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Improved outcome from substituting methotrexate with epirubicin: Results from a randomised comparison of CMF versus CEF in patients with primary breast cancer

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ABSTRACT

We compared the efficacy of CEF (cyclophosphamide, epirubicin, and fluorouracil) against CMF (cyclophosphamide, methotrexate, and fluorouracil) in moderate or high risk breast cancer patients. We randomly assigned 1224 patients with completely resected unilateral breast cancer to receive nine cycles of three-weekly intravenous CMF or CEF. Patients were encouraged to take part in a parallel trial comparing oral pamidronate 150 mg twice daily for 4 years versus control (data not shown). Substitution of methotrexate with epirubicin significantly reduced the unadjusted hazard for disease-free survival (DFS) by 16% (hazard ratio 0.84; 95% CI; 0.71–0.99) and for overall survival by 21% (hazard ratio 0.79; 95% CI; 0.66–0.94). The risk of secondary leukaemia and congestive heart failure was similar in the two groups.

Overall CEF was superior over CMF in terms of DFS and OS in patients with operable breast cancer without subsequent increase in late toxicities.

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1. Introduction

The benefits of adjuvant chemotherapy were recognised more than 25 years ago.[1–3] The predominant regimen used in these pivotal trials was later named classical CMF and consisted of four-weekly oral cylophosphamide 100 mg/m^2 days one to fourteen in combination with intravenous methotrexate 40 mg/m^2 and 5-fluorouracil 600 mg/m^2 days one and eight.[1,2] The Milan group observed no detrimental effects switching to twelve cycles of three-weekly intravenous CMF (600 mg/m^2, 40 mg/m^2, 600 mg/m^2).[4] Based on indirect comparisons, others have, however, hypothesised that classical CMF might be superior to three-weekly intravenous CMF.[5] A direct comparison in a randomised trial has never been undertaken in the adjuvant setting, but in advanced breast cancer, classical CMF with oral cyclophosphamide has, in a single small phase III trial, been superior to three-weekly intravenous CMF.[6] The meta-analyses performed by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) have confirmed that CMF improves disease-free survival and overall survival in patients with operable breast cancer, but have not explored the possible differences between classical and intravenous CMF.[7]

Anthracyclines are among the most active drugs in advanced breast cancer and several trials comparing CMF-like regimens with regimens including an anthracycline were published between the late 1970s and the early 1990s. Although the results of these, often quite small, trials were conflicting, a statistical overview from five of these trials demonstrated a 31% increase in time to progression and a 20% prolongation of survival time.[8] Similar results were demonstrated in the largest of these trials.[9] Similar efficacy but generally less cardiotoxicity has been observed with epirubicin compared to doxorubicin when administered at equitoxic doses.[10,11] Alongside other groups we decided to evaluate whether the results obtained with anthracyclines in advanced breast cancer could be translated into the adjuvant setting. We used a symmetrical design with randomisation to regimens both consisting of a 3-drug combination given nine times intravenously with 3 week intervals. The dose and schedule of cyclophosphamide and 5-fluorouracil was identical in both groups.

2. Patients and methods

This open-label, randomised, phase III trial involving centres nationwide in Denmark, and three health-care regions in Sweden and Iceland, was conducted in accordance with the Helsinki declaration and was approved by ethical committees with jurisdiction for the participating institutions. The Danish Breast Cancer Cooperative Group prepared the original protocol (DBCG trial 89D). Minor regional modifications were later added in the sub-protocols of the other healthcare regions. Informed consent was obtained before randomisation following oral and written information.

2.1. *Patients*

The study included women who had completely resected unilateral invasive carcinoma of the breast and no signs of distant metastases as determined by physical examination, chest radiography, and bone scintigraphy (if positive, to be confirmed by radiography), or axial bone radiography. Lower axillary clearance (level I and part of level II) in combination with breast-conserving surgery or mastectomy was required. In node positive patients, endocrine trials were run in parallel and three distinct groups of patients were therefore eligible for this trial. Group A: node negative premenopausal patients, independent of hormone receptor status but with malignancy grade II or III and a primary ductal carcinoma 5 cm or less in size; Group B: premenopausal patients with hormone-receptor negative (oestrogen (ER) and progesterone (PgR) receptor negative) or unknown tumours, and either axillary lymph node metastases or tumours with a size larger than 5 cm; and Group C: postmenopausal patients with hormone-receptor negative tumours, and either axillary lymph node metastases or tumours with a size larger than 5 cm. A patient was classified as premenopausal if she had amenorrhoea for less than 2 months, amenorrhoea for less than 12 months and FSH in the premenopausal range, or 50 years of age or younger in the case of hysterectomy.

2.2. *Pathological procedures*

Classification of histological type and grade (ductal carcinomas) according to regional guidelines e.g. WHO or Ackerman, examination of tumour margins, invasion into skin or deep fascia, measurement of gross tumour size, total number of lymph nodes identified and number of metastatic nodes was mandatory. ER and PgR were analysed using immunohistochemical assays or dextran-coated charcoal assays in frozen tissue. Tumours were considered to be receptor positive in the quality controlled and validated biochemical ligand-binding assay as defined by the laboratories in each region. Following immunohistochemical staining, tumours were considered receptor positive if the percentage of ER or PgR positive epithelial cells was 10% or above.

2.3. *Treatment*

Patients were assigned to either CMF (cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2 and 5-fluorouracil 600 mg/m^2) or CEF (cyclophosphamide 600 mg/m^2, epirubicin 60 mg/m^2 and 5-fluorouracil 600 mg/m^2) both given intravenously (i.v.) day one every 3 weeks. Loco-regional radiotherapy was administered according to regional guidelines and patients not assigned to loco-regional radiotherapy received nine cycles of CMF or CEF, while patients assigned to radiotherapy received one or two cycles of CMF or CEF before radiotherapy and one or two cycles of single agent cyclophosphamide (850 mg/m^2) concomitant with radiotherapy followed by CMF or CEF to a total of nine cycles of chemotherapy. The doses were primarily adjusted according to white cell and platelet counts ($\times 10^9$/l) day one of the scheduled cycle as follows: platelets > 100 and WBC > 3.0, 100%; platelets 50–100 or WBC 2.0–3.0, 75% of all three drugs. If platelets < 50 or WBC < 2.0, the treatment was delayed for 1 week. The protocol permitted secondary randomisation to pamidronate 150 mg given orally twice daily for 4 years against control. The use of endocrine therapies was not recommended.

2.4. Follow-up

Symptoms, side effects, and findings on clinical examination were recorded every 12 weeks during the first year, every 6 months during the second through to the fifth year, and thereafter annually to a total of 10 years. A complete follow-up on vital status was obtained in Denmark and Iceland for all patients through linkage to the respective civic registrations. Haemoglobin, white blood cell count, and platelet count, were examined on day one of each chemotherapy cycle. Additional biochemical tests and imaging examinations were done when indicated by existing symptoms or signs.

2.5. Statistical analysis

The study was designed to have an 80% power to detect a 1.20 HR in the CMF versus CEF comparison, under the assumption of no interaction with pamidronate. It was expected that 300 patients yearly could be entered onto the protocol, and that the 5-year DFS would be 50% following CMF. The pre-planned sample size was 1500 patients in 5 years, with an additional 5-year follow-up. Randomisation was stratified according to group and centre; the patients were allocated to treatment by the minimisation method, with a random component of 20%. Patients from Denmark and Iceland were randomised by the DBCG data centre and patients from Sweden were randomised by each regional data centre (Uppsala, Linköping, Umeaa). Data were collected by the respective data centres and subsequently accumulated centrally by the DBCG Registry. The DBCG Data Centre undertook central review, querying, and analysis of data.

Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up. OS was calculated as the elapsed time from randomisation until death, irrespective of cause of death. DFS was defined as the duration of survival without loco-regional recurrence, distant metastases, contralateral breast cancer or death irrespective of cause. OS and DFS were analysed unadjusted by the intention to treat principle and treatment regimens were compared using the log-rank test stratified by centre and group. For multivariate analysis the Cox proportional hazards regression model was applied to assess the per protocol adjusted hazard ratio of treatment regimen, and to explore interactions. Factors included in the multivariate analyses were group (A, B, C), age (<45, 45–49, 50–59, 60–69), tumour size (⩽20 mm, 21–50 mm, >50 mm), nodal status (0, 1–3, 4–10, >10 positive combined with 1–3, >3 examined), histological type and grade (ductal grade I, ductal grade II, ductal grade III, ductal but grade unknown, other histological types), hormone receptor status (ER or PgR positive, at least one negative, both unknown), centre and treatment regimen. Interactions between treatment and the covariates group, age, positive lymph nodes, tumour size and hormone receptor status were investigated in categories as shown in Fig. 3, and in separate models. The assumptions of proportional hazards were assessed by log(–log) S plots, Schoenfeld residuals, and by including in the model a time-dependent component for each covariate. The hazard rate of hormone receptor status was not proportional and therefore stratification was used. Associations between regimen and other characteristics were analysed by chi-square test. P-values are two-tailed. Statistical analyses were done with the SAS 8.2 program package.

3. Results

From January 1990 to January 1998, we recruited 1224 patients nationwide from centres in Denmark, and three health care regions in Sweden and Iceland. Recruitment was closed immediately after the publication of the EBCTCG overviews in 1998 demonstrating a substantial effect of tamoxifen in younger women with breast cancer and superiority of anthracycline-containing chemotherapy regimens compared to regimens without anthracyclines. Twenty were ineligible and 38 patients did not start the assigned adjuvant treatment. A complete follow-up for survival was achieved for all 1224 randomised patients while 25 patients were lost to follow-up for recurrence (Fig. 1). All patients were included in the unadjusted analysis according to the intention to treat principle. The randomisation was well balanced with no significant differences between the two groups (Table 1).

3.1. Study outcome

This analysis was conducted 5 years after closure of recruitment. Median estimated potential follow-up was 10 years both for DFS (9.6 years) and OS (10.2 years). A total of 569 first events were observed; 264 in the CEF group and 305 in the CMF group (Table 2). DFS was significantly improved for the CEF group ($P < 0.04$) and the unadjusted hazard ratio in the CEF group compared with the CMF group was 0.84 (95% CI; 0.71–0.99) (Fig. 2). The unadjusted hazard ratios for DFS were broadly similar in groups A, B and C (0.82, 0.84 and 0.85) and no significant difference was found in a test of equality ($P = 0.99$). Causes of death are shown in Table 2, and the unadjusted hazard ratio for OS in the CEF group compared with the CMF group was 0.79 (95% CI; 0.66–0.94, $P < 0.01$). The

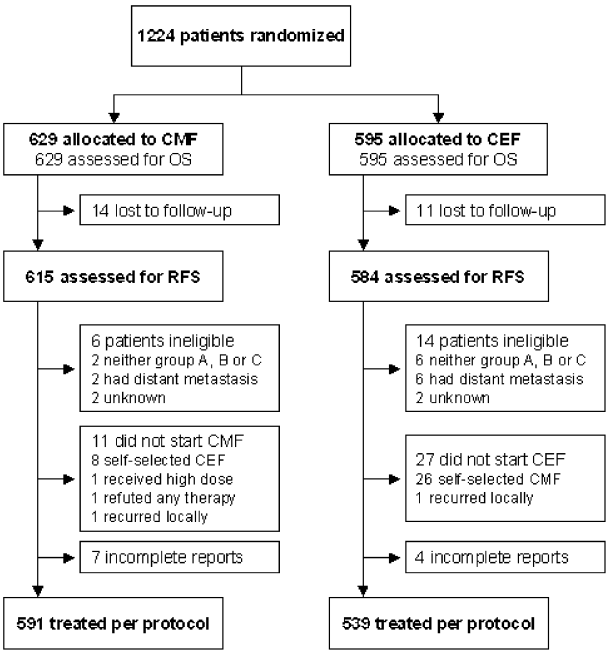

Fig. 1 – Trial profile.

Table 1 – Base-line characteristics of the intention to treat population

	CMF Group (N = 629) No. (%)	CEF Group (N = 595) No. (%)
Age at enrollment		
⩽39 Years	114 (18)	94 (16)
40–49 Years	308 (49)	304 (51)
50–59 Years	133 (21)	127 (21)
60–69 Years	74 (12)	70 (12)
Menopausal status		
Premenopausal	458 (73)	433 (73)
Postmenopausal	171 (27)	161 (27)
Unknown	0 (0)	1 (0)
Loco-regional therapy		
Breast-conserving surgery with irradiation	159 (25)	130 (22)
Mastectomy with irradiation	157 (25)	156 (26)
Mastectomy without irradiation	301 (48)	297 (50)
Mastectomy (irradiation unknown)	10 (2)	10 (2)
Unknown	2 (0)	2 (0)
Nodal status		
Negative	178 (28)	179 (30)
1–3 Positive	236 (38)	227 (38)
⩾4 Positive	185 (29)	164 (28)
Positive, but no. of positive nodes missing[a]	28 (4)	23 (4)
Unknown	2 (0)	2 (0)
Tumour size		
0–20 mm	284 (45)	250 (42)
21–50 mm	294 (47)	295 (50)
>50 mm	43 (7)	37 (6)
Unknown	8 (1)	13 (2)
Histologic type		
Infiltrating ductal carcinoma	552 (88)	532 (89)
Infiltrating lobular carcinoma	17 (3)	20 (3)
Medullar carcinoma	22 (4)	21 (4)
Other carcinomas	11 (2)	4 (1)
Unknown	27 (4)	18 (3)
Malignancy grade (ductal carcinomas only)		
Grade I	34 (6)	39 (7)
Grade II	255 (46)	241 (45)
Grade III	216 (39)	210 (39)
Unknown	47 (9)	42 (8)
Oestrogen-receptor status		
Positive	109 (17)	113 (19)
Negative	368 (59)	331 (56)
Unknown	152 (24)	151 (25)
Progesterone-receptor status		
Positive	72 (11)	65 (11)
Negative	208 (33)	204 (34)
Unknown	349 (55)	326 (55)

a One centre (Umeaa) was unable to report exact no. of positive nodes.

unadjusted hazard ratio for OS was 0.48 (95% CI; 0.28–0.80) in group A compared to 0.84 (95% CI; 0.65–1.08) in group B and 0.86 (95% CI; 0.65–1.13) in group C. This difference was not significant ($P = 0.12$).

Table 2 – End-point events

	CMF Group N = 615 No. (%)	CEF Group N = 584 No. (%)
Events included in analysis of DFS		
Local or regional recurrence only[a]	64 (10)	60 (10)
Distant recurrence	205 (33)	145 (25)
Contralateral breast cancer	18 (3)	33 (6)
Death (without recurrence)	18 (3)	26 (4)
Recurrence, contralateral cancer or death	305 (50)	264 (45)
Second primary non-breast cancer		
Ovaries	4 (1)	2 (0)
AML	1 (0)	1 (0)
MDS	1 (0)	0 (0)
Other	8 (1)	11 (2)
Death	N = 629 No. (%)	N = 595 No. (%)
Any cause	280 (45)	226 (38)
Breast-cancer related	249 (40)	189 (32)
Death (without recurrence)		
Cardio-vascular causes	3 (0)	4 (1)
Other causes	9 (1)	14 (2)
Unknown causes	19 (2)	19 (3)

a Breast, chest wall or regional lymph nodes.

A supportive and unplanned multivariate analysis evaluated CEF compared to CMF in eligible patients treated as assigned according to the protocol (Fig. 3). The prognostic factors included in the Cox model were group, age, nodal status, tumour size, hormone receptor status, and histological type and grade. Type of surgery had no significant impact and was excluded (data not shown). No significant differences were found comparing the adjusted per protocol treatment effects for groups A, B and C with respect to DFS ($P = 0.93$) and OS ($P = 0.16$). The hazard ratio for DFS and OS appeared to be similar irrespective of age, nodal status, tumour size, and receptor status, that is, CEF was not less beneficial in any of these subgroups (Fig. 3).

3.2. *Toxicity*

No treatment related deaths were reported. The mean of the relative cumulative dose (actual/planned mg/m^2) was 0.94 in the CMF group and 0.93 in the CEF group and a similar relative dose per cycle was administered in the two groups (Fig. 4). In both groups the mean of the relative dose intensity (actual/planned mg/m^2 per time unit) was 0.89. Only 38 patients (7%) did not complete all cycles of CEF compared to 28 patients (5%) in the CMF group. Patients who received CEF had had more nausea and vomiting ($P < 0.01$), e.g. 109 (20%) had severe nausea and vomiting at least once compared to 65 (11%) in the CMF group (Table 3). Conjunctivitis or stomatitis was likewise more pronounced in the CEF group. Complete alopecia was uncommon in the CMF group (8%) and frequent in the CEF group (83%).

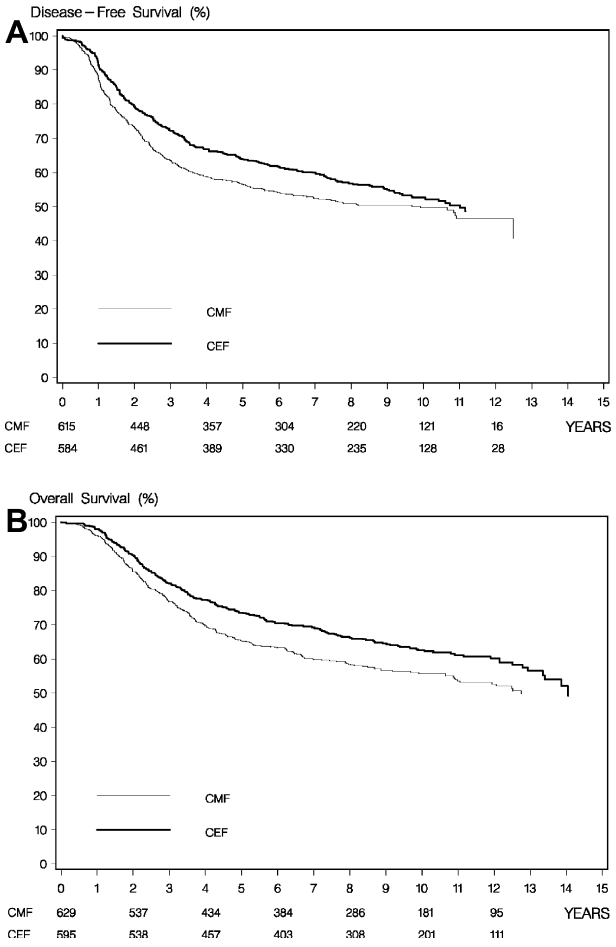

Fig. 2 – Kaplan–Meier estimates of DFS (Panel A) and OS (Panel B).

Among premenopausal patients a permanent cessation of menses was registered in 136 patients (33%) in the CMF group compared to 204 patients (55%) in the CEF group, while the consequences were unclear in 132 patients (32%) and 100 patients (27%), respectively. One patient in each group developed acute myeloid leukaemia (AML) and one patient in the CMF group developed myelodysplastic syndrome (MDS). The overall incidence during treatment or follow-up of cardiac and thromboembolic events was broadly similar in the CEF (4.8%) and CMF (4.3%) groups.

4. Discussion

We report the final and preplanned 10-year results of a symmetrical designed randomised trial comparing CEF with CMF. Substitution of methotrexate with epirubicin provides a prolongation of more than 20% in DFS and OS, which is a significant and clinically meaningful benefit. Apart from a single and preplanned interim analysis at closure of recruitment, these data have not undergone earlier analysis. The benefits from CEF over CMF remained consistent in the three distinct groups of patients selected for this trial, and in the retrospective subgroup analysis regardless of age, nodal status, tumour size, and hormone receptor status.

Randomised trials comparing anthracycline-based and CMF-based regimens have shown mixed results. Only five trials have compared CEF[12,13] or CAF[14–16] with CMF using the same number of drugs, schedule and treatment duration in both regimens. Ten-year results from two of these trials have been published. The Canadian MA.5 trial compared six cycles of classical CMF to CEF with epirubicin 60 mg/m^2 i.v. days one and eight in premenopausal patients without use of tamoxifen.[13] A significant improvement in RFS was achieved with CEF at 5 years, and was maintained at 10-years (52% versus 45%, $P = 0.007$), whereas a significant improvement in OS observed at 5-years not was sustained at 10-years in the stratified log-rank test. In the Intergroup 0102 trial, six cycles of CAF with i.v. doxorubicin 30 mg/m^2 days one and eight was not superior to classical CMF.[16]

A significant difference was not achieved in the four asymmetrical designed trials that compared AC[17,18] or EC[19,20] against classical CMF. With the exception of a small Belgian three-arm trial that used eight causes of EC with 60 or 100 mg/m^2 of epirubicin,[19] these trials compared i.v. anthracycline-based therapy for 12 weeks against 24 weeks of CMF. Heterogeneous results have emerged from trials comparing anthracycline-containing regimens other than CAF/CEF or AC/EC mainly against CMF. Of special interest is a large British/Scottish trial demonstrating superiority of four cycles of three-weekly epirubicin 100 mg/m^2 followed by four cycles of CMF against CMF alone for a similar duration.[21] By assessing individual patient data from 17 trials, not including the recent British/Scottish trial, the EBCTCG meta-analysis[7] has shown a risk reduction for recurrence (11%, $P = 0.001$) and death (16%, $P = 0.00001$). A benefit of the same magnitude as that observed with polychemotherapy compared to no chemotherapy was however achieved in trials (including the current) comparing six to nine cycles of CMF to CEF in a symmetrical design i.e. a 19% reduction in risk of recurrence and a 26% reduction in mortality.[7] Similar reductions were observed when comparing CMF to CAF, while such an effect was not observed from other anthracycline containing regimes. A strong conclusion cannot be derived from the literature, but neither indirect comparisons of the individual trials nor the EBCTCG meta-analysis suggest that CEF for 6 months is inferior to other anthracycline-containing regimens.

Superiority of CEF to CMF was, in the current trial, achieved with the use of epirubicin at a dose of 60 mg/m^2 to a total of at least 420 mg/m^2, and patients in both groups experienced similar haematological toxicity. Outcome was improved in the Belgian trial with high-dose EC (100:830) compared to standard dose EC (50:500), and in a trial by the FASG following escalation of epirubicin from 50 to 100 mg/m^2 in the CEF combination.[19,22] The doses of doxorubicin and cyclophosphamide have been escalated conventionally, not taking individual patient tolerance into account, with no evident benefits.[23–25]

Amenorrhoea occurred more frequently among premenopausal patients in the CEF group (55%) than among patients in the CMF group (33%) ($P < 0.01$), and it has been hypothesised that the efficacy of chemotherapy may partly be mediated through induction of amenorrhoea.[26] In the multivariate analysis however, the superiority of CEF to CMF appears to be independent from age and hormone receptor status. Tamoxifen was not administered, and its use could have

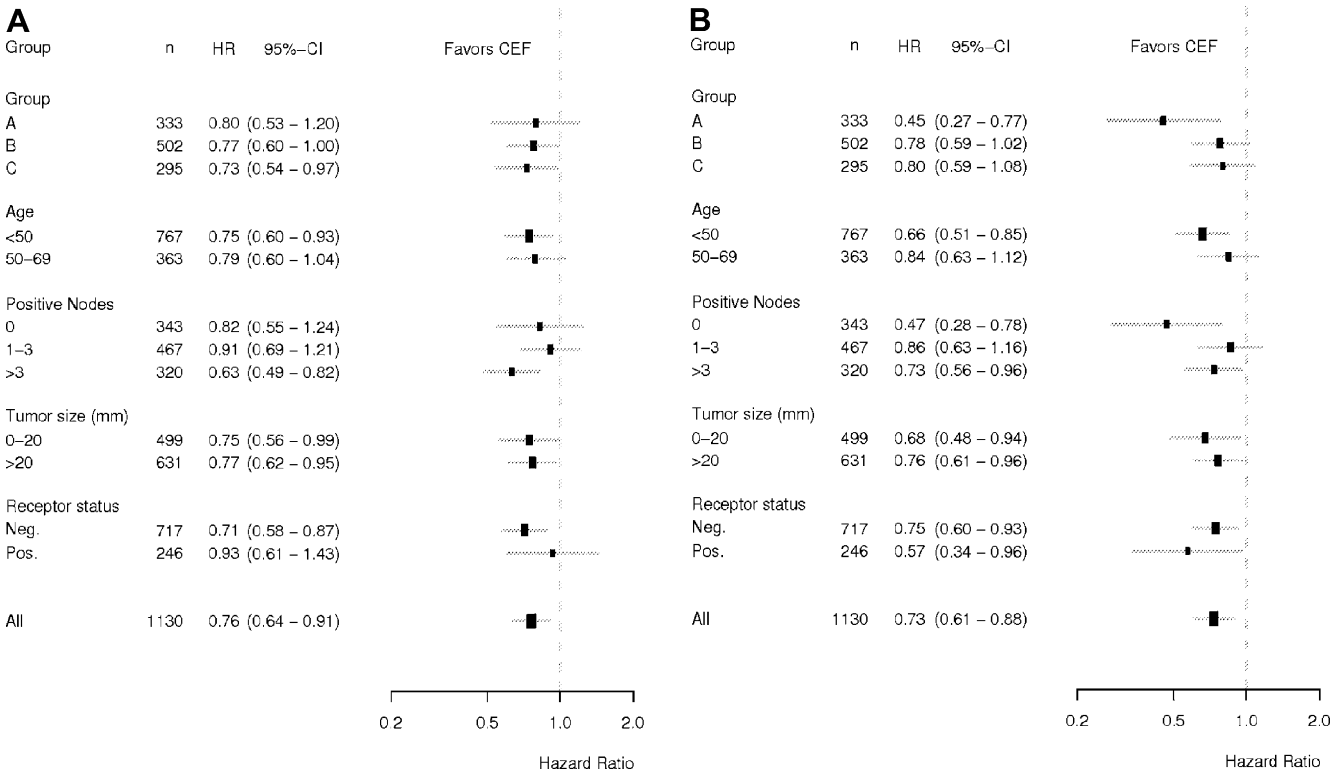

Fig. 3 – Forest plots illustrating proportional hazard models for DFS (Panel A) and OS (Panel B). Hazard ratios refer to adjusted per protocol estimates obtained in the multivariate analysis.

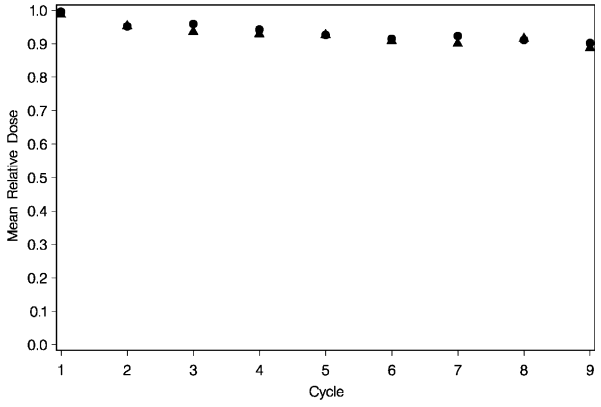

Fig. 4 – Mean relative dose per cycle (actual/planned mg/m^2) of CMF (●) and CEF (▲).

Table 3 – Toxicity

	CMF N = 591 No. (%)	CEF N = 539 No. (%)	P Value
Nausea and vomiting			P < 0.01
None (Grade 0)	179 (30)	105 (20)	
Slight (Grade 1)	187 (32)	172 (32)	
Moderate (Grade 2)	153 (26)	146 (27)	
Severe (Grade 3)	65 (11)	109 (20)	
Unknown	7 (1)	7 (1)	
Conjunctivitis or stomatitis			P = 0.04
None (Grade 0)	325 (54)	249 (46)	
Slight (Grade 1)	172 (29)	196 (36)	
Moderate (Grade 2)	75 (13)	71 (13)	
Severe (Grade 3)	10 (2)	14 (3)	
Unknown	9 (2)	9 (2)	
Alopecia			P < 0.01
None (Grade 0)	238 (40)	31 (6)	
Minimal (Grade 1)	290 (49)	47 (9)	
Complete (Grade 2–3)	48 (8)	446 (83)	
Unknown	15 (3)	15 (3)	

Toxicity was recorded using a simplified translation of the WHO toxicity scale.

influenced the results of this trial. A detrimental outcome has been demonstrated by administration of tamoxifen and chemotherapy concurrently as compared to sequential.

At least one episode with severe vomiting was observed in 20% of the patients in the CEF group compared to 11% in the CMF group. Antiemetics were, however, not used according to modern standards. Aside from alopecia, other acute toxicities were generally acceptable. With a 10-year follow-up only three patients developed AML or MDS and only one of these patients received CEF. Considering the dose per cycle (60 mg/m^2), dose-intensity (20 mg/m^2/week) of epirubicin, and maximal cumulative doses below 540 mg/m^2 of epirubicin and 5900 mg/m^2 of cyclophosphamide, this low incidence of AML/MDS was anticipated.[27] The incidence of cardiotoxicity was not increased in the CEF group, which was anticipated due to the dose-intensity and cumulative dose of epirubicin.[28,29]

The topoisomerase II-alpha protein is the primary target of anthracyclines, and the gene encoding topoisomerase II-alpha (*TOP2A*) is located close to *HER2* and is often co-amplified with *HER2*. A retrospective study of the Danish part of the current trial suggests a positive predictive value of both

TOP2A amplifications and deletions concerning the efficacy of epirubicin.[30] The use of *TOP2A* could reestablish selection criteria for the use of non-anthracycline containing chemotherapy, but must await confirmation in other trials.

In conclusion, this definitive analysis demonstrates a therapeutic advantage of CEF over CMF in primary breast cancer patients. Chemotherapeutic treatment with CEF was associated with an increase in acute but reversible toxicities, but a subsequent increase in late toxicities was not observed.

Conflict of interest statement

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