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Papers

Cathepsin D, Both a Prognostic Factor and a Predictive Factor for the Effect of Adjuvant Tamoxifen in Breast Cancer

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Cathepsin D is a lysosomal protease implicated in cancer metastasis. Its concentration in breast tumours has also been shown to be of prognostic importance, although to what extent this is subject to lymph node status, the use of adjuvant therapy and menopausal status has not been clearly evaluated. At a cut-off level of 45 pmol/mg protein (61% of the 623 samples were classified as high cathepsin D tumours; immunoradiometric assay), we found cathepsin D to be of prognostic importance only among breast cancer patients with lymph node-positive (N+) disease not treated with adjuvant tamoxifen. When the series was stratified according to cathepsin D content of their tumours, progesterone receptor (PgR) status and lymph node involvement, adjuvant tamoxifen was found to have a significant beneficial effect only among patients with N+ and PgR-positive breast cancer whose tumours had a high cathepsin D content.

Key words: cathepsin D, prognosis, treatment prediction, tamoxifen, breast cancer
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INTRODUCTION

THE IDENTIFICATION of breast cancer patients at high risk of relapse is currently one of the most important issues in breast cancer research. Hitherto, histopathological variables, including lymph node involvement, tumour size and morphological pattern, and biological variables, such as steroid receptor status and DNA content in individual breast cancer cells have been shown to be useful for prognostic purposes in the clinical management of breast cancer. More recently, oncogenes and tumour suppressor genes have also been shown to provide prognostic information [1]. Steroid receptor status can be used as a predictive factor in the selection of adjuvant modality (cytotoxic or endocrine). However, we still need better prognostic and treatment predictive instruments than those used today.

Cathepsin D was first described by Westley and Rochefort [2] as a 52-kD glycoprotein, whose secretion in hormone-dependent breast cancer cell lines was increased by oestrogens and inhibited by anti-oestrogens [3]. Cathepsin D was also found to be produced constitutively in hormone-independent breast cancer cell lines. Secreted as a 52-kD precursor, cathepsin D is proteolytically processed in the lysosomes to the mature 34- and 14-kD forms via an intermediate 48-kD form. Owing to its proteolytic and mitogenic properties, cathepsin D has been suggested to be involved in tumour dissemination [3]. Its prognostic importance has also been demonstrated in clinical breast cancer patient series [4–13]. These studies have raised issues which need to be elucidated before the role of cathepsin D in the clinical management of breast cancer can be established. Such issues include the choice of method, optimal cut-off value, the importance of subgrouping with respect to lymph node involvement, steroid receptor content and menopausal status. Moreover, the use of cathepsin D as a prognostic factor and as a predictive factor, namely the effect of adjuvant therapy needs further investigations.

The aim of this study was to further investigate the prognostic importance of cathepsin D in breast cancer. The specific questions addressed in our study were the choice of cut-off value, the importance of lymph node status and the effect of adjuvant anti-oestrogen treatment and whether the level of the cathepsin D concentration is of any predictive value in this respect.

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MATERIAL AND METHODS

Patients and treatment

The series comprised 623 patients (most of them with stage II tumours), 515 of whom were participants in two separate prospective randomised clinical trials launched by the South Sweden Breast Cancer Group (SSBCG), where the effect of adjuvant tamoxifen (TAM) at 20 mg daily was investigated. Trial 1 (in progress since 1985) consisted of TAM given for 2 or 5 years to 297 postmenopausal patients under 75 years of age. Trial 2 (1985–1990) consisted of TAM given for 2 years to 218 premenopausal patients, non-TAM-treated premenopausal patients serving as controls. The remaining 108 patients had not participated in clinical trials (38 TAM-treated and 70 non-TAM-treated). TAM was given independent of steroid receptor status. Local treatment was given in accordance with guidelines adopted by the SSBCG. Surgery consisted either of modified radical mastectomy with axillary dissection, or of conservative segmental resection with axillary dissection in some patients with tumours less than 20 mm in diameter, combined with postoperative radiation of the remaining breast tissue. In general, patients with metastasis to the axillary lymph nodes received postoperative radiation. Of the total number of patients, (n = 623), 398 (64%) received adjuvant treatment with TAM (31% N0 and 69% N+), and 225 (36%) did not (42% N0 and 58% N+). No patient received adjuvant cytostatic treatment. The TAM-treated group were more often N+ (69 versus 58%), >20 mm in diameter (71 versus 57%) and oestrogen receptor (ER) positive (68 versus 59%) than the non-TAM-treated group (χ^2 analysis). No differences were found in progesterone receptor (PgR) and DNA ploidy status, and S-phase function (SPF) between the two treatment groups.

Patient and tumour characteristics are summarised in Table 1. After a median follow-up of 37 months, 168 (27%) of the 623 patients had developed recurrence (150 distant and 18 locoregional).

Table 1. Characteristics of the 623 patients and adjuvant treatment

	n	%
Menopausal status	623	
Pre		40
Post		60
Lymph node status	621	
N0		35
N1–3		39
N4+		26
Tumour size	622	
≤ 20 mm		34
> 20 mm		66
ER status	620	
Negative		35
Positive		65
PgR status	605	
Negative		45
Positive		55
DNA ploidy status	357	
Diploid		32
Non-diploid		68
S-phase fraction	333	
Low		57
High		43
Adjuvant tamoxifen	623	
Not treated		36
Treated		64

Analytical methods

Cathepsin D. At our department, residual cytosol and tumour tissue from steroid receptor analysis are routinely stored at −70°C. In the present study, cathepsin D analysis was performed on such frozen cytosol samples, using an immunoradiometric assay (ELSA-cath-D-kits; CIS-BIO International Gif-sur-Yvette, France). Cathepsin D content was analysed in two subsets of samples. The first subset (n = 198) consisted of supernatant samples after 20 000 g centrifugation from the routine ER and PgR analysis and were analysed for cathepsin D in Montpellier after being recentrifuged at 100 000 g, the cathepsin D content being expressed in pmol/mg protein, determined according to Bradford with bovine serum albumine (BSA) as standard protein [14]. Owing to changed routines, the second subset (n = 425) had already been centrifuged at 100 000 g. In this subset, cathepsin D analysis was performed at Lund, using the protein determination method of Lowry and co-workers ([15], BSA as standard protein). Control analysis showed that the two subsets did not differ significantly with regard to the prognostic importance of cathepsin D, optimal cut-off value or the correlations with other prognostic factors.

ER and PgR. ER and PgR were measured with two different techniques, ER content with isoelectric focusing (IF) in polyacrylamide gels and enzyme immunoassay (EIA), and PgR content with a dextran-coated charcoal method (DCC) with Scatchard analysis and EIA. In a comparison of previous results obtained with different ER and PgR assays in the same breast cancer samples, we found interassay agreement to be highly significant for both ER content (r_s = 0.98, n = 127) and for PgR content (r_s = 0.88, n = 97), although somewhat higher values were obtained with EIA than with IF or DCC. Thus, the cut-off values adopted for defining receptor positivity had to be adjusted according to the different measuring techniques. Samples with ER and PgR concentration values of ≥ 10 fmol/mg protein, obtained with IF and DCC, were classified as positive, and samples with values below this level as negative [16]. The corresponding cut-off level for EIA was 25 fmol/mg protein.

Flow cytometric (FCM) DNA analysis. Residual tumour tissue after the routine ER and PgR analysis was available in 357 (57%) of the 623 cases included in the present series. These tumour samples were used for FCM DNA analysis in an Ortho 50H instrument after staining with propidium iodide [17]. In accordance with the Convention of Nomenclature for DNA Cytometry [18] ploidy status was defined as follows: one DNA cell population = diploid, two or more cell populations = non-diploid. The percentage of nuclei corresponding to the SPF was estimated by a planimetric method [19]. Non-diploid samples with SPF ≥ 12% and diploid samples with SPF ≥ 7% were classified as high SPF, and samples with values below these levels as low SPF [16].

Gene analysis. Gene amplification was analysed with the Southern blot and slot blot techniques as described previously [20]. Filters were sequentially hybridised with probes for the *ERB*B2, *int*2 and c-*myc*-genes, and with probes for control markers located on the same chromosomal arm as the respective proto-oncogenes (i.e. for *ERB*B2 at 17q-myeloperoxidase, for *int*2 at 11q-progesterone receptor and for *myc* at 8q-mos). The degree

of amplification was evaluated with densitometric analysis of slot blot autoradiograms, by comparing the signals from each proto-oncogene and control gene. Tumours were classified as having a single or an amplified (≥ 2) gene copy number of the haploid genome.

Statistics

The existence of correlations between different factors was verified with Spearman's rank correlation test (r_s). Subgroup differences in cathepsin D content were assessed with Pearson's χ^2 analysis. Life-table analysis was performed with the log-rank test [21, 22]. Recurrence-free survival was analysed with Cox's proportional hazards model [23] and with stepwise covariate selection, a P value of 0.15 being adopted as the upper limit for the inclusion of a covariate, RR values denote the relative risk for each covariate ultimately entered into the analysis. In addition, stratified analyses were performed after subgrouping the patients with regard to lymph node status, PgR status and adjuvant endocrine treatment (i.e. given or not). Unless otherwise stated, P values < 0.05 were considered significant.

RESULTS

Choice of the cut-off value for cathepsin D

Using life-table analysis for recurrence-free survival, different cut-off values between 20 and 100 pmol/mg protein were tested. The best separation into two groups in the series as a whole was obtained with cut-off values between 35 and 45 pmol/mg protein (Figure 1). As one of the main purposes of the present study was to investigate the prognostic importance of cathepsin D in relation to adjuvant TAM, the same analysis was performed after subgrouping the patients with respect to TAM, the best cut-off value being found to be 45 pmol/mg protein for the subgroup not treated with TAM and 30 pmol/mg protein for the subgroup treated with TAM (Figure 1). However, at a cut-off level of 30 pmol/mg protein, only 18% (73/398) of the tumours were classifiable as low cathepsin D, a proportion inconsistent with those of 23–68% obtained in studies by others [5, 13]. Thus, in the present series, 45 pmol cathepsin D/mg protein was chosen as the cut-off value, yielding 243 cases (39%) with low tumour cathepsin D content (< 45 pmol/mg protein) and 380 (61%) with high tumour values (≥ 45 pmol/mg protein).

Correlation between cathepsin D and other prognostic factors

As assessed with Spearman's rank correlation test, the cathepsin D concentration was significantly positively correlated both with the number of DNA stemlines (i.e. cell populations), the SPF and amplification of *ERBB2*, and a weak positive correlation with ER and PgR content, patient age and lymph node involvement, but no correlation with tumour size or *int2* and c-*myc* amplification (Table 2).

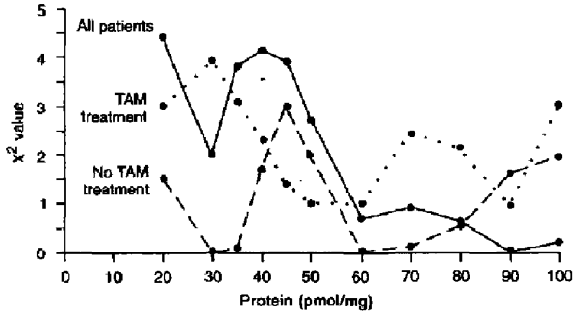

Figure 1. Life-table analysis (log-rank test for recurrence-free survival) of different cut-off values of cathepsin D for three groups of breast cancer patients: (1) all patients, ●——●, (2) patients not treated with adjuvant TAM ●– – –● and (3) patients treated with adjuvant TAM ●· · ·●. The χ^2 value obtained for each cut-off value was plotted against the value itself.

Table 2. Spearman's rank correlation coefficient (r_s) between tumour cathepsin D content and other prognostic factors in breast cancer

Factor	r_s	P value	Number of cases
Lymph node status	0.08	0.02	621
Tumour size	−0.03	0.19	622
Age at operation	0.078	0.027	623
ER	0.08	0.023	620
PgR	0.08	0.033	603
DNA stemlines	0.18	<0.001	357
S-phase fraction	0.16	0.002	333
ERBB2 amplification	0.15	0.032	161
int2 amplification	0.04	0.30	159
c-*myc* amplification	−0.10	0.26	47

ER, oestrogen receptor; PgR, progesterone receptor.

Somewhat similar patterns of correlation were obtained with χ^2 analysis when tumours were subgrouped according to cathepsin D content (i.e. high versus low, with the cut-off level of 45 pmol/mg protein): the proportion of tumours with high SPF values was 51% (102/202) in the high cathepsin D subgroup but only 32% (42/131) in the low cathepsin D subgroup (P = 0.0009). With χ^2 analysis, no relationships were found between cathepsin D and either ER and PgR status, tumour size or menopausal status although there was a weak correlation with lymph node status—high cathepsin D tumours being predominantly N4+, and low cathepsin D tumours predominantly N0 or N1–3 (P = 0.038).

The prognostic importance of cathepsin D in relation to lymph node status

When lymph node status was also considered, the cathepsin D value tended to yield more prognostic information in the N+ group (n = 404) than in the N0 group (n = 217). The recurrence rate for N+ patients was 25% in the low cathepsin D subgroup and 37% in the high cathepsin D subgroup (P = 0.11), the corresponding figures for N0 patients being 15 and 18% (P = 0.52), respectively.

The prognostic importance of cathepsin D in relation to adjuvant endocrine treatment

When investigating the importance of prognostic factors in breast cancer, the importance of adjuvant systemic therapy should be considered, since if adjuvant therapy is given, it is also the predictive value of the factor in relation to the effect of treatment which is studied. In the present study, it was indicated that adjuvant endocrine therapy affected outcome, since in the group not treated with adjuvant TAM (n = 225), the recurrence rate was 22% in the low cathepsin D subgroup and 34% in the high cathepsin D subgroup (P = 0.06), the corresponding figures in the TAM-treated group (n = 398) being 21 and 28% (P = 0.24), respectively.

From the results outlined above, it thus appears that the prognostic importance of the cathepsin D value seemed to be dependent both on N status and whether adjuvant TAM had been given or not. We, therefore, investigated in stratified

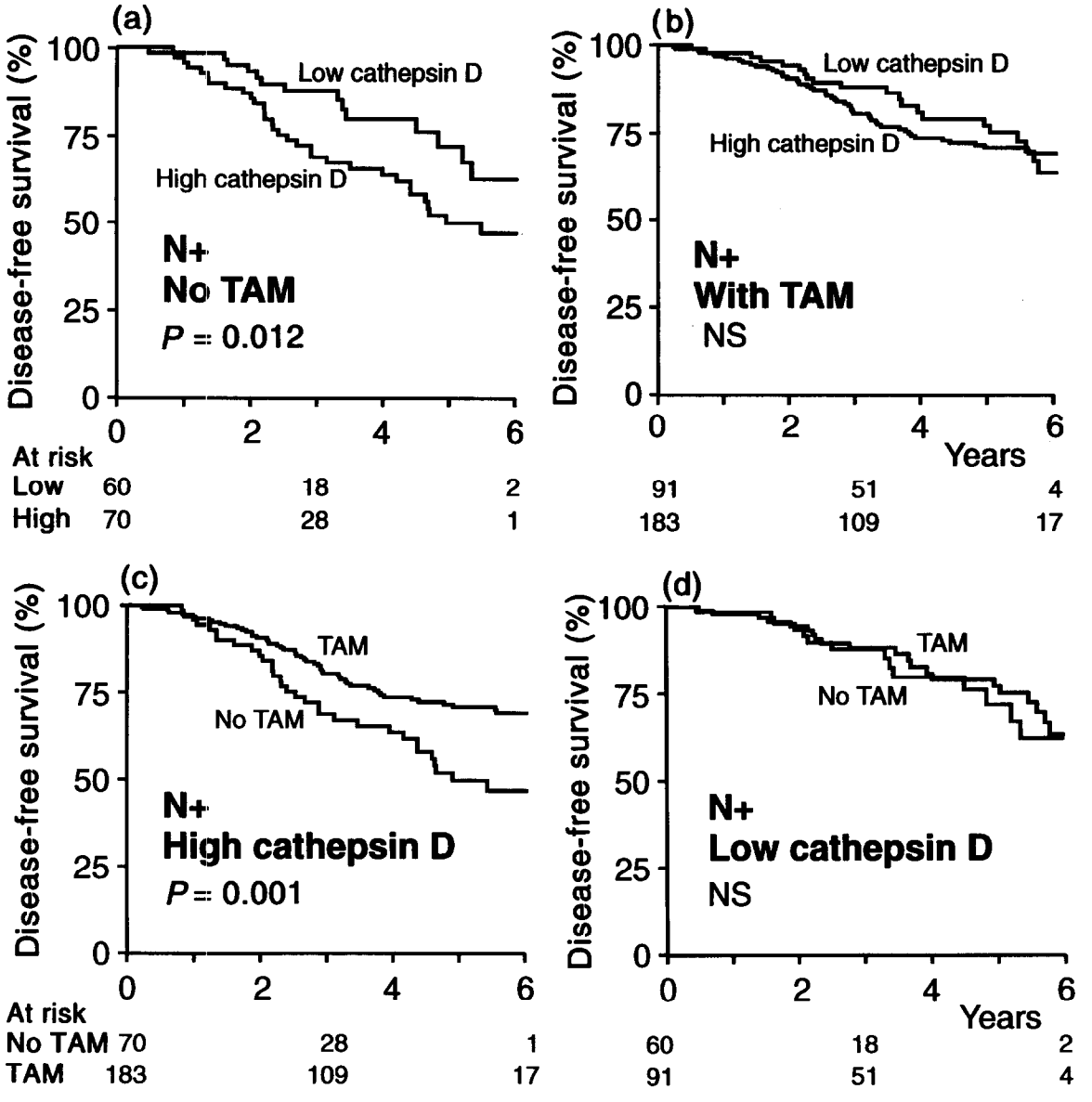

Figure 2. The prognostic importance for recurrence-free survival of tumour cathepsin D content for (a) N+ patients not treated with tamoxifen and (b) N+ patients treated with tamoxifen. The effect of adjuvant tamoxifen for (c) N+ patients with high tumour cathepsin D content and (d) N+ patients with low tumour cathepsin D content.

analyses the prognostic value of cathepsin D when both N status and TAM were taken into consideration. For patients with N+ breast cancer not treated with TAM (n = 130, Figure 2a), the recurrence rate was 23% in the low cathepsin D subgroup and 49% in the high cathepsin D subgroup (P = 0.012). No significant differences were found for the other three combinations of N status and TAM. The corresponding figures were 26 and 33% for N+ patients treated with TAM (P = 0.65; n = 244; Figure 2b), 19 and 19% for N0 patients not treated with TAM (P = 0.99; n = 99), and 13 and 16% for N0 patients treated with TAM (P = 0.71; n = 123).

By combining the results shown in Figure 2a and b, it was found that adjuvant TAM has a beneficial effect for N+ patients with tumours containing high cathepsin D levels. This is further illustrated in Figure 2c. Adjuvant treatment with TAM decreased the recurrence rate from 49 to 33% (P = 0.001) for N+ breast cancer with high cathepsin D levels, whereas it had no beneficial effect for the group of N+ patients with low cathepsin D tumors [Figure 2d; recurrence rate 23% (no TAM) and 26% (with TAM); P = 0.74]. For N0 patients, no statistically significant effect of TAM was obtained, either in the high or in the low cathepsin D subgroup.

The effect of TAM in relation to PgR status

In our breast cancer series as a whole, where the effect of adjuvant TAM has been studied, PgR status has hitherto proved to be a better predictive factor than ER status [24]. We, therefore, investigated the predictive value of PgR status in the subset of N+ breast cancer patients where cathepsin D was evaluated. Treatment with adjuvant TAM improved recurrence-free survival for patients with PgR+ tumours (P = 0.006; n = 224). For patients with PgR− tumours (n = 168), no beneficial effect of TAM was demonstrated (P = 0.33).

The effect of adjuvant TAM in relation to both PgR and cathepsin D status

The results outlined above suggest the level of the cathepsin D concentration to be a prognostic factor, as shown in N+

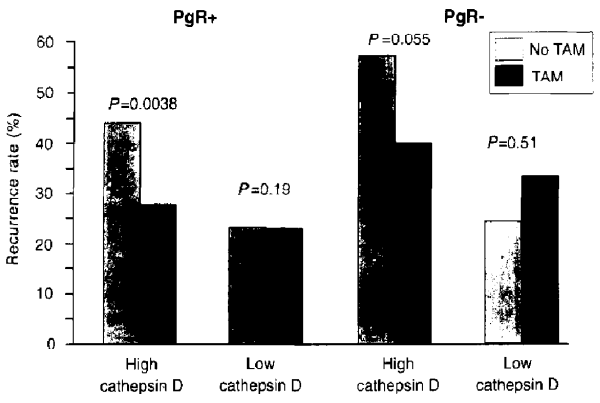

Figure 3. The effect of adjuvant tamoxifen on recurrence-free survival (log-rank test) for N+ patients with different combinations of PgR and cathepsin D status.

patients not treated with adjuvant TAM (Figure 2a). It would also seem to be a predictive factor for the effect of adjuvant TAM—at least in the N+ group—since only patients with tumours containing high cathepsin D levels had any statistically significant benefit of TAM (Figure 2c). As both cathepsin D and PgR status had predictive value for the effect of TAM, the question arose of whether cathepsin D may provide additional information to PgR status (or vice versa) concerning prediction of the effect of adjuvant TAM. To test this, the effect of adjuvant TAM was studied for four different groups of patients with N+ breast cancer: (1) PgR+/high cathepsin D (n = 145), (2) PgR+/low cathepsin D (n = 79), (3) PgR−/high cathepsin D (n = 100), and (4) PgR−/low cathepsin D (n = 68). The results summarised in Figure 3, show that the most profound beneficial effect of adjuvant TAM was seen for PgR+/high cathepsin D patients, thus confirming the cathepsin D level to be a factor of importance for the effect of adjuvant TAM. In addition, in the group of patients with the worst prognosis (PgR− and high cathepsin D level), there was a slight beneficial effect of TAM (P = 0.055), indicating that the cathepsin D level may identify a group of endocrine responsive patients with PgR− tumours.

Multivariate analysis

From the results described above, it is clear that adjuvant TAM is important for the prognostic importance of cathepsin D. Multivariate analysis was, therefore, performed after dividing the patients into two subgroups, one with adjuvant TAM, the other without. For the group of patients not treated with TAM, N status, tumour size and cathepsin D were found to be independent prognostic variables for recurrence-free survival, whereas ER, PgR and menopausal status were not (Table 3a). For the group of patients treated with adjuvant TAM (Table 3b), N status and ER were found to be independent prognostic factors, whereas tumour size, PgR, cathepsin D and menopausal status were not. PgR entered the model as a significant factor if ER was excluded.

Previous results obtained by our group [16] have demonstrated the SPF to be a strong prognostic factor. We, therefore, performed multivariate analysis in the subgroup of patients not treated with adjuvant TAM with tumours analysed for SPF and cathepsin D (n = 111), and found the SPF to enter the model

Table 3. Recurrence-free survival, according to univariate and multivariate analysis (Cox's proportional hazards model) of prognostic covariates for patients not treated or treated with adjuvant tamoxifen

Covariate	Univariate	Multivariate		
	P value	P value	Relative risk	95% confidence interval
(a) Patients not treated with adjuvant tamoxifen (n = 199)				
Axillary lymph nodes				
0, 1–3, 4+	0.0001	< 0.001	1.9	1.4–2.6
Tumour size				
≤ 20 versus > 20 mm	0.009	0.010	2.4	1.2–4.6
PgR status				
Negative versus positive	NS	NS		
ER status				
Negative versus positive	NS	NS		
Cathepsin D				
Low versus high	0.036	0.014	2.0	1.1–3.5
Menopausal status				
Pre- versus postmenopausal	NS	NS		
(b) Patients treated with adjuvant tamoxifen (n = 350)				
Axillary lymph nodes				
0, 1–3, 4+	< 0.0001	< 0.0001	1.9	1.4–2.6
Tumour size				
≤ 20 versus > 20 mm	NS	NS		
PgR status				
Negative versus positive	0.014	NS		
ER status				
Negative versus positive	0.008	0.002	2.1	1.4–3.3
Cathepsin D				
Low versus high	NS	NS		
Menopausal status				
Pre- versus postmenopausal	NS	NS		

NS, non-significant; ER, oestrogen receptor; PgR, progesterone receptor.

(together with lymph node status), whereas cathepsin D lost its independent prognostic value.

DISCUSSION

The level of tumour concentration of cathepsin D was shown to be both a prognostic factor and a predictive factor for the effect of adjuvant TAM. The variable was shown to be of prognostic importance among N+ patients not treated with adjuvant TAM. For patients treated with TAM, a statistical improvement of recurrence-free survival after therapy could only be demonstrated for those with high cathepsin D levels and N+ breast cancer. For the N0 group, no importance of cathepsin D could be demonstrated, irrespective of whether TAM had been given or not. Our finding in the N0 group should, however, be considered preliminary, since the duration of follow-up was relatively short (median 37 months). The differences of some tumour characteristics between the TAM-treated and non-TAM-treated group may, to some extent, influence the results. However, as the TAM-treated group were more often N+ and had large tumours, the difference in prognosis between the two groups should be even more pronounced if only patients in randomised trials were included. This was also indicated when the 108 non-randomised patients were excluded from the analysis (data not shown). In three studies investigating the importance of N status, cathepsin D also appeared to be of greatest value in N+ patients [7–9]. In a fourth study [6], cathepsin D was demonstrated to be of prognostic importance in the N0 group only. Findings in other studies of N0 patients have varied, with the value of cathepsin D as a prognostic factor in this subgroup of patients supported by some [5, 10–12], but not by others [13]. There may be several explanations for these differences in results, including the choice of methods, cut-off value or endpoint (recurrence-free survival or overall survival), the patient characteristics (menopausal status and N status), whether adjuvant therapy has been given or not and duration of follow-up. Moreover, if multivariate analysis is performed, the selection of factors included may also affect the results concerning the independent prognostic value of cathepsin D.

One reason for the discrepancies in results between these studies may have been the differences in the techniques used. Although immunoradiometric assay (ELISA-cath-D kit) was used in several studies [5, 7, 10, 11, 13, present study] as was immunohistochemistry [9, 12], the other methods were used in only one or two studies: enzyme immunoassay [4, 8], western blotting [6], enzymatic activity [10]. However, differences in the choice of technique alone do not completely explain the discrepant results, as discrepancies remain even when the comparison is confined to results obtained with ELISA-cath-D kits, both in overall results for the various series studied, and in the results of subgroup analyses (e.g. N0 versus N+, ER− versus ER+).

In this respect, the explanation may be methodological as different cut-off values were adopted by those using the immunoradiometric assay, ranging from 35 to 70 pmol/mg protein, and resulting in differences in the proportion of tumours with 'high' cathepsin D concentrations, ranging from 23 to 68% [5, 7, 13]. These figures should be compared with that of 61% obtained with a cut-off level of 45 pmol/mg protein in the present study. In the study by Foekens and co-workers, no uniform cut-off point was chosen for the series as a whole, since a continuous trend was found for the recurrence rate to increase with increasing cathepsin D concentration, and the series was divided into four subgroups with regard to tumour cathepsin D content [11]. Both in our study and in some of the others, the criterion for the choice of cut-off value was optimal prognostic separation into high and low cathepsin D groups [6, 7, 13], an approach that should be regarded as hypothesis generating, and requiring substantiation in a new independent series. Others have used the median value [8] or two or three different cut-off values [4, 10, 11].

Further reasons for different conclusions regarding the prognostic importance of cathepsin D may be differences in N status and whether adjuvant systemic treatment has been given or not. The choice of endpoint (recurrence-free survival or overall survival) did not affect the major findings in the present study. When subgrouping our series with regard to N status and adjuvant TAM, cathepsin D was only found to be of prognostic importance for patients not treated with adjuvant TAM, and only in the N+ subgroup. As 87% (113/130) of the patients in this group (N+/no TAM) were premenopausal, the importance of menopausal status for the prognostic importance of cathepsin D could not be evaluated in the present series. Cathepsin D content has been shown to increase with age, values being higher for postmenopausal patients than among premenopausal patients, both in benign breast disease and in breast cancer [4, 25]. Different cut-off values were also adopted for pre- and postmenopausal patients in the latter study [4]. In our study a trend ($P = 0.07$) was found where the proportion of tumours with high cathepsin D values ($\geq$ 45 pmol/mg protein) was greater among postmenopausal patients than among premenopausal patients. The finding by Duffy and co-workers, that cathepsin D was a significant prognostic factor only in the ER-positive subgroup and not in the ER-negative subgroup [13], could not be confirmed in our investigation. The lack of prognostic value of cathepsin D in the N0 group in the present work is in conflict with findings in some studies [5, 6, 10–12], but in agreement with those of others [7–9]. The choice of factors included in multivariate analysis would seem to be a more important consideration, since when SPF was included, the independent prognostic value of cathepsin D was lost. This subgroup analysis should, of course, be confirmed in a larger series.

A more striking finding was the profound effect of adjuvant TAM on the results in our study. When TAM treatment was considered, we showed that cathepsin D is both a prognostic factor (for patients not treated with adjuvant TAM) and a predictive factor for the effect of adjuvant TAM (only those patients with a high cathepsin D content in their tumours responded), and especially for the PgR+ subgroup. When subgrouping with regard to ER status was used instead, similar results were obtained—i.e. adjuvant tamoxifen had a significant beneficial effect only for patients with ER-positive and high cathepsin D tumours. Another finding of considerable interest was that the tumour content of cathepsin D may even identify a group of endocrine responsive patients with PgR− tumours, although again this result was obtained in a small subgroup ($n = 100$), and needs to be confirmed in a larger number series, preferably comprising patients also participating in prospective randomised trials.

The importance of taking adjuvant TAM therapy into consideration when examining the prognostic importance of different variables has previously been demonstrated in our laboratory. The SPF is a strong prognostic factor for breast cancer patients not treated with adjuvant TAM, while it seems to be without importance for clinical outcome among patients treated with adjuvant TAM [26]. The SPF would thus seem to be a

prognostic factor. In contrast, *ERBB2* amplification was an independent factor only among patients treated with adjuvant TAM [27], and would not seem to be just a prognostic factor, but rather a predictive factor for the effect of adjuvant TAM. The results concerning cathepsin D suggest it to be both a prognostic factor and a predictive factor for the effect of TAM. The importance of considering adjuvant therapy has also been indicated by Namer and co-workers, who found cathepsin D to be of prognostic importance only among N+ patients treated with adjuvant chemotherapy [7]. TAM is an oestrogen agonist for cathepsin D induction, both *in vivo* [28] and *in vitro* [29]. Therefore, the reason why TAM is more efficient in tumours with high cathepsin D content is not yet understood. Since many of the aforementioned studies included patients treated with adjuvant TAM or chemotherapy, discrepancies in findings may, at least to some extent, have been due to differences in the treatment given.

In agreement with findings in most other studies, we found the tumour cathepsin D level to be unrelated to tumour size and ER or PgR status. We found cathepsin D to correlate with lymph node involvement, ploidy status, SPF and *ERBB2* amplification. While we found the tumour cathepsin concentration to correlate with *ERBB2* amplification, but not with *int2* and c-*myc* amplification, Brouillet and co-workers found cathepsin D to correlate with c-*myc* amplification, but not with *ERBB2* or *int2* amplification [30]. The reasons for this discrepancy may be the small number of cases analysed for c-*myc* amplification (n = 47) in our series, and the rather weak correlation between cathepsin D and *ERBB2* amplification (r_s = 0.15; P = 0.032).

In conclusion, cathepsin D was shown to be both a prognostic factor—at least for N+ premenopausal patients, and a predictive factor for the effect of adjuvant TAM—at least in the N+ subgroup. The somewhat conflicting conclusions between different studies concerning the prognostic importance of cathepsin D emphasise the need of large prospective studies in which adjuvant therapy and different patient and tumour characteristics (menopausal status, N-status, etc.) should be taken into consideration. It would also seem to be important to compare the prognostic significance of tumour cathepsin D content with that of other factors such as the proliferation rate.

1. Borg Å. Gene alterations in human breast cancer. In *Current Perspectives on Molecular and Cellular Oncology*. Demetrios, Spandidos, eds. 1992, Volume 1, part B, 21–79.
2. Westley B, Rochefort H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. *Cell* 1980, **20**, 353–362.
3. Rochefort H, Capony F, Garcia M, *et al.* Estrogen-induced lysosomal proteases secreted by breast cancer cells: a role in carcinogenesis? *J Cell Biochem* 1987, **35**, 17–29.
4. Thorpe S, Rochefort H, Garcia M, *et al.* Association between high concentrations of M_r 52,000 cathepsin D and poor prognosis in primary human breast cancer. *Cancer Res* 1989, **49**, 6008–6014.
5. Spyratos F, Maudelonde T, Brouillet J-P, *et al.* Cathepsin D: an independent prognostic factor for metastasis of breast cancer. *Lancet* 1989, **ii**, 1115–1118.
6. Tandon AK, Clark GM, Chamness GC, Chirgwin J, McGuire WL. Cathepsin D and prognosis in breast cancer. *N Engl J Med* 1990, **322**, 297–302.
7. Namer M, Ramaioli A, Fontana X, *et al.* Prognostic value of total cathepsin D in breast tumors. A possible role in selection of chemoresistant patients. *Breast Cancer Res Treat* 1991, **19**, 85–93.
8. Pujol P, Maudelonde T, Daures J-P, *et al.* A prospective study of the prognostic value of cathepsin D levels in breast cancer cytosol. *Cancer* 1993, **71**, 2006–2012.
9. Henry JA, McCarthy AL, Angus B, *et al.* Prognostic significance of the estrogen-regulated protein, cathepsin D, in breast cancer. An immunohistochemical study. *Cancer* 1990, **65**, 265–271.
10. Kute TE, Shao Z-M, Sugg NK, Long RT, Rusell GB, Case LD. Cathepsin D as a prognostic indicator for node-negative breast cancer patients using both immunoassays and enzymatic assays. *Cancer Res* 1992, **52**, 5198–5203.
11. Foekens JA, Van Puten WLJ, Portengen H, *et al.* Prognostic value of pS2 and cathepsin D in 710 human primary breast tumors: multivariate analysis. *J Clin Oncol* 1993, **11**, 899–908.
12. Isola J, Weitz S, Visakorpi T, *et al.* Cathepsin D expression detected by immunohistochemistry has independent prognostic value in axillary node-negative breast cancer. *J Clin Oncol* 1993, **11**, 36–43.
13. Duffy MJ, Reilly D, Brouillet J-P, *et al.* Cathepsin D concentration in breast cancer cytosols: correlation with disease-free interval and overall survival. *Clin Chem* 1992, **38**, 2114–2116.
14. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1970, **72**, 248–254.
15. Lowry OH, Roseborough N, Farr L, Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
16. Sigurdsson H, Baldetorp B, Borg Å, *et al.* Indicators of prognosis in node-negative breast cancer. *N Engl J Med* 1990, **332**, 1045–1053.
17. Baldetorp B, Dahlberg M, Holst U, Lindgren G. Statistical evaluation of cell kinetic data from DNA flow cytometry (FCM) by the EM algorithm. *Cytometry* 1989, **10**, 695–705.
18. Hiddemann W, Schumann J, Andreeff M, *et al.* Convention on nomenclature for DNA cytometry. *Cytometry* 1984, **5**, 445–446.
19. Baisch H, Gohde W, Linden WA. Analysis of PCP-data to determine the fraction of cells in various phases of cell cycle. *Rad Environ Biophys* 1975, **12**, 31–39.
20. Borg Å, Tandon AK, Sigurdsson H, *et al.* HER-2/neu amplification predicts poor survival in node-positive breast cancer. *Cancer Res* 1990, **50**, 4332–4337.
21. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
22. Mantel N. Evaluation of survival data and two rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966, **50**, 163–170.
23. Cox DR. Regression models and life-tables. *J R Stat Soc (B)* 1972, **34**, 187–220.
24. Rydén S, Fernö M, Borg Å, Hafström L, Möller T, Norgren A. Prognostic significance of estrogen and progesterone receptors in stage II breast cancer. *J Surg Oncol* 1988, **37**, 221–226.
25. Garcia M, Salazar-Retana G, Pages A, *et al.* Distribution of the M_r 52,000 estrogen-regulated protein in benign breast diseases and other tissues by immunohistochemistry. *Cancer Res* 1986, **46**, 3734–3738.
26. Fernö M, Baldetorp B, Borg Å, *et al.* Better effect of adjuvant tamoxifen in high than in low proliferative, progesterone receptor positive breast cancer. *Breast Cancer Res Treat* 1993, **28**, 87.
27. Borg Å. Gene alterations in human breast cancer. From the Joint Care Program of the Southern Swedish Breast Cancer Study Group. Thesis. Lund, Sweden, Wallin & Dalholm Boktryckeri AB, 1992, 105–122.
28. Maudelonde T, Domergue J, Henquel C, *et al.* Tamoxifen treatment increases the concentration of 52K-cathepsin D and its precursor in breast cancer tissue. *Cancer* 1989, **63**, 1265–1270.
29. Chalbos D, Philips A, Galtier F, Rochefort H. Synthetic antiestrogens modulate induction of pS2 and cathepsin D messenger ribonucleic acid by growth factors and adenosine 3', 5'-monophosphate in MCF7 cells. *Endocrinology* 1993, **133**, 571–576.
30. Brouillet J-P, Theillet C, Maudelonde T, *et al.* Cathepsin D assay in primary breast cancer and lymph nodes; relationship with c-*myc*, c-*erb*-B-2 and *int*-2 oncogene amplification and node invasiveness. *Eur J Cancer* 1990, **26**, 437–441.

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