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Prognostic effect of amenorrhoea and elevated serum gonadotropin levels induced by adjuvant chemotherapy in premenopausal node-positive breast cancer patients

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Abstract

The purpose of the study was to determine the correlation between prognosis and chemotherapy induced amenorrhoea or elevated gonadotropin levels in node-positive breast cancer patients. Since we have previously found a better prognosis in patients with more profound leucopenia induced by adjuvant chemotherapy, we examined whether this effect was mediated through more efficient induction of amenorrhoea. The study population consisted of 126 premenopausal, primarily operable, node-positive breast cancer patients treated with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) adjuvant chemotherapy at the Department of Oncology, Helsinki University Central Hospital between 1990 and 1993. 12 months after the beginning of adjuvant chemotherapy, the patients were divided into groups with respect to their menstrual function (regular menstruation, irregular menstruation or amenorrhoea). Information about menstruation status and serum concentration of follicle stimulating hormone (FSH) and oestradiol were recorded at 12 and 24 months from the beginning of adjuvant chemotherapy. Median follow-up time was 72 months. Women who experienced amenorrhoea or had irregular menstruation after chemotherapy had a significantly better 5-year disease-free survival (DFS) in univariate analysis than women who continued to menstruate ($P=0.02$). Amenorrhoea and irregular menstruation were associated with a better DFS among patients with oestrogen receptor (ER) positive primary tumours ($P=0.007$), whereas no such association was found in ER negative cases ($P=0.86$). 5-year overall survival (OS) in univariate analysis was also better in patients who experienced amenorrhoea (81%) or who had irregular menstruation (90%) after chemotherapy as compared with patients with regular menstruation (68%; 81 versus 68%, $P=0.05$). The serum FSH level did not correlate significantly with outcome irrespective of the cut-off point chosen. Nodal status, tumour size and menstruation status after chemotherapy were also significantly associated with DFS in a multivariate analysis. The menstruation status after chemotherapy lost its significance for OS in a multivariate analysis whilst the number of affected lymph nodes, tumour size and oestrogen/progesterone receptor status retained their impact. There was no association between the degree of leucopenia and induction of amenorrhoea by CMF. Chemotherapy-induced ovarian function suppression (amenorrhoea/irregular menstruation) after chemotherapy had a favourable effect on DFS in premenopausal breast cancer patients. The post-chemotherapy menstruation status is a clinically usable marker for sufficient endocrine effect of chemotherapy in ER/PR-positive patients in all premenopausal age groups. FSH level seemed to be a less reliable indicator of the castration effect of adjuvant chemotherapy in this study.

Keywords: Amenorrhoea; Breast cancer; Chemotherapy

1. Introduction

Adjuvant chemotherapy reduces the annual rates of recurrence and death in breast cancer [1]. The average incidence of permanent amenorrhoea induced by cyclophosphamide, methotrexate and 5-fluorouracil (CMF) adjuvant chemotherapy (cyclophosphamide administered intravenously (i.v.) on day 1 or orally during days 1–14) is 68% [2], but the rate depends on age at treatment. Since adjuvant cytotoxic chemotherapy has consistently been more effective in premenopausal than in postmenopausal breast cancer patients, it is conceivable that the effect of adjuvant chemotherapy might partly be mediated through chemical castration [3]. In several studies patients who develop amenorrhoea or have irregular menstruation after adjuvant chemotherapy have had better survival than those who continue to

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menstruate [3–13]. However, some studies have failed to demonstrate the association between chemotherapy-induced amenorrhoea and a better survival rate [14, 15]. The controversy on that subject could at least partly be explained by the lack of uniform definitions of menopause status and amenorrhoea [2]. Especially in early studies, the definition of amenorrhoea status varied, and patients with amenorrhoea or irregular menstruation were not always reported separately. In only a few of the earlier studies were gonadotropin and oestradiol hormone levels followed-up in addition to the menstrual status. The favourable effect of ovarian suppression on prognosis is mainly found in patients with a hormone receptor-positive cancer and in those younger than 40 years of age [8]. Here we report the results of an analysis of the relationship between chemotherapy-induced amenorrhoea, FSH (follicle stimulating hormone) levels and outcome in 126 premenopausal, node-positive, breast cancer patients treated with CMF.

In two previous studies we found that a low leucocyte nadir during adjuvant chemotherapy was associated with a long distant disease-free (DFS) and overall survival (OS) [16, 17]. Therefore, we also investigated whether the favourable effect of a more prominent haematological toxicity was due to a more efficient castration effect.

2. Patients and methods

The study population comprised 126 premenopausal, primary operable, node-positive breast cancer patients with T1-3, N1-2, M0 disease treated with CMF adjuvant chemotherapy at the Department of Oncology, Helsinki University Central Hospital during 1990–1993. The patients took part in a prospective randomised trial which investigated the effect of adjuvant clodronate therapy on bone metastases. Clodronate therapy did not affect results in this study and the results of the clodronate trial will be published elsewhere. Premenopausal status at entry was defined as having the last normal menstrual cycle within 3 months before starting chemotherapy. All patients except 1 had serum FSH levels within the premenopausal range (< 30 IU/l). The patients were divided into groups with respect to the menstrual function (regular menstruation, irregular menstruation and amenorrhoea groups) 12 months after the beginning of adjuvant chemotherapy. Amenorrhoea was defined as no menstruation within the previous 6 months.

The patients had either mastectomy or breast conserving surgery, with axillary nodal dissection and were treated with postoperative radiotherapy. Staging investigations included clinical investigation, liver chemistry, chest X-ray, liver ultrasound and bone scintigraphy. All patients received adjuvant chemotherapy which consisted of six 3-weekly cycles of cyclophosphamide (600 mg/m^2), methotrexate (40 mg/m^2) and 5-fluorouracil (600 mg/m^2) given i.v. on day 1 of the cycle. 52 patients also received oral chlodronate therapy 1600 mg daily. The median interval between surgery and the first cycle of chemotherapy was 40.5 days (range: 11–80). Menstruation status was recorded and serum concentrations of FSH and oestradiol were measured at 12, 24 and 36 months from the beginning of adjuvant chemotherapy. In survival analyses, we used the information of menstruation status at 12 months from the beginning of adjuvant chemotherapy. Haematological toxicity caused by chemotherapy and the lowest leucocyte nadir value during chemotherapy were also recorded.

After treatment the patients were followed up at 3- to 4-monthly intervals for the first 2 years and thereafter at 6-monthly intervals at least for 5 years. After the fifth year of follow-up, the control visits were scheduled once a year. The control examinations included clinical investigation and liver enzymes at 3 to 6 monthly intervals. Bone scan, liver ultrasound and chest X-ray were performed only at suspicion of recurrence. Patients were interviewed regarding menopausal status before chemotherapy and every 12 months thereafter.

Of the 126 eligible patients, data from 10 patients were excluded from the analyses: 9 because of missing information on menstrual status and 1 because of irregular menstruation and the FSH level > 30 IU/l before starting chemotherapy.

2.1. Statistical methods

Frequency tables were analysed using the chi-square test. Age and FSH distributions in amenorrheic and non-amenorrheic patients were compared with Mann–Whitney's test. For testing of the prognostic impact of amenorrhoea, patients with irregular menstruation or amenorrhoea were combined and compared with those having regular menstruation after chemotherapy. The DFS was defined as the length of time from surgery to distant or local relapse or death, whichever occurred first. The Kaplan–Meier method was used to estimate survival distributions for DFS and OS [18], and the two-sided logrank test was used to measure the statistical significance of differences in DFS and OS [19]. Cox proportional hazards regression model was used for multivariate analyses of DFS and OS [20]. Only factors significantly associated with DFS or OS in univariate analyses were included in the multivariate model.

3. Results

The baseline characteristics of the patients are shown in Table 1. The three menstruation groups were similar with respect to tumour size, nodal involvement, dose

Table 1
Pretreatment characteristics and dose intensities of chemotherapy of 116 breast cancer patients treated with CMF[a]

	Regular menstruation[b] $n=25$ (22) n (%)	Irregular menstruation[b] $n=39$ (34) n (%)	Amenorrhoea[b] $n=52$ (45) n (%)
Tumour size			
< 2 cm	8 (32)	13 (33)	17 (33)
2.1–5 cm	16 (64)	25 (64)	31 (60)
> 5 cm	1 (4)	1 (3)	4 (8)
No. of positive nodes			
1–3	19 (76)	31 (79)	39 (75)
> 3	6 (24)	8 (21)	12 (23)
ER/PR status			
Positive	14 (56)	24 (62)	31 (60)
Negative	10 (40)	11 (28)	16 (31)
Unknown	1 (4)	4 (10)	5 (10)
	Median (range)	Median (range)	Median (range)
Age (years)	36 (23–44)	43 (28–50)	48 (41–56)
S-oestradiol (nmol/L)	0.3 (0.02–1.1)	0.2 (0.03–0.8)	0.3 (0.02–3.6)
S-FSH (IU/L)	3.9 (0.3–9.7)	5.1 (1.5–15.1)	6.8 (1.8–83.4)
Dose-intensity of cyclophosphamide	95% (73–106)	95% (71–101)	95% (73–101)
Dose-intensity of 5-fluorouracil	95% (73–106)	95% (68–101)	95% (73–101)
Dose-intensity of methotrexate	96% (79–118)	94% (79–116)	96% (69–103)

[a] S-oestradiol, the oestradiol level in nmol/l before chemotherapy; S-FSH, FSH level in IU/l before chemotherapy; ER, oestrogen receptor; PR, progesterone receptor.
[b] Menopausal status 12 months after chemotherapy.

intensity of chemotherapy and serum base-line oestradiol concentration. 52 (45%) patients became amenorrheic, 39 (34%) had irregular menstruation and 25 (22%) retained regular menstruation after chemotherapy. Of the 25 patients who continuated to menstruate at 12 months after chemotherapy 13/25 (52%) had regular menstruation, 4/25 (16%) had irregular menstruation, 3/25 (12%) were amenorrheic and 5/25 (20%) had missing information about menstrual status 24 months after chemotherapy. Of the 39 patients who had irregular menstruation at 12 months after chemotherapy 12/39 (31%) had regular menstruation, 14/39 (36%) had irregular menstruation, 10/39 (26%) were amenorrheic and 3/39 (8%) had missing information about the menstrual status 24 months after therapy. Of the 52 patients who were amenorrheic at 12 months after chemotherapy 46/52 (88%) were still amenorrheic, 1/52 (2%) had regular menstruation and 10/57 (17%) had missing information about the menstrual status at 24 months after therapy. The median oestradiol level at 12 months after therapy was 0.28 nmol/l, (range: 0.02–0.95) in the regular menstruation group, 0.22 nmol/l, (range: 0.02–0.81) in the irregular menstruation group and 0.2 nmol/l, (range: 0.02–0.46) in the amenorrhoea group. Median FSH levels were 3.8 IU/l, (range: 0.1–30.8), 13.8 IU/l, (range: 2–108.6) and 63.2 IU/l, (range: 10–97.9) respectively.

Patients who retained regular menstruation were younger at the time of the diagnosis (median, 36 years, range: 23–44) than patients rendered amenorrheic (median, 48 years, range: 41–56, $P<0.005$) or those with irregular menstruation after chemotherapy (median, 43 years, range: 28–50, $P<0.005$). Women who experienced amenorrhoea had higher serum FSH concentrations before chemotherapy (median, 6.8 IU/l, range: 1.8–83.4) than women who had irregular menstruation after chemotherapy (median, 5.1 IU/l, range: 1.5–15.1, $P=0.014$) or women who continued to menstruate (median, 3.9 IU/l, range: 0.3–9.7, $P<0.005$).

DFS according to chemotherapy-induced amenorrhoea is shown in Fig. 1. 5-year estimated DFS was 74% in the irregular menstruation group, 62% in the amenorrhoea group, and 44% in the regular menstruation group ($P=0.04$). Women who experienced amenorrhoea or had irregular menstruation after chemotherapy had a significantly better 5-year DFS (67%) than women who continued to menstruate (44%, $P=0.02$). Amenorrhoea and irregular menstruation were associated with better 5-year estimated DFS among patients with ER-positive primary tumours ($n=69$, 73% versus 43%, $P=0.007$; Fig. 1b), while no association was found with ER-receptor negative patients ($n=37$, 48% versus 50%, $P=0.86$; Fig. 1c). 5-year overall survival was also better in patients who experienced amenorrhoea (81%) or had irregular menstruation (90%) as compared with patients who had regular menstruation after chemotherapy (68%, $P=0.05$; Fig. 2).

There was no significant association between elevated serum FSH levels and favourable prognosis ($P=0.32$, when tested as tertiles and $P=0.28$, when the median was used as the cut-off point). The leucocyte nadir values were similar in all three menstruation groups (median 2.1×10^9/l, range: 0.7–4.0 in amenorrhoea group, median 2.1×10^9/l, range: 1.3–4.0 in irregular menstruation group and median 1.9×10^9/l, range: 0.6–6.1 in regular menstruation group).

Variables tested in Cox univariate analysis for DFS were age ($P=0.16$); receptor status ($P=0.09$); nodal status ($P<0.005$); tumour size ($P=0.009$); menstruation status ($P=0.02$); FSH after chemotherapy ($P=0.21$, tested as a continuous variable); leucocyte nadir ($P=0.58$, tested as a continuous variable); and dose intensity of the chemotherapy ($P=0.52$ for cyclophosphamide, $P=0.55$ for methotrexate and $P=0.31$ for 5-fluorouracil). Variables tested in Cox univariate analysis for OS were age ($P=0.19$); receptor status ($P=0.002$); nodal status ($P<0.005$); tumour size ($P=0.02$); menstruation status ($P=0.04$); FSH after chemotherapy ($P=0.78$, tested as a continuous variable); leucocyte nadir ($P=0.64$, tested as a continuous variable); and dose intensity of the chemotherapy ($P=0.50$ for cyclophosphamide, $P=0.11$ for methotrexate and $P=0.72$ for 5-fluorouracil).

All variables which were significantly associated with DFS (nodal status, tumour size and menstruation status) or OS (nodal status, tumour size, menstruation status and receptor status) in univariate analysis were tested in a multivariate analysis. Nodal status ($P=0.001$), tumour size ($P=0.03$) and menstruation status after chemotherapy

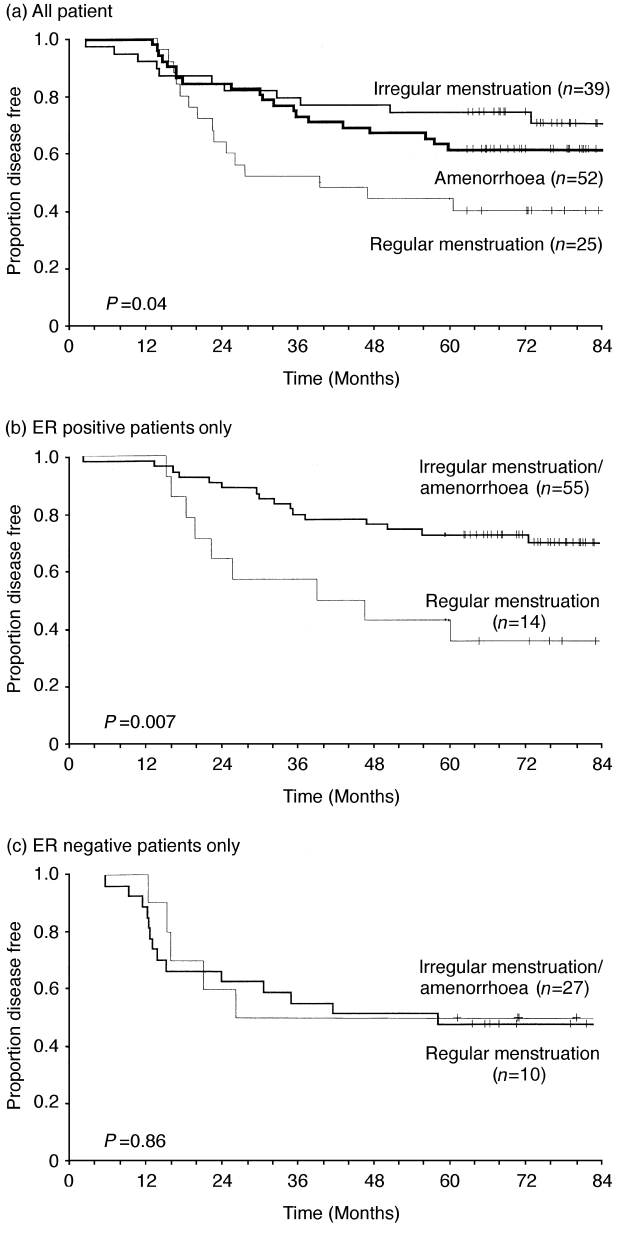

Fig. 1. Kaplan–Meier plots for disease-free survival according to chemotherapy-induced amenorrhoea. (a) All patients; (b) ER-positive patients and (c) ER-negative patients. Patients still alive are shown by the vertical bars.

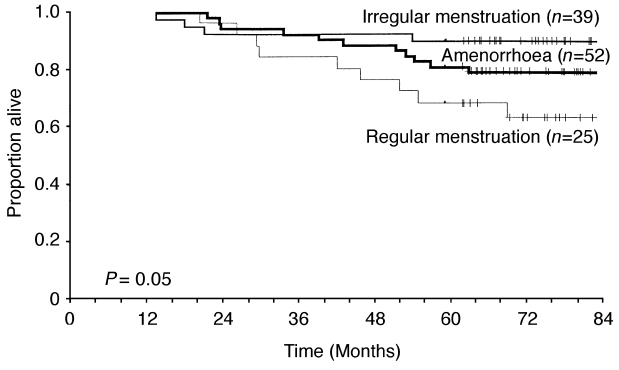

Fig. 2. Kaplan–Meier plots for OS according to chemotherapy-induced amenorrhoea. Patients still alive are shown by the vertical bars.

Table 2
Disease-free and overall survival in Cox multivariate analysis[a]

	DFS			OS		
Factor	RR	(95% CI)	*P* value	RR	(95% CI)	*P* value
Tumour size						
T1	1			1		
T2, T3	1.9	1.5–5.1	0.03	3.3	1.3–8.5	0.01
Nodal status						
1–3	1			1		
⩾ 4	2.8	1.1–3.5	0.001	2.4	1.0–5.8	0.04
Menstruation status						
Ovarian dysfunction	1			1		
Regular menstruation	2.1	1.2–4.2	0.01	2.2	0.9–5.2	0.09
ER/PR status						
Positive				1		
Negative				4.0	1.6–9.8	<0.005

[a] RR, relative risk; CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor.

($P = 0.01$) were also significantly associated with DFS in a multivariate analysis (Table 2). When age as two groups with a cut-off point at the median was entered into the multivariate analysis of DFS, menstruation status still retained its significance. In analyses of OS, the number of affected lymph nodes ($P = 0.04$), tumour size ($P = 0.01$), oestrogen/progesterone receptor status ($P < 0.005$) were independent prognostic factors, whilst menstruation status lost its statistical significance ($P = 0.09$).

4. Discussion

Our study is consistent with earlier findings that drug-induced amenorrhoea is associated with better DFS after adjuvant chemotherapy in breast cancer [3, 7–11]. The significant difference in DFS between those with and without regular menstruation who were oestrogen receptor (ER) positive patients (44% versus 73%, $P = 0.007$) respectively, which was not seen in oestrogen negative patients, supports the fact that association between long DFS and amenorrhoea is based on the castration effect.

Drug-induced amenorrhoea is strongly age related [2, 15] and is seen especially among women who are close to the menopause [7, 9]. The finding that amenorrhoea retained its significance on prognosis even in a multivariate model including age supports the conclusion that this association is due to the castration effect rather than age *per se*. A previous study by Tormey and colleagues [10] also indicated that not only age but also the menstrual status has an effect on prognosis.

Pagani and colleagues found recently that even a temporarily suppressed ovarian function with menses cessation provides some benefit in terms of DFS [11]. Our finding supports this since the group with irregular menstruation had DFS at least as favourable as the patients rendered amenorrheic. Only one third of the patients with irregular menses at 12 months had regained regular menstruation 24 months after beginning of chemotherapy.

We have previously reported that a lower leucocyte nadir during adjuvant chemotherapy is associated with a more favourable prognosis. However, this effect did not seem to be mediated through the castration effect, since the leucocyte nadir values were similar in all three menstruation groups. This also implies that the favourable prognostic effect of amenorrhoea is due to its endocrine consequences, not because amenorrhoea is a biological marker of cytotoxic drug efficacy. The fact that the favourable effect of amenorrhoea is seen only in ER-positive tumours supports this interpretation.

Amenorrhoea induced by cytotoxic chemotherapy is usually accompanied by elevated serum concentrations of FSH and leutinising hormone (LH), and decreased concentrations of oestradiol to postmenopausal levels as a consequence of ovarian failure, whilst patients who continue to menstruate during chemotherapy have no significant changes in hormone levels [7, 21–25]. This was also true in our study. In the irregular menstruation group FSH levels were elevated and oestradiol levels lowered but not to the postmenopausal levels in all patients. Elevated FSH levels unlike clinical menstrual status did not have a significant correlation with longer DFS ($P = 0.21$). This result suggests that menstruation status is a better marker for drug-induced amenorrhoea than FSH measurements. These hormone determinations do not seem to be necessary to distinguish those patients who might benefit from ovarian ablation after chemotherapy.

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