dose intensity achieved with the classical regimen and this, together with its better tolerability, has important implications for the use of CMF as postoperative adjuvant systemic treatment for operable breast cancer.

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Prognostic Relevance of Cathepsin D versus Oestrogen Receptors in Node Negative Breast Cancers

G. Granata, D. Coradini, V. Cappelletti and G. Di Fronzo

The concentration of total cathepsin D in cytosols of 199 node negative women with primary breast cancer in a 10-year retrospective cohort was assayed. Cathepsin D status alone was unable to predict disease-free or overall survival. However, those patients with receptor positive tumours who were cathepsin D positive had longer disease-free ($P = 0.02$) and overall survival ($P = 0.01$) than cathepsin D negative patients. Therefore, measurement of cathepsin D appears to provide additional prognostic information for the prediction of disease-free and overall survival in patients with node negative breast cancer.
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INTRODUCTION

OESTROGEN RECEPTOR STATUS has been used for over a decade as a prognostic index for node negative breast cancer patients. Its prognostic relevance has been consistently reported in many studies. Whereas it is clear that most oestrogen receptor negative (ER^-) patients will have recurrences, a smaller percentage of oestrogen receptor positive (ER^+) patients is also likely to have recurrences.

This emphasises the need for additional prognostic variables to discriminate the subset of ER+ patients at highest risk for recurrence. Several oestrogen-stimulated proteins and their proliferative activities have been studied, and increasing attention has been paid to an oestrogen-regulated protein first discovered as a secretion product from oestradiol-stimulated MCF-7 cells: cathepsin D, a 52 kD protein [1]. The protein seems to be a promising prognostic tool since its proteolytic activity might well be involved in the process of tumour invasion and metastatic spread.

In the present study we retrospectively analysed the impact of the presence of cathepsin D and oestrogen receptors on disease-free (DFS) and overall survival (OS) in 199 node negative breast cancer patients.

PATIENTS AND METHODS

Breast biopsy specimens were obtained from 199 patients with operable primary node negative breast cancer who entered the Istituto Nazionale Tumori between January 1980 and September 1983. The samples, collected at time of surgery of the primary tumour, were snap-frozen and stored in liquid nitrogen. There was histological confirmation of the diagnosis for all cases. None of the patients had any treatment before or after surgery until or unless there was a recurrence.

Tumours 2.5 cm or less in diameter accounted for 69.4% and those more than 2.5 cm in diameter for 30.5%. The median age of the patients was 51 years; 45.5% of the cases were

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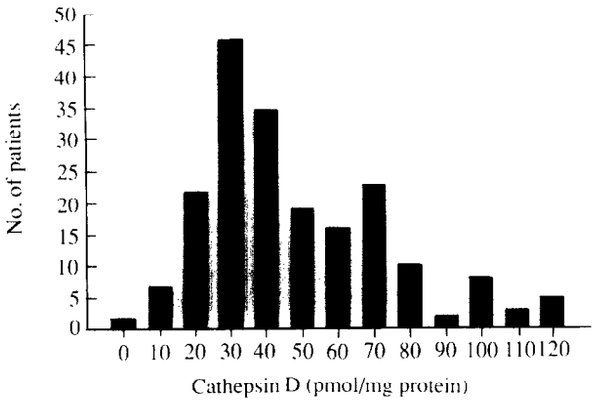

Fig. 1. Distribution of the concentration of cathepsin D in node negative breast tumours (n = 199).

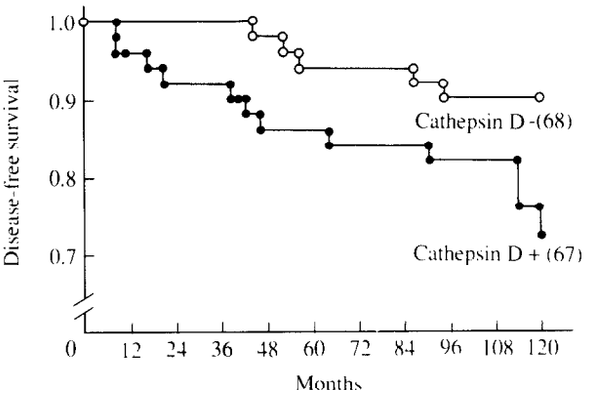

Fig. 2. Disease-free survival of patients with ER$^+$, node negative breast cancer as a function of cathepsin D status (P = 0.02).

premenopausal, and the median follow-up at the time of analysis was 87 months.

The dextran-coated charcoal technique used to measure oestrogen receptors has been described [2]. Results were expressed as specific binding sites per milligram of cytosol protein (fmol/mgP), and tumours containing more than 10 fmol/mgP were defined as ER$^+$. According to these criteria, 148 (74.4%) and 51 (25.6%) tumours were ER$^+$ and ER$^-$, respectively. The total amount of cathepsin D (52 kDa, 48 kDa and 34 kDa proteins) in the same cytosol used for receptor determination was assayed by a solid-phase, two-site immunoradiometric assay using a ELSA-CATH D monoclonal kit (CIS). The total protein concentration was measured by the method of Bradford [3], with minor variations. The impact of the biological variables on DFS (defined as the interval between surgery and the first recurrence) and OS was analysed by the logrank test.

RESULTS

Figure 1 shows the distribution of cathepsin D concentrations in 199 breast tumours. In these samples the median value was 40 pmol/mgP. Lower and upper quartiles divided the patients into groups with low (< 30 pmol/mgP), intermediate (between 30 and 65 pmol/mgP) and high concentrations of cathepsin D (> 65 pmol/mgP). The median value was chosen as the cut-off value for prognostic studies.

We found no significant associations between cathepsin levels and ER status, tumour size, patient age or menopausal status (Table 1). All these variables were considered in a multivariate analysis according to the Cox model and none of them reached statistical significance and was therefore predictive of clinical outcome. However, the relative risk of recurrence was 1.8 for cathepsin D positive vs. cathepsin D negative patients.

When the data were analysed by life-table analysis (at 120 months), cathepsin D status alone did not predict either DFS or OS, although as shown in Fig. 2, cathepsin D was a powerful discriminator of DFS within the subset of ER$^+$ tumours at 120 months of follow-up (P<0.02). ER$^+$ patients who were cathepsin D positive also had significantly shorter OS (P<0.01) than cathepsin D negative patients (Fig. 3). In fact no statistically significant difference was observed between cathepsin D positive patients who were ER$^+$ or ER$^-$ in DFS and OS.

*Table 1. Levels of 52 kDa cathepsin D as compared with other prognostic factors**

Factor	Level of 52 kDa cathepsin D (%)		
	Low (< 30 pmol/ mgP)	Moderate (30–65 pmol/ mgP)	High (> 65 pmol/ mgP)
Oestrogen receptor status			
< 10 fmol/mg protein	31.4 (16/ 51)†	43.1 (22/ 51)	25.5 (13/ 51)
> 10 fmol/mg protein	22.3 (33/148)	51.3 (76/148)	26.4 (39/148)
Tumour size			
< 2.5 cm	22.0 (26/118)	66.1 (78/118)	11.9 (14/118)
> 2.5 cm	21.2 (11/ 52)	59.6 (31/ 52)	19.2 (10/ 52)
Patient's age			
< 50 yr	27.3 (18/ 66)	51.5 (34/ 66)	21.2 (14/ 66)
> 50 yr	22.1 (27/122)	49.2 (60/122)	28.7 (35/122)
Menopausal status			
Premenopausal	29.9 (23/ 77)	45.5 (35/ 77)	24.6 (19/ 77)
Postmenopausal	23.9 (22/ 92)	47.8 (44/ 92)	28.3 (26/ 92)

*No significant differences along rows within factors.
†Number of patients/total number.

DISCUSSION

In recent years, many studies have been published on the putative prognostic relevance of cathepsin D, but results are still contradictory. Spyratos *et al.* [4] and Thorpe *et al.* [5] found a significant predictive value for high concentrations (70–78

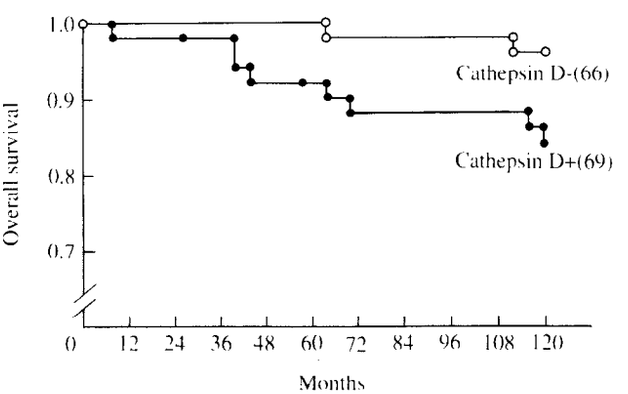

Fig. 3. Overall survival of patients with ER$^+$, node negative breast cancer as a function of cathepsin D status (P = 0.01).

pmol/mgP) of cathepsin D, whereas we found that the best cut-off to discriminate patients with good or bad prognoses was 40 pmol/mgP, the median value, in agreement with Namer *et al.* [6]. Another controversial point is whether cathepsin D is a prognostic variable only in node negative patients [4, 7] or also in node positive ones [5]. This study confirms the prognostic relevance of cathepsin D in node negative women but restricted to the subgroup of ER^+ tumours only.

In agreement with the results of the first clinical studies of the prognostic value of cytosol cathepsin D [8, 9], we found no correlation between the 52 kDa protein and other prognostic markers.

The lack of a correlation of cathepsin D with ER status may be due to the high intrinsic mRNA level observed in ER^- cell lines [10]. There have been some reports of a weak correlation between ER status and cathepsin D but only in premenopausal patients [5]. Our study indicated that cathepsin D identifies high-risk group of ER^+ patients in terms of DFS and OS, regardless of the menopausal status. This variable therefore is an important prognostic factor, although the correlations were not quite significant in the multivariate analysis.

Furthermore, the lack of correlation with steroid receptors (data not shown) and the discriminant power within the group of ER^+ cases are further support for the need to include the cathepsin D assay in the set of variables used for prognostic assessment of node negative breast cancer patients. In our study the ER status alone was not statistically significant, this may have been due to the relatively small sample.

The results of the present study and those reported in the literature were obtained in retrospective studies. If prospective studies on larger patient series confirm these observations, the addition of cathepsin D to the already established prognostic variables may provide an accurate way to predict which 30% of node negative patients will have recurrences and might therefore benefit from aggressive adjuvant therapy.

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