

Prognostic value of prostaglandin F₂α concentrations in breast carcinoma

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SUMMARY Prostaglandin F₂α (PGF₂α) concentrations were measured by radioimmunoassay in homogenised primary tumours from 57 patients with breast cancer. These patients were followed up from 60 to 78 months (median 63 months) after surgery and PGF₂α concentrations were related prospectively to metastatic spread and survival. The amounts of PGF₂α varied greatly in the different tumours (range 0-90 ng/mg protein), but no significant association was found between PGF₂α concentrations and disease free survival, time of relapse, site of recurrence, or overall survival. It therefore seems unlikely that measurement of PGF₂α in breast carcinoma is important in the prognosis of the disease.

Considerable attention has been given to the possible role of prostaglandins in the natural history of breast cancer: human mammary carcinomas produce higher amounts of "prostaglandin-like material" than normal breast tissue.¹⁻² In vitro and animal experiments suggest that prostaglandins might have a role in tumour initiation, the immune response, tumour metastasis and tumour associated hypercalcaemia.³⁻⁶

Views on the role of prostaglandins on tumour growth and metastasis and the prognostic value of tumour prostaglandin concentrations in breast cancer, however, are still controversial. In a previous investigation we showed that PGF₂α concentrations were significantly increased in breast carcinoma when compared to normal breast tissue and benign breast disease. High PGF₂α concentrations were also positively correlated with tumour differentiation, positive hormone receptor state, and low mitotic index. Tumours with a good prognosis (<2.0 cm, negative lymph nodes, and well to moderately differentiated) showed higher PGF₂α concentrations than tumours with a poor prognosis (>2.0 cm, positive lymph nodes, and undifferentiated).²

Our previously reported patients have now been followed for 60-78 months and their disease free survival and overall survival examined in relation to

the PGF₂α concentrations in the primary tumour. This prospective investigation was conducted to ascertain whether measurement of PGF₂α by radioimmunoassay in breast tumours provides useful information on the dissemination and prognosis of breast cancer.

Material and methods

Patient details, histological, and biochemical material were obtained from a previous study. The 57 patients were treated between October 1981 and March 1983 for primary carcinoma of the breast. Before surgery they had a chest x-ray, a liver and skeleton scintigram, and were staged according to the rules of UICC.⁷ None of them had evidence of metastatic disease at the time of surgery.

All patients had a modified radical mastectomy as primary treatment. The mean age was 56.6 years (range 31-80 years). Twenty three patients were premenopausal and 34 postmenopausal. According to the pathological TNM system⁷ patients were classified as follows: pT1aNO (n = 16); pT1aN1a (n = 1); pT1aN1b (n = 6); pT1aN2 (n = 3); pT1bNO (n = 1); pT2aNO (n = 11); pT2aN1a (n = 1); pT2aN1b (n = 10); pT2aN2 (n = 2); pT3aN1b (n = 5); and pT4N1b (n = 1).

Most patients (n = 46) had follow up examinations in our hospital. Every three months they had a

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physical examination and a blood test (blood count, liver function tests, and measurement of serum carcinoembryonic antigen) and every six months a chest x-ray and total bone and liver scintigraphy until 24 months after mastectomy. Thereafter these tests were performed once a year. When there was an abnormal bone scintigram, the patient had a bone x-ray survey.

Local relapse was defined as relapse in the mastectomy scar or in the ipsilateral axillary region and in all cases was confirmed histologically. Distant metastases were, whenever possible, confirmed by histological or cytological findings.

Data on patients who were followed up elsewhere were obtained from their general practitioners or gynaecologist. Whether they were alive or not, the date and site of recurrence, and the cause of death were recorded in March 1988. No patients were lost to follow up.

RADIOIMMUNOASSAY OF PGF2α AND HISTOLOGICAL PROCESSING

Tissue sampling and PGF2α radioimmunoassay were performed as described by Vergote *et al.*² In brief, at surgery tumour samples were divided into two representative parts and immediately immersed either in acetone cooled by solid carbon dioxide at -70°C for prostaglandin determination or in Bouin's fixative for pathological examination. The tissue samples for PGF2α investigation were stored at -30°C until radioimmunoassay was performed. The acetone was evaporated under nitrogen. TRIS buffer was added and the tissue was sonicated for 90 minutes. Ice was regularly added to the bath fluid of the sonification apparatus to keep the temperature below 10°C. The supernate was separated from the tissue after centrifugation at 10 000 × g. Samples were run in an adapted radioimmunoassay according to the method of Granstrom and Kindahl,⁸ using an antiserum that we produced.² A precipitate was formed with bovine globulin after adding polyethyleneglycol (PEG) 4000. Radioactivity was counted in a Packard 460 scintillation counter. The protein content of the breast cancer extracts was measured by the method of Bradford,⁹ and the PGF2α content expressed as ng PG/mg protein.

The histological slides were independently reviewed. Tumours were classified according to the WHO classification.¹⁰ There were 52 infiltrating ductal carcinomas and five lobular carcinomata.

As the distribution values for PGF2α were skewed, non-parametric tests (Wilcoxon's rank test, one way analysis of variance, and regression analysis) were used. Statistical differences are given by the p values indicated in the text, and a difference of p = < 0.05 was regarded as significant.

Table 1 *Tumour prostaglandin F2α concentrations according to presence and site of recurrence**

State	(n =)	PGF2α (ng/mg protein) (Mean SD)
Disease free†‡§	32	21.7 (27.1)
Recurrent disease (total)†	25	15.0 (19.0)
Local recurrence†	11	13.6 (24.4)
Distant metastases§	14	15.8 (12.3)

*Median follow up 63 months.
†p = 0.5; ‡p = 0.17; §p = 0.8 Wilcoxon.

Results

The range of values for PGF2α was 0 to 90 ng/mg protein (median 15 ng/mg protein). Median follow up was 63 months, varying from 60 to 78 months. During this period 25 (43%) patients had recurrent disease and 32 (57%) seemed to have no disease. The PGF2α tissue concentrations tended to be higher in the disease free patients, compared with the group of patients with relapse, but this difference was not significant (p = 0.5, Wilcoxon) (table 1). There was no correlation between the PGF2α tissue concentration and the time to relapse (r = 0.038, p = 0.8, regression analysis).

Fifteen patients died in the study period—13 because of breast cancer, one of intercurrent disease, and one of an unknown cause. As outlined in table 2, PGF2α concentrations of the primary tumour did not differ significantly between the patients dying from their breast carcinoma and the overall group of survivors, or the disease free survivors, respectively.

Discussion

Results of investigations on the possible prognostic importance of determining tumour prostaglandins are not consistent. The reason for this discrepancy is not completely clear, but differences in methodology and laboratory techniques, the studied prostaglandin, and population characteristics may all be important variables.

Initially Bennett *et al.*, using bioassay techniques to measure prostaglandins, showed that tumours from patients with breast cancer showing evidence of bone metastasis on skeletal scanning produced more PGE2

Table 2 *Tumour prostaglandin F2α concentrations according to survival*

State	(n =)	PGF2α (ng/mg protein) (Mean SD)
Alive†	42	17.8 (23.2)
Dead†	15	21.3 (24.5)

*Median follow up 63 months.
†p: 0.66 (Wilcoxon).

than did patients whose tumours showed no evidence of bone metastasis.¹ These findings, however, could not be confirmed in a second study with an extended follow up.¹¹

Rolland *et al* concluded that a high PGE2 production occurs very early in the development of a malignant tumour, and that an increased prostaglandin production seems to be associated with metastasis. The presence of tumour cell embolism and the presence of metastatic axillary lymph nodes seems to be strongly related to a high PGE2 production, particularly in T1 and T2 lesions. This association did not seem to hold for PGF2 α concentrations.¹²

Malachi *et al* found no correlation between PGE2 tumour concentrations and survival, histological type, and stage.¹³ These data were confirmed by Watson *et al*, who measured PGE2 and PGF2 α by gas liquid chromatography-mass spectrometry in extracts of primary tumours from 78 patients with early breast cancer, and showed that they had no prognostic value.¹⁴ Recently Bennett *et al* observed no association between the amounts of prostaglandin-like material (mainly PGE2) extracted from 141 breast carcinomas and the length of survival by means of univariate and multivariate analysis.¹⁵

Although we found in a previous study that high PGF2 concentration correlated with good prognostic variables,² adequate prospective long term follow up of these patients detected no significant association between PGF2 concentrations and disease free survival, site of recurrence, time of relapse and overall survival.

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