# CHAPTER - III

## PLASMA PROLACTIN IN BREAST CARCINOMA : ITS RELATIONSHIP TO CLINICAL PARAMETERS AND AS AN INDICATOR OF DISEASE STATUS

### INTRODUCTION

Prolactin has been established as an important factor in induction and propagation of mammary carcinoma in experimental animals (Wang and Bulbrook, 1977 ; Welsch and Nagasawa, 1977). Published reports, have produced data to show an aetiologic association between prolactin and breast carcinoma and have suggested that this hormone may have a role as a late stage promoter (Kwa et al, 1981 ; Bruning, 1987). It is well known, that the hypothalamic-pituitary-gonadal axis unifies estrogens, androgens and prolactin hormones that have been shown to have a regulatory control on mammary epithelial function ( Mittra et al, 1974). Thus, any event that disturbs this homeostasis, could potentially alter breast epithelial function and growth characteristics, probably leading to neoplastic proliferaton. Inspite of impressive data from experimental studies, the importance of prolactin in human breast carcinoma is less clear. There is now some published evidence which incriminates high circulating prolactin levels to poor prognosis, both in early and advanced breast carcinoma (Jeffcoate, 1978 ;

Dowsett et al, 1983 ; Holtkamp et al, 1984 ; Wang et al, 1986 ; Dowsett et al, 1987 ; Wang et al, 1987 ).. These studies point towards a significant role of prolactin in the pathogenesis of human breast carcinoma.

In our previous publications, we have reported : (1) hyperprolactinaemia in our set up, (2) a linear correlation with histologic grade and (3) an inverse correlation with estrogen-and progesterone-receptors and survival (Bhatavdekar et al, 199Øb). Prolactin was also estimated sequentially and an excellent correlation was observed between prolactin levels and disease course. In case of non-responders, a rise in prolactin preceded clinical symptoms (Bhatavadekar et al, 1989 ; 199Øa).

The present study investigated correlations of circulating prolactin with stage, nodal status, histologic grade and disease outcome in pre-menopausal patients. An attempt was made to study relation between the changes in plasma prolactin and disease progression/regression; existence of lead time from a rise in prolactin level to the appearence of recurrence of disease as well as effect of adjuvant therapy on prolactin levels. Moreover, we also have tried to correlate plasma prolactin to circulating levels of estradiol, progesterone and their receptors (ER and PR) in pre-menopausal breast carcinoma.

STUDY DESIGN

Blood samples from a total of 111 pre-menopausal breast carcinoma patients and controls (N=33) were collected between 9.Ø to 11.Ø A.M. pretherapeutically in ethylenediaminetetraacetic acid (EDTA), disodium salt (1-2 mg/ml) coated tubes. Serial blood samples were obtained from 52/111 patients pretherapeutically to obtain base line level of individual patient and at intervals of 3-6 months for stage II and at monthly/bimonthly intervals for advanced patients. The plasma was separated within 1-2 hours, aliquoted and stored at -7Ø C until assayed usually within two weeks. Studies were performed retrospectively using frozen plasma. The clinical data and follow-up schedule of these patients was as described in the previous Chapter.

PROLACTIN (PRL) ASSAY :

Plasma prolactin (PRL) was assayed using double antibody RIA kits procured from Diagnostic Products Corporation, Los Angeles, USA which used WHO/RP 75/5Ø4 as a standard. The assays were performed in duplicate with an intraassay and an interassay coefficient of variation (CV) of 3% to 5% and 5% to 8% respectively along with internal quality controls. The sensitivity of the kit was 3.7 ng/ml. The normal range for pre-menopausal patients was from undetectable to 2Ø.Ø ng/ml plasma.

PATHOLOGIC STAGING :

UICC p TNM staging scheme (UICC, 1980) was used. The histologic grading was done according to Bloom and Richardson (1957) and was expressed on a scale of I-well differentiated, II-moderately differentiated and III-poorly differentiated tumors.

THERAPY :

The priamry treatment offered to the patients was surgery followed by radiotherapy and/or CMF (N=22) ; bilateral oophorectomy and/or Tamoxifen (TMX) (N=8) and chemoendocrine therapy (CMF) followed by bilateral oophorectomy and/or TMX (N=11). The treatment schedules were implemented by the Medical Oncology units of the Institute as described in Chepter II.

ASSESSMENT OF DISEASE ACTIVITY :

The assessment of disease activity was done following standard criteria (Hayward et al, 1977). Blood samples were collected from the patients before assessment of disease activity. The assessment of response was carried out after a minimum of two chemotherapy cycles or a minimum of two months of hormone therapy. Patients with > 30 ng/ml PRL were considered hyperprolactinaemic.

The breast cancer patients were further classified based on their disease status into :(i) patients who developed

recurrent disease and (ii) patients who responded to various therapeutic modalities and remained relapse free at the end of two years. Patients' characteristics were described in Chapter II.

STATISTICS :

Significance was calculated using an exact contingency table test for order data and the Fisher's two sided exact test (Mehta and Patel, 1983). P values less then Ø.Ø5 were considered as significant.

CORRELATION OF PROLACTIN :

Spearman's non-parametric rank correlation coefficient was determined between (i) PRL and estradiol (ii) PRL and progesterone at diagnosis, in patients who developed recurrence as well as in responders. Moreover, PRL levels were also correlated with the ER and PR.

ASSESSMENT OF PROLACTIN IN BREAST CARCINOMA DIAGNOSIS AND MONITORING :

Prolactin was assessed in breast carcinoma monitoring with the computation of sensitivity, specificity, predictive value and diagnostic accuracy (Tondini et al, 1988 ; Caponigro et al, 199Ø) with the following definitions :

True +ve : Patients in whom prolactin increased with progression.

False -ve : Patients in whom prolactin decreased with progression.

True -ve : Patients in whom prolactin decreased with response.

False +ve : Patients in whom prolactin increased with response.

$$\text{Sensitivity (\%)} = \frac{\text{True +ve ( \# of patients )}}{\text{True +ve + False -ve ( \# of patients)}}$$

$$\text{Specificity (\%)} = \frac{\text{True -ve ( \# of patients )}}{\text{True -ve + False +ve ( \# of patients)}}$$

$$\text{Predictive value (\%) of positive test} = \frac{\text{True +ve ( \# of patients )}}{\text{True +ve + False +ve ( \# of patients)}}$$

$$\text{Predictive value (\%) of negative test} = \frac{\text{True -ve ( \# of patients )}}{\text{True -ve + False -ve ( \# of patients)}}$$

$$\text{Diagnostic efficiency (\%)} = \frac{\text{True +ve + True -ve ( \# of patients )}}{\text{Total \# of patients.}}$$

RESULTS

Plasma prolactin was significantly elevated in pre-menopausal breast carcinoma patients as compared to controls ($P < Ø.Ø1$; Table - 1). 58/111 (52.2%) patients

exhibited prolactin levels above upper limit of normal ( > 20 ng/ml) and hyperprolactinaemia ( > 30 ng/ml) was evidenced in 46/111 (41.4%) patients at diagnosis.

8/10 (80.0%) nulliparous and 48/98 (48.9%) parous breast cancer patients had prolactin above normal limits. These differences were statistically non-significant owing to small number (Table - 2).

RELATIONSHIP OF PLASMA PRL TO DISEASE STAGE :

Prolactin gradually increased as stage advanced. Advanced breast cancer patients (Stage III + IV) exhibited higher prolactin levels in comparison to stage II patients. The prolactin elevations, however, were statistically non-significant due to wider range of sample values. 12/27 (44.4%), 29/57 (50.8%), 9/16 (56.2%) and 8/11 (72.7%) patients evidenced prolactin above normal limits amongst stages II, III, IV and patients presenting with relapse respectively. 38/73 (52.0%) advanced breast cancer patients as opposed to 12/27 (44.4%) stage II breast cancer patients evidenced prolactin above normal limit. These differences were statistically insignificant (Table - 3; Fig. 1).

DISTRIBUTION OF PROLACTIN IN STAGE IV PATIENTS:

Prolactin distribution in stage IV patients according to metastatic site revealed that in patients with bone involvement had lowest circulating PRL levels whereas liver

involvement resulted in highest PRL levels (Table - 4).

Moreover, Ø/3 (Ø.Ø%), 2/5 (4Ø.Ø%), 2/3 (66.6%) and 5/5 (1ØØ.Ø%) patients exhibited prolactin above normal with bone, liver, lungs and more than one metastatic site involvement respectively. Statistical significance of these findings cannot be assessed because of the small number of patients involved.

RELATIONSHIP OF PROLACTIN TO CIRCULATING STEROIDS ACCORDING TO STAGE:

At diagnosis, a statistically non-significant trend of decrease in estradiol ($E_2$) and progesterone (Pg) and an increase in PRL was observed as stage advanced (Table - 5 A). Dotted lines indicate lower and upper normal limits (Figs. 2-3).

Moreover, amongst the patients who developed recurrent disease, an inverse correlation of PRL and $E_2$ ($r = -Ø.13$) was observed before clinical presentation of recurrent disease (Table - 5 B). Conversely amongst responders, an inverse correlation of PRL and $E_2$ was observed at diagnosis ($r = -Ø.19$) while a linear correlation of the two ($r = +Ø.22$) was observed at the end of 2 years. These correlations however, were statistically non-significant. Furthermore, PRL and Pg were inversely correlated before progression ($r = -Ø.36$; $P < Ø.Ø5$) and at progression

(r = -Ø.32) in patients who developed recurrent disease (Table - 5 B).

RELATIONSHIP OF PROLACTIN TO NODAL STATUS:

Significantly higher prolactin levels were demonstrated amongst node positive patients in comparison to node negative patients (Table - 6; P < Ø.Ø2). 46/89 (51.6%) node positive and 4/11 (36.3%) node negative patients exhibited prolactin levels above normal limit.

RELATION OF PROLACTIN TO HISTOLOGIC GRADE:

The mean values of prolactin were elevated with advancement of histologic grade (Table - 7). The difference in prolactin between histologic grade II + III tumors was statistically significant when compared with histologic grade I tumors. Patients with poorly differentiated tumors had higher PRL levels than those with well differentiated tumors (Table - 7). 3/8 (37.5%), 23/37 (62.1%) and 15/23 (65.2%) patients manifested prolactin above normal amongst well, moderate and poorly differentiated tumors respectively.

HYPERPROLACTINAEMIA IN RELATION TO DISEASE OUTCOME:

2Ø/32 (62.5%) patients who developed recurrent disease had hyperprolactinaemia as opposed to 12/32 (37.5%) patients with responsive disease (Table - 8).

PRETHERAPEUTIC PROLACTIN IN RELATION TO SITE AT RELAPSE:

Pretherapeutic prolactin levels were highest in the patients who developed visceral metastasis (Table - 9). On the other

hand, patients who developed local recurrence and/or axillary nodal metastasis had lower pretherapeutic prolactin levels than patients who developed bone metastasis. None of the above differences were statistically significant.

PROLACTIN IN RELATION TO DISEASE STATUS:

Preoperative prolactin levels were significantly elevated in patients who later developed metastasis. On sequential follow-up the prolactin levels were significantly reduced at response (Table - 1Ø; Fig. 4) whereas with appearance of metastatic disease, the prolactin levels increased before clinical presentation of the recurrent disease. We observed that the rise in prolactin preceded disease progression by 3-4 months. Moreover, prolactin levels also remained elevated throughout the course of disease in patients who did not respond to adjuvant therapy (Figs. 5-11).

PROLACTIN IN BREAST CARCINOMA MONITORING:

The sensitivity and specificity of prolactin estimation in pre-menopausal breast carcinoma monitoring was 93.54% and 95.23% respectively. Moreover, the predictive value of a positive test (at recurrence) was 96.66% and the predictive value of a negative test (at response) was 9Ø.9Ø% (Table - 11). Additionally, the diagnostic efficiency of prolactin was 94.23% in pre-menopausal breast carcinoma.

CHANGES IN PROLACTIN LEVELS WITH TREATMENT:

A decline of prolactin was observed after treatment

(Table - 12). The lowering of prolactin was statistically significant only with endocrine manipulations ( $P < Ø.Ø2$).

CORRELATION OF PROLACTIN AND STEROID RECEPTORS:

Statistically significant correlation ( $P < Ø.Ø2$) was observed amongst hyperprolactinaemic $ER^-$ patients as compared to non-hyperprolactinaemic $ER^-$ patients. Such a correlation was not observed with $PR^-$ patients. Significant correlation of prolactin expression was observed amongst hyperprolactinaemic $ER^+$ ($P < Ø.Ø2$) and $PR^+$ ($P < Ø.ØØ1$) patients in comparison to non-hyperprolactinaemic $ER^+$ and $PR^+$ patients (Table - 13; Figs. 12-13).

DISCUSSION

In previously published reports (Bhatavdekar et al, 1987; 1989; 199Ø a,b), we have correlated prolactin in advanced breast carcinoma (pre- and post-menopausal) with nodal status, histologic grade, estrogen- and progesterone-receptors, survival and disease status. In the present study, we addressed this question only to pre-menopausal patients and correlated circulating prolactin levels to clinically important prognosticators. We have observed elevated prolactin levels in pre-menopausal breast cancer patients with concomitant low levels of estradiol and progesterone (Chapter II). Our results correlated with those

of Sheth et al (1975) and Cole et al (1977). Dowsett et al (1983) and Holtkamp et al (1984) have defined hyperprolactinaemia (1000 mIU/L = 30.8 ng/ml) in advanced breast cancer. In our study, the incidence of hyperprolactinaemia in advanced breast cancer was 41.4% as compared to 8% reported by Holtkamp et al (1984).

Higher prolactin levels were found in patients with axillary nodal metastasis than those with no such involvement and poorly differentiated tumors than well differentiated tumors. Wang et al (1986) and Bani et al (1986) also reported similar findings in advanced breast cancer.

Furthermore, high PRL levels were more frequent in non-responders. Holtkamp et al (1984) and Dowsett et al (1987) also observed that when the preoperative level of prolactin was very high, there was a lesser likelihood of a response to endocrine treatment or to chemotherapy. Additionally, hyperprolactinaemic patients experiencing remission after therapy exhibited a return of their prolactin level to normal (L'Hermite and L'Hermite-Baleriaux, 1988)

Although, findings similar to the present study have been published before, to our knowledge this study demonstrates for the first time the relative significance of prolactin as

an index of tumor aggressiveness and that its levels significantly increased with progression. We have obtained best correlations with the various clinicopathologic variables in these patients. The high prolactin levels were significantly reduced to almost normal levels amongst the patients who remained in remission.

All these data indicate that prolactin may have a role in the pathogenesis of advanced breast cancer. Evidence from in vitro experiments supports such a hypothesis. Prolactin induced augmentation of growth and DNA synthesis in a proportion of breast cancer cases mediated by prolactin receptors has been shown by Peyrat et al (1984) and Waseda et al (1985). Moreover, an absence of correlation between prolactin receptors and steroid receptors in pre-menopausal breast cancer patients was reported by Bonneterre et al (1986).

In addition to the above, it is known that immunoreactive 'little prolactin' forms approximately 85% of circulating prolactin (Garnier et al, 1978) and can be cleaved by tissue proteolytic enzymes resulting into a 16 K fragment (Clapp, 1987) which is thought to contain mitogenic potential contributing to pathogenesis of breast cancers. This suggests that elevated prolactin has to do with metabolic processes of the metastatic tumor. It might be possible that

prolactogenic hormones like estrogens stimulate prolactin-dependent tumor cells via prolactin release (Wander et al, 1983). Thus, prolactin probably modulates the effect of other hormones on tumor growth in advanced breast carcinoma (Bhatavdekar et al, 1990a).

An early rise in prolactin in advanced breast carcinoma is an important finding and may offer a sensitive means to predict the presence of occult disease which is often difficult to evaluate. This view of prolactin being an indicator of progressive disease is supported by the fact that in case of non-responders a rise in prolactin preceded clinical symptoms. However, the mechanisms which lead to high prolactin in breast cancer are unknown (Holtkamp et al, 1984). It is a fact that high prolactin is associated with progression of the disease and that the level increases with tumor progression. The prolactin estimations have demonstrated a sensitivity 93.54%, specificity 95.23%, predictive values for positive and negative tests of 96.66% and 90.90% respectively and diagnostic efficiency 94.23% in breast carcinoma monitoring. Thus, serial prolactin estimation may be a more sensitive indicator for assessing a response to treatment. Serial estimations of rising prolactin levels are useful in early diagnosis of recurrence in progressive disease.

We also have tried to correlate pretherapeutic levels of prolactin with serum estradiol and progesterone. The levels of estradiol and progesterone were inversely proportional to prolactin levels as stage advanced.

Steroid receptors have long been acknowledged as important determinants of breast cancer biology. We therefore, correlated prolactin levels with steroid receptors. No correlations were observed between prolactin levels and the ER or PR status. Further, the disagreement between these findings and those of Marugo et al (1988) may be due to the fact that majority of our cases presented with advanced breast carcinoma, whereas a substantial proportion of their patients had localized disease.

In breast cancer patients undergoing adjuvant chemotherapy, CMF does not effect pituitary function ( Dnistrian et al, 1985). Hyperprolactinaemia was documented in 41.4% patients before initiation of therapy but levels decreased significantly after treatment. This decrease in plasma prolactin was the most consistent observation concerning prolactin in this investigation.

Preliminary evidence from published studies suggest that Tamoxifen has a definite antitumor effect in pre-menopausal patients with metastatic breast cancer with a response rate

similar to that obtained with bilateral oophorectomy (Manni, 1987). We have observed that long term antiestrogen treatment did not affect the circulating levels of prolactin (Tables - 13 A and B).

Hypophysectomy has been used as a form of therapy in breast cancers not responding to other standard treatment modalities (surgical and medical steroid hormone manipulation or cytotoxic chemotherapy). In view of the evidence produced by Ward (1977) and the current study, there appears some justification in using antiprolactin measures (surgical, medical) in a restricted subgroup of pre-menopausal breast cancer patients who are hyperprolactinaemic either at presentation or subsequently.

ABSTRACT

It is well known that the hypothalamic-pituitary-gondadal axis unifies estrogens, androgens and prolactin, the hormones which are shown to have a regulatory control on the development, growth and function of mammary gland. There are considerable data in the literature to show an aetiologic association between prolactin and breast cancer. This chapter deals with prolactin estimations in breast carcinoma. The incidence of prolactin was subgrouped taking parity, stage, nodal status and tumor differentiation into

consideration. 8/10 (80.0%) of nulliparous breast cancer patients had prolactin levels above upper limit of normal as compared to 48/98 (48.9%) patients who had one or more children. The prolactin levels of breast cancer patients at diagnosis were significantly elevated ($P < 0.001$) than controls. The mean values of pretherapeutic prolactin were higher in (i) stage IV patients in comparison to stage II patients and (ii) in non-responders as compared to responders.

Section B of the chapter discusses the utility of prolactin in monitoring the course of breast cancers. The patients were grouped into (i) who responded to the treatment and remained relapse free (N=21) and (ii) who had progressive disease (N=31). It was observed that the prolactin levels in non-responders before clinical progression were significantly reduced ($P < 0.05$) in comparison to pretherapeutic levels. The prolactin levels were significantly elevated ( $P < 0.001$) with clinical progression. Similarly amidst responders, the prolactin at last follow-up was significantly reduced ( $P < 0.01$) in comparison to pretherapeutic levels. Both the mean $\pm$ standard error values of prolactin (ng/ml) as well as the percentage variation in hormone with change in disease status were taken into account. Moreover, the sensitivity,

specificity and predictive value of prolactin in breast cancer monitoring is discussed. Also presented in the section are some graphic representations of changes in prolactin levels during the course of breast carcinomas.

Section C discusses hyperprolactinaemia in relation to overall and relapse free survival. It was observed that 2Ø/32 (62.5%) patients evidenced progression who had prolactin levels > 3Ø ng/ml in contrast to only 12/32 (37.5%) patients who remained in remission.

It would be interesting to note the correlation between circulating prolactin and steroid receptors. An attempt is made in section D to correlate prolactin with steroid receptors - the known prognosticators of the disease.

Section E describes the effects of therapy (chemo- and/or hormone therapy) on prolactin levels.

REFERENCES :

Bani IA, Williams CM, Boulter PS, Dickerson JWT.
Plasma lipids and prolactin in patients with breast cancer.
Brit J Cancer 1986 ; 54 : 439-446.

Bhatavdekar JM, Giri DD, Shah NG, Trivedi SN, Patel DD, Karelia NH, Balar DB, Bhaduri A, Vora HH, Ghosh N, Shukla MK.
Prolactin in advanced breast carcinoma : An Indian Experience.
Breast Disease 1990 (b) ; (In press).

Bhatavdekar JM, Shah NG, Trivedi SN, Karelia NH.
Peptide and steroid hormone levels in pre- and post-menopausal breast carcinoma.
Neoplasma 1987 ; 34 : 95-99.

Bhatavdekar JM, Shah NG, Balar DB, Patel DD, Bhaduri A, Trivedi SN, Karelia NH, Ghosh N, Shukla MK, Giri DD.
Plasma prolactin as an indicator of disease progression in advanced breast carcinoma.
Cancer 1990 (a) 65 ; 2028-2032.

Bhatavdekar JM, Trivedi SN, Shah NG, Patel DD, Karelia NH, Shukla MK,Ghosh N, Vora HH.
Comparison of prolactin, CA 15-3 and TPA in breast carcinoma.
Int J Biol Markers 1989 ; 4 : 15Ø-156.

Bloom HJG, Richardson WW.
Histological grading and prognosis in breast cancer.
Brit J Cancer 1957 ; 11 : 359-377.

Bonneterre J, Peyrat JP, Beuscart R, Demaille A.
Correlation between prolactin receptors (PRL-R) estradiol (ER) and progesterone receptors (PgR) in human breast cancer.
Eur J Cancer Clin Oncol 1986 ; 22 : 1331-1336.

Bruning PF.
Prolactin and breast cancer. In: Klijn JGM, Paridaens R, Foekens JA (eds) : Hormonal manipulation of cancer: peptides, growth factors, and new (anti) steroidal agents.
Raven Press, New York 1987 ; 167-173.

Clapp C.

Analysis of proteolytic cleavage of prolactin by the mammary gland and liver of the rat: Characterization of cleaved and 16K forms.
Endocrinology 1987 ; 121 : 2055-2064.

Cole EN, England PG, Sellwood RA, Griffiths K.
Serum prolctin concentrations throughout the menstrual cycle of normal women and patients with recent breast cancer.
Eur J Cancer 1977 ; 13 : 677-684.

Caponigro F, Iaffaioli RV, Pagliarulo C, De Placido S, Frasci G, Ungaro B, Matano E, Bianco AR.
CA 15-3 in human breast cancer. Comparison with tissue polypeptide antigen (TPA) and carcinoembryonic antigen (CEA).
Int J Biol Markers 1990 ; 5 : 73-76.

Dnistrian AM, Greenberg EJ, Dillon HJ, Hakes TB, Fracchia AA, Schwartz MK.
Chemohormonal therapy and endocrine function in breast cancer patients.
Cancer 1985 ; 56 : 63-70.

Dowsett M, McGarrick GE, Harris AL, Coombes RC, Smith IE, Jeffcoate SL.
Prognostic significance of serum prolactin levels in advanced breast cancer.
Brit J Cancer 1983 ; 47 : 763-769.

Dowsett M, McGarrick GE, Harris AL, Coombes RC, Smith IE, Jeffcoate SL.
The prognostic significance of hyperprolactinaemia in breast cancer. In : Klijn JGM, Paridaens R, Foekens JA (eds) Hormonal manipulation of cancer: peptides, growth factors, and new (anti) steroidal agents.
Raven Press, New York 1987 ; 175-181.

EORTC Breast Cancer Group.
Clinical trial of 2-Br-α-erogocryptine (CB 54) in advanced breast cancer.
Eur J Cancer 1972 ; 8 : 155-156.

Garnier PE, Aubert ML, Kaplan SL, Grumbach MM.
Heterogeneity of pituitary and plasma prolactin in man: Decreased affinity of 'big' prolactin in a radioreceptor assay and evidence for its secretion .
J Clin Endo Metab 1978 ; 47 : 1273-1281.

Hayward JL, Carbone PP, Heuson J-C, Kumaoka S, Segaloff A, Rubens RD.
Assessment of response to therapy in advanced breast cancer.
Cancer 1977 ; 39 : 1289-1294.

Holtkamp W, Nagel CA, Wander H-E, Rauschecker HF, Heyden D.
Hyperprolactinaemia is an indicator of progressive disease and poor prognosis in advanced breast cancer.
Int J Cancer 1984 ; 34: 323-328.

Jeffcoate SL.
Diagnosis of hyperprolactinaemia.
Lancet 1978 ; ii : 1245-1247.

Kwa HG, Cleton F, Wang DY, Bulbrook RD, Bulstrode JC, Hayward JL, Millis RR, Cuzick J.
A prospective study of plasma prolactin levels and subsequent risk of breast cancer.
Int J Cancer 1981 ; 28 : 673-676.

L´ Hermite M, L´ Hermite-Baleriaux M.
Prolactin and breast Cancer.
Eur J Cancer Clin Oncol 1988 ; 24 : 955-958.

Manni A.
Tamoxifen therapy of metastatic breast cancer.
J Lab Clin Med 1987 ; 109 : 290-299.

Marugo M, Fazzuoli L, Bernasconi D, Berta S, Giordano G.
Relationship between PRL levels and steriod receptors in breast cancer.
Anticancer Res 1988 ; 8 : 819-824.

Mehta CR, Patel NR.
A network algorithm for Fisher's exact test in RxC contingency tables.
J Am Stat Assoc 1983 ; 78 : 427-434.

Mittra I, Hayward JL, MacNeilly AS.
Hypothalamic-pitutary-prolactin axis in breast cancer.
Lancet 1974 ; ii : 889-891.

Peyrat JP, Djiane J, Bonneterre J, Vandewalle B, Vennin Ph, Delobelle A, Depadt G, Lefebvre J.
Stimulation of DNA synthesis by prolactin in human breast tumor explants: Relation to prolactin receptors.
Anticancer Res 1984 ; 4 : 257-262.

Peyrat JP, Vennin PH, Bonneterre J, Hecquet B, Vandewalle B, Kelly PA, Djiane J.
Effect of bromocriptine treatment on prolactin and steroid receptor levels in human breast cancer.
Eur J Cancer Clin Oncol 1984 ; 2Ø : 1363-1367.

Sheth NA, Ranadive KJ, Suraiya JM, Sheth AR.
Circulating levels of prolactin in human breast cancer.
Brit J Cancer 1975 ; 32 : 16Ø-167.

Tondini C, Hayes DF, Gelman R, Henderson IC, Kufe DW.
Comparison of CA 15-3 and carcinoembryonic antigen in monitoring the clinical course of patients with metastatic breast cancer.
Cancer Res 1988 ; 48 : 41Ø7-4112.

UICC.
TNM classification of malignant tumors.
UICC technical report series 198Ø ; 51.

Wander HE, Blossy ch, Kobberling J, Nagel GA.
Hochdosiertes Medroxyprogesteronacetat beim metastasierenden mammakarzinom: Beziehung Zwischen krankheitsverlauf und hormonprofilen.
Klin Wschr 1983 ; 61 : 553-56Ø.

Wang DY, Bulbrook RD.
Hormones and neoplasia. In : Raven RW (ed), Principles of surgical oncology.
Plenum Medical Book Co, New York, and London 1977 ; 227-261.

Wang DY, De Stavola BL, Bulbrook RD, Allen DS, Kwa HG, Verstraeten AA, Moore JW, Fentiman IS, Chaudary M, Hayward JL, Gravelle IH.
The relationship between blood prolactin levels and risk of breast cancer in premenopausal women.
Eur J Cancer Clin Oncol 1987 ; 23 : 1541-1548.

Wang DY, Hampson S, Kwa HG, Moore JW, Bulbrook RD, Fentiman IS, Hayward JL, King RJB, Millis RR, Rubens RD, Allen DS.
Serum prolactin levels in women with breast cancer and their relationship to survival.
Eur J Cancer Clin Oncol 1986; 22: 487-492.

# TABLES

Table 1 : Prolactin in premenopausal breast carcinoma (M ± SE)

| | N | PRL ng/ml |
|---|---|---|
| Controls | 33 | Ø8.43 ± ØØ.7Ø * |
| Breast cancer patients | 111 | 77.16 ± 18.14 * |
| % elevation ( > 2Ø ng/ml) | | 58 / 111 (52.2%) |

* - P < Ø.Ø1

Table 2 : Prolactin in relation to parity in pre-menopausal breast carcinoma (M ± SE)

| | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| Nulliparous breast cancer patients | 1Ø | 57.87 ± 14.61 | 8/1Ø (8Ø.Ø%) |
| Parous breast cancer patients | 98 | 8Ø.73 ± 2Ø.8Ø | 48/98 (48.9%) |

Table 3 : Prolactin in relation to stage (M ± SE)

| Stage | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| II | 27 | Ø59.43 ± 17.96 | 12/27 (44.4%) |
| III | 57 | Ø54.92 ± 1Ø.39 | 29/57 (5Ø.8%) |
| IV | 16 | 139.17 ± 99.91 | 9/16 (56.2%) |
| Entered at relapse | 11 | 145.69 ± 96.72 | 8/11 (72.7%) |
| III ± IV | 73 | Ø73.39 ± 23.2Ø | 38/73 (52.Ø%) |

Table 4 : Prolactin in relation to distant metastatic sites in stage IV patients (M ± SE)

| Stage IV Patients | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| Lung | 3 | Ø32.39 ± Ø14.ØØ | 2/3 (Ø66.6%) |
| Bone | 3 | ØØ8.55 ± ØØ4.83 | Ø/3 (ØØØ.Ø%) |
| Liver | 5 | 386.59 ± 312.Ø5 | 2/5 (Ø4Ø.Ø%) |
| > 1 site | 5 | Ø34.19 ± ØØ5.13 | 5/5 (1ØØ.Ø%) |

Table 5 A : Prolactin in relation to circulating steroids (M ± SE)

| Stage | N | PRL pg / ml | $E_2$ ng / ml | Pg ng / ml |
|---|---|---|---|---|
| II | 27 | Ø59.43 ± 17.96 | 11Ø.2Ø ± 19.39 | 3.63 ± 1.Ø3 |
| III | 57 | Ø54.92 ± 1Ø.39 | Ø85.19 ± 14.55 | 1.85 ± Ø.36 |
| IV | 16 | 139.17 ± 99.91 | Ø88.72 ± 23.38 | 1.44 ± Ø.45 |
| Entered at relapsed | 11 | 145.69 ± 96.72 | Ø77.9Ø ± 16.31 | 4.34 ± 1.65 |
| III±IV | 73 | Ø73.39 ± 23.2Ø | Ø85.97 ± 12.38 | 1.76 ± Ø.29 |
| Controls | 3Ø | ØØ8.43 ± ØØ.7Ø | 167.2Ø ± 24.18 | 1.38 ± Ø.34 |

Table 5 B : Correlation of prolactin*

| | | |
|---|---|---|
| (A) With E 2 | | |
| Breast cancer patients | N = 111 | - Ø.Ø5 |
| I Patients who developed recurrence | N = 31 | |
| At diagnosis | | - Ø.Ø4 |
| Before progression | | - Ø.13 |
| At progression | | ± Ø.Ø1 |
| II Responders | N = 21 | |
| At diagnosis | | - Ø.19 |
| At last F/U | | + Ø.22 |
| (B) With Pg | | |
| Breast cancer patients N = 111 | | + Ø.Ø2 |
| I Patients who developed recurrence | N = 31 | |
| At diagnosis | | - Ø.Ø1 |
| Before progression | | - Ø.36@ |
| At progression | | - Ø.32 |
| II Responders | N = 21 | |
| At diagnosis | | - Ø.Ø2 |
| At last F/U | | + Ø.15 |

@ - P < Ø.Ø5

* - Correlation expressed as Spearman's rank correlation coefficient.

Table 6 : Prolactin in relation to nodal status (M ± SE)

| | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| Node negative | 11 | 24.64 + Ø7.Ø5 * | 4/11 (36.3%) |
| Node positive | 89 | 75.18 ± 19.64 * | 46/89 (51.6%) |

* - P < Ø.Ø2

Table 7 : Prolactin in relation to histologic grade (M ± SE)

| Histologic grade | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| I | Ø8 | Ø38.34 ± 18.28 | 3/8 (37.5%) |
| II | 37 | Ø72.Ø9 ± 16.89 | 23/37 (62.1%) |
| III | 23 | 147.65 ± 69.16 | 15/23 (65.2%) |
| II + III | 6Ø | 1Ø1.Ø5 ± 28.52 | 38/6Ø (63.3%) |

Table 8 : Hyperprolactinaemia at diagnosis in relation to disease outcome

| | Who developed progression | Who responded |
|---|---|---|
| PRL | | |
| < 3Ø ng / ml (44) | 23/44 (52.2%) | 21/44 (47.7%) |
| > 3Ø ng / ml (32) | 2Ø/32 (62.5%) | 12/32 (37.5%) |
| | * PRL ng/ml | |
| < 3Ø ng / ml | Ø13.47 ± Ø1.49 (23) | Ø13.33 ± Ø1.28 (12) |
| > 3Ø ng / ml | 224.97 ± 9Ø.37 (2Ø) | 1ØØ.84 ± 14.42 (21) |

* Values expressed as Mean ± SE

Figures in parenthesis show number of patients

Table 9 : Pretherapeutic prolactin in relation to site at relapse (M ± SE)

| Site at relapse | N | PRL ng/ml | Patients above normal limit |
|---|---|---|---|
| Soft tissue only | 11 | Ø88.15 ± Ø21.79 | 7/11 (63.6%) |
| Bone | 9 | 182.36 ± 12Ø.46 | 6/9 (66.6%) |
| Viscera | 4 | 426.66 ± 395.7Ø | 3/4 (75.Ø%) |
| > 1 site | 7 | Ø43.Ø7 ± Ø15.36 | 4/7 (57.1%) |

Table 1Ø : Prolactin in relation to disease status (M ± SE)

| Patients who developed recurrence | N = 31 | Responders | N = 21 |
|---|---|---|---|
| At diagnosis | 149.39 ± 6Ø.73 @ | At diagnosis | 47.49 ± 11.8Ø * |
| Before prograssion | Ø19.48 ± Ø4.34 @,$ | | |
| At Progression | Ø6Ø.86 ± 1Ø.4Ø $ | At last F/U | Ø7.98 ± Ø1.1Ø * |

@ - P < Ø.Ø5 * - P < Ø.Ø1 $ - P < Ø.ØØ1

Table 11 : Sensitivity, Specificity and Predictive value of prolactin in pre-menopausal breast carcinoma monitoring

| | N = 52 |
|---|---|
| Sensitivity | 93.54 % |
| Specificity | 95.23 % |
| Predictive value (+ ve test) | 96.66 % |
| Predictive value (- ve test) | 90.90 % |
| Diagnostic efficieny | 94.23 % |

Table 12 : Prolactin in relation to steroid receptors (M ± SE)

| | ER + PRL ng / ml | ER - PRL ng / ml |
|---|---|---|
| PRL < 30 | Ø13.88 ± Ø1.Ø5 (4Ø)@ | Ø13.Ø8 ± Ø1.51 (25)* |
| PRL > 3Ø | 16Ø.52 ± 55.92 (28)@ | 177.1Ø ± 61.Ø7 (18)* |

| | PR + PRL ng / ml | PR - PRL ng / ml |
|---|---|---|
| PRL < 3Ø | Ø13.4Ø ± Ø1.Ø2 (42)$ | Ø13.88 ± Ø1.62 (23) |
| PRL > 3Ø | 155.57 ± 37.6Ø (3Ø)$ | 188.45 ± 97.19 (16) |

*,@ - P < Ø.Ø2 $ - P < Ø.ØØ1

Figures in parenthesis show number of patients

Table 13 A : Changes in prolactin levels with treatment

| Therapy | N | PRL ng/ml Pretherapeutic | After treatment |
|---|---|---|---|
| Chemo | 22 | 122.Ø2 ± Ø49.99 * | 3Ø.69 ± 8.83 * |
| Endocrine | Ø8 | Ø71.17 ± Ø21.37 | 12.23 ± 2.41 |
| Chemo-Endo | 11 | 173.18 ± 144.67 | 17.37 ± 4.65 |

* - P < Ø.Ø2

Table 13 B : Changes in prolactin following treatment (M ± SE)

| Therapy | N | Pretherapeutic | After treatment |
|---|---|---|---|
| Responsive Disease : | | | |
| Chemo | Ø5 | Ø27.84 ± Ø12.Ø4 | 11.31 ± Ø4.28 |
| Endocrine | Ø5 | Ø47.Ø8 ± Ø37.84 | Ø8.58 ± Ø2.ØØ |
| Chemo-Endocrine | Ø2 | ØØ7.53 ± ØØ2.33 | Ø8.95 ± Ø1.45 |
| Progressive Disease : | | | |
| Chemo | 17 | Ø37.34 ± ØØ9.88 | 36.4Ø ± 11.Ø5 |
| Endocrine | Ø3 | Ø47.87 ± Ø24.49 | 18.32 ± Ø3.37 |
| Chemo-Endocrine | Ø9 | 197.57 ± 177.63 | 19.24 ± Ø5.53 |

Fig. 13

Prolactin and Progesterone-receptors in pre-menopausal breast carcinoma patients.

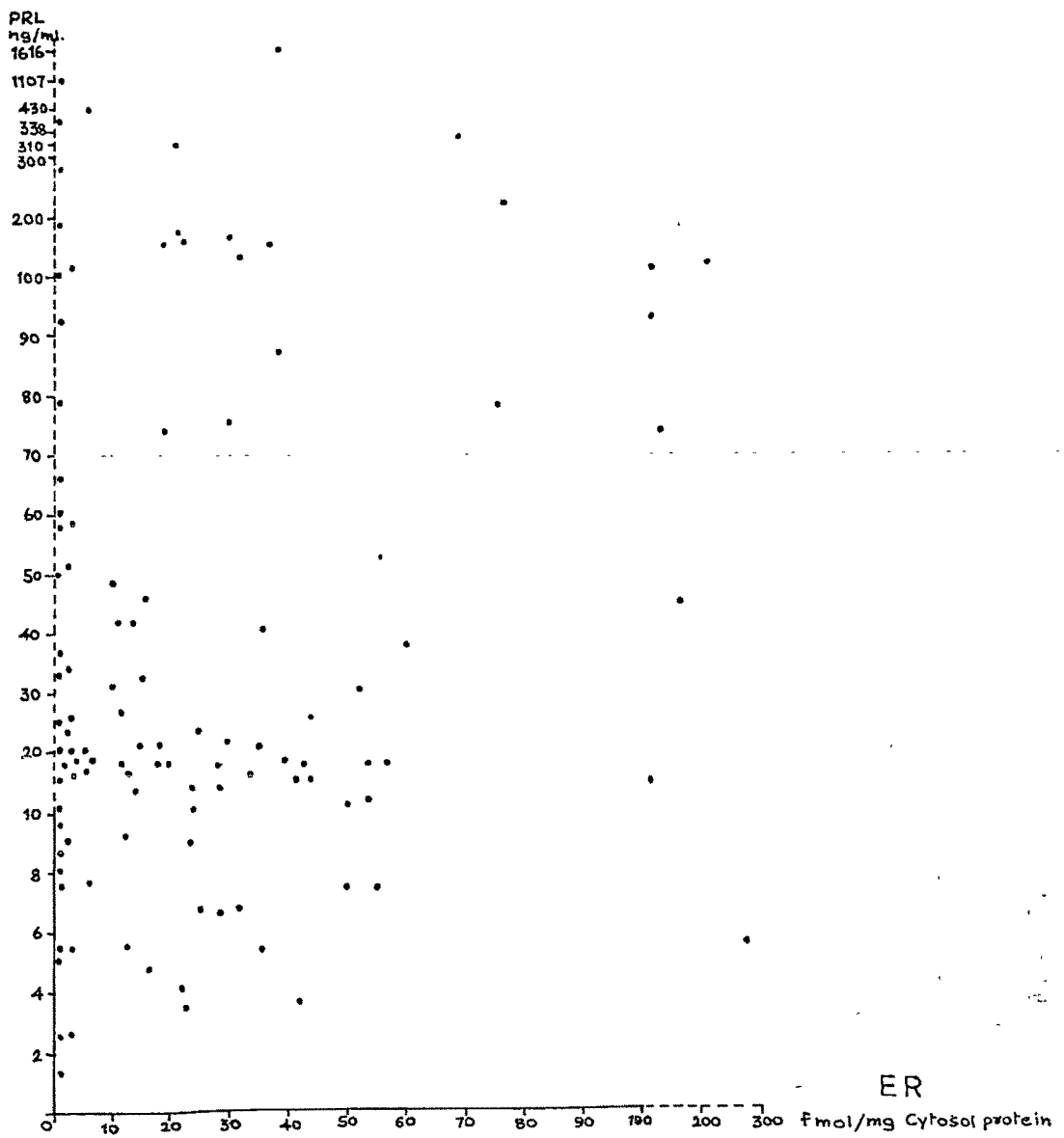
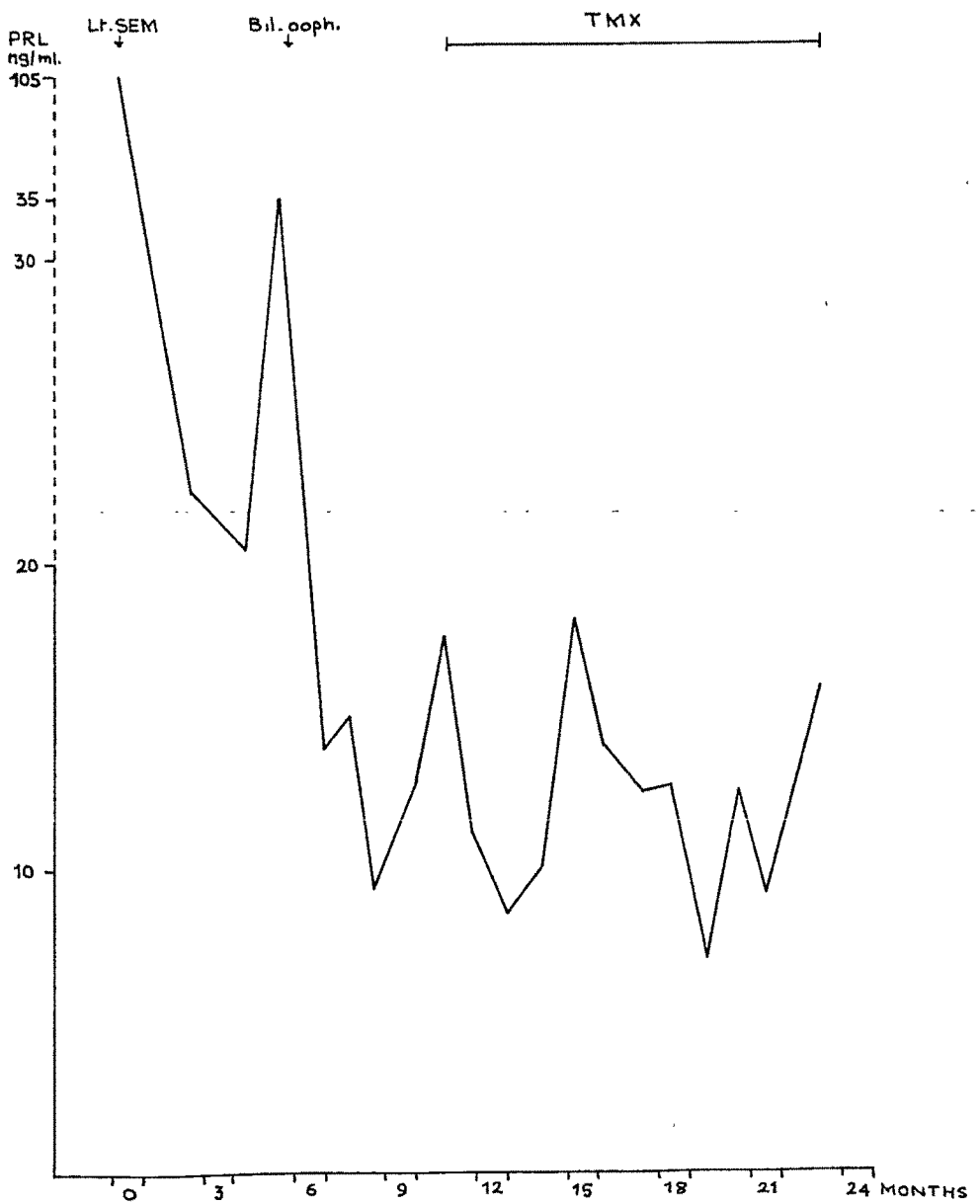

PRL
ng/ml.
1616
1107
430
338
310
300
200
100
90
80
70
60
50
40
30
20
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
100
200
300
ER
fmol/mg Cytosol protein

Fig.12

Fig. 12

Prolactin and Estrogen-receptors in pre-menopausal breast carcinoma patients.




Stage III
HG II

Fig 11

**Fig. 11**

The patient has stage III disease and treated with surgery followed by bilateral oophorectomy. Thereafter, she was treated with Tamoxifen. She responded to the treatment and remained relapse free at the end of two years.

Stage II
HG II

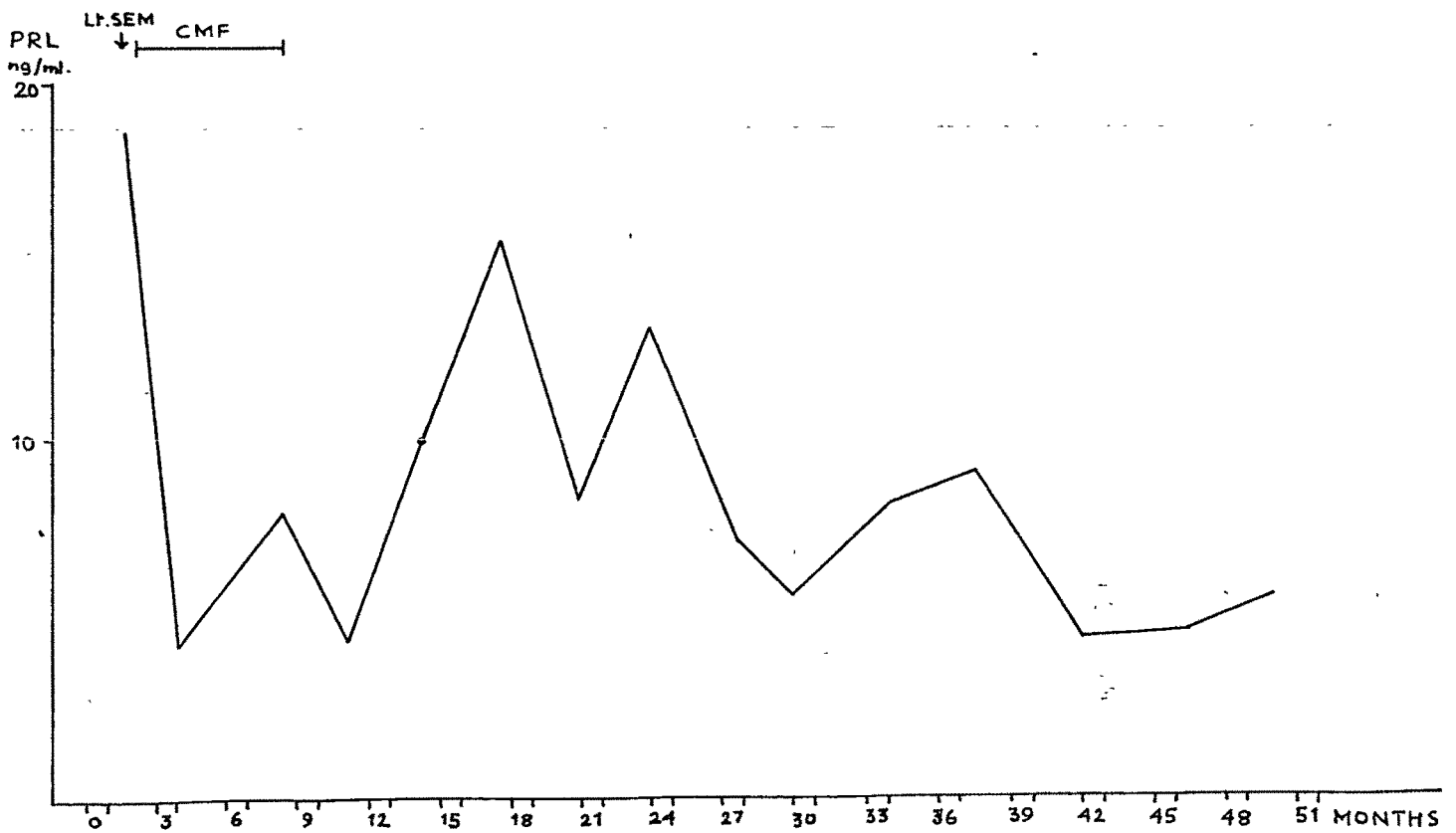
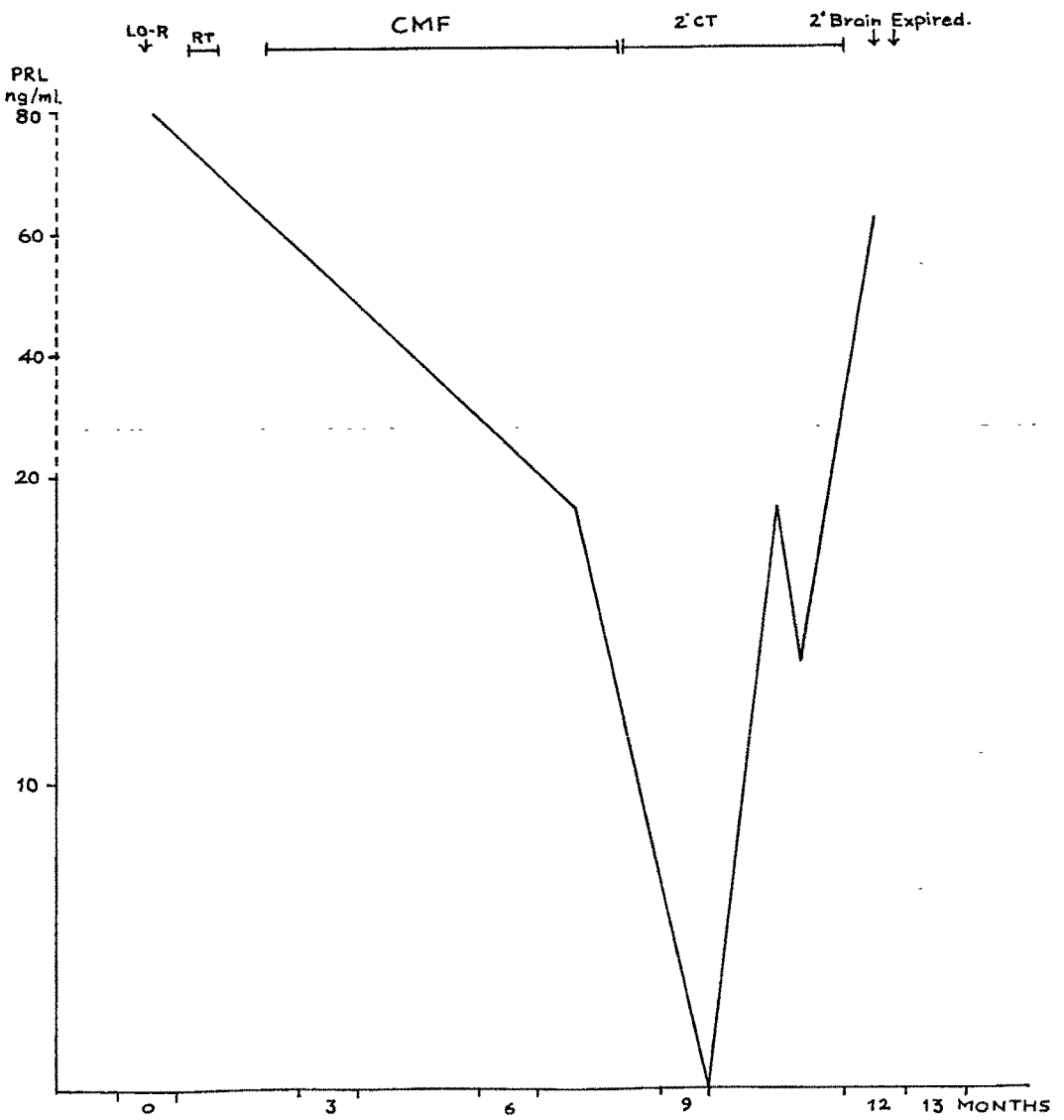



Fig.10

RESPONDERS (Figs. 1Ø-11):

Fig. 1Ø

The patient with stage II disease was treated with surgery followed by CMF. The patient responded to the treatment and remained relapse free at the end of two years.



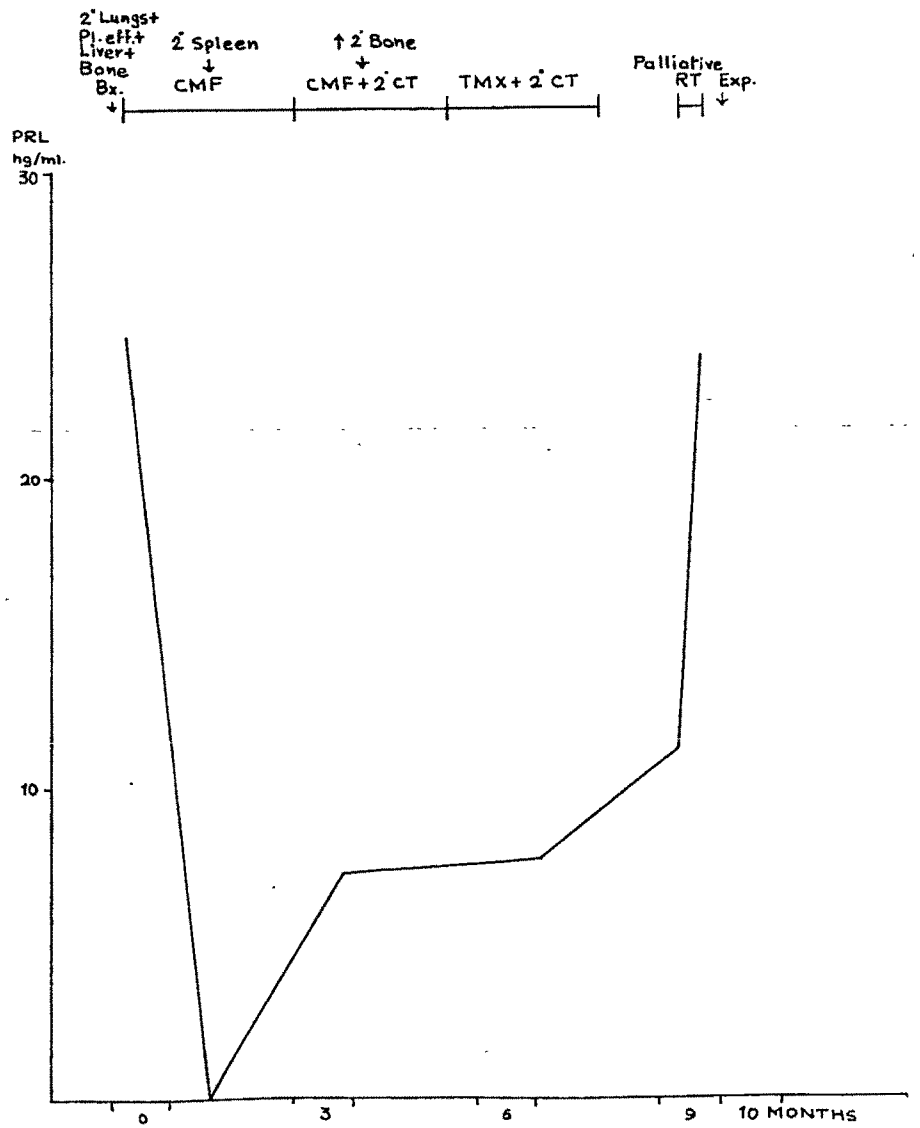

Rec. Ca.
HG II

Fig.9

Fig. 9

Patient came with locally recurrent disease with grade II tumor. She was treated with axillary clearence followed by radiotherapy and CMF and responded to it. After 2.5 months, she developed brain metastasis and expired.

Prolactin levels accurately correlated with progression of the disease.




Stage IV
HG III

Fig. 8

Fig. 8

Stage IV, HG III patient had multiple metastases in the axilla, lungs with malignant pleural effusion, liver and bones. Patient was treated with CMF followed by second line chemotherapy and Tamoxifen. She did not respond to the treatment and died at the end of 9 months. In the beginning, patient responded to the treatment which can be seen by decreased prolactin levels but subsequently high prolactin titres were correlated with the disease progression .

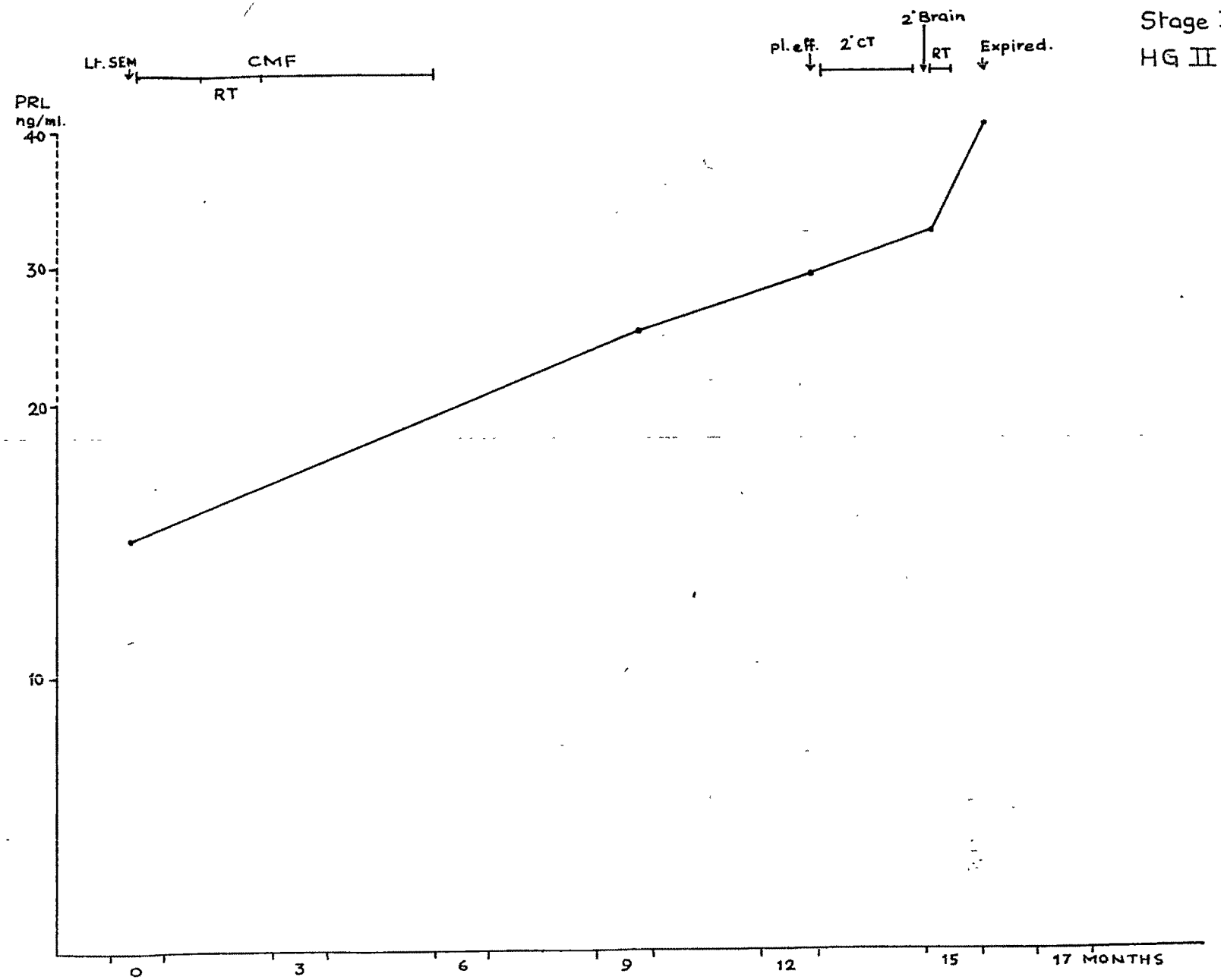

Stage III
HG II
LF. SEM
CMF
RT
pl. eff.
2° CT
2° Brain
RT
Expired.
PRL
ng/ml.
40
30
20
10
0
3
6
9
12
15
17 MONTHS

Fig.7

Fig. 7

Stage III, HG II patient who was treated with surgery followed by CMF and radiotherapy. Patient responded to the treatments. She developed malignant pleural effusion and was treated with second line chemotherapy. She did not respond to it and developed brain metastasis. She died in spite of treatment. Prolactin showed increased titres through out the disease course. Thereby showing an excellent correlation with the disease status.

Stage II
HG II

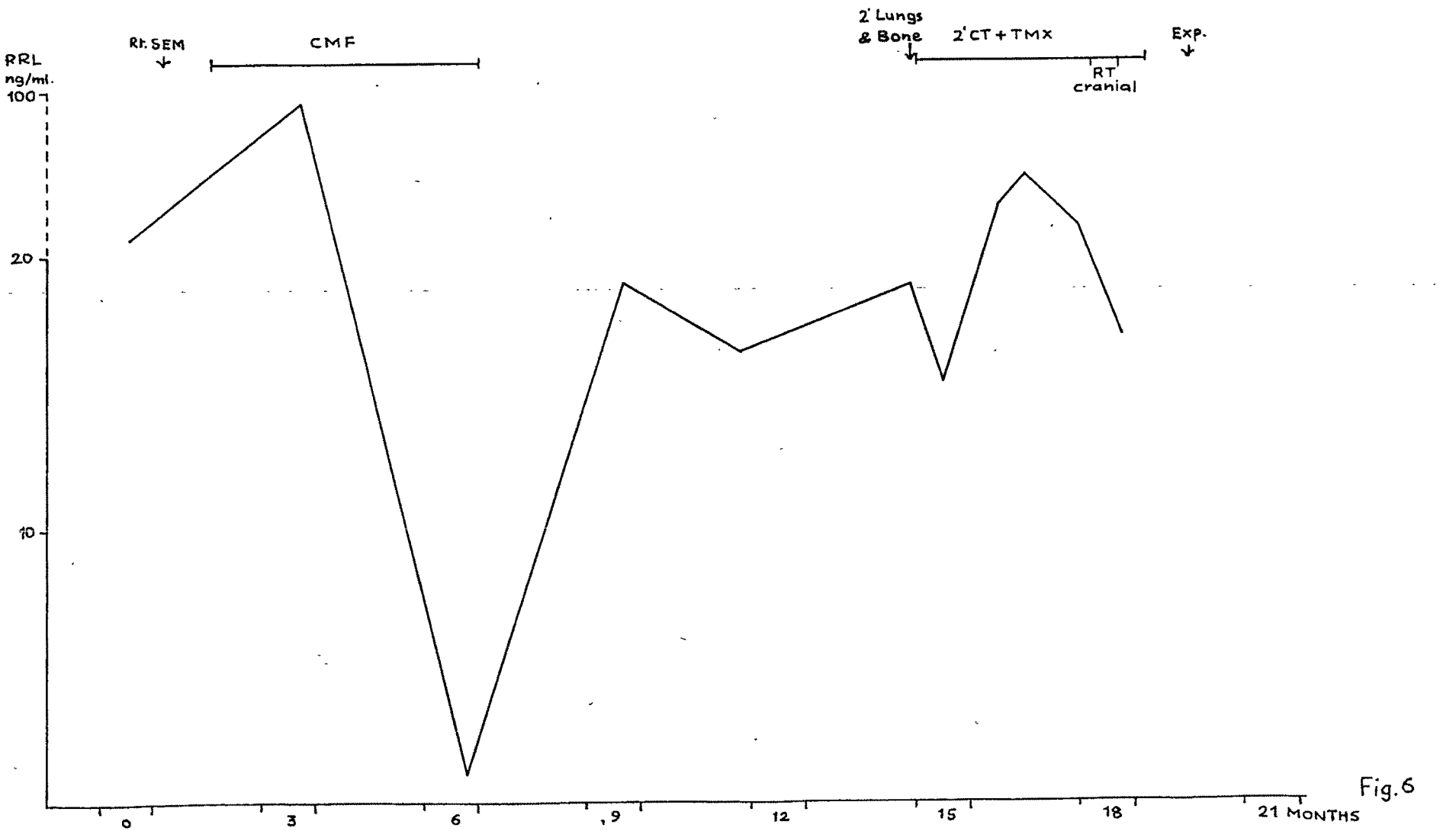



Fig.6

Fig. 6

Stage II, grade II patient treated with surgery. Post-mastectomy sample resulted into elevated prolactin. The patient was treated with CMF. She responded to chemotherapy but as soon as she completed the chemotherapy prolactin showed rising titres. Prolactin remained high for nearly 5 months. Thereafter, she developed metastases in the lungs and bone. She was treated with second line chemotherapy and Tamoxifen. Initially she responded to the treatment which was consistent with prolactin levels. However, within a short time, she developed brain metastasis. She was again treated with cranial radiotherapy. She did not respond to it and finally died by the end of 19th month.

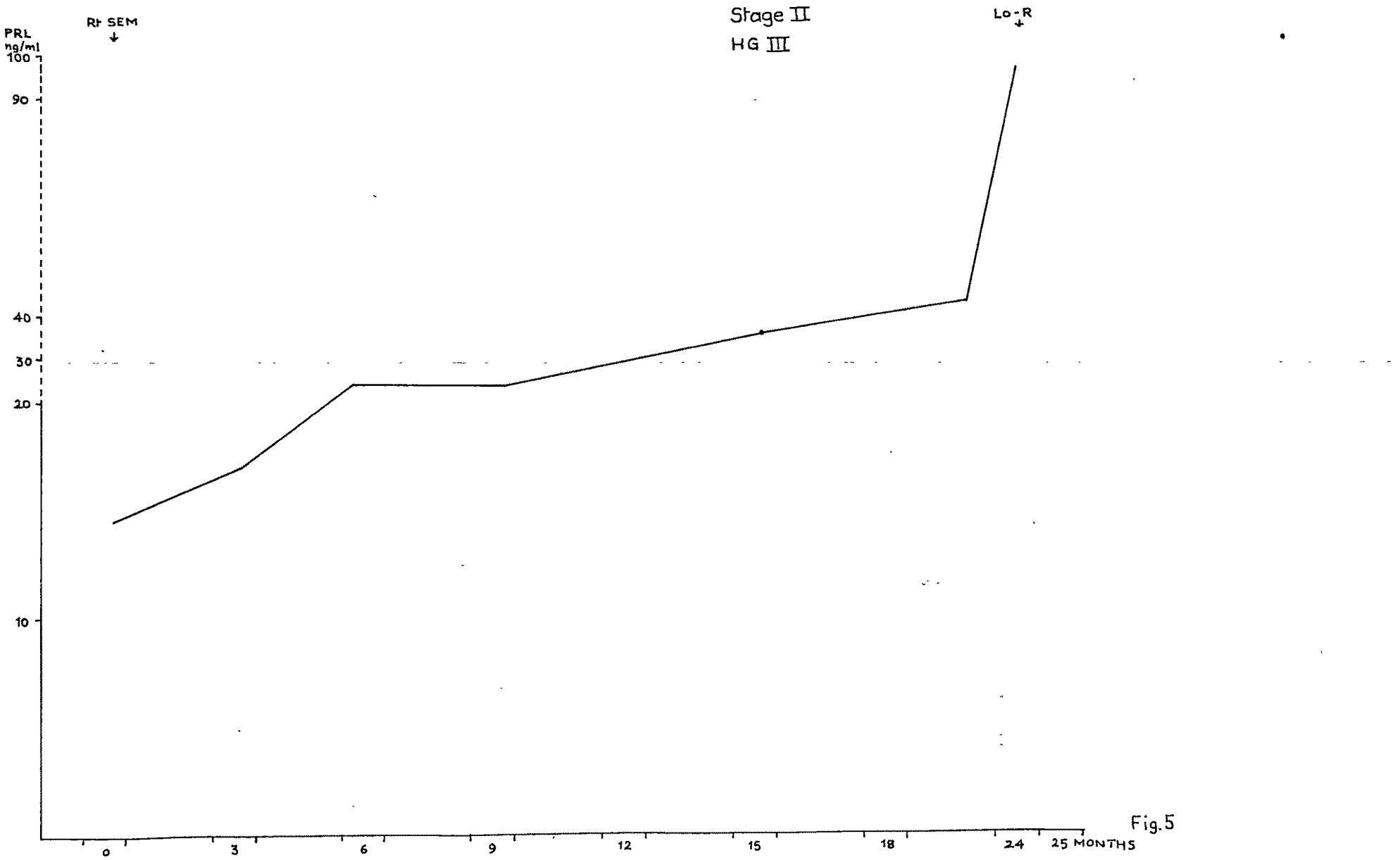

Rf SEM
Stage II
HG III
Lo-R
PRL
ng/ml
100
90
40
30
20
10
0
3
6
9
12
15
18
24
25 MONTHS

Fig. 5

PATIENTS WHO DEVELOPED RECURRENT DISEASE (Figs. 5-9):

Fig. 5

Stage II, grade III patient treated with surgery. The patient developed local recurrence at the end of two years. The prolactin level resulted into significant increase throughout the follow-up period showing lead time by 1Ø months.

Fig. 4

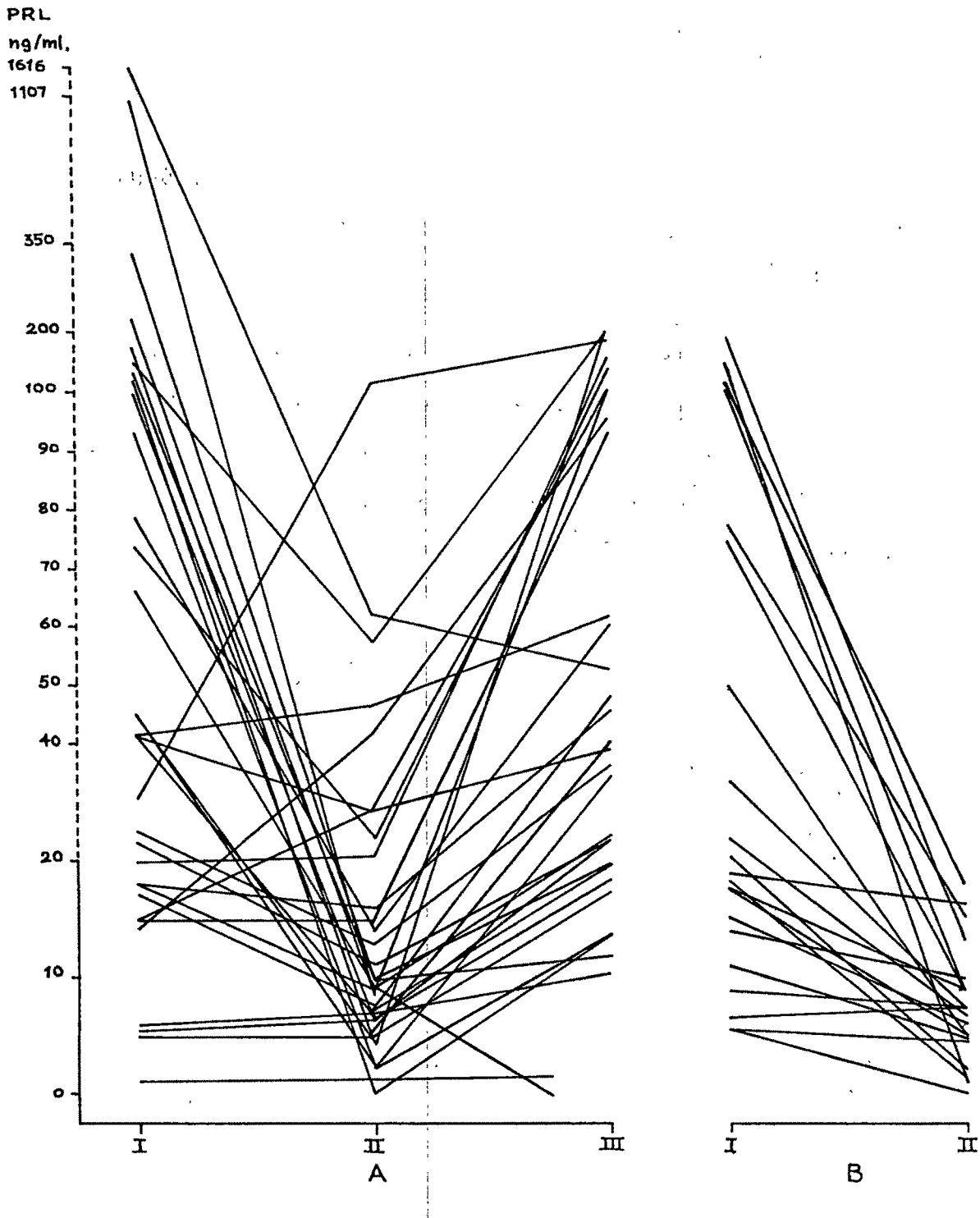

PRL
ng/ml.
1616
1107
350
200
100
90
80
70
60
50
40
20
10
0
I
II
III
A
I
II
B

fig. 4

A. Prolactin in patients who developed progressive disease.

I Pretherapeutic prolactin levels.

II Prolactin levels at progression.

III Prolactin levels at progression.

B. Prolactin levels in Responders.

I Pretherapeutic prolactin levels.

II Prolactin levels at last f/u.

Fig. 3

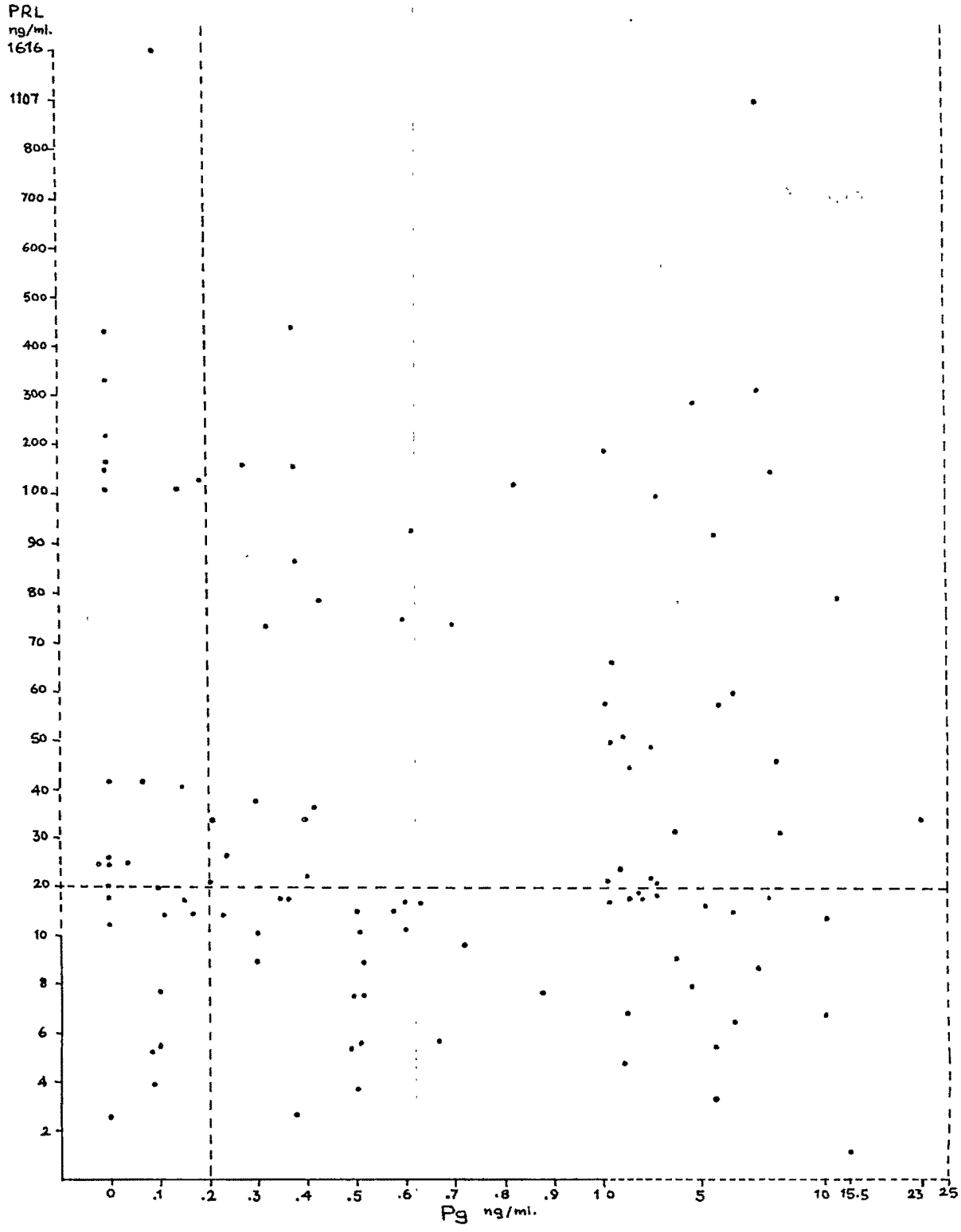

PRL
ng/ml.
1616
1107
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
8
6
4
2
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1 o
5
10
15.5
23
25
Pg ng/ml.

fig. 3

Prolactin and Pg in pre-menopausal breast carcinoma patients.

Dotted lines show normal limit for prolactin and lower and upper normal limit for Pg.

Fig. 2

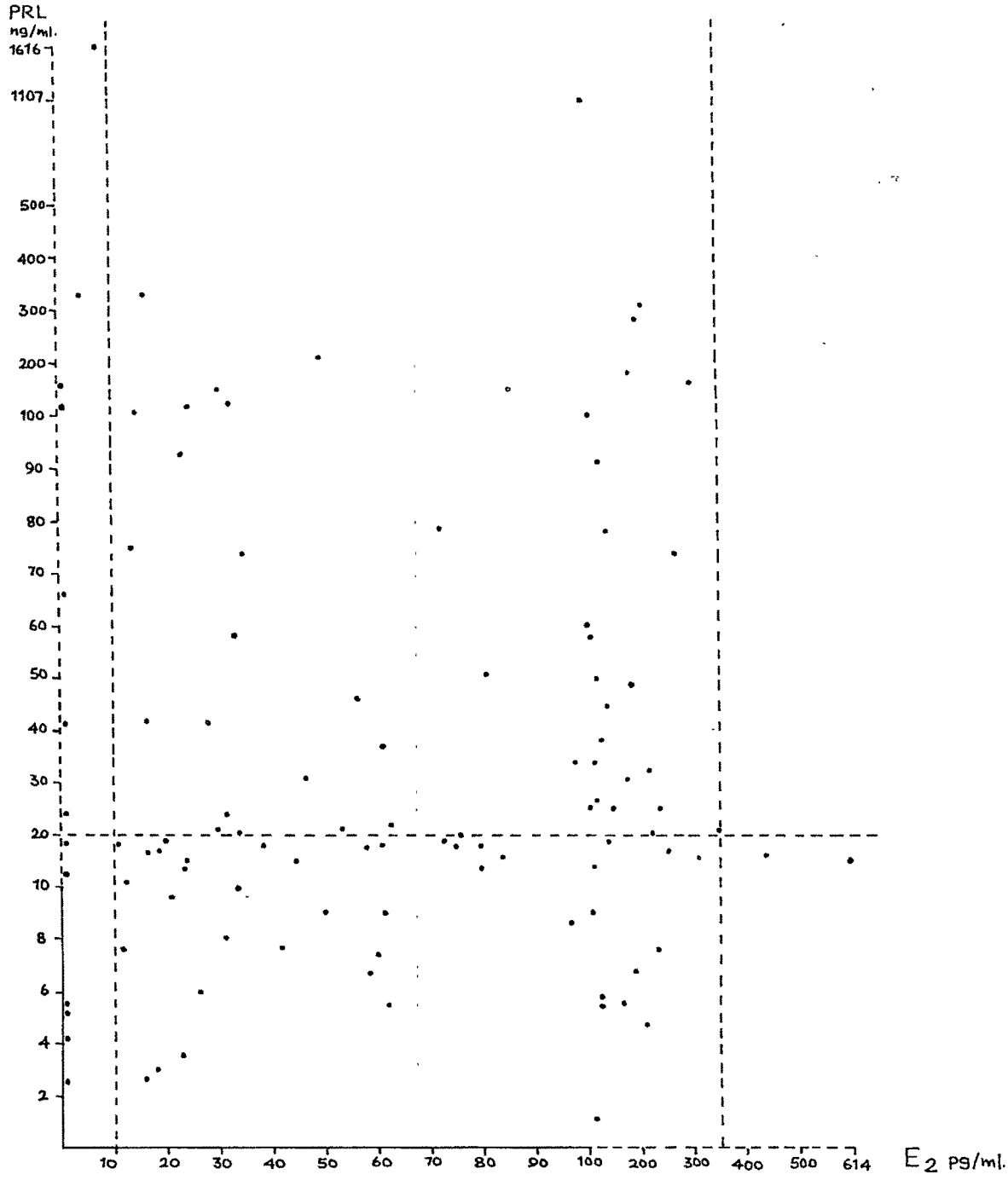

PRL
ng/ml.
1616
1107
500
400
300
200
100
90
80
70
60
50
40
30
20
10
8
6
4
2
10
20
30
40
50
60
70
80
90
100
200
300
400
500
614
E2 pg/ml.

Fig. 2

Prolactin and $E_2$ in pre-menopausal breast carcinoma patients.

Dotted lines show normal limit for prolactin and lower and upper normal limits for $E_2$

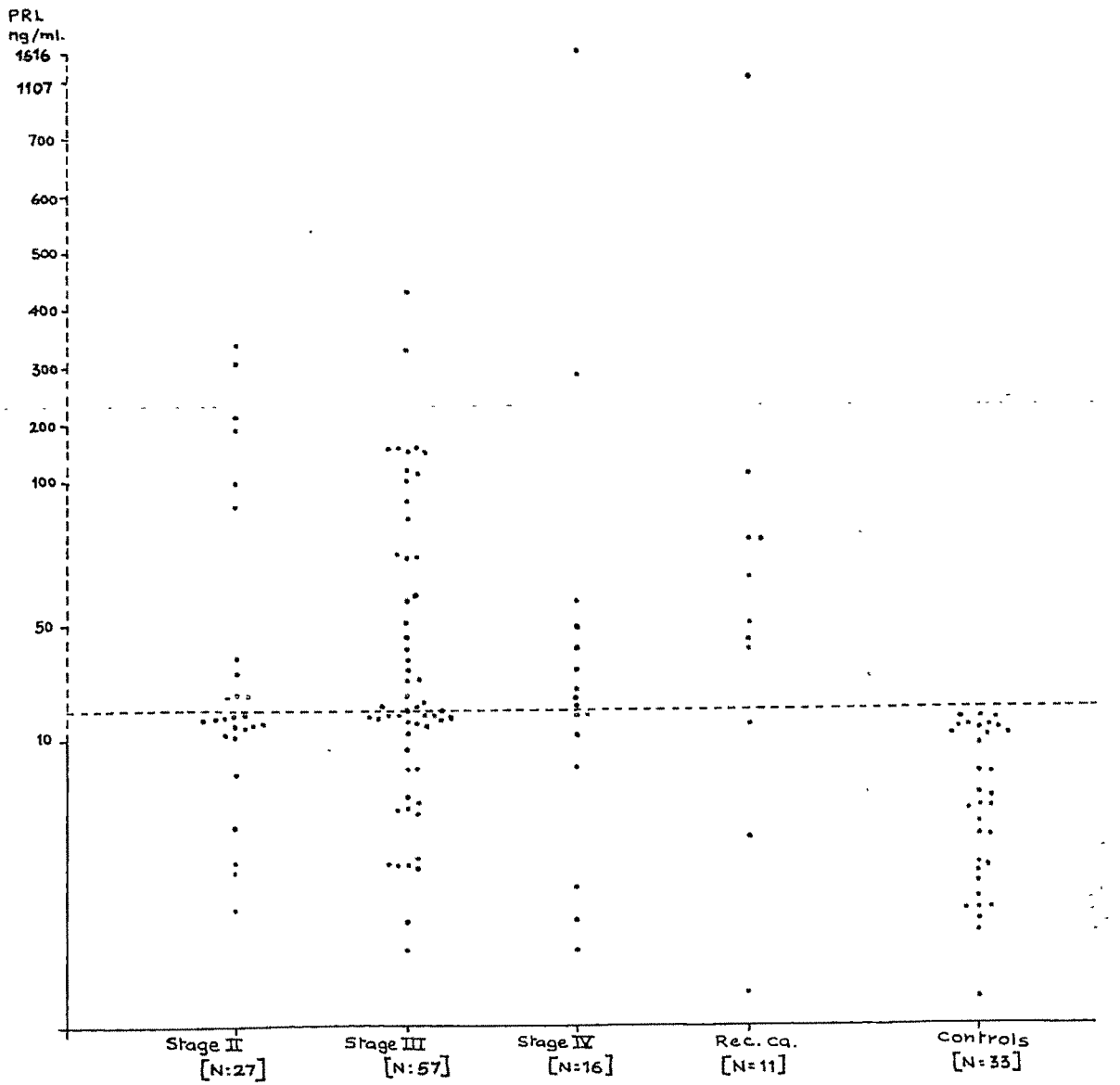

PRL
ng/ml.
1616
1107
700
600
500
400
300
200
100
50
10
Stage II
[N:27]
Stage III
[N:57]
Stage IV
[N:16]
Rec. ca.
[N=11]
Controls
[N=33]

Fig. 1

Fig. 1

Prolactin in pre-menopausal breast carcinoma patients and controls.

Dotted line show normal limit.

# FIGURES

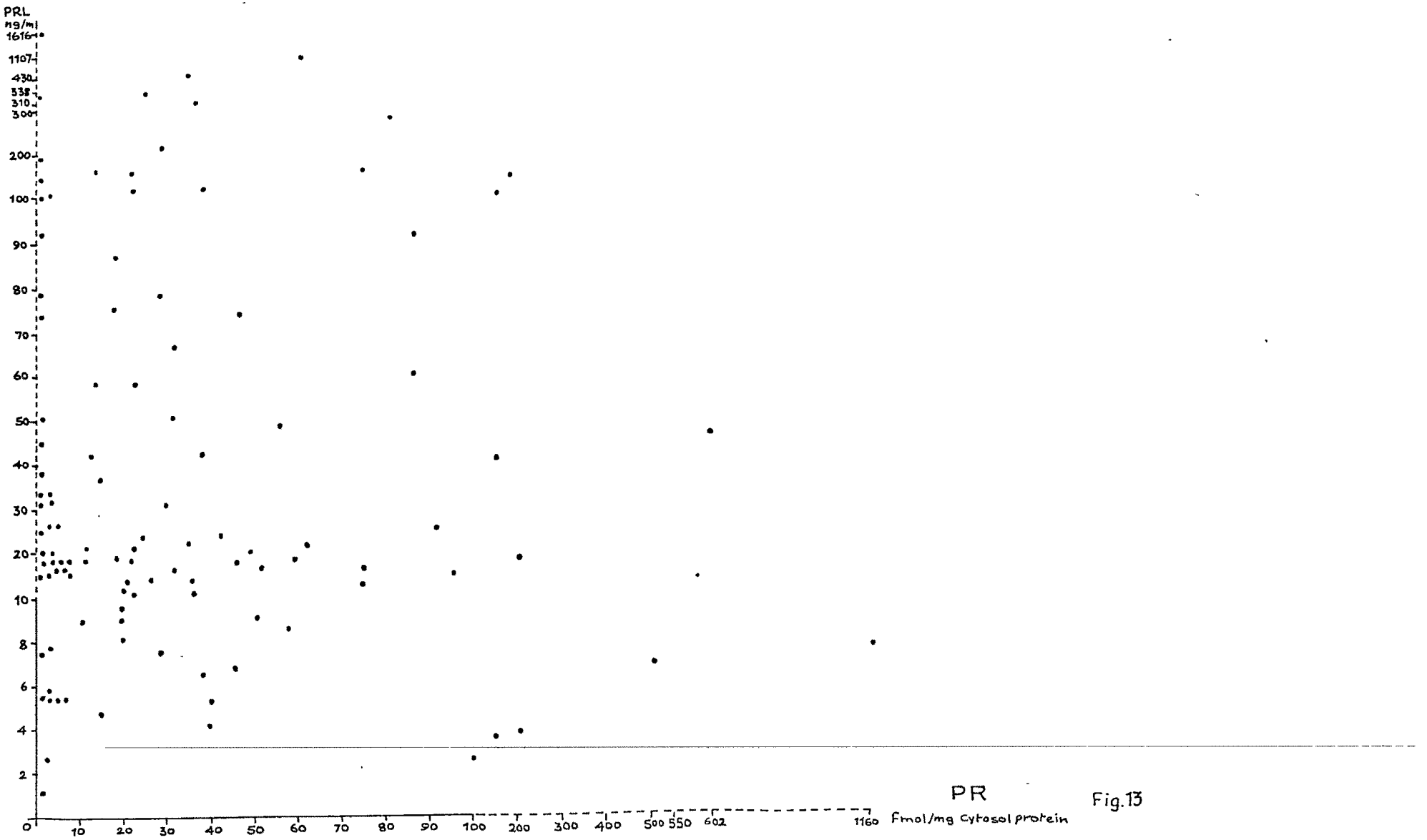

PRL
ng/ml
1616
1107
430
338
310
300
200
100
90
80
70
60
50
40
30
20
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
100
200
300
400
500
550
602
1160
Fmol/mg cytosol protein
PR

Fig.13