Pergamon

European Journal of Cancer Vol. 31A, No. 5, pp. 671–677, 1995

0959–8049/95 $9.50+0.00

0959-8049(94)00532-X

Relationship Between Cathepsin D and Other Pathological and Biological Parameters in 1752 Patients With Primary Breast Cancer

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The relationship between cathepsin D and other pathological or biological prognostic parameters has not yet been defined through systematic studies in breast cancer. The aim of the present investigation was to define the relationship between cathepsin D and nodal status, tumour size, steroid receptors and tumour grade in a wide patient series. Cytosol cathepsin D was assayed with an immunoradiometric assay in tumour samples from 1752 patients. A statistically significant, but not biologically meaningful association was found between cathepsin D and both tumour size and grade. Cathepsin D was significantly higher in node-positive than in node-negative tumours. However, cathepsin D is not of great use in order to predict the risk of axillary metastases in individual patients, due to overlapping of cathepsin D values between node-positive and node-negative cases. A significant, direct association was found between cathepsin D and both oestrogen receptor and progesterone receptor cytosol levels. Nevertheless, preliminary data indicate that cathepsin D and steroid receptors provide independent prognostic information.

Key words: cathepsin D, breast cancer, nodal status, tumour size, oestrogen receptor, progesterone receptor, prognosis

Eur J Cancer, Vol. 31A, No. 5, pp. 671–677, 1995

INTRODUCTION

IT HAS recently been suggested by a meta-analysis on the relationship between pathological and biological characteristics of breast cancer that pathological stage is probably an indicator of the age of the tumour rather than of its intrinsic biological aggressiveness [1]. A wide array of biological parameters have been studied in breast cancer, which show a broad spectrum of biological actions [2]. Among them, much interest has focused on cathepsin D, a lysosomal endoprotease widely distributed in mammalian tissues which belongs to the family of aspartic proteinases and has a probable physiological role in the catabolism of intra-cellular and extra-cellular endocytosed proteins [3]. Cathepsin D was shown to be specifically secreted under oestrogen control in MCF-7 breast cancer cell lines, being, however, also intrinsically expressed in oestrogen-unresponsive breast cancer cell lines [4]. Further studies showed that the regulation of cathepsin D synthesis and secretion is a complex phenomenon in which both steroid and proteinaceous growth factors are implicated [5]. Cathepsin D is synthesised as an inactive precursor (52 kD) that is then processed into the mature form (34 kD + 14 kD) through an intermediate 48-kD moiety [5]. Cathepsin D was overexpressed in breast cancer cell lines and in human breast cancer tissue in comparison to normal mammary epithelial cells [6] and normal glandular breast cancer tissue [7], respectively.

A putative role of cathepsin D in breast carcinogenesis has been suggested. Cathepsin D could be responsible for cancer development or progression through two possible mechanisms: (1) the protease activity of overexpressed cathepsin D could improve the metastatic potential of the tumour [8]; or (2) cathepsin D could act extracellularly through direct or indirect autocrine mechanisms [9]. These findings are the biological base for a possible prognostic role of cathepsin D in breast cancer. The prognostic value of cathepsin D has been evaluated in several investigations, in which more than 4000 patients were studied. In general, high cathepsin D is related to a poor prognosis [10–19]. However, the results of different investigators do not agree on the subgroups of patients in which

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This study was carried out on behalf of Comitato Italiano per il Controllo di Qualità del Laboratorio in Oncologia (Chairman Prof. Adriano Piffanelli) (see acknowledgements).
Revised 17 Nov. 1994; accepted 9 Dec. 1994.

cathepsin D provides a significant prognostic indication. Moreover, of major concern, in spite of the bulk of available data, is that conflicting results are reported concerning the association between cathepsin D and other major prognostic factors (N, T, G and receptor status) [10–26].

Methodological bias could be in part responsible for the conflicting data so far reported. Indeed, five different methodological approaches have been used in the different patient series so far evaluated.

The aim of the present investigation is, therefore, to study the relationship between cathepsin D and nodal status, tumour size, steroid receptors, histological type and tumour grade in a wide patient series, in which tumour samples have been processed and cathepsin D assayed in the same laboratory with the same methods, in order to establish the association between the marker and the major disease characteristics.

PATIENTS AND METHODS

Patients

From 1983 to 1992, 2524 patients with untreated primary breast cancer entered the study in five institutions. Patients were enrolled from two institutions until 1983, from three institutions until 1987, from four institutions until 1989 and from five institutions until 1992. Inclusion criteria were as follows: stage 1–3 infiltrating breast carcinoma; no previous or concomitant malignancies of other organs; no irradiation or chemotherapy before the surgery. Patients with inflammatory breast cancer were excluded. Pathological findings were classified according to the criteria of the World Health Organization. Menopausal status was classified as follows: premenopausal, patient with regular menstrual cycle; perimenopausal and postmenopausal, those whose last regular menses occurred less than and more than 2 years before, respectively.

All the participating institutions followed a standardised protocol for tissue collection and storage. Tissue samples were stored in liquid nitrogen until assayed.

Cytosol preparation

Tissue samples were pulverised using a microdismembrator and homogenised with phosphate buffer. Low-salt extract (cytosol) was prepared by centrifugation at 100 000 *g* for 1 h at 4°C [27].

Cathepsin D immunoassay

Cathepsin D was measured using a solid phase sandwich immunoradiometric assay (IRMA) based on two monoclonal antibodies, D7E3 and M1G8, which detects the total amount of cathepsin D (52-kD, 48-kD and 34-kD proteins) (CIS Bio international, Gif-sur-Yvette, France), following the instructions of the manufacturer.

Oestrogen receptor (ER) and progesterone receptor (PgR) radioligand binding assay

ER and PgR were measured in the low-salt extract using the dextran-coated charcoal (DCC) method recommended by the EORTC [27].

The assays of steroid receptors and cathepsin D were monitored using both intra-laboratory and inter-laboratory quality control programmes [28, 29].

Protein assay

The total protein in the cytosol (c.p.) was measured by the Coomassie brilliant blue colorimetric assay (Bio-rad, Anheim, California, U.S.A.).

Statistical evaluation

Data were analysed with Anova, Fisher's exact test, Kruskal–Wallis test, regression analysis using both data and logarithms of data, Spearman correlation, Kaplan–Meier method (Mantel–Cox test) and Cox multivariate analysis.

RESULTS

Cathepsin D method validation

Precision. The precision of cathepsin D IRMA method was satisfactory both in intra- (coefficient of variation, c.v., lower than 4.9%) and in inter-assay setting (c.v. lower than 8.3%), as well as in inter-laboratory evaluation (c.v. lower than 14.4%). The yearly distribution of cathepsin D found in samples collected from 1983 until 1992, stored at −80° for variable time spans, was not significantly different. Therefore, it was concluded that storage of cytosol samples should not significantly affect cathepsin D concentration. In addition, no significant differences were found among cathepsin D levels in samples collected in the five different institutions participating in the study (data not shown).

Accuracy. Dilution tests performed using phosphate buffer showed a good linearity (recovery ranging from 96 to 107%) over a wide range of dilutions (from 1:10 to 1:2000) (data not shown).

Cathepsin D in patients with breast cancer

Cathepsin D was measured in 2524 tissue samples of primary breast cancer. The relationship between cathepsin D and nodal status, tumour size and receptor status was evaluated in 1752 patients in which these parameters were all available in every individual patient (selected cases). Menopausal status and tumour grade were known in 1464 and 743 patients, respectively. The values of cathepsin D found in the overall patient series and in selected cases did not differ significantly (data not shown). The clinical and pathological characteristics of selected cases (Table 1) were not significantly different from those of the overall group. The distribution of cathepsin D values found in breast cancer is shown in Figure 1. Cathepsin D was evaluated both as a continuous variable and after subdivision into three groups: below the 40th percentile value (<31 pmol/mg of cytosol protein), between the 40th and 70th percentile value (from 31 to 47 pmol/mg of cytosol protein) and above the 70th percentile value (>47 pmol/mg of cytosol protein). The value of 31 pmol/mg of cytosol protein, corresponding to the 40th percentile value, was used as a positive/negative cutoff point for a preliminary prognostic evaluation since, in a previous study, it was shown to be the most effective prognostic threshold [30].

Cathepsin D, age and menopausal status (data not shown)

Cytosol concentration of cathepsin D did not show significant variations according to patients' age at the time of the surgery. The frequency distribution of low, intermediate and high cathepsin D values was comparable when patients were divided into age groups of 5 years. Cathepsin D levels did not show variations related to menopausal status.

Cathepsin D, tumour size and axillary status

Cathepsin D levels tended to be higher in larger tumours (Kruskal–Wallis test, $P = 0.056$), but the weak association was not clinically meaningful. Cathepsin D was significantly higher in tumours with axillary metastases (median 38.6, interquartile 27.2–53.5 pmol/mg) than in those with negative lymph nodes (median 32.9, interquartile 22.7–47.7 pmol/mg,

Table 1. Clinical and pathological characteristics of evaluated patients

Median age in years (range)	59 (25–89)
Menopausal status	
Premenopausal	331 (22.6%)
Perimenopausal	78 (5.3%)
Postmenopausal	1055 (72.1%)
Tumour size (cm)	
< 2	874 (49.9%)
2–5	826 (47.1%)
> 5	52 (3.0%)
No. of positive lymph nodes	
0	916 (52.3%)
1–3	442 (25.2%)
> 3	394 (22.5%)
Histological type	
Intraductal carcinoma	45 (2.6%)
Ductal infiltrating carcinoma	1404 (80.1%)
Lobular infiltrating carcinoma	165 (9.4%)
Medullar carcinoma	48 (2.7%)
Other types	90 (5.1%)
Tumour grade	
G1	156 (21.0%)
G2	285 (38.4%)
G3	302 (40.6%)
Receptor status	
ER + PgR +	1106 (63.1%)
ER + PgR −	195 (11.1%)
ER − PgR +	154 (8.8%)
ER − PgR −	297 (16.9%)

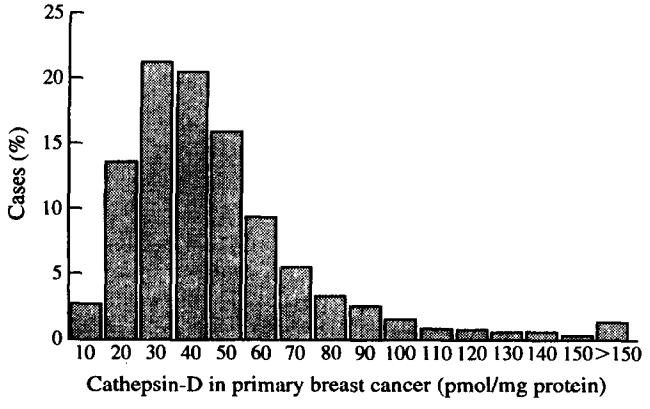

Figure 1. Distribution pattern of cathepsin D levels in 2524 breast cancer samples (numbers in abscissa are the upper limit of each class).

Kruskal–Wallis test, $P < 0.0001$). The difference between the frequency of samples with low and high cathepsin D was evident between cases without positive lymph nodes and those with one to three positive lymph nodes, but not between those with one to three and those with four or more positive lymph nodes (Table 2).

Considering that tumour size and axillary status are closely associated [1], we evaluated the relationship between cathepsin D and axillary status after subdivision of patients according to tumour size in the 1491 cases in which the tumour diameter was reported in addition to pT. In 138 cases with tumours 1 cm or less, cathepsin D levels were not significantly different in node positive (N+) (37 cases) and node negative (N−) (101 cases) (Kruskal–Wallis test, $P = 0.323$). However, in 613 cases with tumours between 1.1 and 2 cm, cathepsin D was significantly higher in N+ (243 cases) than in N− (370 cases) (Kruskal–Wallis test, $P = 0.0012$). In 740 tumours larger than 2 cm, cathepsin D was still significantly higher in N+ (443 cases) than in N− cases (297 cases) (Kruksall–Wallis test, $P = 0.0162$) (Figure 2). These data suggest that cathepsin D is a possible indicator of the tendency of the tumour to spread to the axilla rather than a parameter related to tumour bulk, as it is also supported by the fact that cathepsin D is more strongly associated to axillary status than to tumour size. Nevertheless, this finding appears speculative and not clinically meaningful, due to the wide overlapping of individual values between N+ and N−.

When both cathepsin D and the number of positive lymph nodes were evaluated as continuous variables using the Spearman correlation for ordinal data, a significant direct association was found ($r = 0.121, P < 0.001$).

Cathepsin D and histological type or tumour grade

No association was found between cathepsin D levels and the histological type of the tumour. Cathepsin D levels were higher in G3 (median 37.0, interquartile 24.1–52.1) and G2 (median 36.4, interquartile 25.3–48.0) than in G1 (median 30.8, interquartile 20.6–42.4; Kruskal–Wallis test, $P = 0.0254$) tumours.

Cathepsin D and receptor status

A significant association was found between cathepsin D levels and both ER (ER = 90 + 0.5 cathepsin D; $r = 0.108$, $n = 1752$, $P < 0.001$) and PgR (PgR = 107 + 0.8 cathepsin D; $r = 0.114$, $n = 1752$, $P < 0.001$). The association pattern was confirmed using the Spearman correlation for ordinal data (ER: $r = 0.122, P < 0.001$; PgR: $r = 0.135, P < 0.001$). However, the scattergrams (data not shown) and the distribution of cathepsin D in relation to ER and PgR (Figures 3 and 4) suggest that the biological association is probably weak. The association between cathepsin D and both ER and PgR was further evaluated using cross-tab examination of cathepsin D distribution. Subdividing ER or PgR using the same cutoffs as for cathepsin D (lower than the 40th percentile, between the 40th and 70th percentile, and higher than the 70th percentile), samples with higher cathepsin D levels tended to occur more frequently in cases with higher ER (Table 3) or PgR levels (Table 4). The association between cathepsin D and both ER and PgR did not show a continuous pattern. Indeed, the rate of high cathepsin D levels was significantly different between samples with ER and PgR above the 70th percentile value and those below this point ($P = 0.0005$ for ER, $P = 0.0001$ for PgR). In contrast, the distribution pattern of cathepsin D levels was not significantly different between cases with ER or PgR lower than the 40th percentile value and those from the 40th to the 70th percentile value.

When subdividing cases according to receptor status assessed by clinically used cutoff points for both ER and PgR (10 and 20 fmol per mg of cytosol protein), the frequency of elevated levels of cathepsin D was significantly higher in cases in which both receptors were positive using either 10 or 20 fmol/mg of cytosol protein as cutoff point (Pearson χ^2 $P < 0.0001$ and $P = 0.0035$, respectively). Cathepsin D and receptor status (ER and PgR positive/negative cutoff point 10 fmol/mg of cytosol protein) were significantly associated in both N− (Pearson χ^2, $P = 0.0013$) and in N+ (Pearson χ^2, $P = 0.0033$). Cathepsin D was also measured in 53 samples of normal glandular breast

Table 2. Frequency distribution of cathepsin D according to the number of positive axillary lymph nodes

No. of positive lymph nodes	Cathepsin D (pmol/mg cytosol protein) < 31* Cases	(%)	31–47† Cases	(%)	> 47‡ Cases	(%)
0	419	(45.8)	258	(28.2)	238	(26.0)
1–3	164	(37.0)	135	(30.5)	144	(32.5)
> 3	120	(30.4)	135	(34.3)	139	(35.3)

Pearson χ^2 statistics 30.94, $P < 0.0001$.
* Lower than 40th percentile. † Between 40th and 70th percentile. ‡ Above 70th percentile value of cathepsin D distribution. The observed frequencies and the row percentages (in parentheses) are reported above.

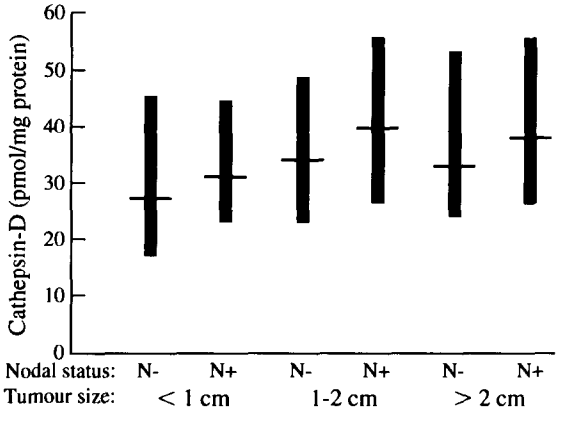

Figure 2. Relationship between cathepsin D and nodal status after subdivision of patients according to tumour diameter (vertical bars represent the interquartile range, horizontal bars represent the median value).

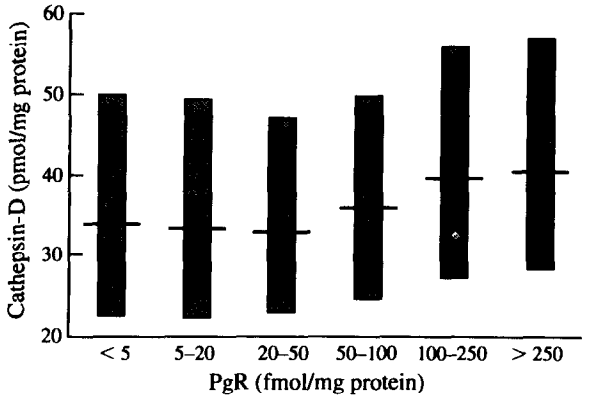

Figure 4. Relationship between cathepsin D and progesterone receptor status (vertical bars represent the inter-quartile range, horizontal bars represent the median value).

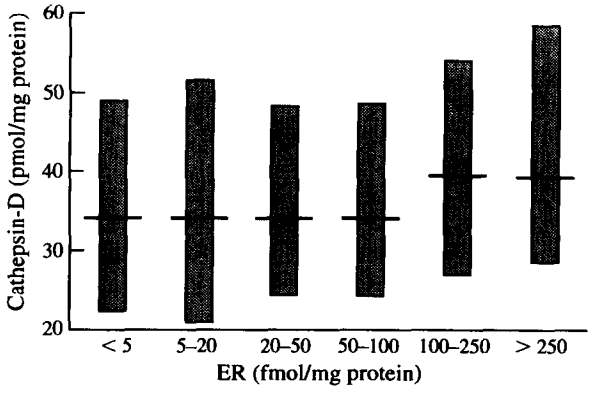

Figure 3. Relationship between cathepsin D and oestrogen receptor status (vertical bars represent the inter-quartile range, horizontal bars represent the median value).

tissue in order to assess any baseline value of cathepsin D "physiologically" expressed. Cathepsin D levels in normal glandular tissue (median 7.2, interquartile 4.3–10.3 pmol/mg of cytosol protein) were significantly lower than in tumour samples ($P < 0.001$). The 95th percentile value of cathepsin D found in normal breast tissue (19.5 pmol/mg of cytosol protein) was used as a cutoff between putative physiologically and pathologically expressed cathepsin D. In breast cancer samples, the association between steroid receptors and cathepsin D was significant only in cases in which cathepsin D was higher than 19.5, while no significant association was found in cases in which the cathepsin D was lower than this cutoff point. Interestingly, cathepsin D and pS2, which is an oestrogen-regulated peptide [31], were also significantly associated in cases with cathepsin D greater than 19.5 pmol/mg protein ($r = 0.196$, $P < 0.001$, $n = 661$) while they were not associated in samples with cathepsin D lower than 19.5 pmol/mg of cytosol protein ($r = 0.155$, $P = 0.133$, $n = 106$). These findings suggest that cathepsin D could be expressed in breast tissue at a baseline level, which is possibly not under oestrogen control in the carcinoma. The positive association between cathepsin D and steroid receptors is puzzling, considering that they should provide opposite prognostic information.

In a subset of patients (489 cases) in which follow-up data were available, the prognostic value of cathepsin D has been investigated (manuscript in preparation). Overall, higher cathepsin D indicates a poorer prognosis [Mantel–Cox test, $P < 0.0004$ for relapse free survival (RFS), $P < 0.0002$ for overall survival (OS)]. Nodal status, pT, cathepsin D, ER and pS2 were independent prognostic indicators when using the Cox's stepwise proportional hazard model. Cathepsin D was the second most effective indicator after nodal status for RFS (Table 5) and the third, after nodal status and ER for OS (Table 6).

Table 3. Frequency of cathepsin D distribution in relation to the concentration of ER in tumour cytosol

ER (fmol/mg cytosol protein)	Cathepsin D (pmol/mg cytosol protein) < 31* Cases	(%)	31–47† Cases	(%)	> 47‡ Cases	(%)
< 24*	308	(44.0)	198	(28.3)	194	(27.7)
24–112†	227	(42.8)	162	(30.6)	141	(26.6)
> 112‡	168	(32.2)	168	(32.2)	186	(35.6)

Pearson χ^2 22.2, $P = 0.0002$.
* Lower than 40th percentile. † Between 40th and 70th percentile. ‡ Above 70th percentile value of cathepsin D or ER distribution. Both cathepsin D and ER are subdivided as lower than the 40th percentile, between the 40th and 70th percentile and above the 70th percentile value. The observed frequencies and the row percentages (in parentheses) are reported above.

Table 4. Frequency distribution of cathepsin D in relation to the concentration of PgR in tumour cysotol

PgR (fmol/mg cytosol protein)	Cathepsin D (pmol/mg cytosol protein) < 31* Cases	(%)	31–47† Cases	(%)	> 47‡ Cases	(%)
< 21*	315	(44.9)	192	(27.4)	195	(27.7)
21–124†	211	(40.9)	164	(31.8)	141	(27.3)
> 124‡	173	(33.1)	166	(31.7)	184	(35.2)

Pearson χ^2 20.6, $P = 0.0004$. * Lower than 40th percentile. † Between 40th and 70th percentile. ‡ Above 70th percentile value of cathepsin D or PgR distribution. PgR status was lacking in 11 patients, therefore the total patients in this analysis was 1741. Both cathepsin D and PgR are subdivided as lower than 40th percentile, between 40th and 70th percentile and above 70th percentile value. The observed frequencies and the row percentages (in parentheses) are reported above.

Table 5. Cox's stepwise proportional hazard model. Relapse-free survival analysis

Covariate	Improvement χ^2	*P* value	Global χ^2	*P* value	Relative risk
Nodal status	16.55	< 0.001	16.94	< 0.001	2.40
Cathepsin D	10.11	0.001	25.85	< 0.001	2.69
pS2	8.09	0.004	34.05	< 0.001	1.99
pT	2.98	0.084	36.71	< 0.001	1.58

Table 6. Cox's stepwise proportional hazard model. Overall survival analysis

Covariate	Improvement χ^2	*P* value	Global χ^2	*P* value	Relative risk
Nodal status	11.83	0.001	11.95	0.001	2.98
Oestrogen receptor	8.72	0.003	20.99	< 0.001	1.99
Cathepsin D	4.45	0.035	24.54	< 0.001	2.07
pS2	3.80	0.051	29.19	< 0.001	1.93

However, the prognostic role of cathepsin D was restricted to node-positive patients only. Cathepsin D levels were significantly associated to RFS and OS after stratification according to receptor status (Mantel–Cox test, $P < 0.05$).

Concerning the prognostic role of steroid receptors according to cathepsin D status, ER and PgR levels higher than 10 fmol/mg of cytosol protein were significantly associated to a better overall survival in both cathepsin D positive and in cathepsin D negative cases (Mantel–Cox test, $P < 0.05$), while they were not associated to RFS.

DISCUSSION

The lysosomal protease cathepsin D is one of the most extensively studied parameters evaluated for the biological characterisation of breast cancer. However, so far no specific studies have been focused on the evaluation of the relationship between cathepsin D and other well-established prognostic factors.

We studied cathepsin D levels in a large patient series in which several other clinical and pathological parameters were available. Overall, the median value and distribution parameters of cathepsin D found in the present series are comparable with those obtained by other investigators using the same method [13–15, 19]. Higher levels of cathepsin D found in cancer than in normal breast tissue are in agreement with similar findings reported in both breast cancer [6, 7] and other malignancies [32]. In agreement with other studies, cathepsin D is not associated to age [10–14, 17–20, 22, 25, 26], tumour size [10–15, 17–19, 23–26] nor menopausal status.

Quite conflicting data have been reported concerning the relationship between cathepsin D and axillary nodal status. Seven studies showed higher cathepsin D levels in N+ than in N− tumours [12, 15, 18–20, 23, 24], while seven did not identify any relationship [10, 11, 13, 17, 22, 25, 26]. Results from different studies are still conflicting when selecting only the nine investigations, concerning 1998 patients, in which the same immunoradiometric assay was used for cathepsin D determination [11, 13, 15, 19, 22, 23, 25, 26]. In the present study, we demonstrated a statistically significant association between higher cathepsin D and axillary lymph nodal metastases. The association seems related to the tendency of the tumour to metastasise since it depends on the presence or absence of metastases rather than on the number of invaded lymph nodes, as is expected in the case of a bulk-marker. In agreement with these findings, Pujol and associates also found that cathepsin D concentrations were more significantly different between N+ and N− cases than when subdividing patients according to the number of positive lymph nodes [18]. Also, the association between cathepsin D and nodal invasion is conserved after stratification of patients in homogeneous groups according to tumour size. Nevertheless, these findings do not help in predicting the axillary status on the basis of cathepsin D levels in the tumour, due to wide overlapping of individual values between N+ and N− cases (Figure 2).

In the present study, we showed that cathepsin D was higher in G3 and G2 than in G1 tumours. These findings are in contrast with those of six other groups which did not identify any correlation between cathepsin D and tumour grade [10, 11, 17–20]. However, they are in agreement with data by Cazin and associates which found the same trend, showing cathepsin D concentration similar in G3 and G2 tumours, both being higher than G1 tumours [26]. In agreement with these findings, cathepsin D was found to be higher in less differentiated endometrial and cervical cancers [33].

More complex is the evaluation of the relationship between cathepsin D and receptor status. Four studies showed a direct association between cathepsin D and ERs [10, 19–21], eight did not [11, 13, 14, 18, 22–24, 26]. Three authors found a significant association between cathepsin D and PgRs [19, 20, 26], seven did not [10, 11, 13, 18, 22–24]. Tandon and associates found higher levels of cathepsin D in receptor-positive than in receptor-negative cases in N+ patients [12], while Marsigliante and colleagues found higher levels of cathepsin D in patients in which both ER and PgR were positive than in those in which both the receptors were negative [25].

In the present study, we demonstrated a positive association between cathepsin D and both ER and PgR. However, in spite of the statistical significance, the biological meaning of this relationship is less clear. This is not surprising, since the regulation of cathepsin D is the result of a very complex mechanism [5]. Indeed, different regulation patterns seem to occur in different organs, particularly regarding the role of steroid receptors [34].

Nevertheless, the biological classification of receptor-positive and cathepsin D-positive cases is a relevant problem since they should provide opposite prognostic information. Indeed, among the 1752 cases evaluated, 40.1% expressed both ER and cathepsin D as being higher than the cutoff points used for clinical decision (> 10 fmol/mg of cytosol protein and > 31 pmol/mg of cytosol protein, respectively). Similar findings occur for PgR, 39.3% of cases showing both positive PgR and positive cathepsin D values.

The study of the relationship between cathepsin D and the oestrogen-induced protein pS2 did not provide definitive insight. Indeed, a weakly significant correlation was found between the two parameters, which confirms the partial oestrogen dependency of cathepsin D [19, 20], but does not allow for the identification of a possible oestrogen dependency of cathepsin D in individual samples.

In a previous study, on a limited group of patients using the multivariate analysis, we showed that cathepsin D provides independent prognostic information when compared to pS2 and steroid receptors [30]. Preliminary results of a prognostic evaluation of a wider patient series (manuscript in preparation) show that cathepsin D maintains an independent prognostic role in both receptor-positive and, restricted to overall survival, receptor-negative cases. Steroid receptors show a weakly significant association to a longer overall survival in both cathepsin D+ and cathepsin D− patients. These findings suggest that the statistical association between cathepsin D and steroid receptors is probably of no clinical relevance when the prognostic role of the two parameters is considered.

From the results of the present study we can draw the following conclusions: cathepsin D is not associated to age and menopausal status. Cathepsin D is not related to tumour bulk, but possibly to the tendency of the tumour to spread to axillary lymph nodes; however, it is not of great use in order to predict the risk of axillary metastases in individual patients due to the overlapping of cathepsin D values between node positive and negative cases. Cathepsin D is associated to steroid receptor status. The association pattern is, however, weak and seems to appear above a baseline cathepsin D level which could be independent of oestrogen control in breast cancer. In spite of the above association, preliminary data of an ongoing study on the prognostic role of cathepsin D indicate that cathepsin D and steroid receptors provide independent prognostic information.

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Acknowledgements——The present investigation was financially supported by the Regione Veneto, Italy. The authors wish to thank Mr Gino Fungher for his skilled technical assistance and Miss Francesca Ellero for editing the manuscript.

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