Eur J Cancer Clin Oncol, Vol. 23, No. 6, pp. 745–748, 1987.
Printed in Great Britain

0277–5379/87$3.00+0.00

Ovarian Function and Adjuvant Chemotherapy for Early Breast Cancer

N. PADMANABHAN,* D.Y. WANG,† J.W. MOORE† and R.D. RUBENS*‡

**ICRF Clinical Oncology Unit, Guy's Hospital, St Thomas Street, London SE1 9RT and †ICRF Clinical Endocrinology Laboratory, Lincoln's Inn Fields, London WC2A 3PX, U.K.*

Abstract—*The effect of cyclophosphamide, methotrexate and fluorouracil (CMF) on ovarian function has been studied in 74 pre-menopausal patients with operable breast cancer. After median follow-up of 47 months, 50, 70 and 80% of 35 patients receiving CMF became permanently amenorrhoeic within 3, 6 and 12 months respectively; in contrast, only 5 in the no treatment (control) group of 39 patients became permanently amenorrhoeic within 12 months. Younger patients (less than 35 years) were more likely to retain or regain menstrual function while on or after CMF treatment.*

Estimation of ovarian and pituitary hormones in a subset of these women showed that CMF treatment was associated with a decrease in serum oestradiol and progesterone and an increase in serum follicle stimulating hormone and luteinizing hormone to post-menopausal levels. These hormonal changes are consistent with the induction of amenorrhoea during CMF treatment and the absence of resumption of menstrual function after completion of treatment suggests that CMF causes permanent ovarian ablation in a majority of these patients.

INTRODUCTION

ADJUVANT chemotherapy with cyclophosphamide, methotrexate and fluorouracil (CMF) after primary treatment of operable breast cancer results in a significant prolongation of relapse-free and overall survival in pre-menopausal patients [1, 2]. Because the benefit of adjuvant CMF is essentially confined to pre-menopausal patients and adjuvant endocrine treatment is of value in pre-menopausal patients [3], it is pertinent to assess the extent to which CMF modifies ovarian function. For this purpose, serum pituitary and ovarian hormone levels were measured and related to the menstrual history recorded in pre-menopausal patients in a randomized trial of CMF against no treatment (control group) after mastectomy.

PATIENTS AND METHODS

Patients

Seventy-five pre-menopausal patients (last menstrual period within 1 year of entry into the trial or hysterectomy with conservation of the ovaries and less than 50 years of age) with breast cancer and involved axillary nodes were eligible for this study. These patients after mastectomy and axillary clearance were randomized (from October 1979 to June 1982) to receive either CMF (cyclophosphamide 80 mg/sq m orally days 1–14, methotrexate 32 mg/sq m i.v. days 1 and 8 and 5-fluorouracil 480 mg/sq m i.v. days 1 and 8, 28-day cycles) or no treatment (control group). The details of the protocol and the first analysis of this trial have been published [2]. Serum samples were obtained from patients before and 8–10 days after surgery, at 3 and 6 months after commencement of chemotherapy and at 6 weeks after completion of chemotherapy (12 month sample). The timing of samples was identical in the control group. For both groups the time of blood sampling was dependent solely on the date of mastectomy and was not related to the phase of menstrual cycle. Serum samples were aliquoted and preserved at −20° C until analysis. Menstrual history was obtained from patient records for all 39 control patients and for 35 patients treated with CMF (1 patient had had hysterectomy with retention of ovaries and was excluded). Of these patients, serum samples were available from 20 treated and 23 control group patients. The complete set of serial blood samples was available from 9 treated and 11 control group patients.

Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin

Accepted 24 November 1986.
‡Author to whom correspondence and requests for reprints should be addressed.

Table 1. *Patient characteristics*

	All patients		Hormone profile group	
	Controls	CMF	Controls	CMF
Total No.	39	35	23	20
Age (yrs) median and range	46 (28–55)	45 (25–55)	45 (29–54)	44 (31–54)
Within 12 months of entry into the trial				
No menopause	34	8	21	5
Age median (range)	45 (28–53)	35 (25–38)	44 (29–52)	35 (31–38)
% CMF median (range)	—	54 (35–98)	—	60 (40–92)
Menopause	5	27	2	15
Age median (range)	52 (52–55)	46 (35–55)	53 (52–53)	45 (35–55)
% CMF median (range)	—	75 (45–100)	—	75 (45–98)

were determined using a second antibody radioimmunoassay method (Amersham International plc, FSH Cat. No. IM 2070, LH Cat. No. IM 2080, prolactin Cat. No. IM 1061). Serum oestradiol and progesterone were determined using a solid-phase radioimmunoassay method (Diagnostic Products Corporation, Oestradiol Cat. No. TKE 25 and progesterone Cat. No. TKPG 2). Dehydroepiandrosterone (DHEA) and its sulphate ester (DHEAS) were measured by radioimmunoassay [4].

Groups were compared using the 2-sample Wilcoxon-rank test for unpaired data [5]. The log-rank test [6] was used to assess the significance of the difference in the time to permanent amenorrhoea (no resumption of menstrual periods over a median follow-up of 4 yr).

RESULTS

Patient characteristics are detailed in Table 1.

Menstrual function

In contrast to 5/39 patients in the control group, 27/35 patients treated with CMF became amenorrhoeic within 12 months of commencing treatment ($P < 0.001$, Chi-square = 31.09) and had no resumption of menstrual function during subsequent follow up (median 47 months). On plotting the time to menopause, the probability of becoming permanently amenorrhoeic was 50, 70 and 80% at 3, 6 and 12 months respectively for patients treated with CMF and 3, 10 and 17% for patients in the control group. These differences between CMF-treated and control patients were highly significant ($P < 0.001$, Fig. 1H). Of the 8 patients treated with CMF but without induction of amenorrhoea during treatment, 5 had intact menstrual function during and after treatment and in 1 menstrual function resumed 5 months after completion of CMF; in the remaining 2 patients menstrual function ceased 2 and 15 months after completion of CMF treatment. These 8 patients were significantly younger than CMF-treated patients who became amenorrhoeic ($P < 0.01$, Table 1). Although the median cumulative dose of CMF (expressed as % projected dose) received by patients who became permanently amenorrhoeic was higher than in those without permanent amenorrhoea (Table 1), this difference was not statistically significant (2-sample Wilcoxon-rank test and Chi-square test after classifying patients in each group as $> = 65\%$ and $< 65\%$ CMF dose, $0.1 > P > 0.05$). In the control group, patients who attained spontaneous menopause within 12 months were significantly older than patients who did not become menopausal ($P < 0.001$, Table 1).

Hormone profile

In the control group the levels of FSH and LH rose gradually from before operation to 12 months; only the difference between the levels preoperatively and at 12 months was significant ($P < 0.05$, Fig. 1A and B). In contrast, the CMF group levels of FSH and LH rose sharply to post-menopausal levels by 3 months and remained at these levels at 6 and 12 months ($P < 0.001$ preoperative v 3, 6 and 12 months; except for LH at 3 months $P < 0.01$, Fig. 1A and B). The difference in the levels of FSH and LH between control and CMF-treated patients at 3, 6 and 12 months was significant ($P < 0.01$; except for LH at 3 months $P < 0.02$).

There were no significant alterations in the levels of oestradiol and progesterone in the control group

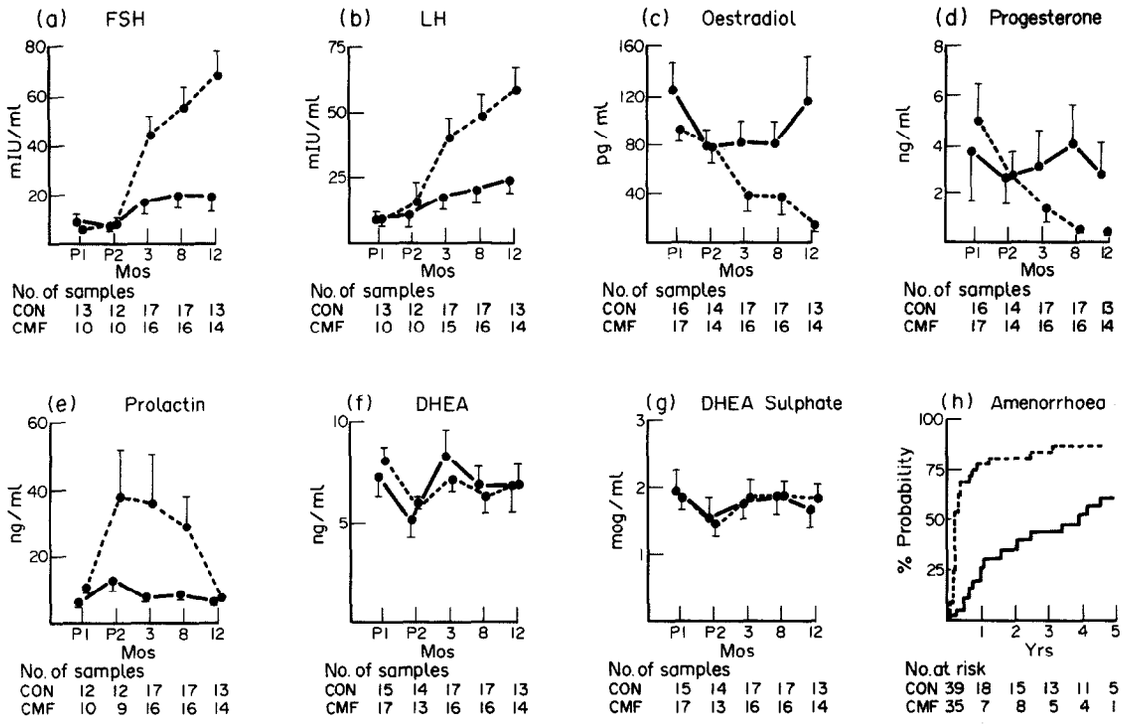

Fig. 1. A comparison of hormone profiles (A–G), mean levels and 1 S.E. (vertical bars), in control (solid line) and CMF treated patients (dotted line). For P values see text (mean + S.E. used for clarity of figures, statistics by 2 sample Wilcoxon-rank test for unpaired data). P1 = Preoperative, P2 = Post-operative, Mos = months, Con = Controls. In H the time to permanent amenorrhoea has been plotted for control (solid line) and CMF-treated (dotted line) patients. Yrs = Years.

of patients from before operation and during the next 12 months (Fig. 1C and D). In contrast, for patients treated with CMF, the levels of oestradiol dropped sharply to postmenopausal levels from 3 months onwards and the difference between the levels preoperatively and at 3, 6 and 12 months was highly significant ($P < 0.001$, Fig. 1C). The levels of progesterone declined less rapidly in patients treated with CMF and the levels at 6 and 12 months were significantly lower than the preoperative levels ($P < 0.01$, Fig. 1D). The difference in the levels of oestradiol between control and CMF-treated patients at 3, 6 and 12 months was significant ($P < 0.01$, Fig. 1C) and for progesterone only the difference at 12 months was significant ($P < 0.01$, Fig. 1D).

The post-operative levels of prolactin were significantly higher than the preoperative levels in both control ($P < 0.05$, Fig. 1E) and CMF-treated patients ($P < 0.01$, Fig. 1E). There was no significant difference between the level of prolactin before operation and at 3, 6 and 12 months (Fig. 1E) in either the control or CMF-treated patients. Patients treated with CMF had higher levels of prolactin compared to controls but this was significant only for the post-operative sample ($P < 0.05$) and at 3 months ($P < 0.05$).

There were no significant alterations in the levels of dehydroepiandrosterone and its sulphate ester in either control or CMF-treated patients (Fig. 1F and G).

DISCUSSION

This study shows that the majority of pre-menopausal patients with early breast cancer treated with adjuvant CMF become amenorrhoeic within 6 months of starting treatment. The hormonal studies in a subset of these patients show that the amenorrhoea is associated with elevated levels of pituitary gonadotrophins and decreased levels of estradiol and progesterone—a pattern consistent with menopause or ovarian failure. The absence of resumption of menstrual function in patients who become amenorrhoeic (median age of 46 years) on CMF treatment when followed up for a median period of 4 years suggests that the ovarian failure associated with CMF treatment is likely to be permanent. Patients younger than 35 years are more likely to retain or regain menstrual function during or after CMF treatment suggesting that, for a given dose of CMF, the age of the patient determines whether or not permanent amenorrhoea is induced.

The mildly elevated levels of FSH and LH at 12 months in the control group of patients can be explained by natural menopause attained by some of the older patients in this group. Surgical stress is known to cause elevations of serum prolactin levels [7] and the higher levels of prolactin in CMF-treated patients compared to controls could have been due to stress associated with cytotoxic treatment and/or the regular use of antiemetics. As dehydroepiandrosterone sulphate and dehydroepi-

androsterone are principally derived from the adrenal glands, the absence of significant changes in the levels of these hormones indicates that CMF has little, if any, effect on adrenal secretion of these hormones.

These findings confirm those of others [8, 9, 10, 11] who have reported a similar incidence of amenorrhoea, similar alteration in the pattern of hormonal profile and the importance of age as a factor determining whether amenorrhoea is induced or not during adjuvant chemotherapy. Furthermore, we find that CMF-induced amenorrhoea is likely to be permanent in a majority of pre-menopausal patients. This observation in conjunction with the findings that benefit of adjuvant CMF treatment is confined to (a) pre-menopausal, but not post-menopausal, patients; (b) pre-menopausal patients with progesterone receptor-positive tumours; and (c) pre-menopausal patients with permanent CMF-induced amenorrhoea [12] suggest that CMF-induced ovarian ablation makes a major contribution to the clinical results of adjuvant CMF therapy.

REFERENCES

1. Bonadonna G, Rossi A, Tancini G, Valagussa P. Adjuvant chemotherapy in breast cancer. *Lancet* 1983, **1**, 1157.
2. Howell A, Bush H, George WD, *et al.* Controlled trial of adjuvant chemotherapy with cyclophosphamide, methotrexate and fluorouracil for breast cancer. *Lancet* 1984, **2**, 307–311.
3. Meakin JW, Allt WEC, Beale FA, *et al.* Ovarian irradiation and prednisone following surgery and radiotherapy for carcinoma of the breast. *Breast Cancer Res Treat* 1983, **3** (suppl 1), 45–48.
4. Wang DY, Moore JW, Thomas BS, *et al.* Plasma and urinary androgens in women with varying degrees of risk of breast cancer. *Eur J Cancer* 1979, **15**, 1269–1274.
5. Swinscow TDV. Statistics at square one—XVII. Some non-parametric tests. *Br Med J* 1976, **2**, 632–634.
6. Peto R, Pike MC, Armitage P, *et al.* Design and analysis of randomised clinical trials requiring prolonged observation of each patient. Part II, analysis and examples. *Br J Cancer* 1977, **35**, 1–39.
7. Wang DY, Hampson S, Kwa HG, *et al.* Serum prolactin levels in women with breast cancer and their relationship to survival. *Eur J Cancer Clin Oncol* 1986, **22**, 487–492.
8. Bonadonna G, Brusamolino E, Valagussa P, *et al.* Combination chemotherapy as an adjuvant treatment in operable breast cancer. *N Engl J Med* 1976, **294**, 405–410.
9. Recchione C, Rossi A. Hormonal study in patients developing amenorrhoea during adjuvant chemotherapy for breast cancer. *Tumori* 1979, **65**, 93–97.
10. Rose DP, Davis TE. Ovarian function in patients receiving adjuvant chemotherapy for breast cancer. *Lancet* 1977, **1**, 1174–1176.
11. Dnistrian AM, Schwartz MK, Fracchia AA, Kaufman RJ, Hakes TB, Currie VE. Endocrine consequences of CMF adjuvant therapy in premenopausal and postmenopausal breast cancer patients. *Cancer* 1983, **51**, 803–807.
12. Padmanabhan N, Howell A, Rubens RD. Mechanism of action of adjuvant chemotherapy in early breast cancer—an hypothesis. *Lancet* 1986, **ii**, 411–414.