available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

The risk of amenorrhoea after adjuvant chemotherapy for early stage breast cancer is related to inter-individual variations in chemotherapy-induced leukocyte nadir in young patients: Data from the randomised SBG 2000-1 study

Mikkel Rosendahl [a,*], *Johan Ahlgren* [b], *Jørn Andersen* [c], *Jonas Bergh* [d], *Carl Blomquist* [e,f], *Elisabet Lidbrink* [d], *Henrik Lindman* [e], *Henning Mouridsen* [g,h], *Karsten Bjerre* [h], *Michael Andersson* [g]

[a] *The Fertility Clinic and Laboratory of Reproductive Biology, Copenhagen University Hospital – Rigshospitalet, Denmark*
[b] *Department of Oncology, Gävle Hospital, Sweden*
[c] *Department of Oncology, Aarhus University Hospital, Kommunehospitalet, Denmark*
[d] *Radiumhemmet, Karolinska Oncology and Cancer Centre, Karolinska Institute and University Hospital, Stockholm, Sweden*
[e] *Department of Oncology, Uppsala University Hospital, Uppsala, Sweden*
[f] *Department of Oncology, Helsinki University, Helsinki, Finland*
[g] *The Department of Oncology, Copenhagen University Hospital – Rigshospitalet, Denmark*
[h] *Danish Breast Cancer Cooperative Group Secretariat, Copenhagen, Denmark*

ARTICLE INFO

Article history:
Received 11 August 2009
Received in revised form 15 September 2009
Accepted 15 September 2009
Available online 7 October 2009

Keywords:
Breast cancer
Amenorrhoea
Ovarian failure
Menopause

ABSTRACT

Study aim: Amenorrhoea is a common side-effect to chemotherapy of premenopausal women. We examine the association between chemotherapy-induced leucopaenia and the development of amenorrhoea in premenopausal women with breast cancer.

Materials and methods: In a multi-centre, randomised, controlled study, 1016 premenopausal women received seven series of FEC (F: fluorouracil, E: epirubicin and C: cyclophosphamide) for early stage breast cancer.

In the first series, all patients received standard dose (F: 600 mg/m^2, E: 60 mg/m^2 and C: 600 mg/m^2). Patients with leukocyte nadir 1.0–1.9 $\times 10^9$/l continued with standard dose for the remaining six series ($\text{STANDARD}_{\text{REGISTERED}}$, $n = 279$). Patients with leukocyte nadir $\geqslant 2 \times 10^9$/l were randomised to standard ($\text{STANDARD}_{\text{RANDOMISED}}$, $n = 373$) or increased (TAILORED, $n = 364$) dose of E and C. After each series, leukocyte nadir was evaluated. Absent bleeding after the 5th–7th series of FEC was interpreted as amenorrhoea.

Results: The risk of amenorrhoea increased with age. In age-stratified analysis of the STANDARD groups (equal dose, different initial leukocyte nadir) low leukocyte nadir was associated with amenorrhoea for patients in the age-group 25–39 years ($P = 0.010$).

In age-stratified analysis in the randomised groups (different doses, same initial leukocyte nadir) a dose related increased risk of amenorrhoea was found for age-groups 25–39 (RR: 1.15, 95% confidence interval (CI): 1.06–1.24) and 40–44 years (RR:1.21, 95% CI: 1.001–1.47).

Conclusion: Age is the most important risk factor of amenorrhoea after FEC chemotherapy. However, for younger patients, lower leukocyte nadir in response to STANDARD FEC treatment or increased doses of C and E were associated with increased risk of amenorrhoea.

* *Corresponding author:* Tel.: +45 20831320; fax: +45 3545 4946.
E-mail address: mikro@dadlnet.dk (M. Rosendahl).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.09.019

1. Introduction

Chemotherapy-induced amenorrhoea (CIA) affects 52–70% of premenopausal women receiving adjuvant cyclophosphamide and epirubicin-based chemotherapy for early stage breast cancer.[1] Long-term survival after early stage breast cancer is increasing[2] and consequently, loss of fertility after cancer treatment becomes a major concern.

Certain factors are associated with the risk of CIA. The woman's age plays a central role as the pool of resting primordial follicles in the human ovary diminishes over time.[3] With increasing age, the risk of CIA increases in parallel.[1] This is supported by the recent finding that low serum levels of the ovarian reserve markers anti-Müllerian hormone and Inhibin B are associated with a higher risk of persistent amenorrhoea after chemotherapy for early stage breast cancer.[4] Further, the alkylating agents, such as cyclophosphamide which is a cornerstone in many breast cancer adjuvant chemotherapy regimens, have been shown to constitute an important risk factor.[5–7] However, most breast cancer regimens contain more than one drug, and hence the gonadotoxic effect of one single drug may often be difficult to evaluate due to interactions with the other components of the regimen or the effects of endocrine treatment, such as tamoxifen.[8]

Predicting premature ovarian failure in the individual patient therefore remains difficult, and it seems that individual and unknown factors may play a part.

One such factor could be inter-individual variations in pharmacogenetics and following differences in the systemic exposure of a given dose of a cytotoxic substance. Indeed, several clinical studies have suggested that the degree of chemotherapy-induced bone marrow toxicity may be positively related to the prognosis.[9–13] In a retrospective analysis of the G-CSF (granulocyte colony stimulating factor) supported tailored FEC (5-fluorouracil, epirubicin and cyclophosphamide) arm in the randomized SBG 9401 study, the outcome with reference to breast cancer relapse was similar irrespective of given dose-levels, indicating the potential with individually tailored chemotherapy based on toxicity.[14] In other studies, myelotoxicity after the administration of epirubicin was more strongly correlated to epirubicin plasma concentrations than to the administered dose[15] and despite compensation of dosage according to the body surface, patients had marked differences in systemic exposure of the components in the FEC regimen.[16] Such studies indicate that inter-individual variations in pharmacokinetics influence the degree of the toxicity – a mechanism that may not only affect the bone marrow and the malignant cells – but possibly also the ovaries. Pharmacogenetic determinants may influence on the presence of certain members of the cytochrome p450 superfamily and hence genetic differences among cancer patients may actually cause inter-individual variations in response to chemotherapy[17–20] and the presence or absence of certain polymorphism in the cytochrome p450 genes have been shown to influence the risk of chemotherapy-induced ovarian insufficiency.[21,22]

The FEC regimen is one of the most established regimens in the adjuvant treatment of breast cancer[2] and the dosing of FEC is traditionally based on the body surface area.

The *SBG 2000-1* study of the Scandinavian Breast Group[23] was designed to test the hypothesis that the optimal dose of FEC chemotherapy for early breast cancer could be achieved by adjusting the dose according to bone marrow toxicity in addition to the body surface area.

In the present analysis, we investigate the incidence of CIA in the subgroup of premenopausal women in the *SBG 2000-1* trial. By correlating the degree of chemotherapy-induced leucopaenia with the incidence of chemotherapy-induced amenorrhoea, we test the hypothesis that not only the inter-individual variation in the degree of leucopaenia – but also the risk of amenorrhoea – is an expression of variations in the systemic exposure to the chemotherapy.

2. Patients and methods

The *SBG 2000-1* trial was a prospective randomised study involving 33 centres in Sweden and Denmark. Women with stage II breast cancer or high risk stage I disease were eligible. Patients should have normal bone marrow and organ function, no prior malignant disease and no signs or symptoms of metastasis.

All patients were scheduled to receive seven courses of intravenous (i.v.) FEC at 3-week intervals. The first course was given in standard doses: cyclophosphamide 600 mg/m^2, epirubicin 60 mg/m^2 and fluorouracil 600 mg/m^2.

White blood cell count (WBC) was assessed on days 10, 12/13 and 15 after the first series. The lowest WBC was categorised: Grades 0–I: WBC $\geqslant 3.0 \times 10^9$/l. Grade II: 2.0–2.9 $\times 10^9$/l. Grade III: 1.0–1.9 $\times 10^9$/l. Grade IV: $\leqslant 0.9 \times 10^9$/l.

Patients with WBC Grade III after the first series of FEC received standard doses of FEC for the remaining six series. Patients with WBC Grade IV received reduced doses of cyclophosphamide (450 mg/m^2) and epirubicin (45 mg/m^2). These groups were denoted $\text{STANDARD}_{\text{REGISTERED}}$.

Patients with WBC Grades 0–II after the first series of FEC were randomised to receive either continued standard doses (denoted $\text{STANDARD}_{\text{RANDOMISED}}$) or increased doses of cyclophosphamide (900 or 1200 mg/m^2) and epirubicin (75 or 90 mg/m^2), (denoted TAILORED) for the remaining six series of FEC. The dose adjustments for TAILORED were done to obtain a WBC of Grade III.

After each of the following series, WBC was evaluated on the day of lowest value after the first series. Patients with oestrogen-receptor positive tumours initiated anti-estrogenic treatment after the seventh series of FEC. Gonadotrophic agonists or antagonists were not administered. A total of 1534 patients were included in the study. Of the 1077 premenopausal women, 1016 were included in the analyses. Fig. 1 shows the randomisation procedure and the number of premenopausal patients in each treatment group. The ethical committees with jurisdiction over the participating centres approved the study.

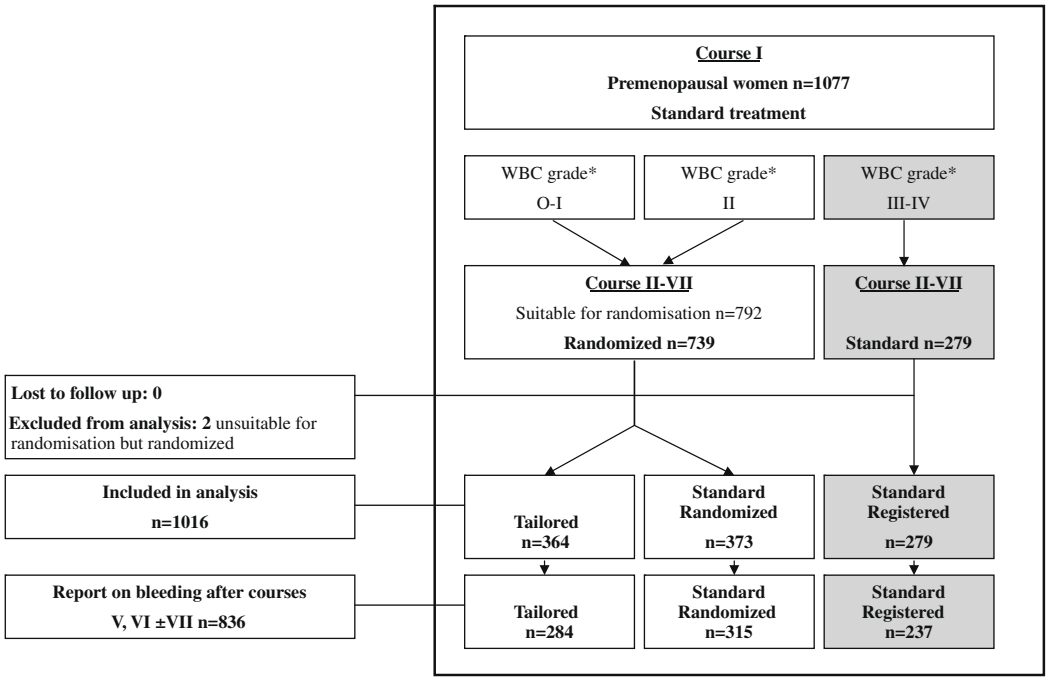

Fig. 1 – Flow chart of randomisation and patients in analyses. STANDARD treatment: cyclophosphamide 600, epirubicin 60, fluorouracil 600 mg/m^2. TAILORED: cyclophosphamide 900 or 1200, epirubicin 75 or 90, fluorouracil 600 mg/m^2. *WBC grade: (leukocytes $\times 10^9$/l) Grades 0–I: $\geqslant$3.0; Grade II: 2.0–2.9; Grade III: 1.0–1.9; Grade IV: $\leqslant$0,9. Lowest values measured on days 10, 12/13 and 15 after first FEC series.

2.1. *Bleeding*

In this study, we investigated the premenopausal women of the *SBG 2000-1* trial, defined as those who had reported a vaginal bleeding in the year prior to inclusion. Three weeks after each of the first six series and three months after the seventh series, patients reported by means of a questionnaire, whether they had had a vaginal bleeding since the previous visit. Lack of bleeding after all the courses V–VII was used as a measure of amenorrhoea.

2.2. *Statistics*

The number of premenopausal women participating in the study was 1077. Totally, 1016 patients were included in the analyses ($STANDARD_{REGISTERED}$ n = 279; $STANDARD_{RANDOMISED}$ n = 373; TAILORED n = 364 (Fig. 1).

According to age at surgery, patients were allocated to age groups (25–39, 40–44, 45–49 and 50–57 years). Cut-points were chosen prior to analysis under consideration of the frequency distribution in treatment groups (Table 1).

In the analyses, the minimum WBC-nadir after FEC series II–VII was categorised in four groups: <1.3; 1.3–<1.7; 1.7–<2.2 and 2.2–5.1 $\times 10^{-9}$/l. Cut-points were chosen prior to analysis based on the frequency distribution of WBC-nadir.

In the analyses focusing on bleeding pattern after series V–VII, report on bleeding after series V and VI was required, whereas a missing answer after series VII was accepted. This was allowed since three months had passed after the seventh series, opposed to 3 weeks after the previous series, and it would therefore be more difficult to recall if a bleeding had occurred. The number of patients who reported after all three series was 559. In addition 277 patients reported after series V and VI only, resulting in a total of 836 patients. The inclusion

Table 1 – Age by treatment group[a], count and column-percent.

Age	Treatment group			Total
	TAILORED	$STANDARD_{REGISTERED}$	$STANDARD_{RANDOMISED}$	
25–39	67 (23.6)	42 (17.7)	59 (18.7)	168 (20.1)
40–44	66 (23.2)	47 (19.8)	89 (28.3)	202 (24.2)
45–49	94 (33.1)	93 (39.2)	114 (36.2)	301 (36.0)
50–57	57 (20.1)	55 (23.2)	53 (16.8)	165 (19.7)
Total	284 (100)	237 (100)	315 (100)	836 (100)

[a] P = 0.089, chisq = 10.98, df = 6.

of patients who only reported after series V and VI did not alter the overall percentage of patients with bleeding or the distribution of age and maximal WBC toxicity. Nor did it alter the proportion of patients in the three study groups (chi-square = 3.33, df = 2, $P = 0.19$).

The dose intensity was calculated as: the delivered dose/the scheduled dose.

The relations between treatment group and age, WBC-nadir, and bleeding and the relation between age and bleeding were evaluated by chi-square tests.

For the equal-dose treatment groups ($STANDARD_{RANDOMISED}$ and $STANDARD_{REGISTERED}$) the association between WBC-nadir and amenorrhoea was evaluated in age-stratified contingency tables. The WBC-nadir and age categories were scored by equally spaced scores. The hypothesis of a linear association between WBC-nadir score and amenorrhoea in at least one stratum was evaluated by the Mantel–Haenszel statistic.

Similarly, the association between treatment and amenorrhoea in the analysis of the randomised treatment groups (TAILORED and $STANDARD_{RANDOMISED}$) was evaluated in age-stratified contingency tables. A two-sided P-value <0.05 was considered statistically significant.

The definition of amenorrhoea was done prospectively; all data were collected as a part of the prospective study, data analysis was planned prospectively and clinical cut-points were determined prior to data analysis.

3. Results

The median dose intensity in the TAILORED group was 95.9, which was lower than that in two groups receiving STANDARD treatment: $STANDARD_{REGISTERED}$ (99.6) and $STANDARD_{RANDOMISED}$ (99.7).

3.1. Bleeding response

The proportion of women who reported on their menstrual bleeding declined with each treatment. Of the 1016 women in the analysis, 836 women reported on menstrual bleeding after FEC series V, VI ± VII. The distribution by treatment groups was $STANDARD_{REGISTERED}$ $n = 237$; $STANDARD_{RANDOMISED}$ $n = 315$; TAILORED $n = 284$, as shown in Fig. 1. In all treatment groups, there was a gradual decrease in the percentage of women who had experienced a bleeding throughout the seven courses. The overall mean percentage of women who experienced a bleeding after each of the seven courses of FEC decreased and was 61%, 60%, 45%, 30%, 18%, 13% and 8% after courses 1, 2, 3, 4, 5, 6 and 7, respectively.

3.2. Age

There was an unequal age-distribution with a tendency of a higher age in the $STANDARD_{REGISTERED}$ group than in the two other groups ($P = 0.089$) (Table 1). The age-distribution of women in TAILORED and $STANDARD_{RANDOMISED}$ did not differ ($P = 0.22$, chisq = 4.40 df = 3), while the age of women in $STANDARD_{REGISTERED}$ tended to be higher than that of the women in $STANDARD_{RANDOMISED}$ ($P = 0.068$, chisq = 7.12 df = 3).

Table 2 illustrates the distribution of the minimal leukocyte count in each treatment group. There was an overall uneven distribution of WBC-nadir values between the treatment groups ($P < 0.0001$, chisq = 487.1, df = 6).

Patients in TAILORED, treated with increased FEC-doses, had lower WBC-nadir values than those in $STANDARD_{RANDOMISED}$ ($P < 0.0001$, chisq = 358.6 df = 3). Patients in $STANDARD_{REGISTERED}$, who had low WBC after the first series of standard dose FEC, had lower WBC-nadir values than those in $STANDARD_{RANDOMISED}$ ($P < 0.0001$, chisq = 203.7 df = 3).

Table 3 shows the incidence of amenorrhoea by age- and patient group. The overall risk of amenorrhoea after FEC series V–VII was 77% however we observed a vast age-related risk of amenorrhoea. In the youngest group (25–39) 67.9% of

Table 3 – Bleeding by age and treatment group. Count and row percentage.

Age[a]	Bleeding	No bleeding	Total
25–39	114 (67.9)	54 (32.1)	168
40–44	54 (26.7)	148 (73.3)	202
45–49	23 (7.6)	278 (92.4)	301
50–57	3 (1.8)	162 (98.2)	165
Treatment[b,c]			
TAILORED	55 (19.4)	229 (80.6)	284
$STANDARD_{REGISTERED}$	50 (21.1)	187 (78.9)	237
$STANDARD_{RANDOMISED}$	89 (28.3)	226 (71.7)	315
Total	194 (23.2)	642 (76.9)	836

[a] $P < 0.0001$, chisqMH = 237.9, df = 1.
[b] Tailored versus Std. rand: $P = 0.011$, chisq = 6.46, df = 1.
[c] Std.rand versus Std. reg: $P = 0.055$, chisq = 3.68, df = 1.

Table 2 – Minimal WBC-nadir (courses II–VII) by treatment group[a], count and column-percent.

WBC-nadir ($\times 10^{-9}$/l)	Treatment group			Total
	TAILORED	$STANDARD_{REGISTERED}$	$STANDARD_{RANDOMISED}$	
<1.3	171 (60.2)	54 (22.8)	11 (3.5)	236 (28.2)
1.3– < 1.7	74 (26.1)	92 (38.8)	23 (7.3)	189 (22.6)
1.7– < 2.2	27 (9.5)	71 (30.0)	90 (28.6)	188 (22.5)
2.2–5.1	12 (4.5)	20 (8.4)	191 (60.6)	223 (26.7)
Total	284 (100)	237 (100)	315 (100)	836 (100)

[a] $P < 0.0001$, chisq = 487.1, df = 6.

Table 4 – Equal-dose treatments ($STANDARD_{RANDOMISED}$ and $STANDARD_{REGISTERED}$). Bleeding versus minimal WBC-nadir during courses II–VII stratified by age[a]. Count and column-percent within age-strata. Patients who received reduced dose (N = 8) have been removed from the analysis.

Age		WBC-nadir ($\times 10^{-9}$/l)				Total	Chi-sqMH	P
		<1.3	1.3– < 1.7	1.7– < 2.2	2.2–5.1			
25–39	Bleeding	9 (60.0)	16 (76.2)	18 (72.0)	36 (92.3)	79 (79.0)	6.63	0.010
	No bleeding	6 (40.0)	5 (23.8)	7 (28.0)	3 (7.7)	21 (21.0)		
40–44	Bleeding	2 (25.0)	7 (25.0)	12 (32.4)	19 (31.1)	40 (29.9)	0.341	0.56
	No bleeding	6 (75.0)	21 (75.0)	25 (67.6)	42 (68.9)	94 (70.1)		
45–49	Bleeding	1 (4.2)	4 (9.8)	7 (10.9)	4 (5.3)	16 (7.8)	0.063	0.80
	No bleeding	24 (96.0)	38 (90.5)	57 (89.1)	72 (94.7)	191 (92.3)		
50–57	Bleeding	0 (0.0)	0 (0.0)	1 (3.0)	1 (2.9)	2 (1.9)	0.805	0.37
	No bleeding	13 (100.0)	24 (100.0)	32 (97.0)	34 97.1)	103 (98.1)		

[a] P = 0.063, chisqMH = 3.47, df = 1.

the patients maintained bleeding opposed to only 1.8% of the oldest group (50–57) (P < 0.0001).

Among the randomised treatment groups, amenorrhoea was more frequent in TAILORED (80.6%) than in $STANDARD_{RANDOMISED}$ (71.7%) (P = 0.011) while the difference in bleeding between the equal-dose treatment groups $STANDARD_{RANDOMISED}$ and $STANDARD_{REGISTERED}$ (78.9%) was not significant (P = 0.055) (Table 3).

The risk of amenorrhoea of the women in the equal dose (STANDARD) treatment groups was analysed in age-stratified analysis of in relation to the minimal leukocyte nadir after FEC series II–VII (Table 4). Patients with reduced dose (N = 8) were not included in the analysis. There was an overall borderline significant association between WBC-nadir and bleeding (P = 0.06, chisqMH = 3.47, df = 1). In particular, for women in the age-group 25–39 years, the risk of amenorrhoea was significantly higher in patients who had experienced a low leukocyte nadir (P = 0.010, chisqMH = 6.63, df = 1) after FEC series II–VII. There was no association between age and WBC-nadir within the STANDARD treatment groups (P = 0.45, chisq = 8.86, df = 9).

Similarly, the risk of amenorrhoea in the randomised patients groups, TAILORED and $STANDARD_{RANDOMISED}$, was analysed in age-stratified analysis (Table 5). There was an overall increased risk of amenorrhoea in the TAILORED group (RR: 1.15, 95% confidence interval (CI): 1.06–1.24), and in addition the relation was significant within the strata 25–39 years (RR: 2.91, 95% CI: 1.57–5.37) and close to significant within the strata 40–44 years (P = 0.055).

4. Discussion

In this study, we have shown that in the youngest patient group there is an association between chemotherapy-induced leukocyte nadir and the risk of chemotherapy-induced amenorrhoea. In addition, the risk was dose-related. Nevertheless, the overall most important risk factor for chemotherapy-induced amenorrhoea after FEC chemotherapy for early breast cancer is the age of the patient.

Several authors have associated amenorrhoea after adjuvant chemotherapy for early breast cancer in premenopausal women with improved prognosis. However, whether amenorrhoea is merely a marker of a generally increased cytotoxic effect or if the chemical castration itself acts as an indirect mediator, remains unclear.[1,24–27]

The *SBG 2000-1* study was a randomised controlled study designed to investigate if bone marrow toxicity-based dose tailoring of FEC could improve distant disease-free survival. In this sub-study, we have investigated a possible connection between amenorrhoea and bone marrow toxicity.

The study consisted of three groups: Two randomised groups which received either standard or increased doses of FEC and one group, which received standard doses of FEC, but had different initial leukocyte toxicities than the other

Table 5 – Randomised patient groups (TAILORED and $STANDARD_{RANDOMISED}$). Bleeding versus treatment stratified by age[a]. Count, column-percent and relative risk (RR) within age-strata.

Age		Treatment		Total	RR	95% CI	Chi-sqMH	P
		$STANDARD_{RANDOMISED}$	TAILORED					
25–39	Bleeding	49 (83.1)	34 (50.7)	83 (65.9)	2.91	(1.57–5.37)	14.45	0.0001
	No bleeding	10 (16.9)	33 (49.3)	43 (34.1)				
40–44	Bleeding	30 (33.7)	13 (19.7)	43 (27.7)	1.21	(1.001–1.47)	3.69	0.055
	No bleeding	59 (66.3)	51 (80.3)	112 (72.3)				
45–49	Bleeding	9 (7.9)	7 (7.4)	16 (7.7)	1.00	(0.93–1.09)	0.0145	0.9
	No bleeding	105 (92.1)	87 (92.6)	192 (92.3)				
50–57	Bleeding	1 (1.9)	1 (1.8)	2 (1.8)	1.00	(0.95–1.05)	0.0027	0.96
	No bleeding	52 (98.1)	56 (92.8)	198 (98.2)				

[a] RR: 1.15 (95% CI: 1.06–1.24), P = 0.0003, chisqMH = 13.04, df = 1.

groups. This particular combination of groups made it possible to perform analyses between the three groups with regard to the risk development of chemotherapy-induced amenorrhoea.

In the analysis of inter-individual variations in leukocyte nadir in the patients who had received standard doses of FEC throughout the study, groups $STANDARD_{RANDOMISED}$ and $STANDARD_{REGISTERED}$, there was only a borderline significant overall effect of leukocyte nadir. However in the age-stratified analysis, we discovered an age-related effect, since amenorrhoea was associated with low leukocyte nadir in the youngest patients but not in the older patient groups. One possible explanation to our findings is that the consequence of an increased dose and more pronounced bone marrow suppression is less important in the older, premenopausal patients, presumably since these patients have an already diminished ovarian oocyte reserve, which becomes exhausted even with low doses of FEC chemotherapy.

This hypothesis was supported by the analysis of the groups, which had equal WBC grade after the first series of FEC but had received different doses of chemotherapy (TAILORED versus $STANDARD_{RANDOMISED}$), showing an overall association between the dose of chemotherapy and the risk of amenorrhoea. This finding was expected and was a confirmation of other studies.[6,7,28]

However, in the age-stratified table analysis, we again discovered that the phenomenon was age-dependent. In the two oldest age groups, the relative risk of amenorrhoea between the two randomised groups was 1.00 but in the two youngest groups, the relative risks of amenorrhoea after tailored FEC chemotherapy were 1.21 and 2.91 respectively. As for the analysis of the STANDARD groups, this suggests that the younger patients have a higher oocyte reserve[3] and we hypothesise that an increase in the doses of C and E will further reduce the oocyte pool, thus rendering more women amenorrhoeic. Contrarily, the older patients, who have an already diminished pool of oocytes in the ovaries, very easily become amenorrhoeic in response to standard doses of C and E and consequently neither a dose escalation nor a higher bioavailability of the substances will result in further damage. This hypothesis of course requires confirmation by other studies.

We used bleeding after each series of chemotherapy as a marker to establish the degree of chemotherapy-induced amenorrhoea and the observed incidence of CIA in 77% of the women correlates well with current knowledge.[1] As anti-estrogenic treatment was not initiated until after the seventh series of FEC, and since the percentage of patients with oestrogen-receptor positive cancer would be the same in the three groups, this is not believed to influence on the results.

For some of the women, amenorrhoea is only temporary, but even though a proportion of the patients with amenorrhoea after the last three series of chemotherapy are expected to regain normal menses,[8,29] temporary amenorrhoea during chemotherapy is associated with higher risk of premature menopause.[30] The validity of the marker was supported by a decreasing number of patients experiencing bleeding with increasing patient age and dose and furthermore, the uniform and persistent reduction of the proportion of patients with bleeding in each of the three patient groups throughout the seven courses of FEC, indicates that the method is indeed able to detect any deviation from a regular bleeding pattern. Nevertheless, a more precise long-term estimate of the percentage of patients with persistent amenorrhoea could probably be obtained by a follow-up questionnaire one or two years after the end of chemotherapy. However, information obtained at this time would be influenced by the use of anti-oestrogenics in a proportion of the patients as well as by other external factors. Finally, measurement of serum levels of Anti-Müllerian hormone, which is produced in the granulosa cells of the small ovarian follicles[4] and has been shown to be a splendid marker of ovarian reserve, would potentially more accurately reflect the isolated impact on ovarian reserve after the seven series of FEC.

This is the first time that the role of inter-individual variations in leukocyte nadir after FEC chemotherapy has been investigated in relation to the risk of chemotherapy-induced amenorrhoea in a randomised-controlled trial. We have shown that, in the younger patients, a more suppressed bone marrow, presumably caused by a higher bioavailability of FEC, is associated with an increased risk of amenorrhoea. We have further confirmed that the risk of chemotherapy-induced amenorrhoea after FEC-treatment in premenopausal women with breast cancer is strongly associated with the age of the patient and the dose of chemotherapy. The findings of the present analysis will be taken into account in the main analysis of the *SBG 2000-1* study.

Bearing in mind the recent developments in fertility preservation in cancer patients[31–33] and the potential increase of the proportion of women with breast cancer experiencing chemotherapy-induced ovarian failure due to the use of individually tailored doses, it is important to address the risk of infertility and possible steps to preserve fertility early in the management of premenopausal breast cancer patients.

Conflicts of interest statement

None declared.

Acknowledgements

We wish to thank the many people, who helped making the SGG 2000-1 study a reality by including patients and gathering data.

REFERENCES

1. Walshe JM, Denduluri N, Swain SM. Amenorrhea in premenopausal women after adjuvant chemotherapy for breast cancer. *J Clin Oncol* 2006;**24**(36):5769–79.
2. Early Breast Cancer Trialists' Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**(9472):1687–717.
3. Faddy MJ. Follicle dynamics during ovarian ageing. *Mol Cell Endocrinol* 2000;**163**(1–2):43–8.
4. Anders C, Marcom PK, Peterson B, et al. A pilot study of predictive markers of chemotherapy-related amenorrhea

among premenopausal women with early stage breast cancer. *Cancer Invest* 2008;**26**(3):286–95.
5. Meirow D, Assad G, Dor J, Rabinovici J. The GnRH antagonist cetrorelix reduces cyclophosphamide-induced ovarian follicular destruction in mice. *Hum Reprod* 2004;**19**(6):1294–9.
6. Yuce MA, Balkanli KP, Gucer F, et al. Prevention of cyclophosphamide-induced ovarian damage by concomitant administration of GnRHa in mice: a dose-dependent relationship? *Eur J Gynaecol Oncol* 2004;**25**(5):628–31.
7. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. *Hum Reprod Update* 2001;**7**(6):535–43.
8. Petrek JA, Naughton MJ, Case LD, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. *J Clin Oncol* 2006;**24**(7):1045–51.
9. Saarto T, Blomqvist C, Rissanen P, Auvinen A, Elomaa I. Haematological toxicity: a marker of adjuvant chemotherapy efficacy in stage II and III breast cancer. *Br J Cancer* 1997;**75**(2):301–5.
10. Poikonen P, Saarto T, Lundin J, Joensuu H, Blomqvist C. Leucocyte nadir as a marker for chemotherapy efficacy in node-positive breast cancer treated with adjuvant CMF. *Br J Cancer* 1999;**80**(11):1763–6.
11. Colleoni M, Price K, Castiglione-Gertsch M, et al. Dose-response effect of adjuvant cyclophosphamide, methotrexate, 5-fluorouracil (CMF) in node-positive breast cancer. International Breast Cancer Study Group. *Eur J Cancer* 1998;**34**(11):1693–700.
12. Mayers C, Panzarella T, Tannock IF. Analysis of the prognostic effects of inclusion in a clinical trial and of myelosuppression on survival after adjuvant chemotherapy for breast carcinoma. *Cancer* 2001;**91**(12):2246–57.
13. Cameron DA, Massie C, Kerr G, Leonard RC. Moderate neutropenia with adjuvant CMF confers improved survival in early breast cancer. *Br J Cancer* 2003;**89**(10):1837–42.
14. Wilking N, Lidbrink E, Wiklund T, et al. Long-term follow-up of the SBG 9401 study comparing tailored FEC-based therapy versus marrow-supported high-dose therapy. *Ann Oncol* 2007;**18**(4):694–700.
15. Jakobsen P, Bastholt L, Dalmark M, et al. A randomized study of epirubicin at four different dose levels in advanced breast cancer. Feasibility of myelotoxicity prediction through single blood-sample measurement. *Cancer Chemother Pharmacol* 1991;**28**(6):465–9.
16. Sandstrom M, Freijs A, Larsson R, et al. Lack of relationship between systemic exposure for the component drug of the fluorouracil, epirubicin, and 4-hydroxycyclophosphamide regimen in breast cancer patients. *J Clin Oncol* 1996;**14**(5):1581–8.
17. van Schaik RH. Cancer treatment and pharmacogenetics of cytochrome P450 enzymes. *Invest New Drugs* 2005;**23**(6):513–22.
18. van Schaik RH. Implications of cytochrome P450 genetic polymorphisms on the toxicity of antitumor agents. *Ther Drug Monit* 2004;**26**(2):236–40.
19. Moore MJ. Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet* 1991;**20**(3):194–208.
20. Innocenti F, Ratain MJ. Update on pharmacogenetics in cancer chemotherapy. *Eur J Cancer* 2002;**38**(5):639–44.
21. Singh G, Saxena N, Aggarwal A, Misra R. Cytochrome P450 polymorphism as a predictor of ovarian toxicity to pulse cyclophosphamide in systemic lupus erythematosus. *J Rheumatol* 2007;**34**(4):731–3.
22. Takada K, Arefayene M, Desta Z, et al. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. *Arthritis Rheum* 2004;**50**(7):2202–10.
23. Edlund P, Gunnarsdottir K, Anderson H, et al. Side effects in treatment with FEC (5-fluorouracil, epirubicin and cyklophosphamide) in standard doses compared to tailored doses, based on hematological toxicity, for primary breast cancer. Results from the Scandinavian Breast Group 2000-1 Study. San Antoni Breast Cancer Symposium, poster no. 2057; 2005.
24. Del ML, Venturini M, Sertoli MR, Rosso R. Amenorrhea induced by adjuvant chemotherapy in early breast cancer patients: prognostic role and clinical implications. *Breast Cancer Res Treat* 1997;**43**(2):183–90.
25. Pagani O, O'Neill A, Castiglione M, et al. Prognostic impact of amenorrhoea after adjuvant chemotherapy in premenopausal breast cancer patients with axillary node involvement: results of the International Breast Cancer Study Group (IBCSG) Trial VI. *Eur J Cancer* 1998;**34**(5):632–40.
26. Vanhuyse M, Fournier C, Bonneterre J. Chemotherapy-induced amenorrhea: influence on disease-free survival and overall survival in receptor-positive premenopausal early breast cancer patients. *Ann Oncol* 2005;**16**(8):1283–8.
27. Parulekar WR, Day AG, Ottaway JA, et al. Incidence and prognostic impact of amenorrhea during adjuvant therapy in high-risk premenopausal breast cancer: analysis of a National Cancer Institute of Canada Clinical Trials Group Study–NCIC CTG MA5. *J Clin Oncol* 2005;**23**(25):6002–8.
28. Minton SE, Munster PN. Chemotherapy-induced amenorrhea and fertility in women undergoing adjuvant treatment for breast cancer. *Cancer Control* 2002;**9**(6):466–72.
29. Berliere M, Dalenc F, Malingret N, et al. Incidence of reversible amenorrhea in women with breast cancer undergoing adjuvant anthracycline-based chemotherapy with or without docetaxel. *BMC Cancer* 2008;**8**(1):56.
30. Partridge A, Gelber S, Gelber RD, et al. Age of menopause among women who remain premenopausal following treatment for early breast cancer: long-term results from International Breast Cancer Study Group Trials V and VI. *Eur J Cancer* 2007;**43**(11):1646–53.
31. Andersen CY, Rosendahl M, Byskov AG, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod* 2008;**23**(10):2266–72.
32. Donnez J, Martinez-Madrid B, Jadoul P, et al. Ovarian tissue cryopreservation and transplantation: a review. *Hum Reprod Update* 2006;**12**(5):519–35.
33. Oktay K, Buyuk E, Davis O, et al. Fertility preservation in breast cancer patients: IVF and embryo cryopreservation after ovarian stimulation with tamoxifen. *Hum Reprod* 2003;**18**(1):90–5.