Original article

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Postoperative dose-dense sequential chemotherapy with epirubicin, followed by CMF with or without paclitaxel, in patients with high-risk operable breast cancer: a randomized phase III study conducted by the Hellenic Cooperative Oncology Group

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Purpose: The aim of this study was to explore the effect of dose-dense sequential chemotherapy with or without paclitaxel primarily on disease-free survival (DFS) and secondarily on overall survival (OS) in patients with high-risk operable breast cancer.

Patients and methods: From June 1997 until November 2000, 604 patients with $T_{1-3}N_1M_0$ or $T_3N_0M_0$ tumors were randomized to three cycles of epirubicin 110 mg/m^2 followed by three cycles of paclitaxel 250 mg/m^2 followed by three cycles of 'intensified' CMF (cyclophosphamide 840 mg/m^2, methotrexate 47 mg/m^2 and fluorouracil 840 mg/m^2) (group A), or to four cycles of epirubicin followed by four cycles of CMF, as in group A (group B). All cycles were given every 2 weeks with granulocyte colony-stimulating factor support.

Results: A total of 595 patients were eligible. Median follow-up was 61.7 months for group A and 62 months for group B. The 3-year DFS was 80% in group A and 77% in group B. Survival rates were 93% and 90%, respectively. The effect of treatment on the hazard of death was different according to hormonal receptor status. More specifically, in patients with negative receptor status the hazard of death was significantly higher for group B (hazard ratio 2.42). Both regimens were well tolerated and severe acute side-effects were infrequent. No cases of severe cardiotoxicity or acute leukemia were recorded.

Conclusions: The present study failed to demonstrate a significant difference in DFS or OS between the two treatment groups. However, our study has shown clearly that high-dose paclitaxel can be safely incorporated to dose-dense sequential chemotherapy.

Key words: breast cancer, dose-dense chemotherapy, E-CMF, paclitaxel, randomized

Introduction

Clinical research on the treatment of breast cancer has recently focused not only on the development of new active drugs such as the taxanes [1], but also on new treatment strategies such as increased dose density [i.e. increasing dose intensity (DI) by reducing the interval between cycles] and sequential chemotherapy. These new strategies were the result of the application of mathematical models of cell growth kinetics, such as the Norton–Simon extension of the Skipper–Schobel–Wilcox model [2, 3], into the clinic. The feasibility and safety of these novel concepts of dose-dense and/or sequential adjuvant chemotherapy have been successfully tested during the past 15 years, in women with operable breast cancer [4–6].

The first study to document the beneficial role of sequential adjuvant chemotherapy in high-risk patients with operable breast cancer was reported by Bonadonna et al. [7], from the Milan group. In a randomized trial they showed that four cycles of doxorubicin followed by eight cycles of CMF yielded superior results compared with alternating administration of doxorubicin and CMF in patients with four or more positive nodes.

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The corresponding 5-year survival rates for the two groups were 78% and 62%, respectively.

Over a series of clinical trials, colleagues at the Memorial Sloan-Kettering Cancer Center (MSKCC) developed a regimen consisting of three cycles of doxorubicin 90 mg/m^2 every 2 weeks followed by three cycles of paclitaxel (Taxol®) 250 mg/m^2 every 2 weeks and three cycles of cyclophosphamide 3 g/m^2 every 2 weeks [8]. Granulocyte colony-stimulating factor (G-CSF) was administered prophylactically between treatments. When this dose-dense sequential chemotherapy schedule was applied to a group of high-risk patients with stage II–IIIA breast cancer, encouraging results were observed after a median follow-up of 3 years. Various other dose-dense regimens have subsequently been reported [9, 10]. Moreover, the Hellenic Cooperative Oncology Group (HeCOG) developed a similar regimen based on our previously published experience of administering high-dose epirubicin monotherapy in a dose-dense fashion in patients with breast cancer and more than nine involved axillary nodes [11], and on the results obtained from the previously mentioned studies [7, 8]. In our regimen, three cycles of high-dose epirubicin 110 mg/m^2 were followed by three cycles of high-dose paclitaxel 250 mg/m^2 and finally by three cycles of 'intensified' CMF (cyclophosphamide 840 mg/m^2, methotrexate 57 mg/m^2 and fluorouracil 840 mg/m^2). All cycles were given every 2 weeks with G-CSF support. The selection of CMF following sequential treatment with epirubicin and paclitaxel was influenced by the similar studies mentioned previously [7, 8]. Intensification of CMF was preferred to high-dose cyclophosphamide, as used by the MSKCC group [8], mainly because the latter was accompanied by a high incidence of acute toxicity, which was considered to be mutagenic [12]. This treatment was given to 42 patients with operable breast cancer and more than nine positive nodes, with excellent tolerability. The 3-year disease-free survival (DFS) rate using this regimen was 72% with the 3-year overall survival (OS) rate being 90% [13].

Motivated by this information, we designed a phase III study (HE 10/97) in high-risk patients with operable breast cancer, in order to evaluate the role of paclitaxel when integrated in dose-dense sequential chemotherapy. The primary objective of the study was to compare DFS of patients treated with dose-dense sequential chemotherapy with epirubicin and intensified CMF with or without the incorporation of paclitaxel. Secondary study end points were OS, acute toxicity and quality of life (QoL).

Patients and methods

Eligibility criteria

Women were eligible for the study if they met the following requirements: histologically confirmed epithelial breast cancer; pathological stage $T_{1-3}N_1M_0$ or $T_3N_0M_0$ [14]; Eastern Cooperative Oncology Group performance status 0–1; normal cardiac function; and adequate bone marrow, hepatic and renal function. Patients with a history of serious cardiac disease, other serious medical illness or inability to comply with the treatment plan and follow-up visits were excluded from the study. Postmenopausal patients with one to three positive axillary nodes and positive hormonal receptor status were also excluded. The clinical protocol and the companion translational research studies were approved by the HeCOG Protocol Review Committee and by the Institutional Review Board of Kyanous Stavros Hospital and AHEPA University Hospital. Written informed consent was obtained from all patients.

Pretreatment evaluation included medical history, physical examination, chest X-ray and liver ultrasound (or computed tomography scan in case of more than nine positive nodes), bone scan, ejection fraction measured by nuclear gated heart scan or by echocardiogram, complete blood count (CBC) and biochemistry. CBC and biochemistry were repeated prior to each cycle and ejection fraction after the completion of all cycles.

Treatment

Stratified randomization balanced by center was performed at the HeCOG Data Office in Athens, using the following stratification factors: menopausal status (pre- versus postmenopausal), hormonal receptor status (positive versus negative) and number of positive nodes (zero versus one to three versus four or more). Postmenopausal were considered patients without menses for the last 2 years or patients >50 years of age who underwent a hysterectomy for non-malignant reasons.

Patients randomized to group A were treated with three cycles of epirubicin 110 mg/m^2 followed by three cycles of paclitaxel 250 mg/m^2 over 3 h and three cycles of intensified CMF (cyclophosphamide 840 mg/m^2, methotrexate 57 mg/m^2, fluorouracil 840 mg/m^2) (E-T-CMF). The interval between cycles was 2 weeks.

Prophylactic treatment with G-CSF (filgrastim; 5 μg/kg) was administered on days 3–10 of each cycle. Patients randomized to group B received four cycles of epirubicin every 2 weeks followed by four cycles of intensified CMF (E-CMF) at the same doses and intervals as in group A, with G-CSF support. Ondansetron ± dexamethasone were used as antiemetics in all patients. Tamoxifen 20 mg daily was prescribed for 5 years to all patients with estrogen and/or progesterone receptor positive status or those of unknown status. Additionally, all premenopausal patients underwent ovarian suppression with monthly intramuscular injections of 2.5 mg triptoreline for 1 year.

Radiation therapy (RT) was mandatory for all patients with breast conserving surgery or for those with four or more positive lymph nodes and/or tumor size ≥5 cm (irrespective of the initial operation type). RT included irradiation of the chest wall or the breast using two tangential photon fields and supraclavicular fossa (in case of four or more positive nodes or ≥5 cm tumors) to 50–55 Gy (1.8–2 Gy per fraction). This was followed by a 10–15 Gy boost to the tumor bed in patients treated with breast conserving surgery or to the mastectomy scar. Minor deviations from this technique according to local institutional guidelines were allowed, provided that each center specified them before participating in the study.

Treatment with tamoxifen, ovarian suppression treatment and RT were initiated after the completion of chemotherapy.

Dose modification

Blood counts were carried out before each treatment. If granulocytopenia or thrombocytopenia was observed, treatment was delayed until absolute neutrophil count was ≥1500/μl and platelets ≥100 000/μl. In the event of grade 3 or 4 granulocytopenia and/or thrombocytopenia, the dose of all drugs was reduced by 25% and 40%, respectively, in all subsequent cycles. For grade 2–3 mucositis the doses of epirubicin or CMF were reduced by 40% and that of paclitaxel by 25%. For grade 2 neurotoxicity the dose of paclitaxel was reduced by 40%. Treatment was interrupted prematurely in the event of grade 4 non-hematological toxicity. Finally, in the event of grade 3 neurological toxicity, treatment with paclitaxel was interrupted and the patient continued treatment with CMF. Toxicity criteria were those adopted by the World Health Organization.

Follow-up

Median follow-up was 61.7 months for group A and 62 months for group B. Patients were followed at the clinic with physical examination, CBC, biochemistry and CA 15-3 determination every 3 months for the first 2 years and every 6 months thereafter. Chest X-ray, ultrasonography of the abdomen and bone scan were repeated every 6 months for the first 3 years and annually thereafter. Mammography was repeated annually. A bone scan was not routinely performed after the third year, except when it was clinically indicated. QoL was assessed at baseline and at the end of chemotherapy using the European Organization for Research and Treatment of Cancer QLQ-C30 questionnaire [14].

Statistical analysis

For a two-sided test at the 5% level of significance and power of 80%, the number of patients required to detect a 15% difference in DFS rate, to a baseline rate of 60% at the 5-year time point, was 410 patients. The study accrual rate was estimated at 150 patients per year. Taking into consideration a 3% withdrawal rate, 420 patients were planned to enter the study. Actual accrual was higher than expected and the targeted sample size was increased to 600 patients to achieve a power of 80% for detecting a 12.5% difference in DFS rate. At the interim analysis (80 events, April 2001), based on the O'Brien–Fleming boundary values, no significant differences in DFS were detected and the study was continued to completion.

OS was measured from the date of randomization until death from any cause. Surviving patients were censored at the date of last contact. DFS was measured from randomization until local recurrence, distant relapse, or death from the disease without relapse. Time to event distributions were estimated using Kaplan–Meier curves and compared using the log-rank test. The Cox proportional hazards models were used to assess the strength of association of DFS and OS with various clinical and histological variables. A backward selection procedure with removal criterion $P > 0.10$ identified the subclass of significant variables among the following: treatment group (group A versus group B), age, menopausal status (pre- versus postmenopausal), nuclear grade (I–II versus III–IV), receptor status (negative versus positive), tumor size (≤2 cm versus 2–5 cm versus >5 cm) and number of positive nodes (zero to three versus four or more). The Wald χ^2-test and the corresponding P values were used to determine significance. Analysis was conducted according to the intention-to-treat principle.

Differences in QoL between the two study groups at baseline and at the end of chemotherapy, as well as changes between baseline and end of chemotherapy within each group were compared using Wilcoxon tests. For ease of interpretation, all scale and item scores were linearly transformed to a 0–100 scale. For the five functional scales and the global QoL scale, item responses were recorded so that higher scores represented a better level of functioning. For the symptom-oriented scales and items, a higher score corresponded to a higher level of symptoms. All statistical tests were two-sided and performed at a significance level of 0.05.

Results

From June 1997 until November 2000, 604 patients were randomized to the study. Nine patients were found non-eligible. Reasons for non-eligibility were the presence of metastatic disease at the time of randomization (six patients), history of previous cancer, histological diagnosis of tumor other than mammary carcinoma and insufficient records (one patient each). Selected patient and tumor characteristics are depicted in Table 1. There were no significant differences in major characteristics between the two treatment groups with the exception of tumor

Table 1. Selected patient and tumor characteristics

	Group A (E-T-CMF) [*n* (%)]	Group B (E-CMF) [*n* (%)]
n	298	297
Age (years)		
Median	50	50
Range	24–76	22–78
No. of nodes removed		
Median	18	18
Range	3–59	4–53
No. of positive nodes		
Median	6	6
Range	0–54	0–49
0 nodes	6 (2)	5 (2)
1–3 nodes	72 (24)	80 (27)
4–9 nodes	123 (41)	125 (42)
>9 nodes	97 (33)	87 (29)
Menopausal status		
Premenopausal	158 (53)	163 (55)
Postmenopausal	140 (47)	134 (45)
Type of operation		
Modified radical mastectomy	220 (74)	231 (78)
Breast conserving surgery	78 (26)	66 (22)
Interval from operation (weeks)		
<2	23 (8)	25 (8)
2–4	145 (49)	125 (42)
>4	130 (44)	147 (49.5)
Receptor status		
Negative	68 (23)	71 (24)
Positive	225 (75)	225 (76)
Unknown	5 (2)	1 (0.3)
Tumor size (cm)		
≤1	13 (4)	14 (5)
1.1–2	74 (25)	84 (28)
2.1–3	79 (26)	85 (29)
3.1–5	88 (29)	67 (23)
>5	44 (15)	47 (16)
Nuclear grade[a]		
I	18 (6)	8 (3)
II	108 (36)	155 (52)
III	169 (57)	133 (45)
IV	2 (1)	0 (0)
Unknown	1 (0.3)	1 (0.3)

[a]The two treatment groups are not balanced in terms of grade ($P < 0.001$).
E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.

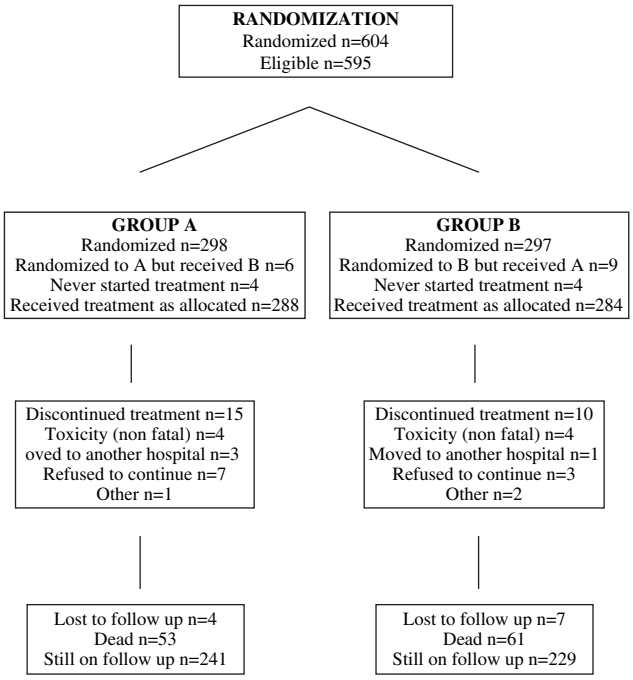


Figure 1. Progress through the various stages of the trial. Survival status update on September 2004.

grade ($P < 0.001$). The progress of patients through the various stages of the trial is shown in Figure 1 according to the Consolidated Standards of Reporting Trials [16]. Four patients in each group never started protocol treatment and thus are not included in the treatment characteristics and toxicity analysis. Twenty-five patients (15 in group A versus 10 in group B) discontinued chemotherapy. Reasons for treatment discontinuation were toxicity (four versus four), continuation of treatment in another hospital (three versus one), withdrawal of consent (seven versus three) and other (one versus two).

Selected treatment characteristics are shown in Table 2. Most of the cycles were given on time and at full doses (Table 2). Treatment was delayed mainly because of hematological toxicity. Severe (grade 3–4) hematological and non-hematological side-effects are shown in Table 3. Patients randomized to group A had a higher incidence of peripheral neuropathy ($P < 0.001$) and hypersensitivity reaction ($P = 0.006$), attributed to the addition of paclitaxel.

Patient compliance to chemotherapy, acute toxicity and OS were compared according to age at registration (<65 versus ≥65 years). Seventy-five patients (13%) were ≥65 years of age. After adjusting for treatment group it was found that older patients completed chemotherapy at a significantly lower rate in group A (95% <65 years versus 77% ≥65 years; $P = 0.003$), but not in group B (95% <65 years versus 98% ≥65 years; $P = 0.48$). Moreover, older patients had a significantly higher incidence of severe toxicities, such as neutropenia (10% <65 years versus 23% ≥65 years; $P = 0.003$), thrombocytopenia (0.6% <65 years versus 4% ≥65 years; $P = 0.03$) or fatigue (0% <65 years versus 4% ≥65 years; $P = 0.002$). OS did not differ between the two age groups.

Table 2. Selected treatment characteristics

	Group A (E-T-CMF)	Group B (E-CMF)
n	297	290
No. of cycles delivered	2599	2287
Median	9	8
Range	1–11[a]	1–8
% of E cycles given at full dose[b]	92	90
% of T cycles given at full dose[b]	86	–
% of CMF cycles given at full dose[b]	86	82
% of cycles given with a delay	23	26
Median interval between cycles (days)	14	14
Median delivered DI		
E	54	54
T	123	
C	412	406
M	28	27
F	412	406
Median RDI		
E	0.99	0.97
T	0.98	
C	0.98	0.97
M	0.98	0.96
F	0.98	0.97

[a]One patient received an extra cycle of CMF, while another patient received two extra cycles of CMF (protocol violation).
[b]At least 90% of the dose defined in the protocol.
E, epirubicin; T, paclitaxel (Taxol®); C, cyclophosphamide; M, methotrexate; F, fluorouracil; DI, dose intensity (mg/m^2/week); RDI, relative dose intensity.

Overall, 15 patients (seven in group A versus eight in group B) developed febrile neutropenia. There were no treatment-related deaths. Nineteen patients (10 in group A versus nine in group B) were hospitalized. Even though severe cardiotoxicity was not reported in our study, one patient in group A discontinued treatment following an episode of angina during the third infusion of epirubicin.

After a median follow-up of 62 months (range 0.1–86.5+), 189 patients (32%; 91 in group A and 98 in group B) had relapsed and 114 (19%; 53 in group A and 61 in group B) had died. DFS (range 0.1–83+ months) and OS (0.1–86.5+ months) did not differ significantly between treatment groups (log-rank $P = 0.55$ and $P = 0.38$, respectively).

The estimated DFS rates in group A and group B were 93% [95% confidence interval (CI) 90% to 96%] versus 97% (95% CI 95% to 99%) at 1 year, 80% (95% CI 75% to 85%) versus 77% (95% CI 73% to 80%) at 3 years and 70% (95% CI 65% to 75%) versus 68% (95% CI 63% to 73%) at 5 years (Figure 2).

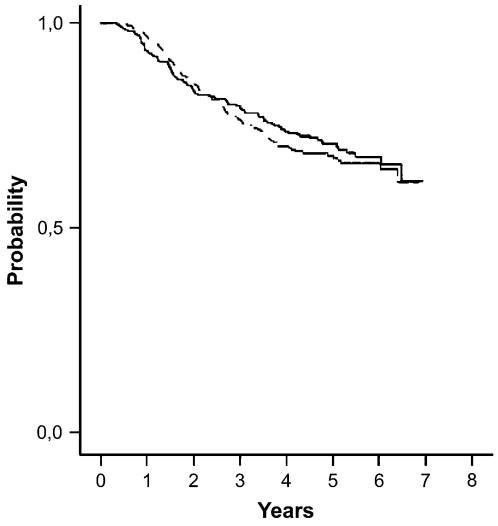
The Cox multivariate regression analysis for DFS (Table 4) revealed that the hazard of disease progression at any time was significantly higher for patients with more than three positive

Table 3. Incidence (%) of severe toxicities

	Group A (E-T-CMF) (n = 297)				Group B (E-CMF) (n = 290)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	n	%	n	%	n	%	n	%
Anemia	3	1.0	0	0	3	1.0	2	0.7
Leukopenia	16	5.4	4	1.3	16	5.5	3	1.0
Neutropenia	24	8.0	11	3.7	17	6.0	15	5.2
Thrombocytopenia	2	0.7	1	0.3	2	0.7	1	0.3
Nausea/vomiting	11	3.7	0	0	11	3.8	2	0.7
Peripheral neuropathy[a]	18	6.0	1	0.3	0	0	0	0
Hepatotoxicity	2	0.7	0	0	0	0	0	0
Hypersensitivity reaction[b]	11	3.7	0	0	1	0.3	0	0
Mucositis	6	2.0	0	0	5	1.7	2	0.7
Fatigue	1	0.3	0	0	2	0.7	0	0
Pain	1	0.3	0	0	0	0	0	0
Athralgia/myalgia	6	2.0	0	0	1	0.3	0	0

[a]$P < 0.001$; [b]$P = 0.006$.

E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.



No. of patients at risk at the beginning of the trial at one, three and five years, respectively

E-T-CMF:	298	276	235	118
E-CMF:	297	284	221	111

Figure 2. Disease-free survival of patients treated with E-T-CMF (solid line) or with E-CMF (dashed line) (P = 0.55). E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.

nodes [four or more versus zero to three: hazard ratio (HR) 2.66; 95% CI 1.75–4.04; $P < 0.001$], higher grade (III–IV versus I–II: HR 1.39; 95% CI 1.03–1.87; P = 0.03) and larger tumor size (2–5 cm versus ≤2 cm: HR =1.48; 95% CI 1.04–2.11; P = 0.03; >5 cm versus ≤2 cm: HR 1.22; 95% CI 0.76–1.97; P = 0.41). Positive receptor status significantly decreased the hazard of disease progression by 29% compared with negative receptor status (negative versus positive: HR 0.71; 95% CI 0.51–0.99; P = 0.04). The treatment effect on the hazard of disease progression was not different according to hormonal receptor status (Figure 3A and B).

No death was observed at 1 year, while the estimated OS rates were 93% (95% CI 90% to 96%) versus 90% (95% CI 87% to 93%) at 3 years and 84% (95% CI 80% to 88%) versus 81% (95% CI 76% to 86%) at 5 years in groups A and B, respectively (Figure 4).

Results of the Cox regression analysis for OS (Table 4) revealed that several prognostic factors including tumor size (2–5 cm versus ≤2 cm: HR 1.41; 95% CI 0.88–2.24; P = 0.15; >5 cm versus ≤2 cm: HR 1.72; 95% CI 0.97–3.06; P = 0.06), grade (III–IV versus I–II: HR 1.41; 95% CI 0.96–2.07; P = 0.08) and number of positive nodes (four or more versus zero to three: HR 2.72; 95% CI 1.57–4.71; $P < 0.001$) were related to significantly poorer survival.

In addition, statistically significant evidence exists that treatment effect on the hazard of death was different according to hormonal receptor status (P = 0.03). More specifically, in patients with negative receptor status, the hazard of death was significantly higher for group B (HR 2.42; 95% CI 1.17–4.99) (Figure 5A), while in those with positive receptor status no significant difference between treatment groups was found (HR 0.96; 95% CI 0.62–1.50) (Figure 5B). The treatment effect on OS in patients with negative receptor status was present when limiting the analysis to breast cancer-related deaths. Furthermore, the observed effect cannot be attributed to other possible confounding factors such as the administration of hormonal treatment, first-line chemotherapy for advanced disease or treatment with trastuzumab, which were found to be balanced between the two treatment groups. Additionally, the confounding effect of the lower cumulative doses of epirubicin and CMF for the E-T-CMF arm and the worse tumor grade recorded in these patients should drive the survival difference in the opposite direction.

Table 4. Estimated HRs and 95% CIs for disease-free and overall survival: multivariate analysis

	HR	95% CI	Wald χ^2 *P* value
Disease-free survival			
Treatment group			
E-T-CMF	1		
E-CMF	1.16	0.87–1.55	0.31
Hormonal receptor status			
Negative	1		
Positive	0.71	0.51–0.99	0.04
Tumor size (cm)			
≤2	1		
2–5	1.48	1.04–2.11	0.03
>5	1.22	0.76–1.97	0.41
Nuclear grade			
I–II	1		
III–IV	1.39	1.03–1.87	0.03
No. of positive nodes			
0–3	1		
≥4	2.66	1.75–4.04	<0.001
Overall survival			
Treatment group			
E-T-CMF	1		
E-CMF	2.42	1.17–4.99	0.02
Hormonal receptor status			
Negative	1		
Positive	1.2	0.62–2.35	0.59
Tumor size (cm)			
≤2	1		
2–5	1.41	0.88–2.24	0.15
>5	1.72	0.97–3.06	0.06
Nuclear grade			
I–II	1		
III–IV	1.41	0.96–2.07	0.08
No. of positive nodes			
0–3	1		
≥4	2.72	1.57–4.71	<0.001
Treatment group by receptor status interaction	0.4	0.17–0.93	0.03

HR, hazard ratio; CI, confidence interval; E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.

Nine patients (five in group A and four in group B) developed secondary malignancies including contralateral invasive breast cancer in two patients in group A, thyroid cancer in two patients in group B, and endometrial cancer, colon cancer, small-cell lung cancer, cervical cancer and glioblastoma multiforme in one patient each. Additionally, one patient in group B developed myelodysplastic syndrome (MDS) in the fourth year following completion of chemotherapy.

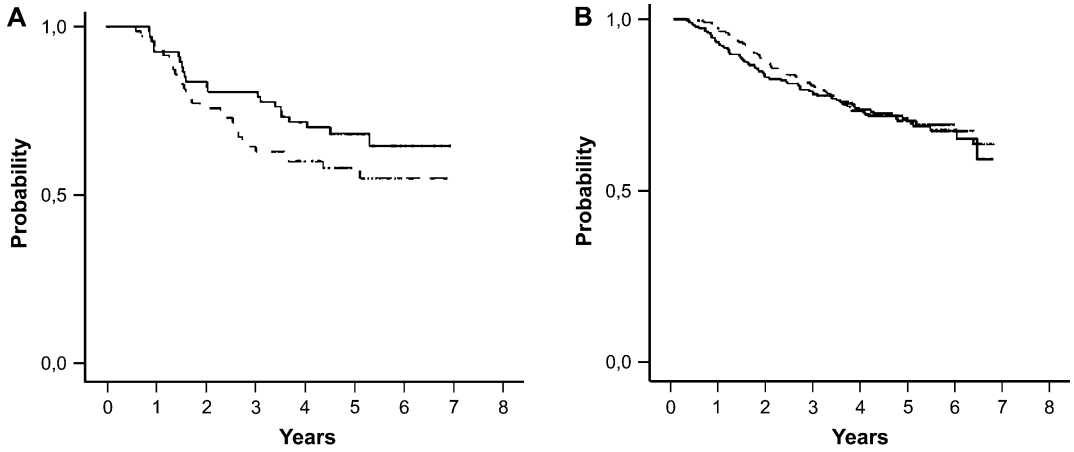
Of the 595 patients, 139 (23%) (72 in group A, 67 in group B) had both baseline and end of chemotherapy QoL assessment. Comparison of QoL outcomes between the two groups is shown in Table 5. No differences were found between the two treatment arms either at the beginning or at the end of chemotherapy ($P > 0.05$ in all cases). Comparison of baseline and end of chemotherapy mean scores within each group showed a significant increase in nausea and vomiting for both groups (from 7.6 to 17.8, $P = 0.007$, for group A and from 8 to 16.9, $P = 0.015$, for group B). On the contrary, the social functioning was significantly decreased (from 82.4 to 71.3; $P = 0.003$) in group A only, while emotional functioning and pain were significantly improved in group B only (from 67.3 to 72.6, $P = 0.031$, and from 23.9 to 14.9, $P = 0.007$, respectively).

Discussion

The present study is one of the first randomized clinical trials that has attempted to improve the efficacy of adjuvant chemotherapy in operable breast cancer with the use of a dose-dense sequential chemotherapy regimen with or without paclitaxel. The importance of the use of dose-dense and/or sequential chemotherapy in high-risk patients with breast cancer was recently highlighted by two pivotal studies, the Intergroup trial 0148 [17] and the Intergroup trial C9741 [18]. The first demonstrated that the addition of paclitaxel to a conventional sequential chemotherapy regimen had a significant impact on DFS and OS. The hazard reduction was 17% for recurrence and 18% for death. The 5-year DFS was 70% and 65%, and the 5-year OS was 80% and 77% with or without the addition of paclitaxel, respectively. In the second trial, it was shown that at 4 years, dose-dense sequential treatment significantly improved DFS compared with conventional sequential treatment (82% versus 75%, respectively). The potential benefit from taxane and anthracycline combinations has been explored in seven randomized studies in patients with advanced breast cancer [19–25]. Four trials [19, 20, 22, 24] reported higher response rates and two trials longer median time to progression for the arm including a taxane [19, 20]. Notably, in one trial, survival was in favor of the taxane-containing arm [19].

Our study was designed to evaluate primarily the impact on DFS of the addition of paclitaxel to a dose-dense regimen of high-dose epirubicin and CMF. Epirubicin and CMF were administered in both treatment arms at the same dose levels and the same dose intensities. However, the number of treatment cycles with these drugs was four in the E-CMF arm, compared with three in the E-T-CMF arm. This was due to the reluctance of study investigators at the time of study design to participate in a study with a treatment arm containing less than four cycles of epirubicin without the 'reassuring' incorporation of paclitaxel.

The optimal duration of anthracycline-containing adjuvant chemotherapy in breast cancer, remains unclear. Four cycles of doxorubicin and cyclophosphamide are considered standard treatment for most patients with operable breast cancer in the US, since there are no randomized trials supporting longer duration of chemotherapy, known to be associated with increased



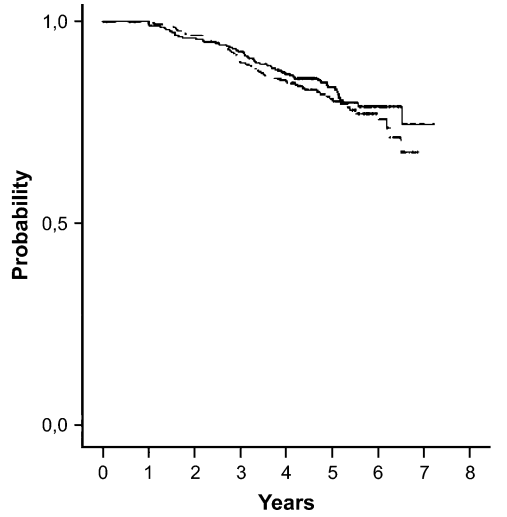
No. of patients at risk at the beginning of the trial at one, three and five years, respectively

E-T-CMF:	68	62	54	25
E-CMF:	71	66	45	19

No. of patients at risk at the beginning of the trial at one, three and five years, respectively

E-T-CMF:	225	209	177	90
E-CMF:	225	217	176	92

Figure 3. Disease-free survival of patients with (**A**) negative hormonal status treated with E-T-CMF (solid line) or with E-CMF (dashed line) (Wald χ^2 $P = 0.09$) or (**B**) positive hormonal status treated with E-T-CMF (solid line) or with E-CMF (dashed line) (Wald χ^2 $P = 0.89$). E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.



No. of patients at risk at the beginning of the trial at one, three and five years, respectively

E-T-CMF:	298	296	273	142
E-CMF:	297	294	260	136

Figure 4. Overall survival of patients treated with E-T-CMF (solid line) or with E-CMF (dashed line) ($P = 0.38$). E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.

risk of cardiotoxicity [26]. It is not well established that the same holds true when epirubicin is substituted for doxorubicin. In a randomized study in which premenopausal patients with involved axillary nodes were treated with three or six cycles of 5-fluorouracil, epirubicin and cyclophosphamide, a significant difference in DFS and OS was evident favoring the patients that received longer treatment [27]. At any rate, four cycles of doxorubicin given in a dose-dense sequential schedule followed by paclitaxel and cyclophosphamide are apparently sufficient, as clearly demonstrated in the Intergroup C9741 trial [18]. In our study, a further reduction in the number of cycles was attempted by incorporating an anthracycline (in this case epirubicin) with the addition of paclitaxel. Whether this minimization of the cumulative dose of anthracycline and the introduction of paclitaxel may have a beneficial effect with regard to the long-term toxicity profile without compromising survival remains to be seen with longer follow-up.

Of note, a study of the Arbeitsgemeinschaft fuer Gynaekologische Onkologie (AGO) compared a similar to ours dose-dense sequential regimen [three cycles of epirubicin (150 mg/m^2) followed by three cycles of paclitaxel (225 mg/m^2) followed by three cycles of cyclophosphamide (2.5 g/m^2) (E-T-C) every 2 weeks] with four cycles of conventional doses of epirubicin/cyclophosphamide (90/600 mg/m^2) followed by four cycles of paclitaxel (175 mg/m^2) every 3 weeks. According to an early report on 1284 patients at a median follow-up of 28 months, presented at the 40th Annual Meeting of the American Society of Clinical Oncology [28], the dose-dense regimen significantly improved DFS and OS. The 3-year DFS and OS rates with E-T-C were almost identical to those observed with E-T-CMF in our study. However, only patients <65 years of age with more than three positive nodes were enrolled in the AGO study [28]. The age of breast cancer patients at the time of enrolment in a study testing an intensified treatment may be a significant factor for issues such as compliance to the protocol and ultimately long-term outcome. In our study, patients of more than 65 years of age were at higher risk for treatment interruption than younger patients in the E-T-CMF arm and for severe myelotoxicity and/or fatigue development in both treatment arms. Our data suggest that when patients of this age group participate, as they should, in this type of trial, meticulous counselling, closer monitoring and increased care are mandatory.

A number of significant prognostic factors for OS were identified by Cox regression analysis in our study, including tumor size, nuclear grade and number of infiltrated axillary nodes.

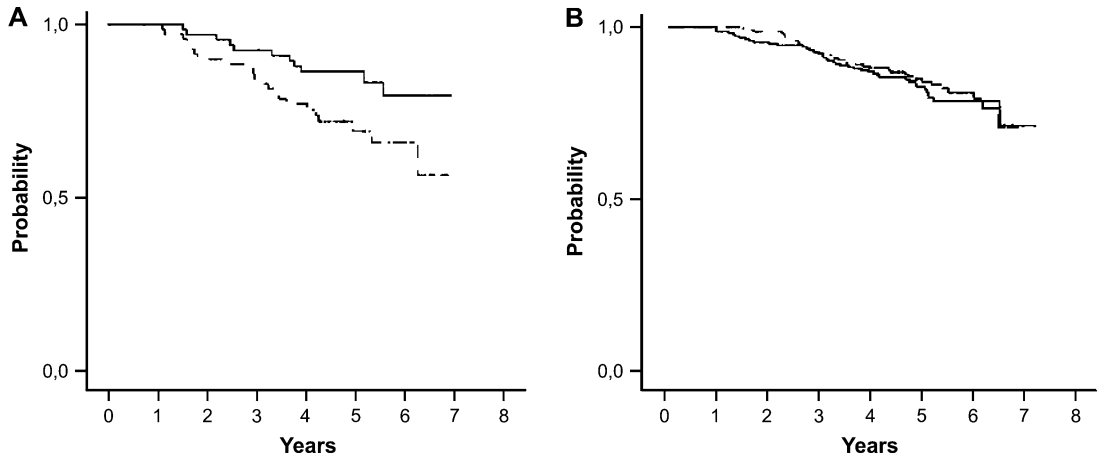


Figure 5. Overall survival of patients with (**A**) negative hormonal status treated with E-T-CMF (solid line) or with E-CMF (dashed line) (Wald χ^2 $P = 0.02$) or (**B**) positive hormonal status treated with E-T-CMF (solid line) or with E-CMF (dashed line) (Wald χ^2 $P = 0.87$). E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil.

Table 5. Quality of life comparisons

Functioning scales	Before treatment		After treatment		Difference (after – before)		*P* value
	Group A (E-T-CMF)	Group B (E-CMF)	Group A (E-T-CMF)	Group B (E-CMF)	Group A (E-T-CMF)	Group B (E-CMF)	
Physical	76.9	75.8	71.9	78.2	−5.0	2.4	NS
Role	66.2	66.7	66.7	67.2	0.5	0.5	NS
Cognitive	88.6	91.5	87.0	91.5	−1.6	0.0	NS
Emotional	68.7	67.3	67.9	72.6	−0.8	5.3	0.098
Social	82.4	82.1	71.3	76.4	−11.0	−5.7	NS
Global quality of life	63.8	63.9	63.1	67.8	−0.7	3.8	NS
Symptom scales and items							
Fatigue	28.1	26.2	35.2	30.3	7.1	4.1	NS
Nausea and vomiting	7.6	8.0	17.8	16.9	10.1	8.9	NS
Pain	25.7	23.9	18.7	14.9	−6.9	−8.9	NS
Dyspnea	18.0	17.4	23.6	24.4	5.5	6.9	NS
Sleep disturbance	25.5	24.9	30.1	27.9	4.6	3.0	NS
Appetite loss	15.3	15.4	19.9	16.9	4.6	1.5	NS
Constipation	13.0	22.4	18.0	22.9	5.1	0.5	NS
Diarrhea	7.4	4.0	6.9	6.0	−0.5	2.0	NS
Financial impact	16.7	13.4	12.0	10.9	−4.6	−2.5	NS

E, epirubicin; T, paclitaxel; C, cyclophosphamide; M, methotrexate; F, fluorouracil; NS, not significant (*P* value >0.10).

Furthermore, the analysis revealed that in patients with negative receptor status the hazard of death was significantly reduced by 59% when these patients were treated with paclitaxel. Survival benefit from the addition of paclitaxel in such patients was also observed in the Intergroup 0148 trial [17]. However, such findings have not been demonstrated in the replica B-28 trial of the National Surgical Adjuvant Breast and Bowel Project [29] or in the Intergroup C9741 trial [18]. It must be mentioned that, in contrast to the Intergroup 0148 and C9741 trials, our study was designed with hormonal receptor status as a stratification factor.

As far as acute toxicity is concerned, both regimens were well tolerated and there were no treatment-related deaths. No significant differences in the incidence of severe toxicities were seen between the two groups of patients, with the exception of

peripheral neuropathy and hypersensitivity reactions occurring more frequently in the E-T-CMF arm. The incidence of febrile neutropenia and other major severe toxicities recorded with E-T-CMF in our study were very similar to those reported in the Intergroup C9741 trial with A-T-C chemotherapy, even though it appears that patients treated with doxorubicin experienced more pronounced nausea/vomiting than our patients who received epirubicin instead of doxorubicin. No cases of acute leukemia were reported among our patients. Only one case of MDS was seen in a patient who was free of disease at 4 years postchemotherapy. This low incidence of secondary hematological malignancies is in accordance to the 3-year incidence of acute myelogenous leukemia or MDS reported at 0.17% in the Intergroup 0148, and at 0.18% in Intergroup C9741 trials. Nevertheless, it has to be kept in mind that indirect comparisons across trials could be misleading and should be considered in the context of hypothesis-generating observations rather than conclusions.

The results of the current study may be confounded by the different number of cycles or the different cumulative doses of epirubicin and CMF between the two treatment arms and the bias of worse tumor grade in the E-T-CMF arm. Despite these weaknesses there are several key issues that have been addressed in this study. At the trial design stage, information that any potential benefit from ovarian ablation following chemotherapy would be restricted to premenopausal patients with positive hormonal receptor status was not available from the 1995 overview [30] or any other clinical trial. This is one of the few trials with dose-dense chemotherapy in which not only hormonal receptor status was a stratification factor, but also the confounding variable of chemotherapy-induced endocrine effects was eliminated by the suppression of ovarian function for 1 year in all premenopausal patients. Moreover, scheduling hormonal therapy and RT after the completion of chemotherapy prevented any potential interactions between chemotherapy and these treatment modalities.

The E-T-CMF regimen, as given in our study, compared with the dose-dense sequential arm in the Intergroup C9741 trial [18] and that of the paclitaxel-containing arm in the Intergroup 0148 trial [17], was administered for a shorter period of time, i.e. 4 months instead of 5.5 months, without compromising the short-term survival.

In addition, since QoL data are limited in patients treated with dose-dense sequential chemotherapy, an attempt was made in the present study to collect such data. Unfortunately, only a small proportion (23%) of patients participating in the study had a complete assessment of QoL. It is important to note, however, that QoL assessment was encouraged but was not a primary objective of the study. Furthermore, issues of QoL may be of less interest in adjuvant trials where patients do not exhibit intense disease-related symptoms and thus investigators are not as keen in collecting QoL data from their patients. Therefore, no definite conclusions can be drawn from this study on the impact of paclitaxel on QoL when integrated in dose-dense sequential chemotherapy.

In conclusion, the present study failed to demonstrate a statistically significant benefit either in DFS, the primary study end point, or OS from the addition of paclitaxel to dose-dense sequential chemotherapy with high-dose epirubicin and CMF. This result is to be interpreted in the context of the size of the difference the study was powered to detect. For patients with negative receptor status, a statistically significant benefit in DFS for the E-T-CMF arm was not found, while in OS a significant benefit was demonstrated. It remains to be seen whether these findings, based on a small number of events, will hold with longer follow-up. Most importantly, it has been demonstrated clearly in the present study that high-dose paclitaxel can be safely incorporated in a dose-dense schedule, and that the incidence of cardiotoxicity or secondary leukemia was not increased with either dose-dense regimen. Other important issues such as the optimal dose or the optimal sequence of the drugs, and the importance of dose intensity, cumulative dose or duration of treatment, remain to be elucidated by future randomized trials. Our group has recently completed a randomized trial (HE 10/00) in which the E-T-CMF treatment arm of the present study was compared with four cycles of epirubicin (83 mg/m^2) and paclitaxel (187 mg/m^2) every 3 weeks followed by three cycles of CMF every 2 weeks (ET-CMF). Duration of chemotherapy and cumulative dose of all drugs were identical in both treatment arms, while the dose intensities of paclitaxel and epirubicin were approximately double in the E-T-CMF arm.

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