CHAPTER - II

SERUM STEROID HORMONE PROFILE IN PRE-MENOPAUSAL BREAST CANCER PATIENTS.

INTRODUCTION

In the previous Chapter, we have described the significance of estrogen- and progesterone-receptors in pre-menopausal breast carcinoma patients which were also correlated with other important prognosticators. Owing to the importance of the receptor determinations for the management of individual breast cancer patients, detailed knowledge is needed concerning the factors affecting receptor levels that are measured in tumor cytosol. A number of hormonal factors can influence the receptor concentration in the tumor cytosol (Longacre and Bartow, 1986). Moreover, it is well known that the existence of harmonious ovarian cycle function ensures perfect mammary development. Several physiological conditions and external factors that could influence hormones and their possible relation to the development of breast cancer are worth considering. The role of hormonal imbalance as risk factors for human breast cancer is still controversial. Key and Pike (1988) excellently reviewed the role of estradiol and progesterone in the epidemiology and prevention of breast carcinoma.

In our earlier publication, we have described profile of peptide and steroid hormones in pre- and post-menopausal breast carcinoma patients and concluded that high levels of prolactin and altered ratio of androgen : estrogen possibly plays a major role in the development of breast carcinoma (Bhatavdekar et al, 1987). There is considerable interest in exploring the effects of cytotoxic drugs on endocrine function in breast cancer patients (Jordan et al, 1987). Several studies have indicated that cytotoxic chemotherapy results in amenorrhea, secondary to primary ovarian failure in pre-menopausal patients (Dnistrian et al, 1983, 1985; Manni, 1987 ; Secreto et al , 1989).

In the present study, we have estimated circulating levels of estradiol, progesterone, testosterone and its major precursor androstenedione in pre-menopausal breast carcinoma patients taking stage, nodal status, histologic grade and disease course into consideration. Moreover, we also have investigated the endocrine consequences of chemo- and/or endocrine therapy in these patients. Furthermore, circulating estradiol levels were correlated with estrogen receptor content.

STUDY DESIGN

The pre-menopausal breast carcinoma patients (N=111) and age matched healthy controls (N=3Ø) were randomly selected for the study. The clinical data was as described in the previous Chapter.

The accurate details of the menstrual cycle length in breast cancer patients could not be procured and hence, phase of the menstrual cycle was not considered at the time of hormone estimation.

PATIENT SAMPLING :

In a serial follow-up programme executed at Endocrinology Division, venous blood samples were collected in the morning between 9.Ø - 11.Ø AM pretherapeutically to obtain base line level of individual pateints and at intervals of every 3-6 months for stage II and at monthly/bimonthly intervals for advanced patients. These hormones were estimated sequentially to investigate : (1) whether these hormones can be used as disease monitor and (2) to observe the endocrine consequences of combination therapy. Serum was separated within 2 hours, aliquoted and preserved at -7Øo C till analysis. The assays of steroid hormones were performed within 1 month. Studies were performed retrospectively using frozen sera.

PATHOLOGIC STAGING :

Pathologic staging (UICC, 1980) system was employed in the present study. Histologic grading was done according to Bloom and Richardson (1957) and expressed on a scale of I- well differentiated, II- moderately differentiated, III- poorly differentiated.

THERAPY :

The primary treatment offered to the patients was surgery simple extended mastectomy, radical mastectomy (SEM, RM) followed by radiotherapy (RT) to control local disease. The adjuvant chemotherapy (N=22) comprised of CMF (cyclophosphamide, methotrexate and 5-fluorouracil). The hormonal manipulations (N=8) included bilateral oophorectomy and/or Tamoxifen (TMX). The chemohormonal therapy (N=11) introduced was CMF followed by TMX and/or bilateral oophorectomy. The treatment schedules were implemented by Medical Oncology units of the Institute. The CMF regimen was cyclophosphamide, 100 mg/sqm orally, days 1 to 14; methotrexate, 40 mg/sqm and 5-fluorouracil, 600 mg/sqm intravenously, days 1 and 8. An identical cycle of chemotherapy was repeated every two weeks subsequently. Drug doses were modified according to toxic side effects. The dose of TMX was 10 mg, twice daily. The second line chemotherapy given was MMC-10 mg/sqm, VLB-10 mg/sqm and

adriamycin-40 mg/sqm either singly or in combination with Tamoxifen.

STEROID HORMONE ASSAYS :

Serum estradiol (E_2), progesterone (Pg) , testosterone (T) and androstenedione (Andro.) were estimated using commercially available RIA (Radioimmunoassay) kits procured from Diagnostic Products Corporation, Los Angeles, USA, according to the manufacturer's instructions. All the estimations were performed in duplicate with an intraassay and an interassay coefficient of variation (CV) of 3% to 5% and 5% to 8% respectively alongwith internal quality controls. The sensitivity of the assays were : 8 pg/ml for E2 , 0.05 ng/ml for Pg, 0.11 ng/ml for T and 0.02 ng/ml for Andro. The normal range of these steroid hormones for premenopausal controls was : 10 - 355.0 pg/ml -E_2 , 0.2 - 25.0 ng/ml - Pg, 0.15 - 1.1 ng/ml T and 0.8 - 4.0 ng/ml - Andro.

ASSESSMENT OF DISEASE ACTIVITY :

During the follow-up period, the assessment of disease activity was carried out from time to time using standard criteria (Hayward et al, 1977). The patients treated with surgery were immediately followed by combination therapy and hence the pretherapeutic levels of these hormones were compared to those after therapy. The assessment was carried

out after a minimun of two chemotherapy cycles or a minimum of two months of TMX. Depending on their ultimate outcome, the patients were grouped into (I) the patients who developed recurrent disease and (II) patients who responded to various therapeutic modalities at the end of two years.

I Patients who developed recurrent disease (N=31) :

The steroid hormone levels at diagnosis amongst patients who developed recurrent disease were compared to : (i) the levels of preceding blood samples (termed as 'before progression') and (ii) the levels at recurrence (termed as 'at progression').

II Patients who responded to various therapeutic modalities (N=21):

Similarly, the pre-therapeutic steroid hormone levels amongst responders were compared to the levels at last follow-up when the disease was in remission at the end of two years.

CORRELATION OF ESTRADIOL WITH ESTROGEN RECEPTOR:

Pretherapeutic E_2 levels of patients were grouped into : (i) $<$ 100 pg/ml, (ii) 100-200 pg/ml and (iii) $>$ 200 pg/ml and were compared with estrogen receptor concentration with simultaneous consideration of stage amongst patients with ER^+ tumors.

STATISTICAL ANALYSIS:

The differences in steroid hormones were analysed to obtain statistical significance using (i) X^2 - analysis and (ii) an exact contingency table for order data and Fisher's two sided exact test (Mehta and Patel, 1983). P values less than Ø.Ø5 were considered to be significant.

RESULTS

STEROID HORMONES IN PREMENOPAUSAL BREAST CARCINOMA PATIENTS:

Circulating steroid hormone levels in breast cancer patients were compared with controls. E_2 was significantly reduced ($P < Ø.Ø1$) and Pg was significantly elevated ($P < Ø.Ø5$) in breast cancer patients, while circulating T and Andro. showed a statistically non-significant decline in patients as compared to controls. Moreover, a statistically significant increased Andro.:E_2 ratio was observed amongst breast cancer patients in comparison to controls (Table - 1).

Furthermore, 45/111 (4Ø.5%) patients demonstrated circulating steroids within normal limits and 66/111 (59.4%) patients exhibited abnormal steroid levels. In patients noted with abnormal circulating steroids, 4Ø/66 (6Ø.6%) expressed abnormality of any one of them while 17/66 (25.7%), 8/66 (12.1%) and 1/66 (1.5%) patients indicated abnormality of two, three and four circulating steroids respectively.

STEROID HORMONES IN RELATION TO STAGE:

A statistically non-significant trend of decrease in circulating steroids except Andro. was observed as stage advanced (stage II to IV). On the contrary, pg amongst patients entered at relapse showed elevated levels as compared to stage II (Table - 2). The difference however, was statistically non-significant (i.e. pretherapeutic - stage II, III and IV vs patients entered at relapse).

The abnormality of circulating steroids was uniformly distributed amongst all stages of breast cancer patients. Amongst stage II, 12/27 (44.4%) patients demonstrated normal steroid levels while 15/27 (55.5%) patients exhibited abnormal circulating steroids. 10/15 (66.6%), 3/15 (20.0%) and 2/15 (13.3%) patients displayed abnormality of any one, two or three circulating steroids respectively.

Amidst stage III, 21/57 (36.8%) and 36/57 (63.1%) patients indicated normal and abnormal circulating steroids respectively and 22/36 (61.1%), 11/36 (30.5%), 3/36 (8.3%) patients had anomalous levels of one, two or three steriods respectively.

6/16 (37.5%) and 10/16 (62.5%) stage IV patients presented normal and abnormal circulating steroids respectively. 6/10 (60.0%), 1/10 (10.0%), 2/10 (20.0%) and 1/10 (10.0%)

patients substantiated abnormal levels of any one, two, three or all four steriods respectively.

STERIOD HORMONES IN RELATION TO NODAL STATUS:

A statistically non-significant trend of decreased E_2 and Pg and increased T and Andro. was observed amongst node positive patients in comparison to node negative patients (Table - 3).

5/11 (45.4%) node negative and 35/89 (39.3%) node positive patients demonstrated normal steriods contrary to 6/11 (54.5%) node negative and 54/89 (60.6%) node positive patients who exhibited abnormal steriod levels. These differences did not attain statistical significance.

STERIOD HORMONES IN RELATION TO HISYOLOGICAL GRADE:

Circulating E_2 and Pg levels did not show significant change amongst patients with histological grade III tumors as compared to patients with histological grade I tumors. On the other hand, T and Andro. exhibited an increase for similar comparisons. The increase in T was statistically significant ($P < 0.05$) while the increase of Andro. amongst patients with HG III tumors was non-significant in comparison to patients with HG I tumors (Table - 4).

STERIOD HORMONES IN RELATION TO DISEASE STATUS:

Pretherapeutic circulating Pg and T were elevated while circulating E_2 and Andro. levels were decreased amongst

responders as compared to the patients who developed recurrence. The differences however, were statistically insignificant (Table - 5).

Moreover, at diagnosis 7/31 (22.5%) patients exhibited normal and 24/31 (77.4%) patients evidenced abnormal circulating steriods amongst those who developed recurrence as opposed to 9/21 (42.8%) patients who demonstrated normal and 12/21 (57.1%) patients who showed abnormal circulating steriods amidst responders. These differences however, could not attain significance.

Amidst patients who developed recurrence, a statistically significant decline in E_2 and Pg and a non-significant decline of Andro. was observed at progression while T levels showed a non-significant increase. On the other hand, a significant decline in E_2, a non-significant decrease in Pg and T and a non-significant elevation of Andro. was demonstrated amongst responders at the end of 2 years (Table - 5). From our sequential data,it is clear that these hormones can not be used as disease monitor (Figs. 1-5).

CHANGES IN STEROID HORMONE PROFILE AFTER THERAPY:

Menstrual function:

In the present study, we have performed estimations of E_2, Pg, T, Andro, PRL, FSH and LH to obtain a hormonal profile.

It was observed that at diagnosis, only 8/111 (7.2%) premenopausal breast carcinoma patients exhibited all these hormones within normal limits thereby indicating normal ovarian function. Out of 8 patients 5 had stage II and 3 had stage III disease. The abnormal levels observed in the remaining patients may be due to observed hyperprolactinaemia (> 30.0 ng/ml; Chapter III) in 46/111 (41.4%) as well as it may be due to the fact that 75% of the patients had advanced breast carcinoma.

PATIENTS TREATED ONLY WITH CHEMOTHERAPY:

Chemotherapy consequenced into reduction in E_2, Pg, T and Andro.. The lowering of E_2 only shown to be statistically significant while the decline of Pg, T and Andro. after chemotherapy was statistically non-significant (Table - 6). The majority of patients developed amenorrhea within 4-6 months (Figs 1 to 4).

PATIENTS TREATED WITH ENDOCRINE MANIPULATION:

Endocrine manipulation resulted into a reduction in E_2, Pg, T and Andro. levels after treatment as compared to pretherapeutic levels. The decline of only T was statistically significant and the reductions in E_2, Pg and Andro. were statistically non-significant (Table - 6; Fig 5).

PATIENTS TREATED WITH CHEMOENDOCRINE THERAPY:

The low E_2 levels towards the lower normal limit at

diagnosis itself might be due to ovarian failure which might have resulted from the advanced stage of the disease. Therefore, it may be possible that a further decrease in E_2 levels after treatment was not observed, so the patients treated with chemoendocrine therapy did not exhibit any change in serum E_2 levels contrary to the chemotherapy alone. A decline in Pg and T levels resulted into similar decline as that of CMF and endocrine therapy alone. In contrast to chemo- and endocrine therapy, the combined therapy consequenced into an increase in Andro. levels (Table -6). None of the differences of circulating steriods amongst patients treated with chemoendocrine therapy were statistically significant (Fig 1).

TYPE OF THERAPY IN RELATION TO RELAPSE FREE SURVIVAL:

The relapse free survival of patients who developed recurrence was noted and it was observed that though statistically non-significant, the remissions obtained with endocrine manipulations resulted into longer relapse free survival. The shortest relapse free survival was obtained with chemoendocrine therapy (Table - 7). Significant difference was not observed in the relapse free survival as majority of our patients had advanced disease.

CORRELATION OF SERUM ESTRADIOL AND ESTROGEN RECEPTORS:

We have observed significantly high E_2 levels with low ER

values. However, the difference was statistically non-significant. On the other hand, no such trend was observed in advanced stage of breast cancer. Only 2/111 of our patients had elevated E_2 (more than 355.Ø pg/ml) while none of our patients had elevated Pg (Table - 8; Figs. 6-7).

DISCUSSION

A number of epidemiologic and endocrinologic investigations have suggested an association between hormones and breast cancer. However, to date no single finding indicates that an endocrine abnomality in terms of hormone production or metabolism is the cause or is related to the growth of breast cancer (Bhatavdekar et al, 1987). The present study, found a significant reduction in E_2 and no change in T and Andro. while Pg showed a significant increase in breast cancer patients when compared with controls. These patients were divided into two groups: (1) stage II patients and (2) advanced breast cancer patients. In stage II patients E_2 was low, no significant change in T and Andro. and slightly high levels of Pg were observed when compared with controls. In advanced stages, there were low levels of E_2 and T with no remarkable change in Pg and Andro.. However, there are controversies regarding the levels of these hormones in breast cancer (England et al, 1974; Malarkey et al, 1977;

Sherman et al, 1979; Drafta et al, 1980; Moore et al, 1982; Bruning et al, 1985; Siiteri et al, 1986; Meyer et al, 1986). Moore et al (1982) and Bruning et al (1985) have affirmed that there is an increase in bio-available E_2 (free E_2 + Albumin bound E_2) in breast cancer patients. Majority of these studies carried out in western countries were on early breast cancers and without considering the menopausal status, while in the present study, 84/111 (75.6%) patients had advanced breast cancer. Moreover, the ratio between Andro: E_2 was significantly higher in breast cancer patients when compared with controls. This clearly suggested an imbalance between androgen and estrogen. (van Landegham et al, 1985; Key and Pike, 1988; Secreto et al, 1989).

The present study showed a non-significant decline in steriod hormones except Andro. as stage advanced, which was in sharp contrast to prolactin (Chapter III). Breast tumors are heterogenous and at an advanced stage they escape hormonal control. We also have observed high levels of prolactin and epidermal growth factor receptors (EGF-R) (unpublished data) with significantly low concentrations of ER and PR in advanced breast cancer. Therefore, from our unpublished data, it appears that high levels of prolactin and EGF-R with increased ratio of Andro:E_2 might have a cumulative effect on tumor aggressiveness.

We know that high nodal positivity and poor differentiation of the tumor is a sign of poor prognosis. We have observed low levels of E_2 and significantly high levels of T and Andro. in node positive and poorly differentiated tumors as compared to node negative and grade I histology respectively (statistically non-significant).

It was observed that chemotherapy resulted into diminished circulating E_2 and Pg levels. These results corroborate with other studies (Rose and Davis, 1977; Dnistrian et al, 1985; Jordan et al, 1987; Padmanabhan et al, 1987) and confirms that the administation of cytotoxic drugs may result in primary ovarian failure in pre-menopausal breast cancer patients. Ovarian failure was documented by a decrease in the estradiol:gonadotropin ratio (Chapter IV), resulting in hormonal profiles characteristic of women after natural menopause. It is also known that the ovarian failure induced by chemotherapy occurs earliar in older premenopausal patients and there is an inverse relation between age and duration of treatment required to induce ovarian suppression (Dnistrian et al, 1985). Drug induced ovarian suppression has been well documented as a frequent side effect of cytotoxic chemotherapy but its possible therapeutic benefit in breast cancer has been difficult to evaluate. This phenomenon was especially true for our

advanced breast cancer patients who developed recurrences within a short period after completion of cytotoxic chemotherapy (Figs. 1-4). Cytotoxic chemotherapy with CMF does not significantly affect adrenal steriod metabolism since androgen secretion is preserved and any decrease in Andro. can be attributed to the ovarian source (Rose and Davis, 198Ø).

Endocrine treatment also resulted into ovarian suppression. We have observed non-significant decline in E_2 which probably suggested that TMX might act as an estrogen than as an antiestrogen at the hypothalamic pituitary level, because it is well known that estrogen like effect seems to prevail when the levels of circulating estrogens are low (Delrio et al, 1986). Contrary to our results, various studies (Sherman et al, 1979 ; Rose and Davis, 198Ø ; Manni and Pearson, 198Ø ; Jordan et al, 1987) revealed an increase in ovarian estrogen production. Majority of their studies were carried out in early breast cancer patients. The chemoendocrine therapy resulted into decreased Pg and no significant change in E_2. The increased levels of Andro. with decreased T levels might be due to an altered adrenal metabolism.

We have observed a decreased ER concentration with an increase in circulating E_2 in stage II breast cancer

patients only. Our result of stage II patients, correlate with Vihko et al (198Ø) who found a variety of correlations between serum estradiol content and tumor cytosol receptor concentrations in pre- and post-menopausal patients.

ABSTRACT

The second chapter features the incidence of circulating Estradiol (E_2), Progesterone (Pg), Testosterone (T) and their major precursor Androstenedione in premenopausal breast carcinoma patients and the levels were compared with controls. The results obtained with these steroid hormones at diagnosis were grouped taking (i) stage and (ii) nodal status into consideration. A statistically non-significant trend of decrease in progesterone was observed as stage advanced. Circulating progesterone in N_1 tumour bearing patients was significantly higher ($P < Ø.Ø1$) than patients with N_3 tumours at presentation.

Section B of the chapter is directed to correlate the changes in sex steroids with change in disease status. The levels of E_2, Pg, T and Androstenedione at presentation were compared with the levels before clinical progression and at clinical relapse. A statistically significant decrease in E_2 ($P < Ø.Ø1$) and Pg ($P < Ø.Ø5$) was observed at clinical relapse. Similarly, the levels of these steroids in

responders were compared with the levels at last follow-up. It was revealed that the levels of estradiol were significantly reduced ($P < 0.05$) amongst responders.

The fluctuations in sex steroids with type of therapy is presented in part C of the chapter. Section D points towards relation of circulating estradiol and progesterone levels with estrogen- and progesterone-receptors.

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TABLES

Table 1 : Serum steroid hormones in premenopausal breast carcinoma patients and controls (M ± SE)

	N	E_2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml	Andro : E2
Controls	15	167.20 ± 24.18 ⁎	1.38 ± 0.34 @	0.63 ± 0.43	2.29 ± 0.55	0.0093 ± 0.003 $
Breast cancer patients	111	090.93 ± 09.53 ⁎	2.49 ± 0.37 @	0.41 ± 0.05	2.00 ± 0.15	0.0380 ± 0.006 $

⁎ - P < 0.01 $ - P < 0.001 @ - P < 0.05

Table 2 : Serum steroid hormones in relation to stage [M ± SE]

	N	E_2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml
II	27	110.20 ± 19.39	3.63 ± 1.03	0.62 ± 0.18	1.87 ± 0.29
III	57	085.19 ± 14.55	1.85 ± 0.36	0.34 ± 0.05	2.12 ± 0.21
IV	16	088.72 ± 23.38	1.44 ± 0.45	0.37 ± 0.07	2.09 ± 0.37
Entered at relapse	11	077.90 ± 16.31	4.34 ± 1.65	0.34 ± 0.06	1.31 ± 0.46
III+IV+Rec	84	084.66 ± 10.95	2.11 ± 0.35	0.35 ± 0.03	2.04 ± 0.17

Table 3 : Steroid hormones in relation to nodal status [M ± SE]

	N	E_2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml
Node negative	11	114.24 ± 41.03	4.79 ± 2.03	0.27 ± 0.08	1.75 ± 0.41
Node positive	89	890.52 ± 10.84	1.94 ± 0.30	0.44 ± 0.06	2.08 ± 0.16

Table 4 : Steroid hormones in relation to histologic grade [M ± SE]

Histologic grade	N	E2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml
				*	
I	08	071.90 ± 23.66	1.71 ± 0.94	0.26 ± 0.06	1.26 ± 0.46
II	37	111.40 ± 20.98	2.59 ± 0.74	0.37 ± 0.09	2.01 ± 0.26
III	23	065.50 ± 19.32	1.27 ± 0.50	* 0,55 ± 0.12	2.31 ± 0.40
II+III	60	094.68 ± 15.17	2.10 ± 0.51	0.44 ± 0.07	2.11 ± 0.22

* - P < 0.05

Table 5 : Steroid hormones according to disease status [M ± SE]

Disease Status	E2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml
I. Patients who developed recurrence N = 31 :				
At Diagnosis	78.14 ± 22.22 *	2.01 ± 0.72 @	0.39 ± 0.06	1.60 ± 0.35
Before Progression	46.68 ± 10.06	1.32 ± 0.39	0.42 ± 0.08	1.93 ± 0.41
At Progression	13.60 ± 02.31 *	0.53 ± 0.15 @	0.72 ± 0.36	1.28 ± 0.27
II. Pesponders N=21 :				
At Diagnosis	76.53 ± 14.20 **	3.19 ± 1.18	0.46 ± 0.16	1.09 ± 0.24
At last F/U	39.38 ± 08.41 **	1.59 ± 0.90	0.36 ± 0.05	1.57 ± 0.27

* - P < 0.01 @,** - P < 0.05

Table 6 : Effect of therapy on steroid hormones [M ± SE]

Therapy	N		E_2 pg / ml	Pg ng / ml	T ng / ml	Andro ng / ml
Chemo	22	Prethera	96.43 ± 29.96 *	2.84 ± 0.81	1.81 ± 0.52	1.81 ± 0.52
		After therapy	21.75 ± 04.43 *	1.80 ± 0.69	0.38 ± 0.09	1.21 ± 0.19
Endocrine	08	Prethera	52.45 ± 30.95	1.92 ± 1.24	0.38 ± 0.10 @	1.37 ± 0.66
		After therapy	41.33 ± 16.10	0.33 ± 0.21	0.12 ± 0.04 @	1.31 ± 0.41
Chemo-Endo	11	Prethera	51.84 ± 15.18	2.56 ± 1.40	0.55 ± 0.31	1.04 ± 0.26
		After therapy	52.27 ± 33.05	0.31 ± 0.20	0.36 ± 0.15	1.35 ± 0.32

* - $P < 0.02$ @ - $P < 0.05$

Table 7 : Relpase free survival in relation to therapy in patients who developed recurrence [M ± SE]

Therapy	N	Relapse free survival in months
Chemo	15	Ø9.5Ø ± 1.33
Endocrine	Ø3	1Ø.41 ± 3.2Ø
Chemo-Endo	Ø9	Ø8.56 ± 1.81

Table 8 : Correlation of circulating estradiol and estrogen receptor [N ± SE]

			Estrogen receptor positive			Estrogen receptor negative	
		N	ER fmol/mg cytosol protein	E2 pg / ml	N	E2 pg / ml	
E2 < 100 pg/ml	Stage II	09	46.61 ± 10.60	038.16 ± 09.44 *	05	048.38 ± 014.14	
	Advanced	36	47,97 ± 09.06	028.33 ± 04.02 $	21	045.07 ± 006.92	
E2 100-200 pg/ml	Stage II	04	37.43 ± 09.88	109.43 ± 28.85	04	141.25 ± 019.08	
	Advanced	08	46.64 ± 19.41	141.18 ± 09.00	09	127.12 ± 010.25	
E2 > 200 pg/ml	Stage II	03	23.33 ± 05.60	290.00 ± 75.00 *	01	244.83	
	Advanced	06	42.98 ± 18.66	271.26 ± 19.92 $	03	286.56 ± 116.09	

* - P < 0.01 $ - P < 0.0001

FIGURES

I. PATIENTS WHO DEVELOPED PROGRESSIVE DISEASE (Figs. 1-3):

Fig.1

The disease course in a stage II, grade II patient is described in Fig. 1. The patient was treated with SEM followed by CMF. All the steroids showed a decline after CMF. Patient was relapse free for 8 months and then she developed 2o deposits in lungs and bone and was instituted second line chemotherapy. When combined chemohormonal therapy was instituted,the Andro. levels initially increased and a decline was evidenced in all steriods. The patient however, did not respond to therapy and ultimately succumbed to death 5 months after detection of relapse.

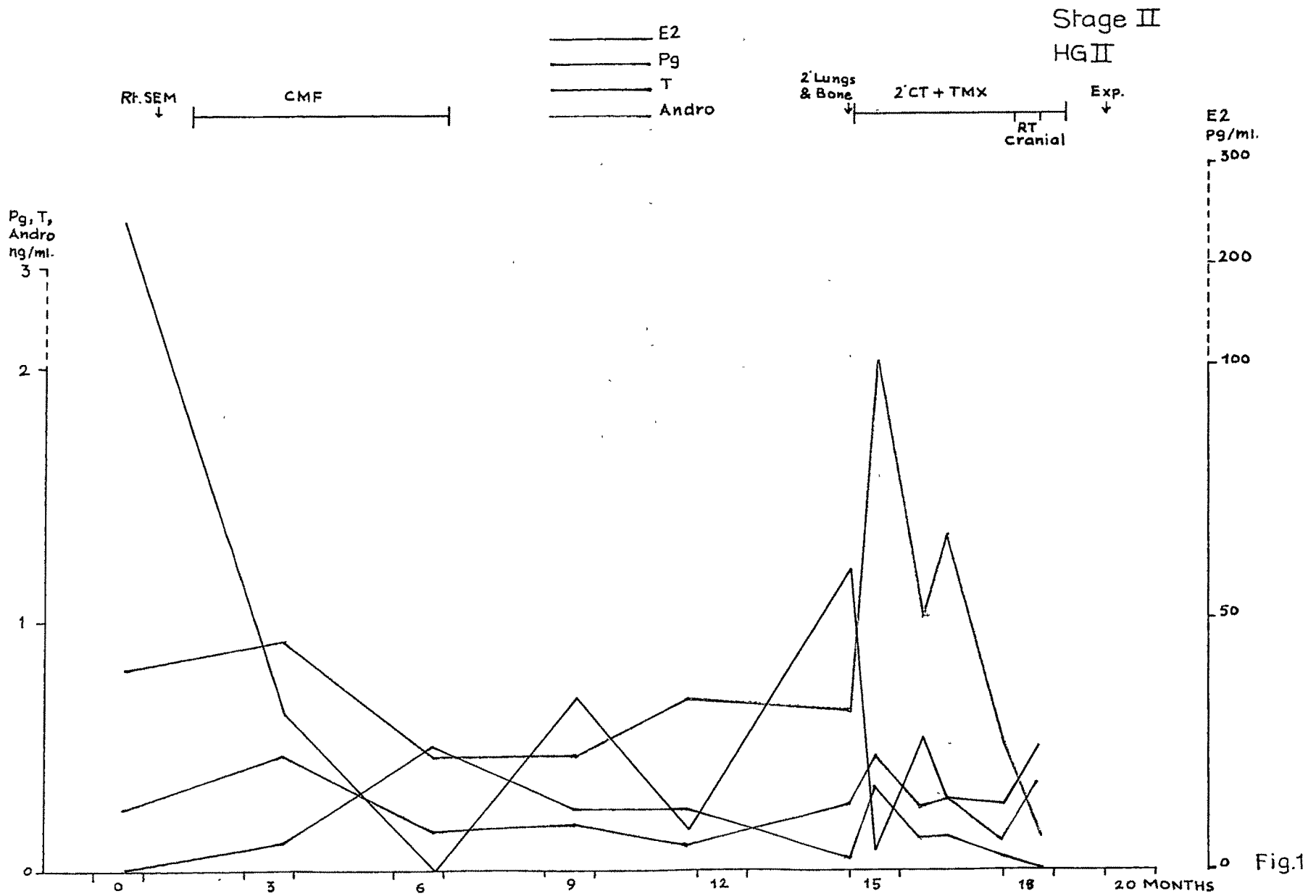

Stage II
HG II
E2
Pg
T
Andro
Rt. SEM
CMF
2' Lungs & Bone
2' CT + TMX
RT cranial
Exp.
Pg, T, Andro ng/ml.
E2 pg/ml.
3
2
1
0
300
200
100
50
0
0
3
6
9
12
15
18
20 MONTHS

Fig.1

Fig. 2

A stage IV patient was treated with SEM followed by CMF. Within 3 months, the patient developed supraclavicular nodes while chemotherapy was being given. The Andro. levels declined throughout the disease course. After the development of supraclavicular nodes, the patient demonstrated an increase in E_2 which reduced in 7 months with dissemination of disease in lungs and brain. All the hormone levels showed a decline with dissemination. The patient died in 3 weeks.

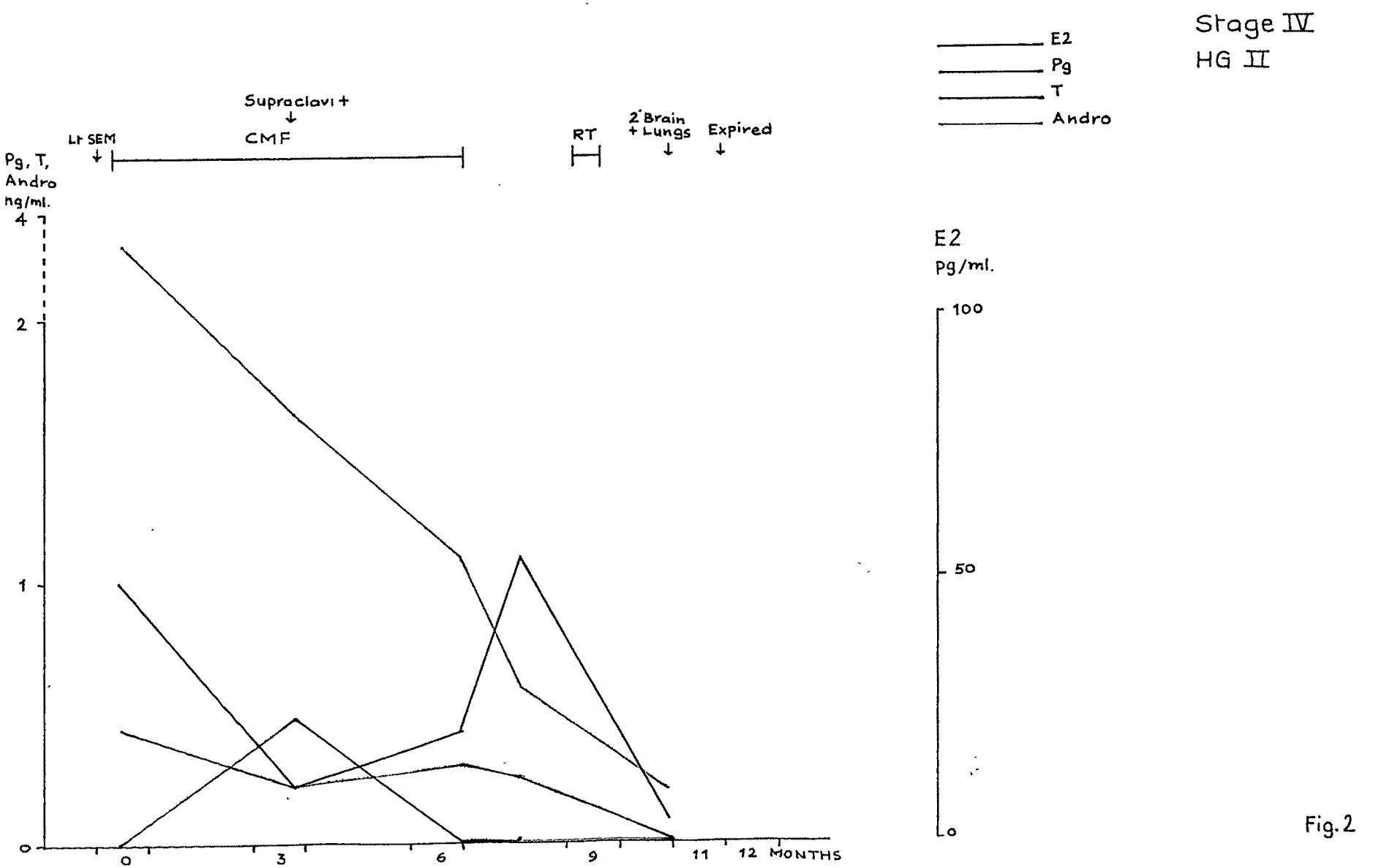
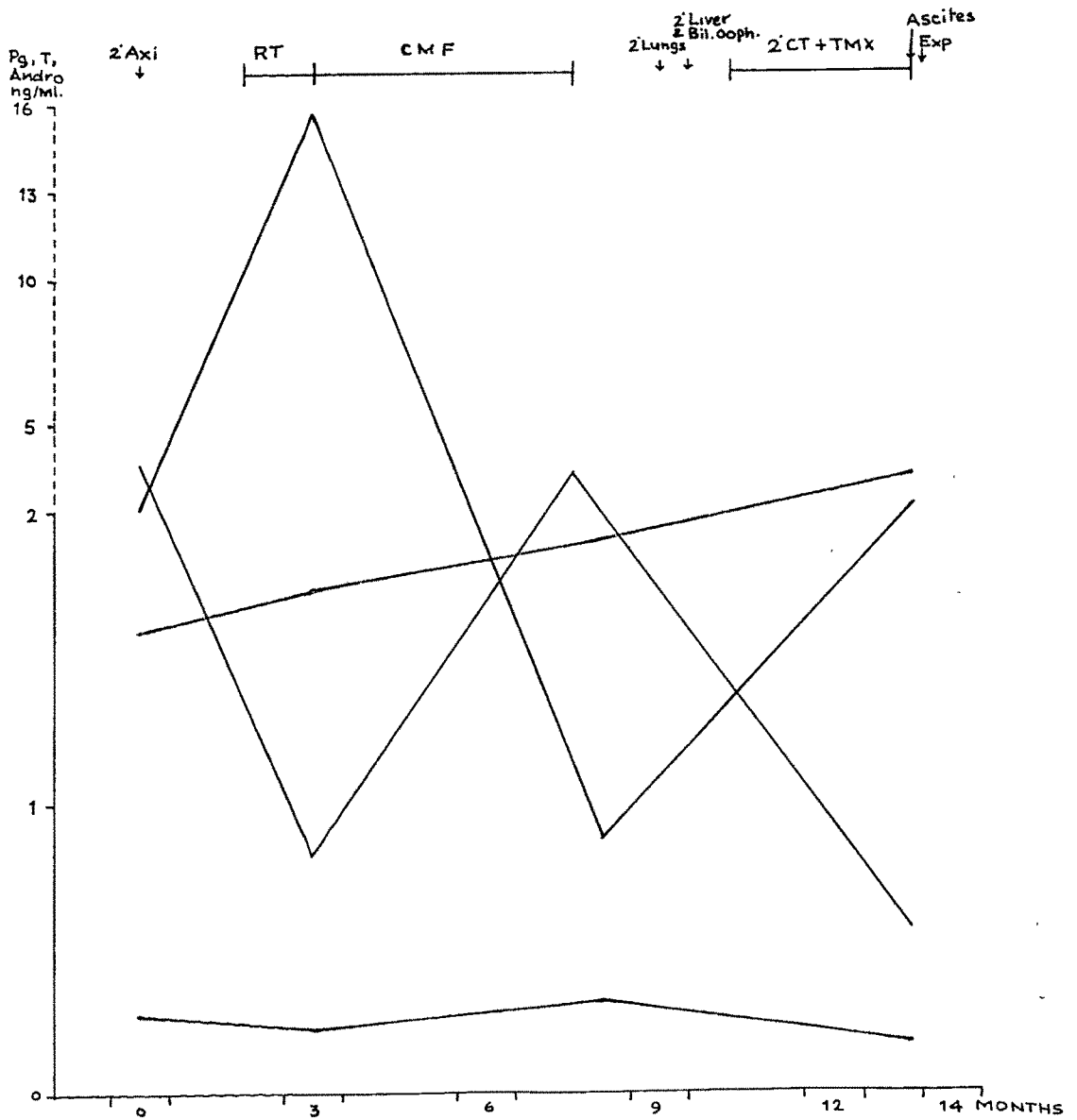

Stage IV
HG II
E2
Pg
T
Andro
Supraclavi +
CMF
Lt SEM
RT
2° Brain
+ Lungs
Expired
Pg, T,
Andro
ng/ml.
4
2
1
0
0
3
6
9
11
12 MONTHS
E2
pg/ml.
100
50
0

Fig. 2

Fig. 3

A patient reported with secondaries in axilla. The treatment offered to the patient was axillary clearance followed by radiotherapy and CMF. The patient developed metastatic disease in lungs and liver after 1.5 months of CMF. The patient was then treated with bilateral oophorectomy followed by second line chemoendocrine therapy. Finally patient developed ascites which culminated into death. The Andro. levels showed an increase during this period also. T levels were low throughout the course of the disease.



Rec. Ca.
HG II

E2
Pg
T
Andro

E2
Pg/ml.
200
100
50
0

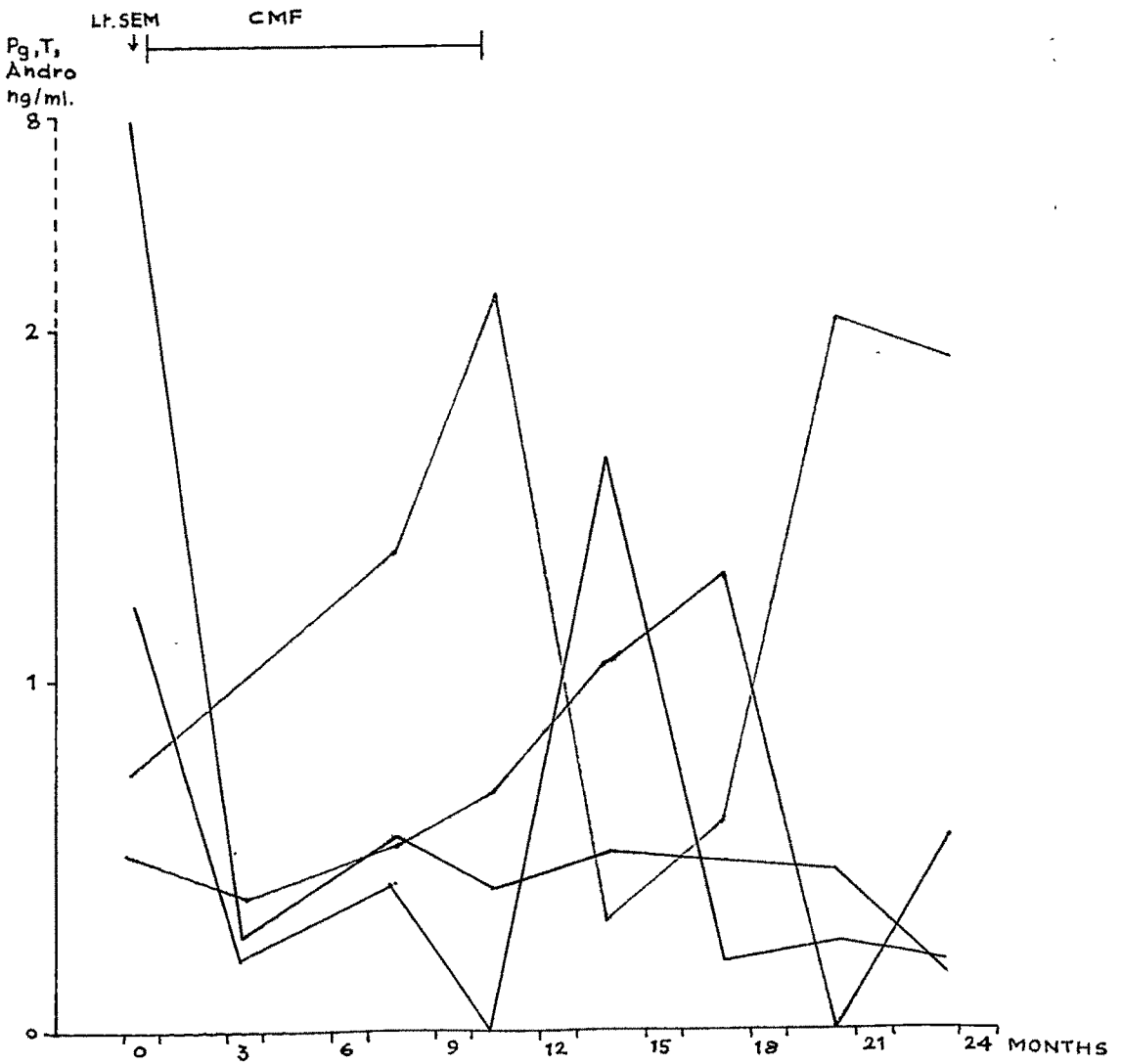
Fig.3

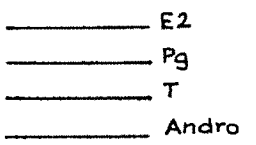
II. RESPONDERS (Figs. 4-5):

Fig.4

A stage II patient was treated with surgery followed by CMF. The patient remained relapse free at the end of 2 years. E2 and Pg levels decreased after CMF. at the end of 2 years, all the four hormones were within normal limits.

Stage II
HG II





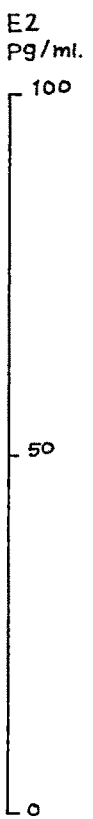
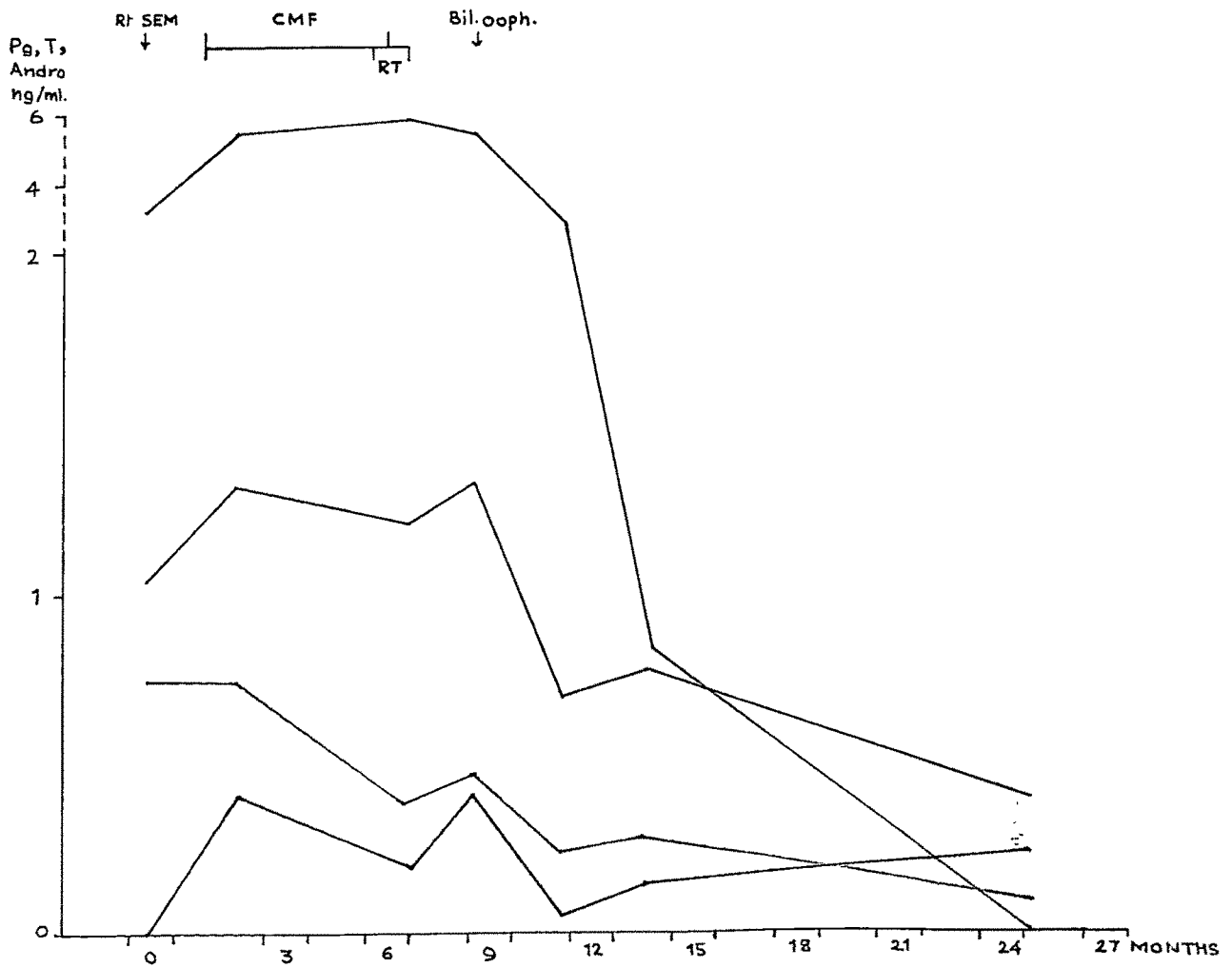


Fig. 4

Fig. 5

A stage III, grade II patient was treated with surgery, CMF, radiotherapy. Bilateral oophorectomy was performed after 1.5 months. The E_2, Pg, T and Andro. levels decreased after therapy. The patient remained relapse free at the end of 2 years.



Stage III
HG II

E2
Pg
T
Andro

E2
Pg/ml.
100
50
0

Fig.5

Fig.6

Scatterogram showing circulating estradiol and estrogen receptor.

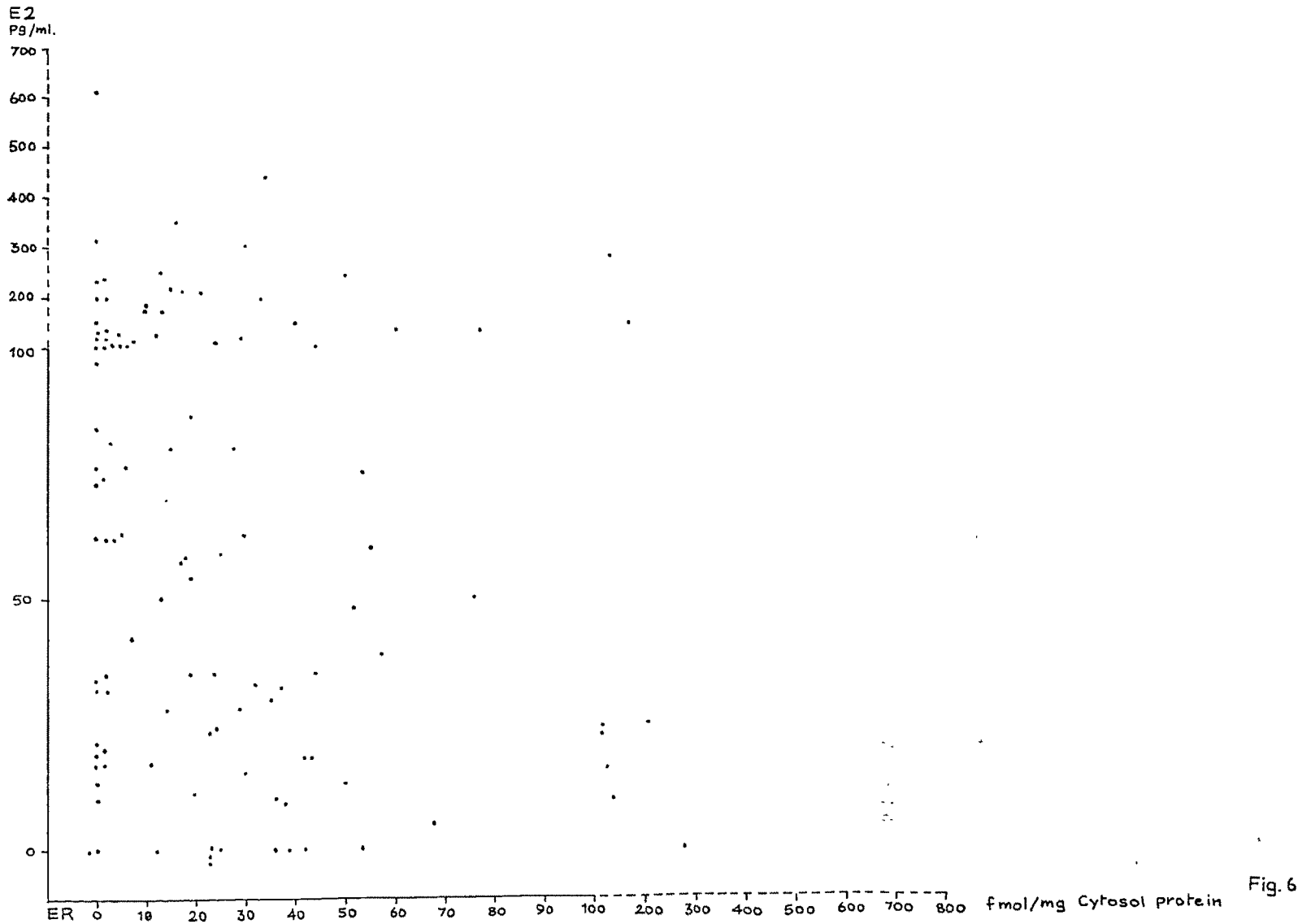

E2
Pg/ml.
700
600
500
400
300
200
100
50
0
ER
0
10
20
30
40
50
60
70
80
90
100
200
300
400
500
600
700
800
fmol/mg Cytosol protein

Fig. 6

Fig. 7

Scatterogram showing circulating estradiol and progesterone receptor.

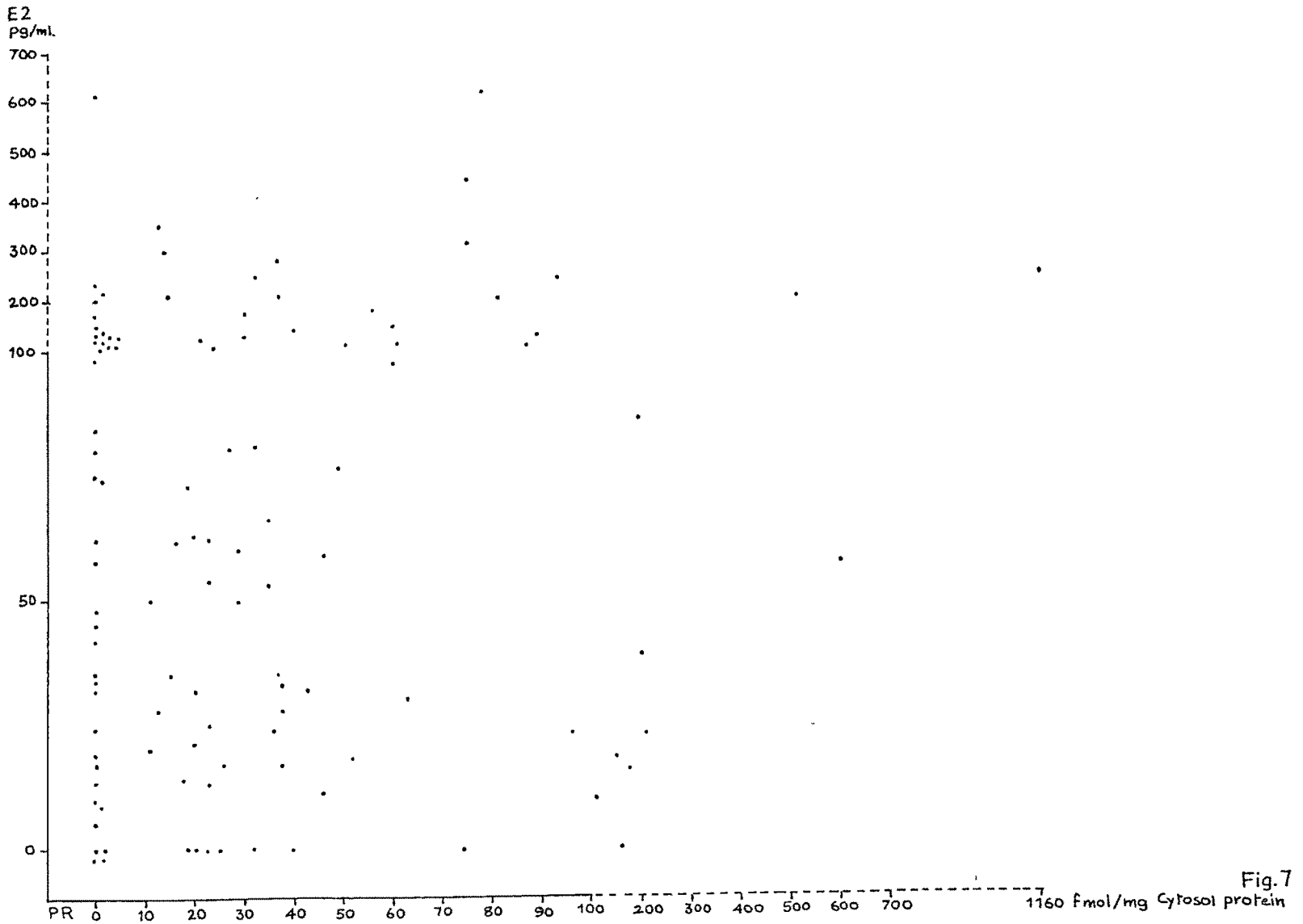

E2
pg/ml.
700
600
500
400
300
200
100
50
0
PR
0
10
20
30
40
50
60
70
80
90
100
200
300
400
500
600
700
1160 fmol/mg Cytosol protein

Fig. 7