Eur J Cancer, Vol. 26, No. 9, pp. 950–953, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Haemostatic Abnormalities and Outcome in Patients with Operable Breast Cancer

Peter McCulloch, Gordon D.O. Lowe, Jessie T. Douglas, Gordon D. Murray and William D. George

The predictive value for cancer recurrence of five measurements of haemostatic activity was studied in 89 patients with operable breast cancer. Neither preoperative nor sequential measurements up to 9 months postoperatively of fibrinopeptide A, fibrin fragment Bβ15-42, fibrinogen and serum fibrin(ogen) degradation products nor the fibrin plate lysis assay correlated with early recurrent disease. Bβ15-42 values were higher preoperatively in patients with oestrogen receptor positive tumours ($P = 0.017$). Mean Bβ15-42 values rose postoperatively ($P = 0.003$), largely because of an increase in patients with oestrogen receptor negative tumours. *Eur J Cancer*, Vol. 26, No. 9, pp. 950–953, 1990.

INTRODUCTION

SUBCLINICAL DISTURBANCES of haemostatic function occur frequently in cancer patients [1–3]. Malignant cells may have novel procoagulant properties, and activate both platelets and the coagulation system [4, 5]. Animal models suggest that the coagulation system may play an important role in the mechanism of haematogenous metastasis [6, 7], whilst clinical studies suggest that levels of fibrinopeptide A (FPA) or fibrin(ogen) degradation products (FDPs) may reflect the presence and extent of malignant disease [8–10].

Previous studies of haemostatic disturbances in cancer patients have been inconsistent. There are few reports of well-characterised populations with one type of malignancy, and fewer reports of patients studied sequentially during treatment. Such studies are essential for the delineation of the natural history of haemostatic changes in malignant disease. Haemostatic measurement is developing rapidly, and modern specific measurements of, for instance, FPA (which is split from fibrinogen by thrombin) and fibrin fragment Bβ15-42 (split from fibrin by plasmin) may clarify previously obscure relations.

Previously, in patients with operable breast cancer, we showed [11] that much of the disturbance of haemostasis found preoperatively was not specific for cancer. We have now examined the value of preoperative and sequential haemostatic measurements as predictors of progression or recurrence in breast cancer.

PATIENTS AND METHODS

Patients

Patients who had initial surgical treatment of operable breast cancer were studied with previously described elegibility criteria [11]. Patients were staged by bone scan, liver ultrasound, transaminase assay and chest X-ray; patients in stages 0–3 were eligible in the absence of previous or current clinical evidence of haemostatic dysfunction. Tumour stage (UICC criteria), and oestrogen receptor (ER) status were recorded. A cytosolic ER level of 5 fmol/l or more was regarded as positive. Local therapy options were lumpectomy or simple mastectomy with axillary clearance, with or without radiotherapy. Lumpectomy patients were given cyclophosphamide, methotrexate and 5-fluorouracil if ER negative or tamoxifen if ER positive. Measurements were made on the morning of surgery and at 3 and 9 months after operation.

Patients were reviewed every 3 months in the first year and every 4 months in the second year after surgery. Staging was only repeated when clinically indicated.

Blood sampling

Atraumatic non-occlusive venepuncture and sample preparation were done at 0800 on the morning of surgery [11]. Postoperative samples were taken mid-morning. Samples were stored at −70°C.

Plasma stored in "Trasylol"/heparin was analysed for FPA and Bβ15-42 by radioimmunoassay (IMCO, Stockholm) [12, 13]. Citrated plasma was analysed for fibrinogen by a method modified from Claus [14] and for plasminogen activators in the euglobulin fraction by the fibrin plate lysis assay method (FPLA) modified from Kluft *et al.* [15] with a human fibrinogen substrate (Kabi, Stockholm). Serum was analysed for FDPs by the Wellcome kit [16].

Study design and statistics

The study was in two parts: (1) the value of preoperative haemostatic parameters for prognosis and (2) the relation between outcome and haemostasis in the 9 months after operation.

Pretreatment results were divided into "high" (above the median) and "low" (at or below the median). Kaplan–Meier survival curves for recurrence were compared with the log-rank test. A similar univariate analysis was done for the effects of stage, ER status, radiotherapy, chemotherapy and tamoxifen. A multivariate analysis was then done with Cox's proportional hazards model, including stage, ER status, FPA, Bβ15-42 and fibrinogen. FPA and Bβ15-42 values were compared in ER positive and negative tumours and in earlier (stage 0–1) and later (stage 2–3) disease by Mann–Whitney *U* tests.

The preoperative and 3 and 9 month postoperative values of each variable were compared by Mann–Whitney *U* test to detect any changes over time in the study population. Subgroups were then compared with repeated measures analysis of variance of log-transformed values, looking separately at linear and

Correspondence to P. McCulloch.
P. McCulloch, G.D. Murray and W.D. George are at the Department of Surgery, Western Infirmary, Dumbarton Road, Glasgow G12, and G.D.O. Lowe and J.T. Douglas are at the University Department of Medicine, Royal Infirmary, Glasgow, U.K.

quadratic effects in time. This analysis detects group differences in the rise or fall with time of the variable studied and the tendency for values either to fall and then rise again or *vice versa*. The subgroups compared were: patients with and without recurrence, patients with ER positive and negative tumours and patients receiving or not chemotherapy, radiotherapy and tamoxifen.

RESULTS

Patients

89 patients were studied. Mean age was 58.8 years (SD 13.6). Median age was 65 (range 30–87). 3 patients had stage 0 cancer, 49 stage 1, 26 stage 2 and 9 stage 3 (2 patients were not staged). 34 patients had ER positive and 48 patients had ER negative tumours; ERs were not recorded in 7 patients. There were 28 smokers, 58 non-smokers and 3 patients whose habits went unrecorded.

Preoperative measurements and prediction of recurrence

20 patients have recurrent disease during a median follow-up of 20 months (maximum 30). Tumour stage had the clearest predictive value for early recurrence; ER status also had some value (Fig. 1). Survival curves for high and low values of FPA and Bβ15-42 are shown in Fig. 2. Similar results were obtained for FPLA, fibrinogen and FDPs. None of these variables had any clear association with early recurrent disease (Table 1). Neither chemotherapy, radiotherapy nor tamoxifen treatment had significant effects on early recurrence in univariate analysis in which selection of patients for different treatments was not considered. Multivariate analysis, including stage, ER status, fibrinogen, FPA and Bβ15-42 failed to show an independent relation between any haemostatic variable and early recurrent disease.

There were no significant differences in haemostatic indices in early and later stage disease, but there was a significant association between higher Bβ15-42 values and positive ER status (median Bβ15-42 value for ER positive patients, 2.95 pmol/ml; for ER negative patients, 2.0; 2-tailed $P = 0.017$).

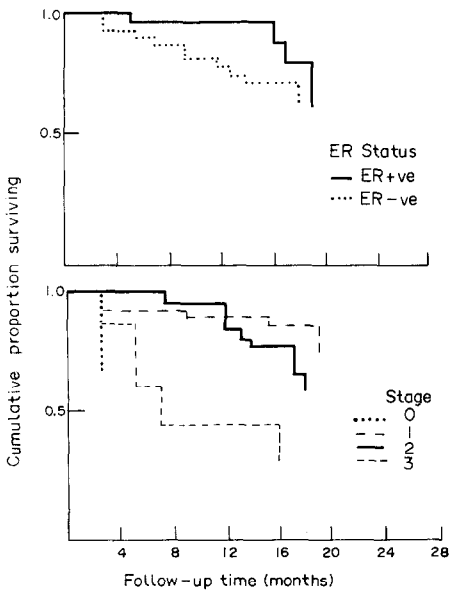

Fig. 1. Life-table analysis of recurrence in breast cancer patients according to stage and ER status. Cumulative proportion surviving = those surviving without recurrent disease. $P = 0.0061$ for null hypothesis that recurrence rates are same in all stages; $P = 0.029$ for hypothesis of no difference in recurrence rate between ER positive and ER negative patients.

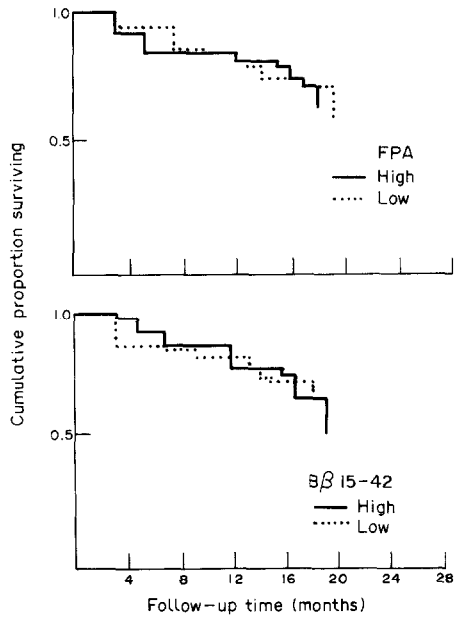

Fig. 2. Life-table analysis of recurrence comparing groups with high and low FPA and Bβ15-42. No significant difference detected between curves for either variables.

Serial measurements of haemostatic function

Between 22 and 26 patients were followed up for the different haemostatic variables. There were no significant changes in

Table 1. Median values of haemostatic variables in breast cancer patients

	Time (mo)		
	0	3	9
FPA (pmol/ml)			
Overall	4.10	4.30	3.60
Recurrent disease	4.30	3	3.60
No recurrence	4.10	4.30	3.75
Bβ15–42 (pmol/ml)			
Overall	2.30	2.80	3.05
Recurrent disease	2.35	2.90	3.40
No recurrence	2.20	2.80	3
FDPs (μg/ml)			
Overall	2.50	2.50	5
Recurrent disease	2.50	2.50	10
No recurrence	2.50	5	5
FPLA (mm)			
Overall	78	92	78
Recurrent disease	78	78	113
No recurrence	78	92	78
Fibrinogen (g/l)			
Overall	2.90	3.06	3.34
Recurrent disease	3.10	3.79	3.77
No recurrence	2.80	3	3.11

Normal range (coefficient of variation): FPA 0.7–2.6 (7%), Bβ15-42 0.6–2.5 (7%), FPLA 50–145 (5%), FDPs < 300 (8%) and fibrinogen 1.5–4.5 (5%).

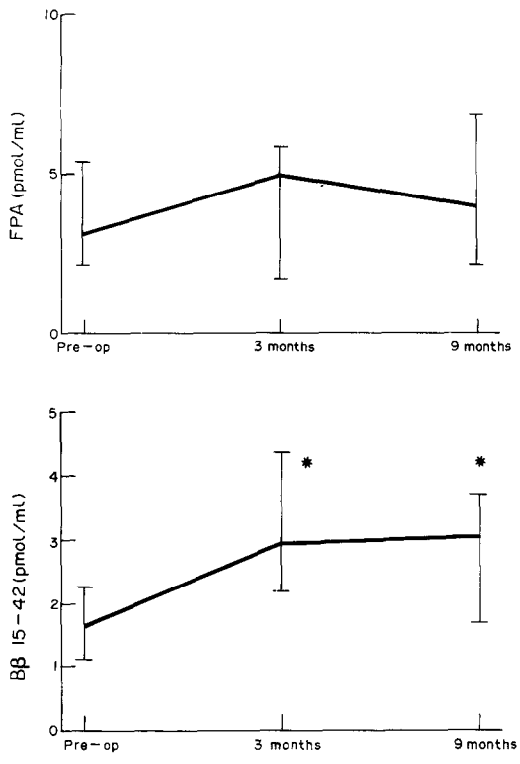

Fig. 3. Changes in FPA and Bβ15-42 after operation for breast cancer (median and interquartile range) *$P = 0.003$ for hypothesis that difference from preoperative value occurred by chance.

FPA, fibrinogen, FPLA or FDPs at 3 and 9 months. The median value of Bβ15-42 rose significantly to 2.9 pmol/ml at 3 months postoperatively compared with 1.65 pmol/ml preoperatively ($P < 0.003$), and remained at this level at 9 months (3.05 pmol/ml). The pattern of change with time for FPA and Bβ15-42 is shown in Fig. 3.

No difference was found between patients with and without recurrent disease in the pattern of any haemostatic variable. Bβ15-42 values after treatment differed significantly in ER positive and ER negative patients: ER positive patients had no average change in Bβ15-42 at 3 months postoperatively (mean value 3.52 pmol/ml preoperatively, 3.24 pmol/ml after 3 months) whilst ER negative patients had a large average rise (1.6 pmol/ml preoperatively and 3.64 pmol/ml postoperatively, $P < 0.05$). Radiotherapy did not affect haemostatic variables, but patients receiving chemotherapy had a significant rise in FPA at 3 months from 2.3 pmol/ml to 9.4 pmol/ml (mean) ($P < 0.05$). Tamoxifen therapy was associated with a persistent fall in fibrinogen levels after surgery.

DISCUSSION

Preoperative measurements of our chosen variables showed no relation with early recurrence of breast cancer. No previous study has formally analysed the predictive value of pretreatment haemostatic measurements, while the evidence on the value of single measurements at any stage is conflicting [3, 8, 9]. We may have failed to detect a real but weak association between one of the variables measured and early recurrence, but such associations are unlikely to be of clinical or scientific value. Longer follow-up, greater patient numbers and elimination of missing values would have improved the statistical power of the study.

Haemostatic function may be altered by clinical events during tumour progression and treament [1, 9, 17], but only one group has reported sequential haemostatic measurements (of FPA) from diagnosis onward in cancer patients [10]. We have attempted to define the natural history of fibrin formation and fibrinolysis in breast cancer patients before, during and after primary treatment. Analysis of FPA, Bβ15-42, FDPs, fibrinogen and FPLA over the first 9 months after diagnosis failed to detect any differences between disease-free patients and those with early recurrence.

FPA and FDPs remained elevated but constant up to 9 months after surgery. Bβ15-42, however, rose significantly after surgery and remained high compared with preoperative values, which suggests that the primary tumour may suppress the normal rise in fibrinolytic activity stimulated by fibrin-forming activity [18, 19]. Plasma fibrinogen and FPLA values were normal throughout the study. The contrast between the FPLA and Bβ15-42 results in the same patients suggests that plasmin activity *in vivo* (Bβ15-42) was not reflected by plasmin activity *ex vivo* (FPLA). The failure of FPA to fall after surgery suggests that the initial elevation was not entirely due to the procoagulant effect of tumour tissue. We have previously shown [11] that non-specific factors such as psychological stress must be partly responsible, which would also help to explain the poor correlation between sequential FPA values and outcome. Two studies have found an association between sequential FPA measurements and early recurrence, although Rickles *et al.* [9] studied chiefly lung cancer and Auger *et al.* [10] defined high FPA levels differently from us. Our patient numbers and sampling intervals may have been inadequate to detect this relation, but its practical value is unlikely to be great since many disease-free patients had persistently high FPA values.

We found an unexpected relation between ER status and Bβ15-42 values. High preoperative Bβ15-42 values correlated well with positive ER status, and only ER negative tumours showed an increase in Bβ15-42 after surgery. The reasons for this were unclear, though one explanation may be that oestrogen can induce the synthesis of plasminogen activators in breast cancer cells [20]. The overall elevation of Bβ15-42 values may mask a suppressive effect from bad prognosis primary tumours.

1. Slichter SJ, Harker LA. Hemostasis in malignancy. *Ann NY Acad Sci* 1974, **230**, 252–261.
2. Sun MCJ, McAfee WM, Hum GJ, *et al.* Haemostatic abnormalities in malignancy: a prospective study of 108 patients. *Am J Clin Pathol* 1979, **71**, 10–16.
3. Mannucci PM, Vaglini M, Maniezzo M, *et al.* Haemostatic alterations are unrelated to stage of tumour in untreated malignant melanoma and breast cancer. *Eur J Cancer Clin Oncol* 1985, **21**, 681–685.
4. Gordon SG, Franks JJ, Lewis B. Cancer procoagulant A: a factor X activating procoagulant from malignant tissue. *Thromb Res* 1975, **6**, 127–137.
5. Gordon SG, Cross BA. A Factor X activating cysteine protease from malignant tissue. *J Clin Invest* 1981, **67**, 1665–1667.
6. Brown JM. A study of the mechanism by which anticoagulation with warfarin inhibits blood-borne metastasis. *Cancer Res* 1973, **33**, 1217–1224.
7. McCulloch PG, George WD. Warfarin inhibition of metastasis; the role of anticoagulation. *Br J Surg* 1987, **74**, 879–83.
8. Peuscher FW, Cleton FJ, Armstrong L, Stoepman-Van Dalen EA, Van Mourik JA, Van Aken WG. Significance of plasma fibrinopeptide A (fpA) in patients with malignancy. *J Lab Clin Med* 1980, **96**, 5–14.
9. Rickles FR, Edwards RL, Barb C, Cronlund M. Abnormalities of coagulation in cancer. *Cancer* 1983, **51**, 301–307.

10. Auger MJ, Galloway MJ, Leinster SJ, McVerry BA, Mackie MJ. Elevated fibrinopeptide A levels in patients with clinically localised breast carcinoma. *Haemostasis* 1987, **17**, 336–339.
11. McCulloch PG, Douglas JD, Lowe GDO, Forbes CD, Murray GM, George WD. *In vivo* measurements of fibrin formation and fibrinolysis in operable breast cancer. Thromb Haemost 1989, **61**, 318–321.
12. Nossel HL, Younger L, Wilner GD, *et al.* Radioimmunoassay of human fibrinopeptide A. *Proc Natl Acad Sci USA* 1971, **68**, 2350–2353.
13. Kudryk B, Robinson D, Netre C, *et al.* Measurement in human blood of fibrinogen/fibrin fragments containing the B-beta 15-42 sequence. *Thromb Res* 1982, **25**, 277–291.
14. Clauss A. Gerrinungsphysiologische schnellmethode zur bestimmung des fibrinogens. *Acta Haematol* 1957, **17**, 237–246.
15. Kluft C, Brakman P, Veldhuyzen-Stolk EP. Screening of fibrinolytic activity in plasma euglobulin fractions on the fibrin plate. In Davidson JF, Samama MM, Desnoyers PC, eds. *Progress in Chemical Fibrinolysis and Thrombolysis*. New York, Raven, 1976, Vol. 2, 57–65.
16. Merskey C, Lalezari P, Johnson AJ. A rapid, simple sensitive method for measuring fibrinolytic split products in human serum. *Proc Soc Exp Biol Med* 1969, **131**, 871–875.
17. Davis RP, Theologides A, Kennedy BJ. Comparative study of blood coagulation changes in patients with cancer and with non-malignant diseases. *Ann Intern Med* 1969, **71**, 67–80.
18. Malone JM, Wangensteen SL, Moore WS, Keown K. The fibrinolytic system: a key to tumour metastasis. *Ann Surg* 1979, **190**, 342–349.
19. Rennie JAN, Ogston D. Fibrinolytic activity in malignant disease. *J Clin Pathol* 1975, **28**, 872–874.
20. Butler WB, Kirkland WL, Gargala TL, Goran N, Kelsey WH, Berlinski PJ. Steroid stimulation of plasminogen activator production in a human breast cancer line. *Cancer Res* 1983, **43**, 1673–1641.

Acknowledgements—This work was supported in part by funds from the Tenovus Foundation for Scotland and from the Bristol-Myers Corporation.

Eur J Cancer, Vol. 26, No. 9, pp. 953–956, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Postoperative Hyperprolactinaemia and Early Recurrence Rate in Breast Cancer

P. Lissoni, A.L. Sormani, G. Tancini, G. Cattaneo, C. Archili, D. Mandelli, S. Crispino, F. Paolorossi and S. Barni

Serum levels of prolactin before and after surgery were measured in 90 women with breast cancer until the 5th postoperative month. Sugery-induced hyperprolactinaemia occurred in 51 patients, without significant correlation to any other clinical variable. After a median follow-up of 39 months, irrespective of each other variable (i.e. nodal involvement, oestrogen receptor status, adjuvant therapies), patients with postoperative hyperprolactinaemia had a significantly lower recurrence rate than those in whom surgery was not followed by an abnormal increase in prolactin secretion (3/51 vs. 13/39, $P < 0.001$). These results suggest that, despite the stimulatory role of prolactin on mammary tumours, the lack of postoperative hyperprolactinaemia is an unfavourable prognostic factor because of its association with a higher relapse rate.
Eur J Cancer, Vol. 26, No. 9, pp. 953–956, 1990.

INTRODUCTION

THE CLINICAL course of breast cancer depends not only on the tumour's biological properties but also on host factors, mainly endocrine status and immune functions. In addition to the stimulatory role of oestrogens, prolactin stimulates experimental breast cancer growth [1–3]. However, the role of prolactin in human breast cancer has still to be established. Preliminary clinical data suggest that this hormone is also stimulatory in human mammary tumours [4–6], since increased prolactin secretion was associated with a less favourable prognosis in patients with advanced breast cancer [5, 6]. Moreover, breast surgery can induce increased prolactin secretion in both premenopausal and postmenopausal women with breast cancer [5, 7, 8]. However, the influence of surgery-induced hyperprolactinaemia on the growth of micrometastases already present at the time of the surgical removal of tumour and on the clinical course of breast cancer has never been investigated. We have studied whether such hyperprolactinaemia is a new prognostic variable, capable of influencing the clinical history of operable breast cancer.

PATIENTS AND METHODS

From December 1985 to July 1987, 90 consecutive women with histologically proven breast carcinoma, stage T1-2, NO-2, MO, entered the study (Table 1). Median age was 57 years (range 33–79). All were inpatients at the First or Second Surgery Division of San Gerardo Hospital, Monza. Patients with high blood levels of prolactin before operation were excluded. None of the patients received any drug affecting prolactin secretion for at least 4 days before blood sampling. Moreover, all patients received the same type of anaesthesia with fluorane. Surgery was followed by adjuvant chemotherapy with (cyclophosphamide/methotrexate/fluorouracil (CMF) in premenopausal patients with axillary node involvement and/or negative

Correspondence to P. Lissoni.
P. Lissoni, G. Tancini, G. Cattaneo, C. Archili, D. Mandelli, S. Crispino, F. Paolorossi and S. Barni are at the Division of Radiation Oncology and A.L. Sormani is at the First Division of Surgery, San Gerardo Hospital, Monza, Milan, Italy.