Prognostic value of prostaglandin F2 α concentrations in breast carcinoma

P A van DAM, I B VERGOTE, G M LAEKEMAN,† G H KEERSMAECKERS,*
F L UYTENBROECK, A G HERMAN†

From the Departments of Gynaecology and *Histopathology, St Camillus Hospital (Antwerp University), Antwerp, and the †Department of Pharmacology, Antwerp University, Wilrijk, Belgium

SUMMARY Prostaglandin F2 α (PGF2 α) 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 PGF2 α concentrations were related prospectively to metastatic spread and survival. The amounts of PGF2 α varied greatly in the different tumours (range 0-90 ng/mg protein), but no significant association was found between PGF2 α concentrations and disease free survival, time of relapse, site of recurrence, or overall survival. It therefore seems unlikely that measurement of PGF2 α 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 PGF2 α concentrations were significantly increased in breast carcinoma when compared to normal breast tissue and benign breast disease. High PGF2 α 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 PGF2 α 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 PGF2 α concentrations in the primary tumour. This prospective investigation was conducted to ascertain whether measurement of PGF2 α 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

Prognostic value of prostaglandin F2 α in breast carcinoma

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 PGF2 α AND HISTOLOGICAL PROCESSING

Tissue sampling and PGF2 α 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 PGF2 α 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 $10\,000 \times g$. 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 PGF2 α 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 PGF2 α 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 F2 α concentrations according to presence and site of recurrence*

State	(n =)	PGF2 α (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 PGF2 α 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 PGF2 α 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 PGF2 α 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, PGF2 α 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 F2 α concentrations according to survival

State	(n =)	PGF2 α (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.

We thank Drs G Albertijn, J Vanderheyden, E Schatteman, J Verkinderen, P Meulyzer, P Dalemans and H Spiessens for kindly providing the tissue specimens or follow up data; and Professors C Hansch and G De Roy for their aid with the histology.

References

- 1 Bennett A, McDonald AM, Simpson JS, Stamford IF. Breast cancer, prostaglandins and bone metastasis. *Lancet* 1975;i:1218-20.
- 2 Vergote IB, Laekeman GM, Keersmaeckers GH, *et al*. Prostaglandin F2 α in benign and malignant breast tumours. *Br J Cancer* 1985;51:827-36.
- 3 Karmali R. Review: prostaglandins and cancer. *Prostaglandin Med* 1980;5:11-28.
- 4 Honn KV, Bockman RS, Marnett LJ. Prostaglandins and cancer: A review of tumour initiation through tumour metastasis. *Prostaglandins* 1981;21:833-63.
- 5 Droller M. Prostaglandins and neoplasia. *J Urol* 1981;125:757-60.
- 6 Goodwin J. Prostaglandins and host defence in cancer. *Med Clin North Am* 1981;65:829-43.
- 7 UICC. (International Union Against Cancer.) *TMN classification of malignant tumours*. Geneva: World Health Organisation, 1983.
- 8 Granstrom E, Kindahl H. Radioimmunoassay of prostaglandins and thromboxanes. *Adv Prostaglandin Thromboxane Res* 1978; 5:119.
- 9 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt Biochem* 1976;42:248-52.
- 10 World Health Organisation. Histological typing of breast tumours. In: *International histological classification of tumours*. No. 2. Geneva: WHO, 1981.
- 11 Bennett A, Berstock D, Carroll M, Stamford I, Wilson A. Breast cancer, its recurrence, and patient survival in relation to tumour prostaglandins. In: *Advances in prostaglandin, thromboxane, and leukotriene research*. Vol. 12. New York: Raven Press, 1983: 299-302.
- 12 Rolland P, Martin P, Jacquemier J, Rolland A, Toga M. Prostaglandin in human breast cancer. Evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells. *JNCI* 1980;64:1061-70.
- 13 Malachi T, Chaimoff C, Feller N, *et al*. Prostaglandin E2 and cyclic AMP in tumour and plasma of breast cancer patients. *J Cancer Res Clin Oncol* 1981;102:71-9.
- 14 Watson DM, Kelly RW, Miller WR. Prostaglandins and prognosis in human breast cancer. *Br J Cancer* 1987;56:367-70.
- 15 Bennett A, Stamford IF, Berstock DA, Dische F, Singh L, A'Hern RP. Breast cancer, prostaglandins and patient survival. *Br J Cancer* 1989;59:268-75.

Requests for reprints to: Dr P A van Dam, Department of Obstetrics and Gynaecology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, England.