cathepsin positive tumors (Figure 2B). There was also no significance of cathepsin D staining when tumors were divided according to vimentin expression. Similarly, cathepsin D had little influence on survival at 60 or 90 months when analyzed separately in node-positive and node-negative subgroups (Figure 3A, B). However, at 20 months in node-positive patients, significantly better survival rates were noted for cathepsin D-positive patients. The association between survival and cathepsin positivity disappeared at later times (Figure 3B).

Among lymph node-negative patients, cathepsin D was present in 64% (38 of 59) of the cases. However, when this group was split according to vimentin expression, an interesting trend was revealed. Patients with vimentin-positive cathepsin-negative tumors had better survival than those with vimentin-positive and cathepsin-positive tumors (Figure 3C). Among patients with vimentin-negative tumors, those positive for cathepsin had a better survival rate than those that were cathepsin-negative (Figure 3D). Although the difference for vimentin-positive patients does not approach statistical significance, for vimentin-negative patients we found a χ^2 of 2.6 where $P < 0.1$ requires 2.7. The survival curves of node-positive patients did not show such a trend for vimentin



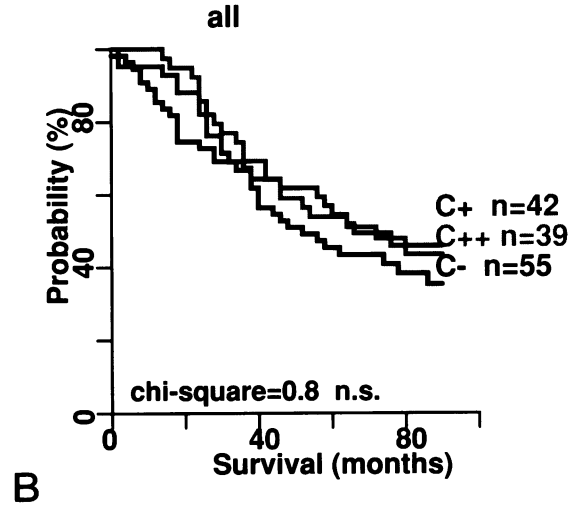


Figure 2. *Survival of patients to immunocytochemical staining for cathepsin D.* **A:** *All patients separated into cathepsin D positive (C+) and cathepsin D negative (C−) with focally positive cases included in the C− group (see text).* **B:** *All patients separated according to intensity of staining for cathepsin D. C+ + strongly positive, C+ positive, C− negative or focally positive; n.s. = not significant.*

negative tumors, and in the vimentin positive subgroup, the numbers were too small to allow a meaningful analysis.

Examination of the data in Figure 3C and D shows that survival of node-negative patients is much worse for those with V+C+ tumors (lower curve Figure 3C) than for those with V−C+ tumors (upper curve Figure 3D), whereas the survival curves for patients with V+C− tumors or V−C− tumors are similar. This analysis of the data suggests that the prognostic risk previously noted for node-negative patients with V+ tumors[15] can be further refined by use of cathepsin. Thus patients with V+C+ tumors seem to have a particularly poor prognosis (Figure 3C).

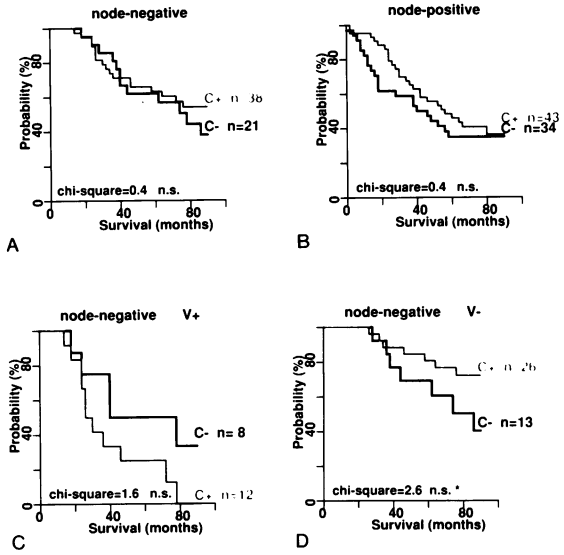


Figure 3. *Survival of patients with node negative and node positive invasive ductal NOS breast carcinoma related to cathepsin D status.* **A:** *Node-negative patients.* **B:** *Node-positive patients.* **C:** *Node-negative vimentin positive patients.* **D:** *Node-negative vimentin negative patients. There is a trend for node-negative patients with vimentin-positive cathepsin-negative tumors to have better survival than those with vimentin-positive cathepsin-positive tumors, but note that numbers of patients in* (C,D) *are small. *chi square = 2.6 where* $P < 0.1$ *requires 2.7* (D); *n.s. = not significant.*

Discussion

Immunohistochemistry of Cathepsin D and Prognosis in Breast Carcinoma

Our data show that when immunohistochemistry with a commercially available polyclonal antibody is used to reveal cathepsin D in invasive ductal NOS breast carcinoma, no significant relationship is seen between cathepsin D expression in cancer cells and survival at 60 or 90 months (Figure 2). This was true not only for all patients considered as group (Table 1) but also when patients were subdivided into those with positive or negative axillary lymph nodes (Tables 2 and 3). By multivariate analysis we found cathepsin D expression to be independent of lymph node status, age, tumor size, histologic grade of the tumor, and survival at 5 years (Table 4).

We found strong expression of cathepsin D in 60% of ductal NOS breast carcinomas. This figure can be compared with immunohistochemical studies using frozen

sections and two antibodies directed against a mature form of cathepsin D, which detected the enzyme in 75% and 64% of breast carcinomas[20,21] and also, to a recent immunohistochemical study[8] using paraffin sections and a polyclonal antibody in which cathepsin D expression was found in 66% of breast carcinomas.

However, when our results on cathepsin D positivity and on survival are compared with those of Henry et al[8] there are some striking differences. Survival curves including all tumors in our study (Figure 2A) show slightly better survival for cathepsin D-positive patients but the differences are small and not statistically significant. In particular we do not see the strongly increased survival for strongly positive cathepsin D patients noted by Henry et al[8] (compare Figure 2B in this study to Figure 5 in reference 8). When lymph node status is considered, we and Henry et al[8] found that cathepsin D status affects survival for node-negative patients. However for node-positive patients, the results of the two studies are different. We found little difference in survival of cathepsin D-positive or -negative patients (Figure 3B), although we noted a prognostic advantage at 20 months. In contrast, Henry et al noted a significant prognostic advantage of cathepsin D immunostaining at all times (Figure 8 in reference 8). Although the survival curves of node-positive cathepsin D-positive patients appeared almost identical in the two studies, those for node-positive cathepsin D-negative patients were different. Thus at 50 months 15/34 (45%) of these patients were alive in our series whereas all 12 such patients in their study were dead within 38 months. When looking for possible differences to explain the different results with respect to survival between the two studies we found that in the study of Henry et al[8] at 36 months only nine node-positive patients were alive. Thus the majority of those surviving belonged to the node-negative group, and since in this group no correlation was seen between cathepsin D and survival, the better survival time in Figure 5 in reference 8 could be attributed to node-negative status of the patients rather than to cathepsin D positivity. In addition there may be differences in the nature of the tumors analyzed in the two studies. Thus although the tumors in our study were all ductal NOS breast carcinomas, the exact histologic type of the tumors in reference 8 was not given. The number of patients in various clinical stages as well as treatment protocols for node positive patients may also differ in the two studies. Finally, different numbers of estrogen positive tumors in node positive groups in both studies may also play a role. This issue cannot be addressed since we do not have ER status data for patients in the current paper, and the percentage of ER positive tumors in node positive and negative groups is not given by Henry.[8]

Cathepsin D in Cytosols and Prognosis in Breast Carcinomas

Results in which cathepsin D has been estimated in cytosols of breast cancer tissue further complicate the interpretation. Although in cytosols high cathepsin D levels have been associated with poor prognosis as manifested by shorter relapse-free survival, recurrence free survival or overall survival,[5–7] the cytosol studies also show discrepancies. Although one study finds a significant correlation between cathepsin D concentration and survival in both lymph node-positive and -negative patients,[5] another finds it greater in node-negative than in node-positive patients,[6] while a third finds it only in node-negative patients.[7] Significant correlation of positive ER status and high cathepsin D levels in cytosols from breast cancer was reported in all pre- and postmenopausal women,[22] only in premenopausal patients,[5] or no association was found between these parameters.[7] Paradoxical findings have been reported. For instance, although higher levels of mature cathepsin D were found in breast cancers that had metastasized to regional lymph nodes, which would suggest a role for cathepsin D in the metastatic potential of tumor cells, nevertheless cathepsin D values did not predict clinical outcome in node-negative patients.[7] It is difficult to compare the results reported on cytosols to those from immunohistochemical studies because they use different cut-off levels, and different antibodies, they do not take into account cathepsin positive macrophages in tumor tissue and most of all because they provide only vague information on the tumor material that was analyzed. In the principal studies that deal with the prognostic significance of cathepsin D in breast cancer, vital detailed information on histologic type of breast carcinoma is not given.[5–7] Only from one report[5] may one infer that 370 ductal carcinomas and 16 other breast cancers were studied. Histologically however, breast carcinoma is a heterogeneous tumor. From a prognostic point of view, it is important to know whether the tumor is *in situ* or an infiltrating carcinoma, whether it is ductal, lobular, or belongs to a special type.[17] Thus inclusion of several medullary or *in situ* carcinomas which have an excellent prognosis may bias the results if one looks for prognostic significance of a single factor.

Cathepsin D in Breast Cancer Stromal Cells

In the stroma of each tumor, we found a variable number of strongly cathepsin D-positive macrophage-like cells and other benign stromal cells (Figure 1D). Similar find-

ings were reported by Henry et al[8] (and Figure 3 in reference 8). Obviously cathepsin D-positive stromal cells will, depending on their number, influence measurements of the concentration of the enzyme in tumor tissue, thus reducing the specificity of such assays. In this series 33% of cathepsin D-negative cancers were heavily infiltrated by large numbers of such cells and would have been included in the cathepsin-positive group by cytosol assays. This leads to an overestimate of cathepsin D-positive tumors (in this study 73% of all cases instead of 60%). This may be one of the reasons why the results of immunohistochemical studies are difficult to compare with studies based on tumor extracts.

Cathepsin D, Vimentin and Prognosis in Breast Carcinomas

Vimentin expression in breast carcinomas has been correlated with several poor prognostic indicators such as low estrogen receptor level, positive EGFR status, high proliferative activity of the tumor, and high histologic grade of ductal NOS carcinoma.[9–14] We have shown that there was a significant inverse relation between vimentin expression and survival at 5 years for node-negative but not for node-positive patients with infiltrative ductal NOS breast carcinomas.[15]

Our data show that a significant ($P < 0.05$) inverse relation exists between cathepsin D expression and vimentin in node-positive infiltrating ductal NOS breast carcinomas (Table 3). Vimentin-positive tumors constituted 29% of cathepsin D-negative tumors but only 9% of those with cathepsin D expression. Such differences were not found in tumors of node-negative patients in whom vimentin was found in 32% of cathepsin-positive and in 38% of cathepsin-negative tumors. Although the number of patients studied is small, there is a trend for node-negative patients with cathepsin D-positive vimentin-positive tumors to have a worse prognosis than for those with cathepsin D-positive vimentin-negative tumors (Figure 3C, D). Our results suggest that, although there is not a correlation between cathepsin D expression and prognosis in all patients, the prognostic risk previously noted for node-negative patients with vimentin-positive tumors can be further refined by use of cathepsin D. Cathepsin D may be of help in stratifying node-negative patients provided the vimentin status is known.

Can Conflicting Results on Cathepsin D and Prognosis be Explained by a Dual Regulation of Cathepsin D?

Estrogens and growth factors influence procathepsin D synthesis. At least two pathways may operate: a direct transcriptional regulation by activated ER and indirect regulation via induction of growth factors by estrogens.[23,24] So, it is likely *in vivo* that the cathepsin D gene is continuously stimulated in ER-positive tumors,[23,24] which are known to have better survival. However, cathepsin D expression is found also in hormone-independent (i.e., ER negative) breast cancer cell lines, perhaps due to continuous stimulation of such cells by autocrine growth factors.[24] Thus in ER-negative tumors cathepsin D may be synthesized in response to continuous stimulation by growth factors. Such tumors may be expected to have an increased proliferation rate. Immunohistochemically detected cathepsin D was found associated with cell proliferation rather than with hormone responsiveness.[21] Since breast carcinomas with low ER levels and a high proliferation rate have a poor prognosis, in this group cathepsin D would be associated with tumors with poor prognosis.

Although ER status was not available for a series of patients in the current study, Henry et al[8] showed that immunohistochemically detected cathepsin D was significantly associated with positive ER status of breast carcinomas and that in patients with ER-positive tumors, cathepsin D was associated with a prognostic advantage. They suggested that "cathepsin D provides additional information on the functional integrity of the estrogen response pathway," and further that "the improved prognosis of patients expressing cathepsin D may be a consequence of functioning estrogen receptor."[8] We show that the mere expression of cathepsin D in breast carcinoma is neither associated with prognostic advantage or disadvantage. But we also find a trend for node-negative patients with cathepsin D-positive vimentin-positive tumors to have worse prognosis than for those with cathepsin D-positive, vimentin-negative tumors. Vimentin expression identifies a population of rapidly proliferating[10] ER-negative[9,10] breast cancer cells that express preferentially EGFR.[9] Cathepsin D in such tumors may have been induced by growth factor rather than by a direct transcriptional pathway. Thus to explain the results of Henry et al,[8] that cathepsin D is associated with better prognosis in patients with ER positive tumors, and our results, that cathepsin D is associated with worse prognosis in vimentin-positive (i.e., mainly ER-negative, EGFR-positive) tumors, we propose that it is not the expression of cathepsin D *per se* that is associated with better or worse survival in infiltrating ductal NOS breast carcinomas, but rather that prognostic information that is associated with cathepsin D expression may depend on the pathway of its synthesis. If cathepsin D expression is due to a direct transcriptional regulation by activated estrogen receptor in an ER-positive tumor, then it may be associated with a favorable prognosis. Alternatively, if cathepsin D expression is due to stimulation by high levels

of autocrine growth factors, then it most likely associates with poor prognosis. Although the two have opposite prognostic influences, both mechanisms result in cathepsin D-positive granules in the cytoplasm of tumor cells. To reveal prognostic significance of these granules, it may be necessary to first identify factors associated with cathepsin D synthesis such as ER or some growth factors. This hypothesis is different from the one suggested by Henry et al[8] but it is in part supported by the results of these authors. Thus when cathepsin-positive tumors are pooled together for a statistical analysis of survival, the two groups of cathepsin positive tumors just discussed have different prognostic consequences. Thus whether a correlation is seen between cathepsin D positivity and survival will depend on the number of cases in each category. This is one possible explanation for the widely different results on cathepsin D and survival in breast carcinomas.[5–8]

In conclusion, empirically cathepsin D alone is not a useful marker of prognosis when applied indiscriminately to all invasive ductal NOS breast carcinomas. However, our results with vimentin, and those of Henry et al with ER suggest that it may help to further stratify patients with ductal NOS breast carcinomas provided the vimentin and ER status is known.

Acknowledgment

We thank Susanne Isenberg for expert technical assistance.

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