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Randomized Trial Comparing Cyclophosphamide, Methotrexate, and 5-Fluorouracil (CMF) Regimen with Rotational CMFEV Regimen (E = Epirubicin, V = Vincristine) as Adjuvant Chemotherapy in Moderate Risk Operable Breast Carcinoma*

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ABSTRACT

Objectives: *The CMFEV* (*cyclophosphamide, methotrexate*, 5-*fluorouracil*, *epirubicin*, *vincristine*) *regimen is an innovative schedule, designed by our Group, aimed at administering five partially or totally no cross-resistant cytotoxic agents in breast carcinoma. It was randomly compared to CMF* (*cyclophosphamide, methotrexate*, 5-*fluorouracil*) *as primary treatment in operable disease and demonstrated a short-term significant increase in clinical complete response rate and a long-term significant locoregional relapse-free survival in premenopausal patients. So, it seemed worth comparing this regimen with CMF as adjuvant chemotherapy in moderate risk operable breast carcinoma. Methods*: *Four hundred and eighty-nine patients with stage I or II moderate risk breast carcinoma were randomized to receive CMF or CMFEV regimen for* 6 *cycles after surgery. Main end points were overall survival* (*OS*), *invasive disease-free survival* (*IDFS*) *and recurrence-free interval* (*RFI*), *as estimated by Kaplan-Meier analyses and log-rank tests. Results*: *At a median observation time of* 7.3 *years* (*range* 5.4 *months*-10.3 *years*), *no significant differences in OS and IDFS were observed between the two arms. Deaths from breast carcinoma were more frequent with CMF* (58.5%) *than with CMFEV regimen* (41.7%) *as well as recurrences from breast carcinoma* (58.8% *with CMF and* 41.2% *with CMFEV*). *These differences were not statistically significant. Conclusion*: *CMFEV appears more effective than CMF in preventing recurrences from primary disease in patients with moderate risk stage I-II breast carcinoma. The lack of statistical significance of the observed differences was probably due to the limited number of patients enrolled which rendered the study underpowdered.*

Keywords*: *Breast Carcinoma*, *Adjuvant Chemotherapy*, *CMF Regimen*, *Epirubicin*, *Vincristine*, *Second Malignancy

1. Introduction

The role of adjuvant systemic therapy in early stage resectable breast carcinoma has been established in a number of prospective randomized studies, and its significant contribution in reducing the odds of relapse and death has been clearly validated by the worldwide overview [1].

The Milan Cancer Institute research group activated the first studies demonstrating and confirming the long

*On behalf of the Italian Oncology Group for Clinical Research (GOIRC), Parma, Italy. The Medical Oncology Units of Palermo, Foligno, Todi and Grosseto contributed with only a few cases.

term efficacy of the combination of cyclophosphamide, methotrexate, 5-fluorouracil (CMF), which became a classical chemotherapy regimen [2,3]. Since the first report, this combination has been modified in a number of ways, but mainly by the addition of other drugs, such anthracycline, sometimes vincristine and, lately, taxanes [4-8].

The CMFEV (cyclophosphamide, methotrexate, 5-fluorouracil, epirubicin, vincristine) regimen is an innovative schedule, compared to CMF, aimed at administering five partially or totally no cross-resistant cytotoxic agents. It was first designed and tested by our Group as a means of late intensification after CMF in metastatic breast carcinoma [9] and then in the neoadjuvant setting of operable breast carcinoma [10,11]. Its rotational strategy is different from that of alternating or sequential schemes as the five agents are administered at full dose but, in order to avoid excessive toxicity and consequent dose reductions, each cycle involves the administration of only four drugs, always including vincristine (V) and epirubicin (E). The regimen is organized in such a way that only two, among the three potentially myelotoxic drugs of the CMF regimen, are rotatively included in the schedule (CMEV, CFEV, MFEV). The planned dosages of C, M and F in each cycle were therefore either 100% or 0%.

The aim of this prospective randomized study was to compare the classical CMF regimen with the CMFEV rotational regimen, both administered postoperatively for 6 cycles, in patients with operable breast carcinoma at a moderate risk of relapse (1 to 3 positive axillary nodes or axillary node negative with at least one biological or morphological risk factor).

2. Patients and Methods

2.1. Eligibility Criteria

The main eligibility criteria were 1) histologically proven operable breast carcinoma recently submitted to a potentially curative surgery; 2) 1 to 3 axillary positive nodes or negative axillary nodes with at least one biological risk factor (estrogen and progesterone receptor negative and/or high proliferative activity (Ki 67, or another proliferative index, higher than 15%) and/or histological grade 3; 3) age ≤70 years; 4) absence of previous or concomitant contralateral breast carcinoma or of previous or concomitant different malignant neoplasm; 5) absence of distant metastases following a complete staging including physical examination, chest X-ray, bone scan, liver echography or computed tomography; adequate bone marrow, kidney, liver, and heart function. Patients with clinical stage III tumours (T3N1; or T4 any N; or any T N2) were not eligible.

2.2. Study Design and Treatment

This was a multi-institutional study carried out by the Medical Oncology Units of Parma, Reggio Emilia, Perugia, Piacenza, Terni, Vigevano, Fermo and Marsciano of the Italian Oncology Group for Clinical Research (GOIRC). The Medical Oncology Units of Palermo, Foligno, Todi and Grosseto contributed with a few cases.

The study design was approved by the ethical committees of the participating institutions and conducted in accordance with the International Good Clinical Practice Guidelines. All patients gave their written informed consent before enrolment in the study. The patients were centrally randomized via phone call to the coordinating office of the GOIRC in Parma. Allocation was made within strata defined by institution, menopausal status (premenopausal vs. postmenopausal), tumour diameter (T1, T2, T3), hormonal receptor status (negative or positive), axillary nodal status (positive or negative). Patients were assigned to receive 6 cycles of CMF regimen or 6 cycles of CMFEV rotational regimen.

Doses and schedules of the CMF combination and of the CMFEV rotational combination are reported in **Table 1**.

Postmenopausal patients, independently from their estrogen receptor (ER) and/or progesterone receptor (PgR) status, received oral tamoxifen 20 mg per day for 5 years, starting at the end of the chemotherapy treatment. Premenopausal patients received this treatment with oral tamoxifen only if they had their estrogen and/or progesterone receptor status positive.

Blood chemistry and liver function tests were repeated on day 1 of each cycle, and complete blood counts were obtained on day 1 and 8. Treatment was delayed of one week if the white blood cell (WBC) count was lower than 4000 and/or platelet count was lower than 120,000. On day 1 after the one week delay, and on day 8, the dosages of C, M, F, and E were reduced by 30% when the WBC ranged from 3900 to 3600 and/or the platelet count ranged from 119,000 to 100,000, and by 50% when the WBC ranged from 3500 to 2500 and/or the platelet count ranged from 99,000 to 70,000. No drugs were administered when the WBC was less than 2500 and/or the platelet count was less than 70,000. No dose reductions were planned for vincristine.

The adjuvant chemotherapy program had to begin no later than 6 weeks from the initial surgery. Radiation therapy, whenever indicated, had to start after the conclusion of chemotherapy program.

Treatment with tamoxifen (20 mg/day for 5 years),

Table 1. CMF and CMFEV regimens: Doses and schedules.

CMF (standard regimen)		
Cyclophosphamide:	600 mg/m^2, iv short infusion	day 1 and 8
Methotrexate:	40 mg/m^2, iv bolus	day 1 and 8
5-Fluorouracil:	600 mg/m^2, iv bolus	day 1 and 8
		(every 4 weeks, 6 cycles)
CMFEV (rotational regimen)		
CMEV combination		
Cyclophosphamide:	600 mg/m^2, iv short infusion,	day 1 and 8
Methotrexate:	40 mg/m^2, iv bolus,	day 1 and 8
Epirubicin:	40 mg/m^2, iv bolus,	day 1 and 8
Vincristine:	1.4 mg/m^2, iv bolus	day 1
		(every 4 weeks, cycles 1 and 4)
CFEV combination		
Cyclophosphamide:	600 mg/m^2, iv short infusion	day 1 and 8
5-Fluorouracil:	600 mg/m^2, iv bolus,	day 1 and 8
Epirubicin:	40 mg/m^2, iv bolus	day 1 and 8
Vincristine:	1.4 mg/m^2, iv bolus	day 1
		(every 4 weeks, cycles 2 and 5)
MFEV combination		
Methotrexate:	40 mg/m^2, iv bolus,	day 1 and 8
5-Fluorouracil:	600 mg/m^2, iv bolus,	day 1 and 8
Epirubicin:	40 mg/m^2, iv bolus,	day 1 and 8
Vincristine:	1.4 mg/m^2, iv bolus,	day 1
		(every 4 weeks, cycles 3 and 6)

when administered, began after the last cycle of chemotherapy Cardiotoxicity was monitored evaluating the left ventricular ejection fraction by radionuclide or ultrasound technique at baseline and, in patients assigned to the CMFEV arm, at the end of the third and of the sixth cycle.

Follow-up visits took place every three months during the first 2 years, every six months during years 3 through 5, and annually thereafter.

2.3. Toxicity Evaluation

Toxicity was evaluated according to the WHO criteria [12], and the patients were classified on the basis of the worst degree of treatment related side effects. .

2.4. Endpoints and Statistical Analyses

The definition of the endpoints selected for this study follows the recommendations of the STEEP system [13].

The primary end point was overall survival (OS), as estimated from the date of random assignment to the date of last contact or death from any cause. Secondary endpoints were the invasive disease-free survival (IDFS) and the recurrence-free interval (RFI). IDFS was estimated from the date of random assignment to the date of occurrence of any of invasive ipsilateral breast tumor recurrence, locoregional recurrence, distant recurrence, invasive contralateral breast cancer, second primary invasive cancer, or death from any cause, whichever came first. RFI was estimated from the date of random assignment to the date of occurrence of any event related to the primary breast tumour, *i.e.* ipsilateral breast, locoregional or distant recurrence, or death from breast cancer. All randomized patients were included in the estimations of OS, IDFS and RFI, according to the intention-to-treat principle.

Others aims of the study were the estimates of the locoregional recurrence-free survival (LRRFS) and of the distant recurrence-free survival (DRFS).

To estimate the LRRFS, the recurrence of invasive carcinoma in the ipsilateral breast, chest wall or skin, or in the ipsilateral axillary, supraclavicular or internal mammary lymph nodes were considered as events. To estimate the DRFS, the first occurrence of metastasis at any distant site was considered as event. In the RFI, LRRFS and DRFS analyses, the patients who developed a contralat-

eral primary breast carcinoma or a second primary non-breast malignancy were censored.

OS, IDFS, RFI, LRRFS and DRFS were obtained from Kaplan–Meier analyses [14], and the primary comparison between the two groups was carried out using the log-rank test.

Cox's model [15] was used for multivariate analyses to assess the independent prognostic role of each prognostic factor, while adjusting for the effect of the other factors. The variables included in the models as covariates were: treatment assigned (CMF or CMFEV), patient age (≤40 years, 41 - 50 years, 51 - 60 years or >60 years), menopausal status (pre or post), clinical T (T1 or >T1), grading (G1/G2 or G3), lymph node status (positive or negative) and hormonal receptor status (ER^-/PgR^-, ER^+/PgR^+ or either one receptor positive). Hazard ratios (HRs) for each variable were obtained by exponentiating the coefficients estimated by the Cox models. Modifications of the relative effect of CMFEV as compared to CMF across the strata of each covariate were assessed by introducing the appropriate interaction terms in the model. These covariates by treatment interaction terms were introduced in the model one at a time. The likelihood ratio test was used to evalu ate the statistical significance of each interaction term. The results of the subgroup analyses are graphically summarized using the Forest plot as indicated by Cuzick [16].

The Pearson Chi-square test and the Fisher's exact test were used to compare the distribution of patient characteristics and toxicities in the two treatment arms.

All statistical tests were two-sided and were carried out using the SPSS package (version 13.0 for Windows). Significance was accepted for P values <0.05.

3. Results

3.1. Patients Characteristics

Between October 1994 and April 2000, 489 patients were randomized to receive CMF (n = 244, 49.9%) or CMFEV (n = 245, 50.1%). One patient, assigned to the CMF arm, was not eligible due to the presence of 7 positive axillary nodes.

Table 2 summarizes the main characteristics of the randomized patients. The median age was 54 years both in CMF arm (range 31 to 70) and in CMFEV arm (range

Table 2. Patient characteristics.

	Total, *n* (%)		CMF, *n* (%)		CMFEV, *n* (%)		*p* #
Age							
Median (y)			54	(31 - 70)	54	(29 - 70)	
≤40 y	54	(11.1)	26	(10.6)	28	(11.4)	0.961
>40 e ≤50 y	135	(27.6)	67	(27.4)	68	(27.8)	
>50 e ≤60 y	187	(38.2)	96	(39.4)	91	(37.1)	
>60 y	113	(23.1)	55	(22.6)	58	(23.7)	
Menopausal status							
Premenop	209	(42.7)	103	(42.2)	106	(43.3)	0.874
Postmenop ≤60 y	167	(34.2)	86	(35.2)	81	(33.1)	
Postmenop > 60 y	113	(23.1)	55	(22.6)	58	(23.6)	
Tumor diameter							
≤2 cm.	260	(53.2)	132	(54.1)	128	(52.2)	0.681
>2 cm.	229	(46.8)	112	(45.9)	117	(47.8)	
Nodal status							
Negative	203	(41.5)	104	(42.6)	99	(40.4)	0.619
Positive	286	(58.5)	140	(57.4)	146	(59.6)	
Histological grade							
G1 + G2	216	(44.2)	113	(46.3)	103	(42)	0.339
G3	207	(42.3)	98	(40.2)	109	(44.5)	
Unknown	66	(13.5)	33	(13.5)	33	(13.5)	
Receptor status							
ER+/PgR+	249	(50.9)	127	(52.1)	122	(49.7)	0.741
ER–/PgR–	139	(28.4)	71	(29.1)	68	(27.8)	
Either one positive	101	(20.7)	46	(18.8)	55	(22.5)	
Proliferative activity (Ki67)							
<15%	98	(20.0)	41	(16.8)	57	(23.2)	0.074
>15%	391	(80.0)	203	(83.2)	188	(76.8)	
Type of surgery							
Breast-conserving	296	(60.5)	154	(63.1)	142	(57.9)	0.243
Mastectomy	193	(39.5)	90	(36.9)	103	(42.1)	

Two hundred forty-four (49.9%) patients in the CMF arm and 245 (50.1%) patients in the CMFEV arm. #Pearson Chi –Square Test for heterogeneity. ER, estrogen receptor; PgR, progesterone receptor.

29 to 70). Two hundred and nine (42.7%) patients were premenopausal; overall, 280 (57.3%) patients were postmenopausal; among those, 167 (34.2%) were ≤60 years and 113 (23.1%) were >60 years. Two hundred and sixty (53.2%) patients had tumour diameter ≤2 cm and 229 (46.8%) >2 cm. Two hundred and three (41.5%) patients were node-negative and 286 (58.5%) patients were node-positive. Histological grade G1 or G2 were found in 216 (44.2%) patients and G3 in 207 (42.3%) patients. ER and PgR were both positive in 249 (50.9%) patients and both negative in 139 (28.4%) patients. Either one of the receptors was positive in 101 (20.7%) of the cases. Tumour proliferative activity was low in 98 (20.0%) patients and moderate/high in 391 (80.0%) patients. Two hundred ninety-six (60.5%) patients received breast conserving surgery, and the remaining 193 (39.5%) underwent mastectomy. No remarkable differences in the patient characteristic distribution between the two study arms were seen (all $p > 0.05$).

3.2. Survival and Events

The distribution of all events (death, recurrence or new malignancy) between the two arms is summarized in **Table 3**.

Sample size estimates, at the time of the design of this trial, were based on unrealistic and outdated projections about survival and effects of the experimental treatment (e.g. 5-year OS = 65%). Posterior power estimates, based on the number of events actually observed, indicate that the study, with 120 relapses, had a power of 89% to detect HR's of 0.6 for IDFS. With 63 deaths, the study had standard power (80%) to detect only risk reductions in excess of 50%.

3.2.1. Overall Survival

The cut-off date for follow-up was July 31, 2006. The median observation time from random assignment to death or censoring was 7.31 years (range: 5.4 months -10.3 years). At the end of the observation period, 426 patients (87.1 %) were alive, with a median follow-up of 7.68 years (7.73 years for the CMF arm and 7.64 years for the CMFEV arm). Among those alive patients, 36 (85.4%) were disease-free and 62 (14.6%) were not. Overall, 63 deaths occurred, 33 (52.4%) in the CMF arm and 30 (47.6%) in the CMFEV arm**.** Among these deaths, 48 (76.2%) were a consequence of the primary breast tumor and 15 (23.8%) were due to other causes. No significant difference in OS was seen between the two arms (**Figure 1**, log rank $p = 0.687$). Cumulative OS at 5 years was 93.0% (95% CI 91.2 - 94.4) in the CMFEV arm and 92.6% (95% CI 90.8 - 94.1) in the CMF arm. At 10 years, these values decreased to 80.5% (95% CI 77.9 - 82.4) in the CMFEV arm and 82.3 (95% CI 79.8 - 84.5) in the CMF arm.

3.2.2. Invasive Disease-Free Survival

Overall, 120 events were observed, 61(50.8%) in the CMF arm and 59 (49.2%) in the CMFEV arm. Among these events, 1 case of death from primary breast cancer without relapse was observed. The 120 events thus included 90 locoregional or distant recurrences and 30 cases of second malingnacy or contralateral breast cancer. IDFS, as estimated for all these events, was not statistically different between the two arms (log rank $p = 0.892$, not shown).

3.2.3. Recurrence-Free Interval, Locoregional Recurrence-Free Survival and Distant Recurrence-Free Survival

When considering the RFI, which evaluates only the events related to the primary breast tumor, we observed that the rate of recurrence was higher in the CMF arm than in the CMFEV arm. Of the 90 recurrences, 53 (58.9%) occurred in the CMF arm and only 37 (41.1%)

Table 3. Events according to treatment.

EVENT	CMF arm *n* (%)		CMFEV arm *n* (%)		TOTAL *n* (%)	
Death						
from breast cancer	28	(58.5)	20	(41.7)	48	(76.2)
from other cause	5	(33.3)	10	(66.7)	15	(23.8)
total	33	(52.4)	30	(47.6)	63	(100.0)
Recurrence						
locoregional	15	(62.5)	9	(37.5)	24	(26.7)
distant[¶]	38	(57.5)	28	(42.4)	66	(73.3)
total	53	(58.9)	37	(41.1)	90	(100.0)
Second malignancies						
contralateral breast	2	(33.3)	4	(66.7)	6	(20.0)
endometrium	3	(50.0)	3	(50.0)	6	(20.0)
leukemia	0	-	3	(100.0)	3	(10.0)
others[§]	3	(20.0)	12	(80.0)	15	(50.0)
total	8	(26.7)	22	(73.3)	30	(100.0)

[¶] including the one patient who died without revealed recurrence; [§] including ovary

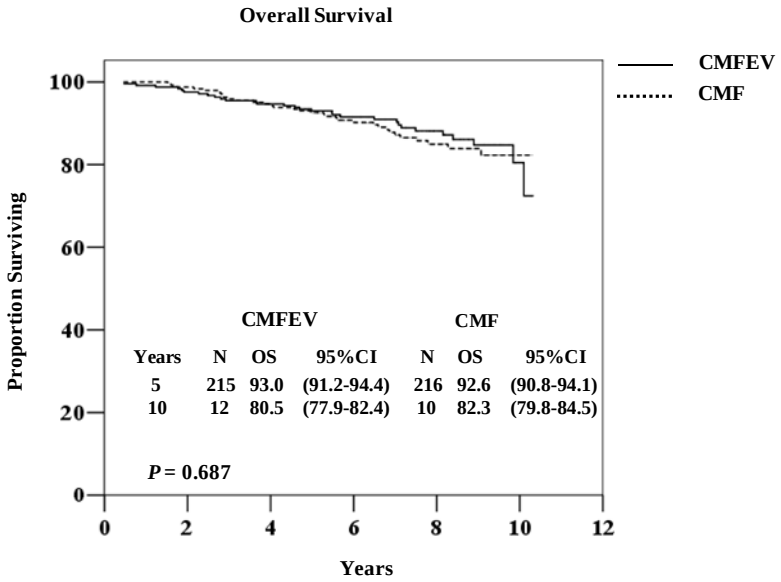

Figure 1. Kaplan–Meier analysis of overall survival. CMF, cyclophosphamide, methotrexate and 5' fluorouracil; CMFEV, CMF, epirubicin and vincristine; N, number of patients at risk; OS, overall survival with 95% confidence interval (CI) in parentheses. *P* value from log-rank test (two-sided) = 0.687.

in the CMFEV arm. This beneficial effect, although not statistically significant, of CMFEV over CMF is shown in **Figure 2** At 10 years, recurrence-free was 80.6% (95% CI 72.1 - 87.5) in the CMFEV arm as compared to 73.5% (95% CI 63.5 - 80.8) in the CMF arm (log rank p = 0.099).

Of these 90 events, 24 were locoregional recurrences and 66 were distant recurrences. Among the 24 locoregional events, 15 (62.5%) were in the CMF arm and 9 (37.5%) in the CMFEV arm. Cumulative 10-year LRRFS was not statistically different between the two arms, being 90.4% (95% CI 88.4 - 92.1) for the CMF arm and 94.0% (95% CI 92.3 - 95.3) for the CMFEV arm (log rank p = 0.472; not shown). Among the 66 distant relapses, 38 (57.5%) were in the CMF arm and 28 (42.4%) in the CMFEV arm. Cumulative 10-year DRFS was similar in the 2 arms, being 78.7% (95% CI 76.1 - 81.1) for the CMF arm and 83.5% (95% CI 81.1 - 85.7) for the CMFEV arm (log rank p = 0.231; not shown).

3.2.4. Second Malignancies

Overall, 30 cases of second malignancies were observed. These events were significantly more frequent in the CMFEV arm than in the CMF arm (Odd ratio = 3.53, 95% CI 1.38 - 9.26, p = 0.003). In the CMF arm, second malignancies occurred in 8 patients (2 contralateral breast cancers and 6 non-breast malignancies); in the CMFEV arm, second malignancies occurred in 22 patients (4 contralateral breast cancers and 18 non-breast malignancies). In the CMF arm, new primary non-breast malignancies were endometrial cancer (3 patients), ovary, lung or kidney cancer (1 patient for each site). In the CMFEV arm, the second non-breast malignancies were melanoma (1 patient), endometrial cancer (3 patients), ovary cancer (3 patients), colorectal cancer (2 patients), thyroid, liver, lung, stomach or pancreas cancer (1 patient for each site), glioblastoma (1 patient) and leukemia (3 patients).

3.3. Multivariate and Subgroup Analyses

In multivariate analyses, nodal status, tumor size and hormonal receptor status were independently associated with OS, IDFS and RFI (not shown) After adjustment for these three factors as well as for patient age, histological grade and menopausal status, we did not find any significant difference in the hazard of death between the CMFEV arm and the CMF arm in the overall population (HR = 0.80, 95% CI 0.48 - 1.35, p = 0.411, **Figure 3**). A similar lack of treatment effect on IDFS was observed (HR = 0.91, 95% CI 0.62 - 1.34, p = 0.645, not shown). By contrast, the hazard of recurrence from the primary breast tumor was lower, with a borderline statistical significance, in the CMFEV arm as compared to the CMF arm (HR = 0.67, 95% CI 0.43 - 1.04, p = 0.073, **Figure 4**). Subgroup analyses of OS comparing the CMFEV arm versus the CMF arm within strata formed by each prognostic factor showed evidence of interaction between the type of adjuvant treatment and the receptor status (**Fig-**

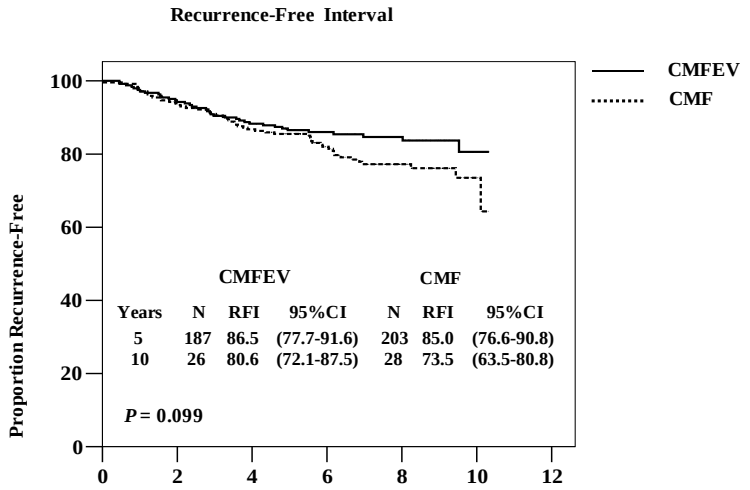

Figure 2. Kaplan–Meier analysis of recurrence-free interval CMF, cyclophosphamide, methotrexate and 5' fluorouracil; CMFEV, CMF, epirubicin and vincristine; N, number of patients at risk; OS, overall survival; EFS, recurrence-free interval, with 95% confidence interval (CI) in parentheses. *P* value from log-rank test (two-sided) = 0.099.

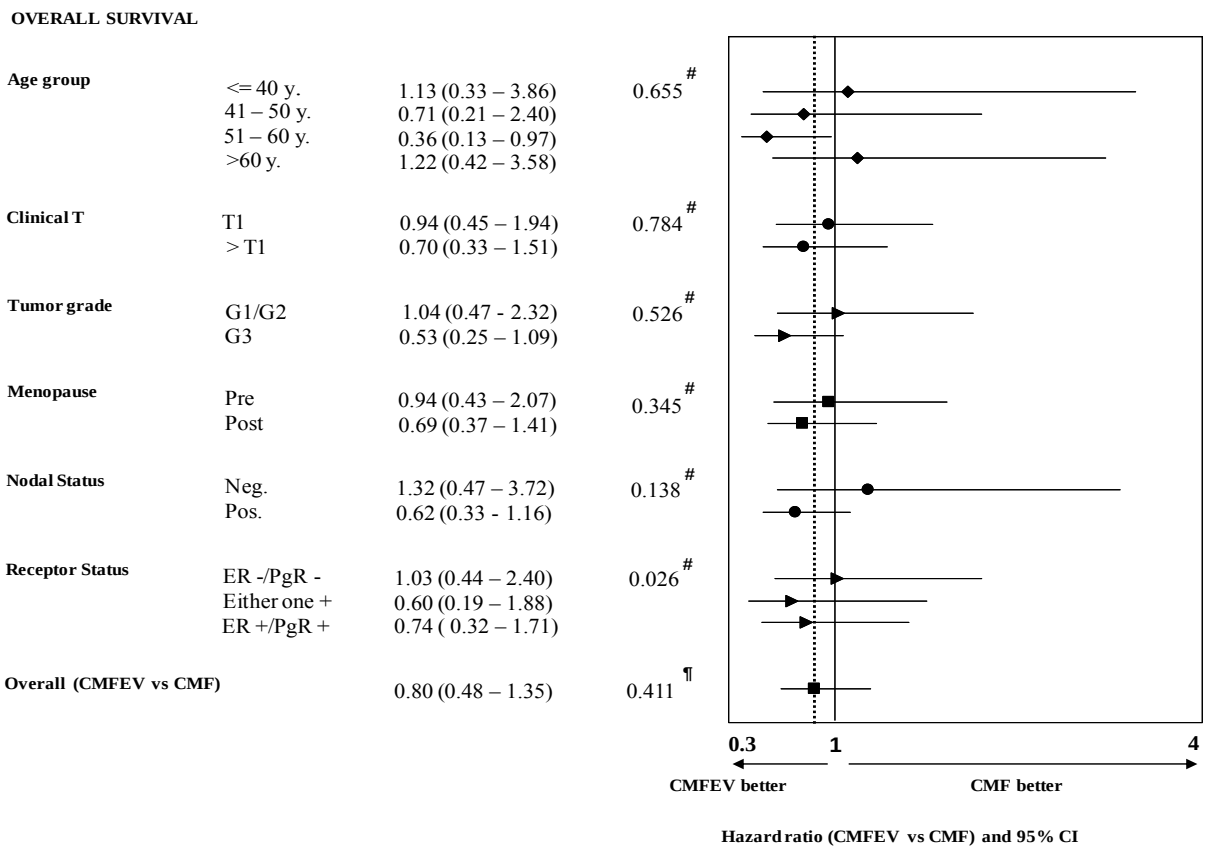

Figure 3. Forest plot of subgroup analysis of OS comparing the CMFEV arm versus the CMF arm within strata formed by each prognostic factor. Hazard ratios and 95% confidence intervals (CI) from a Cox Model in which all covariates significantly contributing to the likehood of the model in the entire dataset were used. Interaction terms assessing the heterogeneity of the effect of treatment regimens across strata for each covariate were introduced in the model one at a time. *P* values are from likehood ratio tests. All statistical tests were two-sided. The plain line shows no effect point and the dotted line shows overall treatment effect for the entire dataset. #test for interaction; ¶overall comparison of CMFEV arm versus CMF arm adjusted for all prognostic factors.

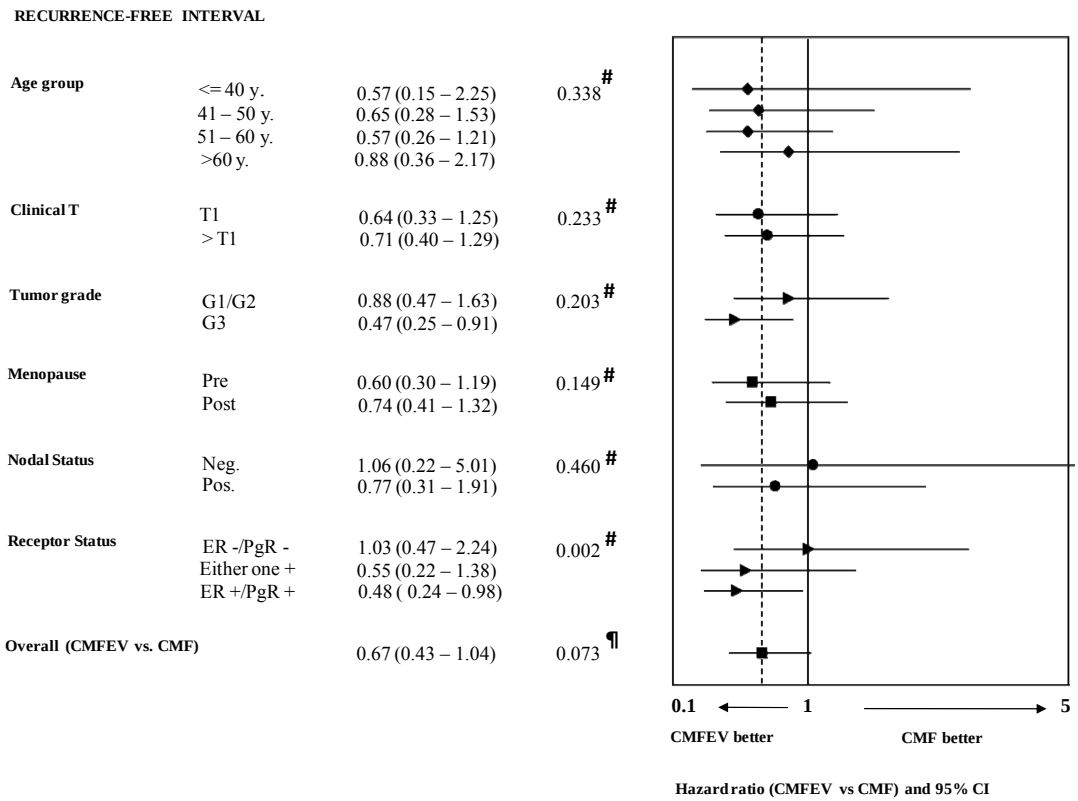

Figure 4. Forest plot of subgroup analysis of RFS comparing the CMFEV arm versus the CMF arm within strata formed by each prognostic factor. Hazard ratios and 95% confidence intervals (CI) from a Cox Model in which all covariates significantly contributing to the likehood of the model in the entire dataset were used. Interaction terms assessing the heterogeneity of the effect of treatment regimens across strata for each covariate were introduced in the model one at a time. *P* values are from likehood ratio tests. All statistical tests were two-sided. The plain line shows no effect point and the dotted line shows overall treatment effect for the entire dataset. #test for interaction; ¶overall comparison of CMFEV arm versus CMF arm adjusted for all prognostic factors.

Table 4. Main toxicities.

	CMF arm		CMFEV arm	
	n	%	*n*	%
Hematological toxicities				
Hemoglobin				
G1/2	34	14	71	29
G3/4	7	3	18	7
WBC				
G1/2	104	43	93	38
G3/4	17	7	44	18
Platelets				
G1/2	4	2	9	4
Non haematological toxicities				
Nausea/vomiting	160	66	179	73
Epigastric pain	27	11	40	16
Constipation	6	2	15	6
Oral	53	22	109	44
Mucositis	71	29	69	28
Skin	10	4	15	6
Kidney	2	1	2	1
Liver	30	12	29	12
Heart	15	6	17	7
Asthenia	27	11	41	17
Mandibular pain	0	0	14	6
Peripheral nervous system	16	7	74	30

ure 3, $p = 0.026$). CMFEV regimen was more favourable when patients presented both estrogen and progesterone receptors positive, or either one receptor positive (HR = 0.74, 95% CI 0.32 - 1.71 and HR = 0.60, 95% CI 0.19 - 1.88, respectively). By contrast, no treatment effect was seen when both receptors were negative (HR = 1.03, 95% CI 0.44 - 2.40) Similar results were obtained for subgroup analyses of RFI (**Figure 4**, $p = 0.002$; HR = 0.48, 95% CI 0.24 - 0.98 for ER+/PgR+; HR = 0.55, 95% CI 0.22 - 1.38 for either one receptor positive; HR = 1.0, 95% CI 0.47-2.24 for ER-/PgR). Regarding IDFS subgroup analysis, no significant treatment effect with respect to positive receptors was observed (not shown).

3.4. Toxicities

Table 4 reports the main toxicities according to treatment. With respect to haematological toxicities, there was a higher incidence of anaemia both grade 1 - 2 and 3 - 4 in the CMFEV arm than in the CMF arm (29% vs. 14%, $p = < 0.001$ and 7% vs. 3%, $p = 0.02$, respectively); there were no relevant differences between the two arms in terms of WBC and platelet toxicities.

With respect to non-haematological toxicities, in the CMFEV arm, compared to the CMF arm, there was a more frequent occurrence of stomatitis (44% vs. 22%; $p < 0.001$), constipation (6% vs. 2%; $p = 0.04$), peripheral nervous system toxicity (30% vs. 7%; $p < 0.001$), mandibular pain (6% vs. 0%).

4. Discussion

4.1. CMFEV Regimen versus CMF Regimen: General Considerations

In the present study, our Group compared for the second time the conventional CMF regimen with the experimental CMFEV regimen in the treatment of non metastatic breast carcinoma. In the first occasion, the comparison was developed in a neoadjuvant settings [10,11]; in the present second occasion, the comparison was developed in an adjuvant settings. Therefore such comparison presents, by itself, some similarities but also some substantial differences. Firstly, these differences are related to the therapeutic scheme utilized, which refers to the different treatment settings. Moreover, in the neoadjuvant settings, the comparison of the therapeutic results could be done both on a short term, based on the evaluation of objective therapeutic response [10], as well as on a long term, based on the outcome [11]. In the adjuvant settings the comparison was possible only on a long term.

In addition, in the present study, some results, which confirmed a superiority of the CMFEV regimen over the CMF regimen, were possibly disturbed and masked by an unexpected increase of second malignancies observed in the CMFEV arm.

For all these reasons, we believe that in a first part of the discussion, it could be useful to summarize in a comparative way the number and the proportions of the main events (relapse and death) we observed in the present study when administering CMF or CMFEV. In a second part of the discussion, we will elaborate a comparative evaluation of the main results observed in our first study of neoadjuvant chemotherapy and in the present study of adjuvant chemotherapy, in terms of efficacy, relationship with endocrine parameters, toxicities. In a third part, we will comment on the unexpected increase of second malignant tumors here observed with CMFEV. In the last part of the discussion, we will compare our results to those reported by other Authors within a comprehensive evaluation of the adjuvant chemotherapy of operable breast carcinoma.

4.2. CMFEV Regimen versus CMF Regimen: Efficacy, Relationship with Endocrine Parameters and Toxicity

The results of the present study showed that the recurrence from the primary tumor was more frequent with CMF regimen (63 events, 58.2%) than with CMFEV regimen (38 events, 41.8%). At 10 years, RFI was 73.5% with CMF and 80.6% with CMFEV. Similarly, deaths due to primary tumor were more frequent in the CMF arm (28, 58.5%) compared to the CMFEV arm (20, 41.7%), even if the OS at 10 years was similar with either regimen (82.3% with CMF, 80.5% with CMFEV).

These differences in terms of recurrence and death, although not statistically significant, offer evidence of a potential major efficacy of the CMFEV compared to the CMF regimen. This efficacy was, at least in part, more clearly showed in our previous study on neo-adjuvant chemotherapy where, on a short term evaluation, the rate of clinical responses, both complete (CR) and complete plus partial (PR), were significantly higher in the subset of pre-menopausal patients treated with CMFEV compared to those treated with CMF [10]. Similarly, on a long-term evaluation, again in the subset of pre-menopausal patients, the proportion of RFS tended to be higher and the proportion of LRRFS was significantly higher in the CMFEV arm compared to the CMF arm, thus mirroring the short-term response results [11].

The reason of the lack of statistical significant differences observed in the present study and of only a few statistically significant differences observed in the pre-

vious study, is probably due to the fact that, in the two studies, different parameters were considered and estimated, and that the eligibility criteria, although similar, were not identical. In any case, both studies may have been under-powered to demonstrate a significant major efficacy of CMFEV over CMF.

Interestingly, both the previous and the present studies showed statistically significant differences between the two chemotherapy regimens according to the menopausal status of the patients or to the biological characteristics of their tumors in terms of ER and/or PgR status. Indeed in the first study, the superiority of CMFEV regimen, as short term objective response, was seen only in pre-menopausal and not in post-menopausal patients; in addition, in a multivariate analysis, a significant interaction was confirmed between the menopausal status and the type of treatment on the probability to obtain CR or CR plus PR [10]. In the present study, a significant correlation was observed between the estrogen and/or progesterone receptor status and the type of regimen; CMFEV was more effective, in terms of OS and RFI, in patients with positive ER and/or PgR tumors but not in patients with both negative ER and PgR tumors. Considering those correlations, it appears that endocrine influences induced by the menopausal status of the patients and/or by the ER and/or PgR status of the tumors may determine the comparative responses of CMF / CMFEV used either as neo-adjuvant or adjuvant chemotherapy in operable breast carcinoma.

As to the main toxicities observed in the present study, the higher proportion of constipation, mandibular pain and peripheral nervous system toxicity on the CMFEV, compared to CMF regimen, could be due to the addition of vincristine. In the first study, a higher proportion of mild neurological side effects was also observed when administering CMFEV as compared to CMF [10]. The anaemia and the stomatitis could also be attributed to vincristine, or to the addition of epirubicin. Overall, the toxicities reported after the administration of CMFEV were rather well tolerated; only in a few patients, the treatment had to be shortened or vincristine administration had to be discontinued.

4.3. CMFEV Regimen versus CMF Regimen: Incidence of Second Malignancies

The significantly higher incidence of second malignancies with CMFEV as compared to CMF was unexpected and deserves some consideration as we did not observed this difference when using these regimens in a neo-adjuvant settings [10] This discrepancy may result either from the higher number of patients in the present study (489 versus 211) or from differences in the number of chemotherapy cycles delivered (4 cycles as neo-adjuvant therapy versus 6 cycles as adjuvant therapy). Moreover, epirubicin and vincristine were administered in 3 and 4 cycles, respectively, in the previous study [10] compared to 6 cycles in the present study.

4.4. CMFEV Regimen versus CMF Regimen: Comprehensive Evaluation of the Adjuvant Chemotherapy of Operable Breast Carcinoma

Two overviews had reported that, in the adjuvant chemotherapy of operable breast cancer, anthracycline containing regimens were more effective than CMF in preventing recurrence and death [1,4]. This concept was particularly stressed by the former study that reported highly statistically significant differences in favour of the anthracycline-containing regimens as compared to CMF [1]. It has to be noted that this overview focused on the anthracycline-containing regimens FAC (fluorouracil, adriamycin/doxorubicin, cyclophosphamide) and FEC (fluorouracil, epirubicin, cyclophosphamide) while the anthracycline-containing regimens AC (adriamycin/, cyclophosphamide) and EC (epirubicin, cyclophosphamide) were not considered [1]. According to the classification adopted by us in 2003 [5], the administration of anthracycline in the AC and EC regimens was substantially "substitutive" as only one of the three CMF agents (cyclophosphamide) was maintained, while in the FAC and FEC regimens the administration of anthracycline was substantially "additive" as two of the three agents of CMF (cyclophosphamide and 5-fluorouracil) were maintained. On the other hand, considering the results of single studies where more than 2000 patients were enrolled, comparisons of CMF with the AC regimen did not provide evidences favouring AC [17,18], while comparisons of CMF with the FAC regimen or with the FEC regimen reported results significantly favouring these two anthracycline-containing combinations [19,20].

In this light, our CMFEV experimental regimen clearly belongs to the "additive" type of combinations but it exceeds FAC or FEC combinations as it involves the conservation of not only two, but of all three CMF agents, although in a rotational strategy. This consideration together with the results we obtained with the CMFEV regimen, both in the first study and in the present one, should encourage the conduction of further studies but the unexpected increase of second malignancies here observed upon CMFEV treatment suggests some cautions. In addition, at the present time, any discussion about the adjuvant chemotherapy of breast car-

cinoma should not exclude the consideration of the addition of taxanes to anthracyclines although results reported so far in this line do not sufficiently encourage this approach [21,22].

4.5. Conclusions

This study provides preliminary evidence suggesting a potentially higher efficacy of the CMFEV regimen as compared to the standard CMF regimen. This result is in line with the overall evidence demonstrating an increased efficacy of anthracycline-containing adjuvant regimens in breast cancer [1], but also lends to support the rationale of using a 5-drugs, rotational regimen. However, before this rationale can be further explored in larger, more focused trials, the increased incidence of cancer at other sites here observed in the CMFEV arm, needs to be thoroughly considered.

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