17. Kelly BW, Morris JF, Harwood BP, Bruya TE. Methods and prognostic value of bone marrow examination in small cell lung cancer. *Cancer* 1984, 53, 99–102.
18. Osterlind K, Hansen HH, Dombernowsky P, Andersen PK. Long term disease-free survival in small cell carcinoma of the lung: a study of clinical determinants. *J Clin Oncol* 1986, **4**, 1307–1313.
19. Stewart P, Buckner CD, Thomas ED, *et al.* Intensive chemotherapy with autologous marrow transplantation for small cell carcinoma of the lung. *Cancer Treat Rep* 1983, **67**, 1055–1059.
20. Bezwoda WR, Lewis D, Livini N. Bone marrow involvement in anaplastic small cell lung cancer. *Cancer* 1986, **58**, 1762–1765.
21. Mansi IL, Berger V, Easton D, *et al.* Micrometastases in bone marrow in patients with primary breast cancer: evaluation as an early predictor of bone metastases. *Br J Med* 1987, **295**, 1093–1096.
22. Berendsen HH, De Ley L, Postmus PE, Ter Haar JC, Poppemans S. The TH: Detection of small cell lung cancer metastases in bone marrow aspiration using monoclonal antibody directed against neuroendocrine differentiation antigen. *J Clin Pathol* 1988, **41**, 273–276.
23. Canon JL, Humblet Y, Labacq-Verheyden AM, *et al.* Immunodetection of small cell lung cancer metastases in bone marrow using three monoclonal antibodies. *Eur J Cancer Clin Oncol* 1988, **24**, 147–150.
24. Sugarbaker EV. Mechanisms of metastasis formation. In: Pilch YH ed. *Surgical Oncology*. New York, McGraw-Hill, 1984, 198.

Eur J Cancer, Vol. 26, No. 11/12, pp. 1145–1147, 1990.
Printed in Great Britain
0277–5379/90 $3.00 + 0.00

Relation between Apocrine Differentiation and Receptor Status, Prognosis and Hormonal Response in Breast Cancer

N.J. Bundred, Helen J. Stewart, D.A. Shaw, A.P.M. Forrest and W.R. Miller

The release of a gross cystic disease fluid protein (GCDFP 15) by tumour explants grown in tissue culture was used to measure apocrine differentiation in 117 women with breast carcinoma. GCDFP 15 was detected by radioimmunoassay in the media from 90% of tumours (range 2–2100 ng/ml, mean 41). Tumour secretion of GCDFP 15 was higher in oestrogen receptor rich (over 20 fmol/mg) tumours ($P < 0.05$) but did not correlate with any other prognostic factors or with survival. Response to hormonal therapy was assessable by UICC criteria in 33 women (6 partial responses, 8 stable disease, 19 progression). Responders had significantly higher tumour oestrogen receptor levels ($P < 0.005$) but a lower GCDFP 15 secretion than non-responders ($P < 0.02$). Apocrine differentiation in breast cancer may be a marker for oestrogen receptor positive tumours that do not respond to hormonal therapy.
Eur J Cancer, Vol. 26, No. 11/12, pp. 1145–1147, 1990.

INTRODUCTION

THE CLINICAL significance of apocrine differentiation in carcinoma of the breast is not well defined. Problems with assessing apocrine change in breast cancer have led to estimates of frequency ranging from 0.3% to 57% [1–3] depending on the histological criteria employed. Gross cystic disease fluid protein 15 (GCDFP 15) is a major protein component of human breast cyst fluid [4]. Expression of the protein in breast cancers correlates closely with apocrine differentiation [5, 6]. Using the presence of GCDFP 15 in the media from cultured tumour explants, we have measured apocrine differentiation in women with breast cancer in relation to long-term follow-up and factors of prognostic importance.

Correspondence to N.J. Bundred, Department of Surgery, Queen Elizabeth Hospital, Edgbaston, Birmingham B15, U.K.
N.J. Bundred, H.J. Stewart, A.P.M. Forrest and W.R. Miller are at the Department of Clinical Surgery, University of Edinburgh, Edinburgh; and D.A. Shaw is at the Department of Medical Statistics, University of Leicester, Leicester, U.K.

PATIENTS AND METHODS

Tumour was obtained at biopsy or mastectomy from 117 women with carcinoma of the breast diagnosed in the Department of Clinical Surgery, Edinburgh University. The tissue was cut into explants measuring 4 × 1 × 1 mm. Four weighed explants were placed on lens paper mounted on stainless steel grids in each of three petri dishes. 2 ml Waymouth's MB752/1 medium containing N-glutamine and 20 mmol/l HEPES was added to each dish which were incubated in 95% oxygen and 5% carbon dioxide at 37°C. Medium was removed and assayed for GCDFP 15 at 24 and 48 h. Culture fluids assayed at 48 h always had low levels of GCDFP 15 and most release had occurred by 24 h; we therefore took values at 24 h as representative of tumour content. Explants were also analysed histologically to confirm presence of tumour and to ascertain cellularity.

GCDFP 15 was measured by radioimmunoassay [5] with purified antigen and antibody donated by D. E. Haagensen. Tumour material adjacent to that taken for culture was also used for histological assessment and oestrogen receptor (ER) measurements [7]. Tumours were considered positive if they contained greater than 20 fmol/ER per mg tissue [7, 8].

Table 1. Patients' details (n = 117)

	Range	Mean
Age (yr)	34–85	58.5
T size (cm)	1.5–12.0	3.9
Node status (% positive)	56.6	—
Oestrogen receptor (fmol/mg)	0–937	46
GCDFP 15 (ng/ml)	0–2100	12.0

Age, menopausal status, parity, T stage, nodal status, tumour grade and cellularity were recorded for all patients, who have now had at least 5 years' follow-up. All patients were routinely staged before surgery by chest X-ray, bone scan and liver scan for the presence of metastatic disease. Lymph-node status was determined by histological assessment of nodes obtained by axillary clearance or node sampling. Women with early breast cancer were treated by mastectomy with either axillary node sampling or axillary clearance.

Primary endocrine therapy in 33 women who either developed metastatic disease (22) or who presented with inoperable advanced local disease (11) was assessed independently by two observers with UICC criteria [9]. Therapy consisted of tamoxifen 20 mg daily in 24 patients, stilboestrol 5 mg daily in 3, aminoglutethimide 250 mg four times a day in 4 and surgical oophorectomy in 1.

Prognostic factors were analysed by constructing Kaplan–Meier curves. Prognostic factors were studied in the following groups: menstrual status was classified as premenopausal (less than 12 months since last menstrual period) or postmenopausal (either more than 12 months since last menstrual period or hysterectomised-ovariectomised or hysterectomised and 50 or more years of age); lymph-node status (all nodes negative or any node positive); stage (T1–T4); and tumour grade and cellularity [8]. Subsequent multivariate analysis was by Cox's proportional hazards model. This method adjusts for the significance of other continuous variables and thus no groupings were used. In the analysis of hormonal response data, the Wilcoxon rank sum test was used.

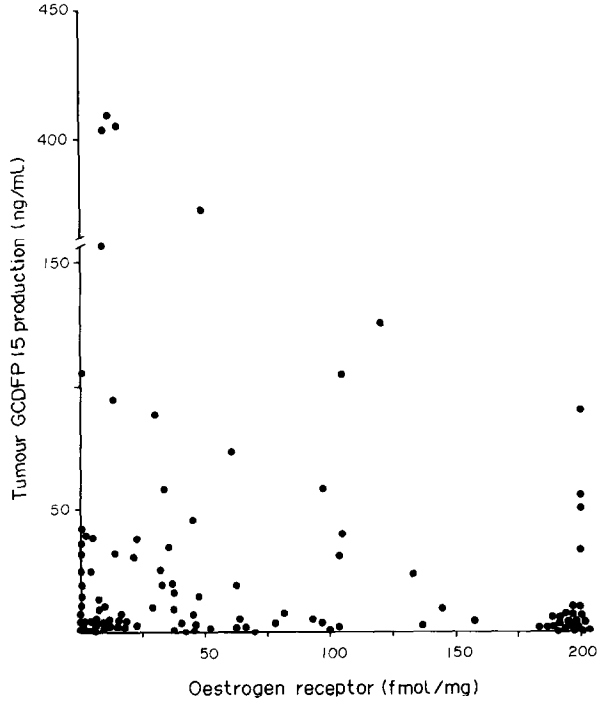

Fig. 1. Correlation between ER level and *in vitro* GCDFP 15 production. $r^2 = 0.026$, $P < 0.05$.

Table 2. Relation between prognostic factors and GCDFP 15 production and disease-free interval and survival in 117 women (univariate analysis)

	DFI	Survival
Age	$P > 0.4$	$P > 0.5$
Menopausal status	$P > 0.5$	$P > 0.5$
Tumour size	$P > 0.2$	$P > 0.1$
Tumour stage (T1,T2,T3)	$P > 0.5$	$P > 0.4$
Node status	$P > 0.4$	$P > 0.5$
GCDFP 15 production	$P > 0.02$	$P > 0.8$
ER level (continuous variable)	$P > 0.2$	$P < 0.02$
ER status		
Negative		
Positive	$P < 0.005$	$P < 0.005$

RESULTS

The clinical characteristics of the women are shown in Table 1. GCDFP 15 was detected in the media of replicate cultures in 90% of tumours. Levels ranged from 0 to 2100 ng/ml, median 12 ng/ml. There was no correlation between the level of protein released and age, menopausal status, tumour size, stage or nodal status. Amounts of GCDFP 15 produced by ER positive or negative tumours did not significantly differ, although within the ER positive group there was a positive correlation between levels of GCDFP 15 and ER level ($P < 0.05$, Fig. 1). Tumour GCDFP 15 secretion did not correlate with either tumour grade or cellularity [5].

97 women had early breast cancer and have been followed up for at least 5 years. There was no significant relation between GCDFP 15 tumour secretion and disease-free interval or survival in either univariate (Table 2) or multivariate analysis. Among the prognostic factors, only ER status proved to be a significant predictor of outcome ($P \leq 0.005$ for survival).

The remaining 20 women had advanced inoperable breast cancer ($n = 12$) or metastases (8) at presentation and were given a combination of hormone therapy (11) or chemotherapy (9). In view of their advanced disease these women were excluded from analysis of the prognostic factors in early breast cancer.

33 women were given hormonal therapy either because they initially presented with advanced disease (5 with bony metastases, 6 with inoperable local tumour) or because they developed recurrent disease (22). The sites of disease and frequency of

Table 3. Hormonal response

Dominant site	No.	Responders*
Visceral	5	3
Soft tissue	16	6
Bone	12	5
Total	33	14 (42%)

*Partial response and stable disease.

Table 4. Hormonal response in 33 patients

	No.	Mean ER level (range, fmol/mg)*	Mean GCDFP 15 production (range, ng/ml)†
Responders			
Partial response	6		
Stable disease	8	22.2 (0–623)	12.46 (0–35.5)
Non-responders (progression)	19	14.2 (0–365)	21.02 (0.5–400)

Significant difference between responders and non-responders: *$P < 0.005$ and †$P < 0.02$.

response are shown in Table 3. 6 patients had a partial response, 8 had stable disease and 19 had no response, showing continued tumour growth. The ER level of those women who responded to therapy (stable disease or partial response) was significantly higher ($P = 0.007$) than those who did not (Table 4). GCDFP 15 secretion was significantly higher in patients failing to respond to hormonal manipulation (Table 4). 9 women had strongly ER positive carcinomas (50 mol/mg) and GCDFP 15 production greater than 5 ng/ml, but only 4 responded to hormonal manipulation, whereas 6 or 7 women with strongly ER positive tumours and GCDFP 15 production less than 5 ng/ml responded.

DISCUSSION

The lack of general agreement about the definition of apocrine differentiation in breast carcinoma has meant that the potential prognostic importance of apocrine change has not been resolved [1–3]. However, investigations with antibody against GCDFP 15 have shown that cyst fluid protein staining may be used as an objective marker of apocrine metaplasia in both benign and malignant disease [6, 10, 11].

We have reported that GCDFP 15 protein production by tumour explants correlated strongly with the degree of histological apocrine differentiation in the tumour [5]. The present study therefore used this method of quantifying tumour apocrine differentiation to determine if a relation existed between tumour prognosis and expression of GCDFP 15. Two studies have suggested a correlation between good tumour grade and the cytosol content [12] and immunohistochemical expression of GCDFP 15 [11] in breast carcinomas. Whilst we could not confirm such an association we did detect a qualitative relation between GCDFP 15 production and ER status in which tumours without GCDFP 15 production were more likely to be ER negative (breast carcinomas expressing GCDFP 15 were equally likely to be positive or negative for ER). No quantitative relation was detected.

The breast tumours examined in this study tended to be larger than would be expected in a typical sample of women presenting with symptomatic breast cancer. This is probably because of the amount of tumour needed to do the study, and this explains the excess of women with positive lymph nodes. No association of GCDFP levels with either tumour size or node status was found. More surprisingly, node status was not itself of prognostic importance in this group of women, which may be because of the small numbers in the study. The presence or amount of GCDFP 15 production in culture by the tumours did not relate to other prognostic factors nor did it predict survival or disease-free interval.

However, in the small subset of women who underwent hormone therapy, responders had a significantly lower tumour GCDFP 15 production and a higher ER level than those who did not respond. In general, patients with hormone responsive breast cancer have histologically well-differentiated tumours with a slower growth rate, better prognosis and longer disease-free interval [13]. These characteristics alone do not completely discriminate for individual patients; ER level does not always correctly predict hormone response. There is therefore a need for a biochemical marker that separates ER positive hormone responsive tumours from non-responsive tumours. GCDFP 15 expression may prove useful in this regard.

Chalbos *et al.* [13] have shown that breast cancer cells in culture produce GCDFP 15 when stimulated by androgens but not oestrogens, and tumours producing GCDFP 15 in culture are usually androgen receptor positive [5]. Thus tumours expressing GCDFP 15 may be driven by androgens and such tumours may require hormone therapy with an antiandrogen rather than the oestrogenic or anti-oestrogenic agents that were used on the women in our study as first-line hormonal therapy. We are currently using an immunohistochemical stain for GCDFP 15 in a much larger group of women whose tumours have undergone hormonal therapy to investigate this hypothesis.

1. Azzopardi JG. *Problems in Breast Pathology*. Major Problems in Pathology Series, Vol II. Philadelphia, Saunders, 1979, 57–67, 341–344.
2. Bonser GM, Dossett JA, Jull JW. *Human and Experimental Breast Cancer*. London, Pitman Medical, 1961.
3. Haagensen CD. *Disease of the Breast*, 3rd ed. Philadelphia, Saunders, 1986, 82–101, 2–266, 474–500.
4. Haagensen DE Jr, Mazoujian GM, Dilley WG, Pedersen CE, Kister SJ, Wells SA Jr. Breast gross cystic disease fluid analysis. 1. Analysis and radioimmunoassay for a major component protein. *J Natl Cancer Inst* 1979, **62**, 239–247.
5. Miller WR, Shivas AA, Franchimont P, Haagensen DE Jr. Breast gross cystic disease protein 15 in human breast cancer in culture. *Eur J Cancer Clin Oncol* 1988, **24**, 223–228.
6. Mazoujian G, Pinkus GS, Davis S, Haagensen DE Jr. Immunohistochemistry of a gross cystic disease fluid protein (GCDFP 15) of the breast. *Am J Pathol* 1983, **110**, 105–112.
7. Hawkins RA, Black RB, Steele RJC, Dixon JM, Forrest AMP. Oestrogen receptor concentrations in primary breast cancer and axillary node metastases. *Breast Cancer Res Treat* **1**, 1981, 245–251.
8. Hawkins RA, White G, Bundred NJ, *et al.* Prognostic significance of oestrogen and progestogen receptor activities in breast cancer. *Br J Surg* 1987, **74**, 1009–1013.
9. Hayward JL, Carbone PP, Henson JC, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. *Eur J Cancer Clin Oncol* 1977, **13**, 89–94.
10. Bundred NJ, Miller WR, Walker RA. An immunohistochemical study of the tissue distribution of the breast cyst fluid protein, Zinc alpha-2 glyco-protein. *Histopathology* 1987, **11**, 603–610.
11. Le Dousall V, Zangerle PF, Collette J, *et al.* Immunohistochemistry of a component protein of the breast cystic disease fluid with mol wt 15 000. *Eur J Cancer Clin Oncol* 1985, **21**, 715–725.
12. Silva JS, Cox CE, Wells SA, *et al.* Biochemical correlates of morphologic differentiation in human breast cancer. *Surgery* 1982, **92**, 443–449.
13. Chalbos D, Haagensen D, Parish T, Rochefort H. Identification and androgen regulation of two proteins released by T47D human breast cancer cells. *Cancer Res* 1987, **47**, 2787–2792.