Ole Møller Jensen Memorial EJC Travel Award

THE *European Journal of Cancer* has established an annual travel award to commemorate the late Dr Ole Møller Jensen, cancer epidemiologist and Director of the Danish Cancer Registry.

The award of £500 is being made each year for the best manuscript published in the *European Journal of Cancer* in the field of cancer epidemiology or a closely related area. The recipient is selected by the Editors of the EJC following advice from members of the Editorial Board.

The winner of the first award, for 1993, is Dr Freda E. Alexander at the Leukaemia Research Centre for Clinical Epidemiology (Universities of Leeds and Southampton), Royal South Hants Hospital, Southampton, U.K. for her paper "Viruses, Clusters and Clustering of Childhood Leukaemia: a New Perspective?" published in *European Journal of Cancer* 1993, vol. 29A, pp. 1424–1443.

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Papers

Steroid Receptors, pS2 and Cathepsin D in Early Clinically Node-negative Breast Cancer

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Four oestrogen-regulated proteins of reported prognostic value, oestrogen receptor (ER), progesterone receptor (PR), pS2 and cathepsin D (Cat D), have been quantified by immunoassays, and the latter studied by immunohistochemistry (IHC) in primary tumours from clinically node-negative early breast cancer patients, entered into a trial of breast conservation therapy in which all the patients received adjuvant tamoxifen. ER, PR and pS2 significantly co-correlated but none correlated with Cat D. ER, PR and pS2, but not Cat D, were significantly associated with tumour size and grade, although Cat D tended to show an inverse relationship with the latter. Cat D (radioimmunoassay) in pmol/mg significantly correlated with the IHC score for Cat D in carcinoma cells as well as the number of Cat D-expressing macrophages. At a median follow-up of only 16 months, recurrence was significantly more common in patients with tumours having negative status for ER, PR and pS2 but was not associated with Cat D status.

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INTRODUCTION

APPROXIMATELY 30% of patients with early, node-negative breast cancer will recur from their disease following primary surgery [1], and identification of this high-risk group may allow specific targeting of adjuvant therapy.

In addition to established prognostic factors, such as tumour size [2, 3] and histological grade [4], measurement of oestrogen-related proteins may provide valuable prognostic information. Negative status for oestrogen receptor (ER) [5], progesterone receptor (PR) [6] and the oestrogen-regulated pS2 protein (pS2) [7, 8] have all been shown to indicate poor prognosis in patients with node-negative breast cancer.

The prognostic role of the lysosomal proteinase cathepsin D (Cat D), which has been shown to be under oestrogen regulation in *in vitro* studies [9], remains less clear. Although some workers, using radioimmunoassay (RIA) [10–13] or quantitative western blotting [14] have suggested that Cat D is a marker of poor prognosis, particularly in node-negative patients, others using RIA have been unable to confirm this in this subgroup [15]. Furthermore, in one immunohistochemical (IHC) study, Cat D expression was found to be a marker of good prognosis and to correlate with ER, but the apparent survival advantage was restricted to node-positive patients [16].

These proteins, being oestrogen regulated, may be of particular value in predicting response to adjuvant tamoxifen. However, there has been no previous study where all the proteins have been measured in the same tumours of patients treated with adjuvant hormonal therapy. We report a detailed study of these proteins in such a group of patients, who were clinically node-negative including a comparison of RIA amd IHC methods of detecting Cat D.

PATIENTS AND METHODS

Patients

A subgroup (n = 83) of patients from the West Midlands Collaborative Trial of Radiotherapy and Adjuvant Tamoxifen in the Conservative Management of Early Breast Cancer (1985–1992) was studied. In this trial, patients with early, clinically node-negative breast cancer, following conservative surgery, were randomised to receive one of two regimens of either postoperative radiotherapy or no radiotherapy at this time. All patients received adjuvant tamoxifen, either further randomised to continuous treatment or for 2 years only. The 83 patients, from the overall trial population of 700, represent patients whose tumour specimens had been sent to our laboratory for steroid receptor assays over the last 3 years of the trial period, during which enzyme immunoassay (EIA) measurements of steroid receptors were performed, and where stored 'cytosol' was available.

Methods

Immunoassays. Fresh tumour specimens were cryopreserved for 0.2 to 5.6 months (median 2.3) in sucrose/glycerol buffer at −20°C [17], which preserves ER levels for at least a year [18]. ER and PR were determined by EIA (Abbott Diagnostics, U.K.) on supernatants of tumours homogenised in buffer containing 0.4 mol/l KCl, as reported previously [18]. Supernatants were stored at −40°C and later assayed for pS2 protein and total Cat D using RIA kits (CIS, U.K.). A KCl concentration of 0.4 mol/l does not affect the assay of either pS2 [23] or Cat D (information from CIS and personal observation). All the immunoassays employed a two-site monoclonal antibody sandwich method of detection. Protein was assayed according to the method of Bradford [19], using the Biorad reagent, and ranged from 0.2 to 3.4 mg/ml (median 1.2). Cut-off levels for positive status for both steroid receptors in our laboratory was taken as 20 fmol/mg. For Cat D (RIA), a cut-off of 35 pmol/mg was used in agreement with previous RIA studies [12, 15]. For pS2, cut-off values have ranged from 0.32 to 11 ng/mg in recent reports [7, 20]. We have arbitrarily used a concentration of 1 ng/mg, although this coincided with the lower level at which the protein could be quantified with precision with the recommended protocol. The precision (coefficient of variation, CV%) of the ER and PR assays for our laboratory has been reported previously [18, 21]. The interassay CV for pS2 over the positive range was 7%, and for Cat D (RIA) was 6.5% for values up to 62 pmol/ml, and 10% for higher concentrations.

IHC. IHC for Cat D was performed on 82 of the 83 tumours studied according to the following procedure. Five micrometre sections of formalin-fixed, paraffin-embedded tissue were cut, dewaxed, rehydrated and incubated in 0.05% pronase E (protease type XIV, Sigma, U.K.) in phosphate buffered saline (PBS) for 10 min at 37°C. All subsequent incubations were carried out at room temperature. After two washes in PBS and incubation in 20% normal goat serum for 20 min, sections were left for 1 h in primary antibody (histoCIS CATH-D murine monoclonal antibody M1G8 from CIS), which was used without further dilution. Following two 5-min washes in PBS, biotinylated goat anti-rabbit/mouse antibody (from SteptABComplex/HRP Duet Kit, Dako, U.K.) was added at a dilution of 1:100 in PBS for 30 min, the sections were washed twice with PBS and incubated with AB complex for 30 min. Colour was developed after two further PBS washes, by adding an aqueous solution of 0.5% 3,3'-diaminobenzidine tetrahydrochloride (Sigma, U.K.) containing 0.03% hydrogen peroxide for 5 min. The sections were lightly counter stained with haematoxylin and mounted. Immunostaining was classified as negative (no epithelial staining), + (weak to moderate staining) and ++ (strong staining), as previously reported by Henry *et al.* [16]. Scoring was performed independently using coded specimens by two of the authors. Intra-observer and interobserver concordances were 94 and 91%, respectively, in agreement with Henry *et al.* [16], and differences were resolved by review.

Macrophage counting

The number of macrophages, as revealed by Cat D staining, was recorded with the help of image analysis using the Optimas v. 3.01 software (Bioscan Inc., U.S.A.). Sections were viewed through a 25x plan apochromatic objective (82.5x final magnification) and distance calibration of the software was made with a 0.01 mm graticule. The full screen image was used for each field measurement, which corresponded to 0.038 mm^2 of section. Macrophages were counted by marking the individual cells with the cursor and software generation of the total number in the field. The number of macrophages per tumour was taken as the mean of five randomly selected fields.

Statistical analysis

The various statistical procedures used, including regression plots and histograms, were performed using the statistical/graphic package StatView 4.0 (Abacus Concepts Inc., U.S.A.) on a Macintosh SE/30, apart from χ^2 for trend which was performed with BMDP on a VAX.

RESULTS

The percentage distribution for menopausal status, histological type, tumour size and grade was very similar in the study group to the overall trial population (Table 1). In particular, unlike many steroid receptor studies, there was no bias towards larger tumours; 55% were 2 cm or smaller in diameter in both the study group and the overall trial population.

Using the above cut-off criteria, the number of tumours with postive status for each of the four proteins was 60 (72.3%) for ER, 47 (56.6%) for PR, 42 (50.6%) for pS2 and 40 (48.2%) for Cat D. The distribution of pS2+ tumours according to steroid receptor status was as follows: ER + PR +, 29/45 (64.4%); ER + PR −, 10/15 (66.7%); ER − PR +, 1/2 (50%); ER − PR −, 2/21 (9.5%). Of the 60 ER+ tumours, 39 (65%) were pS2+, and a similar proportion of PR + tumours were also pS2+ (30/47 or 64%). In contrast, of the pS2+ tumours, 93% (39/42) were ER+ and 71% (30/42) were PR+. With quantitative data, we found ER, PR and pS2 to correlate significantly with each other whether using linear regression analysis of log-transformed data or Kendall's non-parametric rank correlation with correction for ties (Fig. 1). Cat D (RIA), however, showed no correlation with ER, PR or pS2 using parametric or non-parametric analysis (Fig. 2).

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Table 1. Comparison of clinicopathological features of the study group (n = 83) with the overall trial population

Feature	Study group No.	Study group %	Trial population %	χ^2	P
Menopausal status					
Pre	17	20.5	25.9		
Post	61	73.5	69.9	1.52	0.46
Equivocal	5	6.0	4.2		
Histological type					
Ductal	49	59.0	67.6		
Lobular	6	7.2	6.0	2.69	0.44
Other	17	20.5	17.2		
Not known*	11	13.3	9.2		
Tumour size					
≤ 2 cm (T1)	46	55.4	54.8		
2–5 cm (T2)	22	26.5	30.8	1.09	0.58
Not known*	15	18.1	14.5		
Histological grade					
1	10	12.0	10.1		
2	33	39.8	33.2	2.82	0.42
3	16	19.3	18.9		
Not known*	24	28.9	37.9		

*Routine histology reporting was not always complete; a histological review is currently in progress and will be basis of subsequent analysis.

Smaller tumours (≤ 2 cm) were found to be significantly more frequently positive for ER, PR and pS2 than larger tumours whereas values of Cat D (RIA) were equally distributed (Table 2). For ER, PR and pS2, grade 3 tumours were found to be less frequently positive and grade 1 and 2 tumours were more frequently positive, while Cat D (RIA) showed a trend in the opposite direction, although this did not reach significance (Table 3).

Cat D immunostaining is localised to lysosomes of both epithelial cells and stromal macrophages [16]. Epithelial expression of Cat D, as detected by IHC, was found to be significantly related to Cat D (RIA) status of the tumour by χ^2 test for trend (Table 4). Furthermore, as with the RIA, IHC expression of Cat D showed no association with ER, PR or pS2 (data not shown).

The number of macrophages, identified by Cat D staining in the tumour sections, was found to correlate significantly with Cat D (RIA) cytosol levels in pmol/mg (Fig. 3), which would, of course, incorporate Cat D from all cellular components of the tumour. Despite this, the distribution of macrophages (Fig. 4) differed somewhat from that of Cat D (RIA), which was similar to previous reports of larger series of tumours [10–12, 15], particularly for node-negative breast cancer [12]. Although there was a trend towards an association between Cat D (IHC) score and the number of macrophages, the correlation was statistically non-significant (IHC score/median number of macrophages: −/0.6; +/1.6; ++/2.2; Kruskal-Wallace H score = 3.38; $P = 0.18$). However, of nine tumours without macrophages expressing Cat D, only three exhibited carcinoma cell staining for Cat D, whereas 31 of the 41 tumours with macrophages were Cat D (IHC)-positive ($P = 0.022$, Fisher's exact test).

With a limited median follow-up of only 16 months at the time of analysis, there have been 11 recurrences in this sub-set of trial patients. Negative status for ER, PR and pS2 was associated with a significantly increased frequency of recurrence (Table 5). At least two of these three proteins had negative status in 10 of the 11 tumours from the patients who relapsed. Even after this short follow-up, recurrence occurred in 35% of patients with ER tumours. In contrast, no difference in recurrence frequency was found according to Cat D status whether assayed by RIA (Table 5) or IHC (data not shown). All patients were taking tamoxifen at the time of recurrence, and follow-up was very similar for patients with recurrence to those who were disease-free. In view of the short follow-up, more extensive statistical analysis, including multivariate analysis, has not been performed at this stage. An additional 47 patients in the trial have had steroid receptors measured by EIA and the findings for recurrence in these 130 patients confirm the results reported here, with ER being of particular value [22].

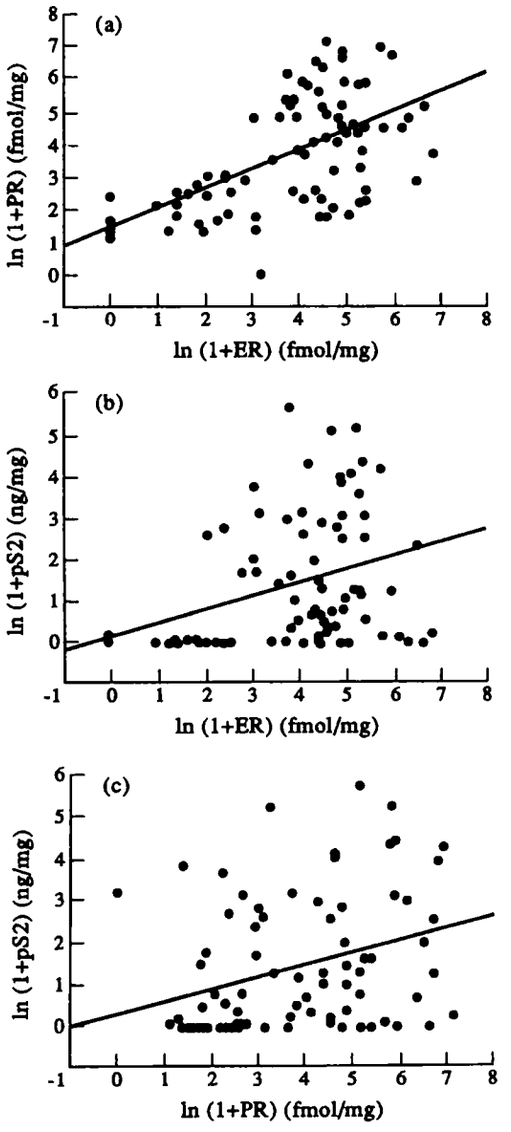

Fig. 1. Relationship between ER, PR and pS2. The data ($n = 83$) are plotted as the natural log (ln) transforms of 1 + assay value to allow for zero values. Pearson's correlation coefficient (r) and Kendall's τ, and equivalent Z score, were as follows: ER versus PR, $r = 0.574$ ($P < 0.0001$), $\tau = 0.364$, $Z = 4.867$ ($P < 0.0001$); ER versus pS2, $r = 0.349$ ($P = 0.0012$), $\tau = 0.261$, $Z = 3.494$ ($P = 0.0005$); PR versus pS2, $r = 0.319$ ($P = 0.0033$), $\tau = 0.270$, $Z = 3.618$ ($P = 0.0003$).

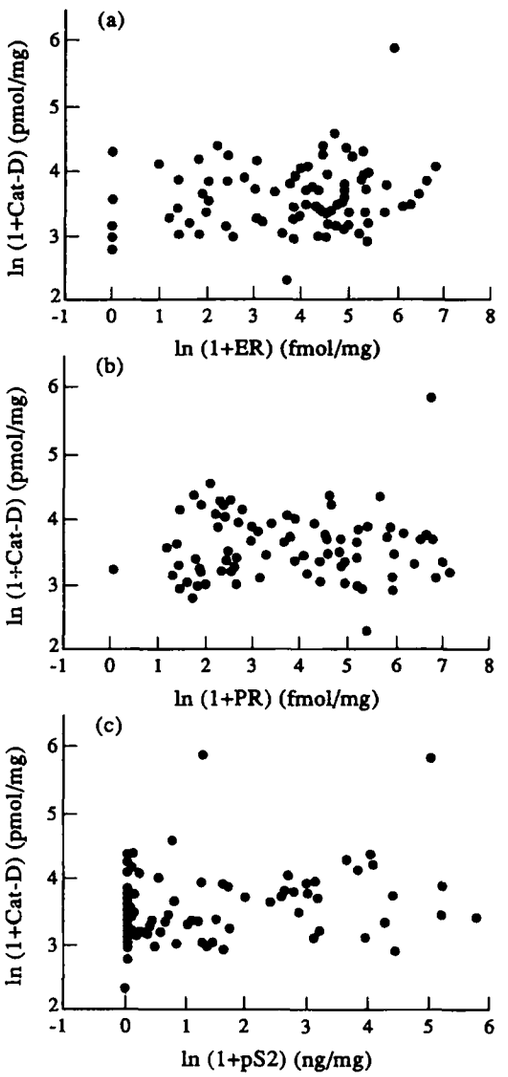

Fig. 2. Relationship between Cat D (RIA) and ER, PR and pS2. The data are displayed and analysed as in Fig. 1. Correlations were not significant: ER, $r = 0.162, \tau = 0.094$; PR, $r = 0.052, \tau = 0.001$; pS2, $r = 0.132, \tau = 0.114$.

Table 2. Relationship between tumour size and positive status for ER, PR, pS2 and Cat D (RIA)

Protein status	Tumour size ≤ 2 cm	Tumour size > 2–5 cm	χ^2	P (df = 1)
ER+	38	11	7.9	0.005
ER−	8	11		
PR+	32	6	10.8	0.001
PR−	14	16		
pS2+	28	5	8.7	0.003
pS2−	18	17		
Cat D+	20	11	0.26	0.61
Cat D−	26	11		

df, degrees of freedom.

Table 3. Relationship between histological grade and ER, PR, pS2 and Cat D (RIA)

Protein status	Tumour grade 1	2	3	χ^2 (df = 2)	χ^{2*} (df = 1)	P
ER+	9	31	4	28.5	18.6	< 0.001
ER−	1	2	12			
PR+	8	21	3	11.97	10.8	< 0.001
PR−	2	12	13			
pS2+	8	17	5	5.86	5.8	0.016
pS2−	2	16	11			
Cat D+	3	13	10	3.30	3.0	0.08
Cat D−	7	20	6			

*χ^2 test for linear trend. df, degrees of freedom.

Table 4. Comparison of Cat D status by RIA and IHC scores

Cat D (IHC) score	Cat D (RIA) (pmol/mg) ≥ 35	< 35
++	23	12
+	12	9
−	5	21
χ^2 (df = 2)	13.69	
χ^2 (df = 1)*	12.35	
P	0.0004	

*χ^2 test for linear trend. df, degrees of freedom.

DISCUSSION

This study has been performed in a representative subset of tumours from clinically node-negative early breast cancer patients entered into a randomised clinical trial of breast conservation therapy, in which all the patients received adjuvant tamoxifen. Importantly, our study population was not biased towards larger or higher grade tumours, the size and grade distributions being almost identical to the trial population.

The relationship between the four proteins assayed has, to our

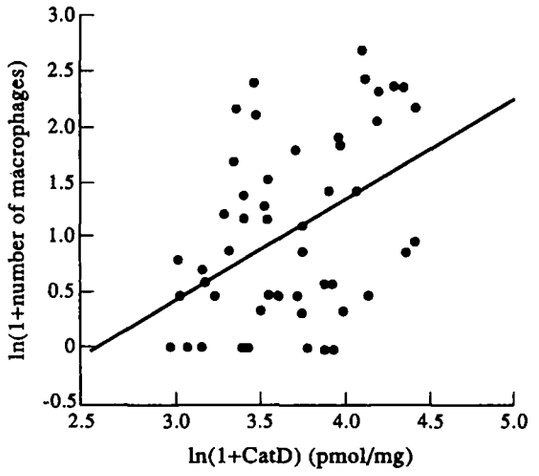

Fig. 3. Relationship between Cat D (RIA) in tumour cytosols and macrophages staining for Cat D by IHC in paraffin sections ($n = 50$). The data are displayed and analysed as in Fig. 1. $r = 0.439$ ($P = 0.0014$), $\tau = 0.279$, $Z = 2.861$ ($P = 0.0042$).

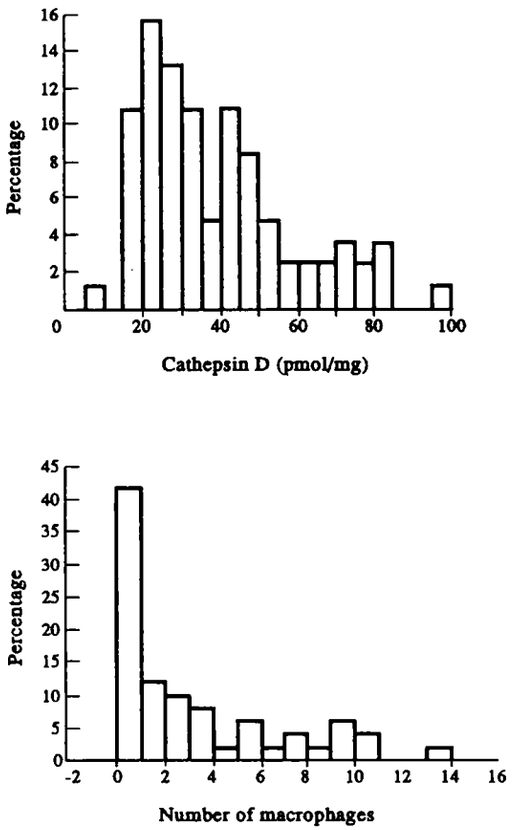

Fig. 4. Distribution of Cat D (RIA) levels in tumour cytosols (n = 83) and macrophages staining for Cat D (IHC) in paraffin sections (n = 50). Note: one tumour with a Cat D (RIA) value of 376 pmol/mg has been omitted from the upper histogram.

knowledge, been reported in only one previous study [23]. In agreement with our results, ER, PR and pS2 were found to correlate significantly with each other, but none of these correlated with Cat D. Other workers have also failed to show a significant relationship between ER and Cat D [11–15] or between pS2 and Cat D [23]. This lack of correlation may be related to the fact that, although Cat D is oestrogen-regulated in ER+ cells lines, it is also constitutively produced by breast cancer cells [9].

ER, PR and pS2 protein behaved similarly in their relationships with tumour size and grade, lending support to the hypothesis that at its inception breast cancer is invariably oestrogen regulated, but with increasing size and de-differentiation the tumour becomes more frequently hormone-independent [24]. Cat D showed no significant relationship with tumour grade or size, confirming other reports of its independence from these risk factors [10–12, 14, 15]. However, compared with other proteins, positive status for Cat D (i.e. higher levels) tended (P = 0.08) to be associated with grade 3 tumours, and lower grade tumours were more frequently Cat D negative.

It has been suggested that higher grade, necrotic tumours are more likely to have high Cat D levels due to the presence of macrophages rich in this lysosomal enzyme [25]. This explanation has also been put forward to account for the lack of concordance in relation to the prognostic value of Cat D between tumour homogenate studies and IHC studies, where macrophage staining can be discriminated and ignored [16]. To answer such suggestions, we performed IHC on the same tumours which had Cat D (RIA) measurements, using one of the monoclonal antibodies (MIG8) used in the RIA.

There have been no previous reports of IHC of Cat D on formalin-fixed, paraffin-embedded tissue using the CIS monoclonal antibodies, which have been used in most of the published cytosol Cat D studies. These antibodies were thought to be reactive only on frozen-sections, unlike the polyclonal antibody used in the Newcastle study [16]. Following protease treatment of the fixed sections, we found immunoreactivities using the monoclonal antibody to be very similar, both in the pattern of staining and in the proportion of positive cases (70%), compared with the polyclonal antibody (66%) [16]. In a very recent report of Cat D in node-negative breast cancer, in which Cat D was detected in paraffin-embedded tumour sections by IHC using a monoclonal antibody from another commercial source, only 36% were scored positive, and a significant association with tumour size, but not with other prognostic factors, was also observed [26].

Contrary to the above hypothesis [16], we found a significant correlation between IHC epithelial staining for Cat D and Cat D levels determined by RIA. Cat D (IHC) also showed no association with ER, PR, pS2, tumour size or grade. Following completion of our study, Maudelonde *et al.* [27] reported on a comparison of cytosolic assay for Cat D by enzyme immunoassay with IHC, using the D7E3 monoclonal antibody from CIS, on frozen sections quantified by computerised image analysis in a series of 34 breast cancers. Our results, in a larger series, confirm their finding of a good correlation between the two methods, using a different CIS monoclonal antibody for the IHC assay.

Although in their series Maudelonde *et al.* [27] found no evidence for an association between high numbers of macrophages and high cytosol Cat D levels, our study clearly shows that cytosol Cat D (RIA) in pmol/mg correlates positively and significantly with the number of macrophages staining for Cat D in paraffin sections of the same tumours. This observation does not, however, allow us to conclude that macrophage Cat D contributes quantitatively to the total Cat D in tumour homogenates. It would seem more likely that both increased expression of Cat D by epithelial cells and the increased number of Cat D-expressing macrophages are both indications of more aggressive or advanced stages of breast cancer. A positive association between Cat D staining of carcinoma cells and the number of macrophages expressing Cat D was observed in the 50 tumours studied here. Significant co-expression of histochemically detected Cat D by carcinoma cells and macrophages in paraffin-

Table 5. Recurrence in relation to status of ER, PR, pS2 and Cat D (RIA)

Protein status	No. with recurrence	No. without recurrence	P*
ER+	3	57	0.0011
ER−	8	15	
PR+	2	45	0.0082
PR−	9	27	
pS2+	2	40	0.0258
pS2−	9	32	
Cat D+	5	35	> 0.999
Cat D−	6	37	

*Fisher's exact test.

embedded sections of tumours from a larger series of node-negative breast cancer patients has recently been reported [26].

Using our cut-off values, approximately two thirds of ER+ and/or PR+ tumours were pS2+, in agreement with several immunohistochemical studies of pS2 [8, 28, 29], while both higher and lower values have been reported using the RIA but with different cut-off levels [7, 23]. The high proportion (93%) of pS2+ tumours which were also ER+ is also consistent with previous reports using the same RIA [7, 23]. It appears that while pS2 is rarely produced by tumours which lack, or express low levels of ER, over 30% of ER+ tumours fail to express pS2 at least at the time of surgical excision. This observation is consistent with the rather poor, albeit significant, correlation between pS2 and ER or PR (Fig. 1), and contrasts with the excellent relationship between the two steroid receptors. This differential may be of prognostic value as some have suggested [7, 20, 30]. In our series of clinically node-negative patients, we found that pS2 correlated significantly with tumour size and grade, which surprisingly agrees with some studies of IHC-detected pS2 [8, 28, 29], but not with reports on pS2 quantified by RIA [7, 20].

In our group of patients, who all received adjuvant tamoxifen, negative status for ER, PR and pS2 was associated with a significantly increased frequency of recurrence at early follow-up. The value of ER in predicting response to hormonal therapy, though of proven value in advanced disease, remains controversial in early breast cancer [4, 30]. Several studies have shown that pS2 has no significant association with time to relapse or overall survival of breast cancer patient populations as a whole [20, 28; 29]. However, it may be of prognostic value in patients with node-negative disease [7, 8], although this may be dependent on ER status [8]. Of particular relevance to the current study is the marked correlation observed between pS2 expression and the outcome of adjuvant tamoxifen therapy reported by Predine *et al.* [20] or response to endocrine therapy on relapse [29]. Our results support the view that the oestrogen-regulated proteins pS2 and PR, together with ER, can predict likelihood of early relapse with adjuvant tamoxifen, although subsequent multivariate analysis is required to determine if these have independent prognostic value. Cat D, which appears functionally unrelated to ER, PR and pS2, was not found to predict early recurrence in this group of patients who were treated with adjuvant tamoxifen, although we cannot exclude it emerging as an independent risk factor with longer follow-up.

1. Early Breast Cancer Trialists Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. *Lancet* 1992, **i**, 1–15 and 71–85.
2. Carter CL, Allen C, Henson DE. Relation of tumour size, lymph node status, and survival in 24,700 breast cancer cases. *Cancer* 1989, **63**, 181–187.
3. Rosen PP, Groshen S, Saigo PE, Kinne DW, Hellman S. A long-term follow-up study of survival in stage I (T1, N0, M0) and stage II (T1, N1, M0) breast carcinoma: a study of 644 patients with median follow-up of 18 years. *J Clin Oncol* 1989, 7, 355–356.
4. Fisher B, Redmond, Fisher ER, Caplan R. Relative worth of estrogen or progesterone receptor and pathological characteristics of differentiation as indicators of prognosis in node negative breast cancer patients: findings from National Adjuvant Breast and Bowel Project Protocol B-06. *J Clin Oncol* 1988, **6**, 1076–1087.
5. Clark GM, McGuire. The clinical usefulness of oestrogen-receptor and other markers of hormone dependence. *Proc Royal Soc Edin* 1989, **95B**, 145–150.
6. Chevallier B, Mosseri V, Dauce IP, Bastit P, Julien JP, Asselain B. A prognostic score in histological node negative breast cancer. *Br J Cancer* 1990, **61**, 436–440.
7. Foekens JA, Rio M-C, Seguin P, *et al.* Prediction of relapse and survival in breast cancer patients by pS2 protein status. *Cancer Res* 1990, **50**, 3832–3837.
8. Thor AD, Koerner FC, Edgerton SM, Wood WC, Stracher MA, Schwartz LH. pS2 expression in primary breast carcinomas: relationship to clinical and histological features and survival. *Breast Cancer Res Treat* 1992, **21**, 111–119.
9. Rochfort H, Augereau P, Briozzo P, *et al.* Structure, function, regulation and clinical significance of the 52K pro-cathepsin D secretion by breast cancer cells. *Biochimie* 1988, **70**, 943–949.
10. Thorpe SM, Rochfort H, Garcia M, *et al.* Association between high concentrations of Mr 52000 cathepsin D and poor prognosis in primary human breast cancer. *Cancer Res* 1989, **49**, 6008–6014.
11. Spyratos F, Maudelonde T, Brouillet JP, *et al.* Cathepsin D: an independent prognostic factor for metastatis of breast cancer. *Lancet* 1989, **ii**, 1115–1118.
12. Granata G, Coradini D, Cappelletti V, DiFronzo G. Prognostic relevance on cathepsin D versus oestrogen receptors in node negative breast cancers. *Eur J Cancer* 1991, **27**, 970–972.
13. Kute TE, Zhi-Ming S, Sugg NK, Long RT, Russell 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.
14. Tandon AK, Clark GM, Chamness GC, Chirgwin JM, McGuire WL. Cathepsin D and prognosis in breast cancer. *New Engl J Med* 1990, **322**, 297–302.
15. Namer M, Ramaioli A, Fontana X, *et al.* Prognostic value of total cathepsin D in breast tumours: a possible role in selection of chemoresistant patients. *Breast Cancer Res Treat* 1991, **19**, 85–93.
16. Henry JA, McCarthy AL, Angus B, *et al.* Prognostic significance of the oestrogen-regulated protein, cathepsin D, in breast cancer. *Cancer* 1990, **65**, 265–271.
17. Crawford DJ, Cowan SK, McMenamin N, Hyder S, Smith DC, Leake RE. A new storage procedure for human tumour biopsies prior to oestrogen receptor measurement. *Cancer Res* 1984, **44**, 2348.
18. Hendrickse CW, Jones CE, Donovan IA, Neoptolemos JP, Baker PR. Oestrogen and progesterone receptors in colorectal cancer and human colon cancer cell lines. *Br J Surg* 1993, **80**, 636–640.
19. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. *Anal Biochem* 1976, **72**, 248–254.
20. Predine J, Spyratos F, Prud'homme JF, *et al.* Enzyme-linked immunosorbent assay of pS2 in breast cancers, benign tumors, and normal breast tissues. *Cancer* 1992, **69**, 2116–2123.
21. Baker PR, Wilton JC, Jones CE, Stenzel DJ, Watson N, Smith GJ. Bile acids influence the growth, oestrogen receptor and oestrogen-regulated proteins of MCF-7 human breast cancer cells. *Br J Cancer* 1992, **65**, 566–572.
22. Stonelake PS, Baker PR, Baker PG, Morrison JM, Spooner D. Steroid receptors in early breast cancer. *Br Med J* 1992, **305**, 51.
23. Goussard J, Lechevrel C, Roussel G, Cren H, Bera O, Sala M. Immunoradiometric assay of pS2 protein in breast cancer cytosols. *Clin Chem* 1991, **37**, 1759–1762.
24. Tinnemas JGM, Beex LVAM, Wobbes Th, Raemaekers JMM, Bernraad Th. Steroid-hormone receptors in nonpalpable and more advanced stages of breast cancer. A contribution to the biology and natural history of carcinoma of the female breast. *Cancer* 1990, **66**, 1165–1167.
25. Cowan WK, Angus B, Henry J, Corbett IP, Reid WA, Horne CHW. Immunohistochemical and other features of breast carcinomas presenting clinically compared with those detected by cancer screening. *Br J Cancer* 1991, **64**, 780–784.
26. 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.
27. Maudelonde T, Brouillet JP, Roger P, Giraudier V, Pages A, Rochefort H. Immunostaining of cathepsin D in breast cancer: quantification by computerised image analysis and correlation with cytosolic assay. *Eur J Cancer*, 1992, **28A**, 1686–1691.
28. Henry JA, Piggott NH, Mallick UK, *et al.* pNR-2/pS2 immunohistochemical staining in breast cancer: correlation with prognostic factors and endocrine response. *Br J Cancer* 1991, **63**, 615–622.
29. Cappelletti V, Coradini D, Scanziani E, Benini E, Silvesrini R, Di Fronzo G. Prognostic relevance of pS2 status in association with steroid receptor status and proliferative activity in node-negative breast cancer. *Eur J Cancer* 1992, **28A**, 1315–1318.

30. Rubens RD. Management of early breast cancer. *Br Med J* 1992, **304**, 1361–1364.

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Interferons Combined with Chemotherapy in the Treatment of Stage III–IV Non-small Cell Lung Cancer—a Randomised Study

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80 patients with previously untreated stage III–IV non-small cell lung cancer (NSCLC) were randomly assigned to receive chemotherapy (CT) alone (arm I: 26 patients) or the same CT combined with either interferon (IFN)-γ (arm II: 27 patients) or with both IFN-γ and IFN-α (arm III: 27 patients). The CT comprised cisplatin 60 mg/m² intravenously (i.v.) day 1 and etoposide 100 mg/m² i.v. days 1, 3 and 5, once every 28 days; the IFN therapy comprised either recombinant IFN-γ1b 0.2 mg/m², subcutaneously, three times a week until day 25, or recombinant IFN-α2c 6 × 10⁶ U given according to the same schedule, and simultaneously with IFN-γ. A maximum of six cycles were given. The treatment was discontinued if progressive disease (PD) was demonstrated. The mean numbers of cycles per patient given in the different arms were 3.6 (arm I), 3.0 (arm II) and 2.9 (arm III). The main reason for discontinuation in all arms was PD. 17 (28%) of the 61 evaluable patients achieved partial responses (35% in arm I, 29% in arm II and 35% in arm III, non-significant). No complete response was recorded. Haematological toxicity was dose-limiting in all arms: leucopenia (WHO grade 3) was observed universally, but more frequently in arm III (in 18% of cycles given). Only two episodes of grade 4 leucopenia were seen (arms II and III) and six episodes of grade 3–4 thrombocytopenia (arm III). Median survival was 6–7 months in all arms. The survival curve for arm II was slightly more favourable (non-significant) than those for other arms. The addition of IFN-γ alone or IFN-α plus IFN-γ to platinum-based CT did not improve response rates nor did it produce any significant survival benefit for patients with NSCLC. Increased haematological toxicity was observed when both IFNs were administered concomitantly with CT.

Key words: cisplatin, etoposide, interferon-α, interferon-γ, NSCLC
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INTRODUCTION

THE REASON for our inability to cure more than 10–15% of all lung cancer patients is the presence of distant metastases at the time of diagnosis. Thus, systemic therapy must be the basis of any successful programme to cure more lung cancer patients, and to improve the survival of those patients who cannot be cured.

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Most drug combinations evaluated in the 1970s comprised inactive agents. These combinations were never shown to prolong the survival of patients with non-small cell lung cancer (NSCLC) at any stage in its course, whether used alone or in combination with surgery or radiotherapy [1].

Recent studies have shown that cisplatin-based chemotherapy improves survival for patients at all stages of NSCLC. For patients with advanced disease, cisplatin-based combinations have improved survival compared with best supportive care [2].

Chemotherapy is most active in patients who have received no prior chemotherapy and who have other well-documented positive prognostic factors. The response rate is not dependent upon the histological variety of NSCLC [3]. New treatment approaches are certainly needed to improve the outcome for the