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Adjuvant systemic therapy for breast cancer in *BRCA1/BRCA2* mutation carriers in a population-based study of risk of contralateral breast cancer

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Abstract

Background—Given the greatly elevated risks of contralateral breast cancer (CBC) observed in breast cancer patients who carry mutations in *BRCA1* and *BRCA2*, it is critical to determine the effectiveness of standard adjuvant therapies in preventing CBC in mutation carriers.

Methods—The WECARE study is a matched, case-control study of 708 women with CBC as cases and 1,399 women with unilateral breast cancer (UBC) as controls, including 181 *BRCA1/BRCA2* mutation carriers. Interviews and medical record reviews provided detailed information on risk factors and breast cancer therapy. All study participants were screened for *BRCA1* and *BRCA2* mutations using denaturing high-performance liquid chromatography (DHPLC) to detect genetic variants in the coding and flanking regions of the genes. Conditional logistic regression was used to compare the risk of CBC associated with chemotherapy and tamoxifen in *BRCA1/BRCA2* mutation carriers and non-carriers.

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Results—Chemotherapy was associated with lower CBC risk both in non-carriers (RR = 0.6 [95% CI: 0.5-0.7]) and carriers (RR = 0.5 [95% CI: 0.2-1.0]; p-value = 0.04). Tamoxifen was associated with a reduced CBC risk in non-carriers (RR = 0.7 [95% CI: 0.6-1.0]; p-value = 0.03). We observed a similar but non-significant reduction associated with tamoxifen in mutation carriers (RR = 0.7 [95% CI: 0.3-1.8]). The tests of heterogeneity comparing carriers to non-carriers did not provide evidence for a difference in the associations with chemotherapy (p-value = 0.51) nor with tamoxifen (p-value = 0.15).

Conclusions—Overall, we did not observe a difference in the relative risk reduction associated with adjuvant treatment between *BRCA1/BRCA2* mutation carriers and non-carriers. However, given the higher absolute CBC risk in mutation carriers, the potentially greater impact of adjuvant therapy in reducing CBC risk among mutation carriers should be considered when developing treatment plans for these patients.

Keywords

adjuvant therapy; *BRCA1*; *BRCA2*; breast cancer; chemotherapy; contralateral; counter-matching; tamoxifen

INTRODUCTION

Carriers of a germline mutation in one of the two autosomal, dominant breast cancer susceptibility genes, *BRCA1* and *BRCA2*, face a risk of breast cancer by age 70 years ranging from 26% to 84%,[1-7] as well as an increased risk of a subsequent contralateral breast cancer (CBC) during their lifetimes.[8-10] The ten-year cumulative incidence rates of CBC among *BRCA1* and *BRCA2* mutation carriers with breast cancer are estimated to range from 18% to 40% compared to a ten-year rate of 3% in breast cancer patients who are non-carriers.[7,10-13]

Chemotherapy and tamoxifen have been observed to reduce the risk of CBC in the general population,[14-17] and in light of the high incidence of CBC observed in *BRCA1/BRCA2* mutation carriers, it is important for clinicians to know if these adjuvant therapies are effective for the prevention of CBC within *BRCA1/BRCA2* mutation carriers to the same extent as within non-carriers. Previous studies have focused largely on *BRCA1* and *BRCA2* carriers from high-risk families and have found a 40-70% reduction in the risk of CBC among carriers who use tamoxifen.[11,12,18,19] A 60% reduction in CBC risk in *BRCA1/BRCA2* mutation carriers associated with chemotherapy use was reported by the Hereditary Breast Cancer Clinical Study.[19] Our study sought to determine the effects of chemotherapy and tamoxifen on the risk of CBC in a population-based setting of breast cancer cases according to *BRCA1* and *BRCA2* mutation carrier status.

MATERIALS AND METHODS

Study population

The WECARE study included 708 cases with asynchronous bilateral breast cancer (for ease, henceforth referred to as contralateral breast cancer [CBC]), and 1399 controls with unilateral breast cancer (UBC). The methods for the WECARE study have been described previously.[14,20] Briefly, the cases were identified from five cancer registries, four in the USA (three Surveillance, Epidemiology, and End Results [SEER] registries in Iowa, Los Angeles [CA], and Seattle [WA], and the Cancer Surveillance Program of Orange County [CA]) and one in Denmark (the Danish Breast Cancer Cooperative Group Registry supplemented with data from the Danish Cancer Registry). Eligibility criteria for the cases in the study included: 1) diagnosis of a first primary breast carcinoma that did not spread

beyond the regional lymph nodes at diagnosis and was diagnosed between January 1, 1985, and December 31, 2000, and a second primary *in situ* or invasive contralateral breast cancer diagnosed at least one year after the first primary breast cancer diagnosis; 2) residence in the same study reporting area for both diagnoses; 3) no previous or intervening cancer diagnosis; 4) under age 55 years at the time of the first primary breast cancer; and 5) alive at the time of contact and able to provide informed consent to complete the interview and a blood sample.

Control participants, who were individually matched to cases, were also identified through the five geographic regions from which their matched cases arose with eligibility criteria including: 1) diagnosis of a first primary breast carcinoma that did not spread beyond the regional lymph nodes at diagnosis and was diagnosed between January 1, 1985 and December 31, 2000; 2) under age 55 years at the time of the first primary breast cancer; 3) alive at the time of contact and able to provide informed consent to complete the interview and a blood sample; 4) no diagnosis of cancer during the at-risk time period (defined as the interval between first diagnosis of UBC and the diagnosis of CBC in the matched case); 5) had an intact contralateral breast at the end of the at-risk interval; and 6) residence in the study reporting area until the end of the at-risk interval. Two control subjects were individually matched to each case on year of birth, year of diagnosis, registry region, and race, and were 1:2 counter-matched on registry-reported radiation treatment of the UBC so that each triplet consisted of one radiation untreated and two radiation treated subjects. This sampling approach is accounted for in the statistical analysis by the inclusion of “sampling weights” in the models. The reference date for cases was the date of diagnosis of the CBC and for controls was the date when the at-risk period ended.

Data collection

All women were interviewed using the same scripted telephone questionnaire, which included questions ascertaining known and suspected breast cancer risk factors occurring during the at-risk period, in addition to risk factors that preceded the at-risk period, including reproductive, family, and medical history. 708 (71% of 988 eligible cases) and 1399 (66% of 2122 eligible controls) women were interviewed and provided blood samples.

Medical records and pathology and hospital reports were obtained in order to determine the treatments used by participants and characteristics of their first tumors. Processing of the blood samples included DNA extraction and lymphocyte cryopreservation. Each data collection center received approval from their Institutional Review Board prior to enrolling women and processing blood samples.

BRCA1 and *BRCA2* carrier status determination

The laboratory methods to determine *BRCA1/BRCA2* carrier status have been described previously.[20] Briefly, *BRCA1/BRCA2* mutation status was determined using a two-staged approach in which denaturing high-performance liquid chromatography (DHPLC) was used to screen for mutations or polymorphic variants in the coding and flanking regions of the genes. The DHPLC variants were sequenced to confirm nucleotide variations. Three laboratories were involved in the detection of *BRCA1* and *BRCA2* genetic variation. Laboratory protocols contained a quality control plan followed by each laboratory that included: an initial blinded screening of a set of 20 positive controls, 5 negative controls, and 21 WECARE Study samples whose mutation status was unknown; blinded rescreening of a randomly selected sample of 10% of all subjects screened within a laboratory; and re-screening by a single laboratory (USC) of a random sample of 10% of all subjects initially screened by all laboratories.

Statistical Analysis

For the primary analyses, chemotherapy and tamoxifen treatments for the first primary breast cancer were categorized as 'Yes/No' and encompassed any breast cancer treatment received prior to the reference date. For both chemotherapy and tamoxifen, use was based on information from the questionnaire and medical record abstraction. The most commonly used chemotherapy regimens were the cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen and cyclophosphamide epirubicin/adriamycin, 5-fluorouracil (CAF/CEF) regimen. The remaining regimens were classified as 'other anthracycline-based regimens' or 'multiple/other regimens.'

Rate ratios (RR) and 95% confidence intervals (CIs) were calculated using conditional logistic regression analysis with the inclusion of a log weight 'covariate' in the model to account for the counter-matched sampling design, where the coefficient of this log weight was fixed at one (i.e., an "offset" in the model).[20,21] The weights were obtained from the numbers of registry reported radiation treated and untreated subjects in the risk set.

Risk factors examined for potential confounding effect included age, BMI, menopausal status, family history of breast cancer, treatments other than the treatment of interest, tumor grade, stage, histology, and estrogen receptor (ER) status. The only factor that altered the risk by more than 10% was age, and it was subsequently included in the conditional logistic regression model. Tests of heterogeneity were conducted using the likelihood ratio test.

Control proportions presented in the tables 2 and 3 were adjusted to reflect the proportions expected in the absence of counter-matching. The adjustments were computed as the weighted average of the crude within-registry reported radiation exposed and unexposed control proportions.

RESULTS

109 mutations in *BRCA1* and 72 mutations in *BRCA2* were detected in the WECARE study population of 705 women with CBC and 1398 women with UBC. Table 1 presents the matched demographic characteristics of CBC and UBC stratified by *BRCA1* and *BRCA2* mutation carrier status. Carriers were substantially more frequent in the younger age groups (p-value = 0.03).

The relative reduction in CBC risk associated with chemotherapy after the first diagnosis of breast cancer was of a similar magnitude for *BRCA1/BRCA2* mutation carriers combined (RR = 0.5 [95% CI: 0.2-1.0]; p-value = 0.04) and non-carriers (RR = 0.6 [95% CI: 0.5-0.7; Table 2]; p-heterogeneity = 0.51). When examined separately, chemotherapy was associated with a reduced CBC risk of a substantial magnitude for *BRCA1* (RR = 0.5 [95% CI: 0.1-1.6]) and *BRCA2* (RR = 0.3 [95% CI: 0.1-1.0]) mutation carriers. Overall, there was no evidence that the relative risk of CBC differed between non-carriers, *BRCA1*, or *BRCA2* mutation carriers (p-heterogeneity = 0.34; Table 2). The reduced relative risk of CBC associated with chemotherapy use did not appear to be modified by time since diagnosis (> 5 years versus ≤ 5 years), menopausal status (pre-versus post-menopausal), or BMI (4^{th} quartile versus lower 3 quartiles) in carriers or non-carriers (data not shown).

With tamoxifen treatment, the relative risk associated with CBC in non-carriers was 0.7 (95% CI: 0.6-1.0) and in *BRCA1/BRCA2* mutation carriers combined it was 0.7 (95% CI: 0.3-1.8). While we observed a statistically significant reduction in risk among non-carriers (p-value = 0.03), we did not observe the same among mutation carriers. The test of heterogeneity did not provide evidence of a difference between the two groups (p-heterogeneity = 0.15). For *BRCA1* mutation carriers the RR was 0.2 (95% CI: 0.0-1.3),

while for *BRCA2* mutation carriers it was 0.9 (95% CI: 0.5-6.9). Again the test of heterogeneity did not provide evidence of a difference in risk associated with tamoxifen between non-carriers, *BRCA1*, and *BRCA2* mutation carriers (p-heterogeneity = 0.72; Table 2). The relative risk for *BRCA1/BRCA2* carriers was not modified by oophorectomy status, regardless of the timing of the oophorectomy (data not shown).

Table 3 presents relative risks associated with chemotherapy stratified by type of regimen. *BRCA1/BRCA2* mutation carriers had a statistically significant reduction in risk associated with CEF/CAF regimens (RR = 0.3 [95% CI: 0.1-0.8]), which the non-carriers did not (RR = 0.8 [95% CI: 0.5-1.2]), but the difference between carriers and non-carriers was not statistically significant (p-heterogeneity= 0.10). We did not observe evidence that the effects differed between carriers and non-carriers for other regimens.

Given that most research on this topic has occurred in high-risk clinical settings, we conducted a subset analysis among women with both early onset disease and a positive family history of breast cancer (172 cases and 249 controls) to approximate a study population arising from high-risk clinics. Within this group, there were 62 *BRCA1* mutation carriers (39 cases and 23 controls, representing 57% of *BRCA1* carriers in this study) and 31 *BRCA2* mutation carriers (22 cases and 9 controls; 43% of the *BRCA2* carriers in this study). Among women diagnosed with a first breast cancer before 45 years of age who had one or more relatives with breast cancer, the risk of CBC associated with chemotherapy among *BRCA1/BRCA2* carriers was 0.6 (95% CI: 0.1-2.9) and the CBC risk associated with tamoxifen among carriers was 0.2 (95% CI: 0.02-1.3).

DISCUSSION

Previously and prior to the availability of *BRCA1/2* carrier status results, we reported substantive reductions in the relative risk of CBC in relation to use of adjuvant chemotherapy and tamoxifen in the WECARE study population as a whole.[14] In this new work which now incorporates *BRCA1/BRCA2* status, we found little evidence that the relative reductions of CBC risk following either adjuvant chemotherapy or tamoxifen treatment for UBC differed among *BRCA1/BRCA2* mutation carriers and non-carriers. However, as *BRCA1* and *BRCA2* mutation carriers have a significantly elevated risk of CBC, 4.5-fold and 3.4-fold respectively,[10] it is important to note that the absolute reduction in CBC risk provided by chemotherapy and tamoxifen would conceivably be much greater in carriers than in non-carriers.

A prior case-control study investigating the effect of chemotherapy among 593 *BRCA1/BRCA2* mutation carriers with bilateral and unilateral breast cancer recruited from 34 high-risk clinics observed findings similar to our results with a 60% (95% CI: 0.3-0.6) reduction in risk of CBC associated with chemotherapy.[19] Two additional studies addressed related but not identical questions to our study. One investigated the risk of CBC associated with chemotherapy in a cohort study of 491 breast cancer cases with a family history of *BRCA1/BRCA2* mutations, although no testing was performed on the individual level (for women with relative(s) with a *BRCA1* mutation: HR=1.04; 95% CI: 0.61-1.76; *BRCA2* mutation: HR= 1.17; 95% CI: 0.45-3.08).[11] The lack of comparability to the reduction in CBC risk observed in the current and prior study could be due to the use of a proxy measure for carrier status at the individual level. Another breast cancer cohort study of 160 *BRCA1/BRCA2* mutation carriers and 445 non-carriers compared CBC risk associated with chemotherapy between *BRCA1/BRCA2* carriers and non-carriers and observed no difference between the two groups (HR=0.99; 95% CI: 0.52-1.91) which is in agreement with our findings.[12]

Laboratory and clinical studies have observed *BRCA1*-positive tumors to be more sensitive to specific chemotherapeutic agents.[22-24] In particular, laboratory studies have indicated that *in vitro BRCA1*-positive tumors may be sensitive to chemotherapeutic agents targeting DNA double strand breaks (including adriamycin), but potentially may be resistant to regimens containing taxanes.[22,25,26] Multiple small-scale clinical studies have observed differential responses to chemotherapy between *BRCA1/BRCA2* carriers and non-carriers in that carriers were more responsive to CA regimens than non-carriers,[24,27] but less responsive to regimens containing taxanes compared to non-carriers.[28] In our study, we observed a suggestion of a greater reduction in CBC risk in *BRCA1/BRCA2* carriers versus non-carriers in relation to CEF/CAF regimens.

We observed no evidence of an overall difference in CBC relative risk reduction associated with tamoxifen use among non-carriers and *BRCA1/BRCA2* mutation carriers. In our study, though, only 15.6% (n=17) of *BRCA1* carriers and 55.6% (n=40) of *BRCA2* carriers were ER-positive, which likely accounts for the small percentage (14.9%) of *BRCA1/BRCA2* carriers in our study who received tamoxifen and contributes to the relatively wide confidence intervals observed for associations with tamoxifen. A preponderance of prior studies investigating treatment effects, including tamoxifen, among *BRCA1/BRCA2*-positive breast cancer patients used data from high-risk clinics. In most of these studies, hormonal treatment was associated with a 40-70% reduction in CBC risk among *BRCA1/BRCA2* mutation carriers.[11,12,18,19] Within our data, there was some suggestion of a greater reduction in the magnitude of the relative risk among the subgroup of patients that met our “high-risk” criteria (i.e., early onset and positive family history) intended to approximate women from high-risk clinics than among *BRCA1/BRCA2* mutation carriers overall. Overall, our findings are in broad agreement with the literature reporting on CBC risk associated with tamoxifen within *BRCA1/BRCA2* mutation carriers.

A notable strength of our study was its population-based design, which allowed for the investigation of treatment effects among a sample of *BRCA1/BRCA2* carriers who are representative of the broad spectrum of carriers with breast cancer in the general population, both with and without family history. This allows for our results to be generalizable to a wider array of *BRCA1/BRCA2* mutation carriers. Additional strengths included comprehensive *BRCA1/BRCA2* mutation screening using DHPLC which is a method with demonstrated high sensitivity and specificity,[29] and the detailed treatment information available on all of the women. A constraint of our study was the limited number of women with *BRCA1/BRCA2* mutations, particularly among women treated with tamoxifen and within specific chemotherapy regimens.

We observed that chemotherapy and tamoxifen reduced the risk of CBC in *BRCA1/BRCA2* carriers and non-carriers to a similar relative degree. Given the high baseline rate of CBC among carriers, the absolute reduction in risk among carriers could be considerable, making this an important reason to consider recommending adjuvant chemotherapy for *BRCA1/BRCA2* carrier patients. Currently, the American Society of Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN) recommendations regarding adjuvant therapy do not distinguish between *BRCA1/BRCA2* mutation carriers and non-carriers because thus far, the evidence to recommend using differential systemic therapy based on *BRCA1/BRCA2* carrier status has been insufficient.[22,25,30-32] Therefore, further studies assessing whether risk reductions among *BRCA1/BRCA2* mutation carriers differ by chemotherapeutic regimens are needed.

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Appendix

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ABBREVIATIONS

BRCA1	breast cancer susceptibility gene 1
BRCA2	breast cancer susceptibility gene 2
CAF/CEF	cyclophosphamide epirubicin/adriamycin, 5-fluorouracil

CBC	contralateral breast cancer
CMF	cyclophosphamide, methotrexate, 5-fluorouracil
DHPLC	denaturing high-performance liquid chromatography
ER	estrogen receptor
UBC	unilateral breast cancer

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Table 1

Demographic characteristics of bilateral and unilateral breast cancer cases by *BRCA1* and *BRCA2* carrier status

		***Non-carriers*[1]**		***BRCA1***		***BRCA2***	
		Bilateral n (%)	**Unilateral n (%)**	**Bilateral n (%)**	**Unilateral n (%)**	**Bilateral n (%)**	**Unilateral n (%)**
Total		597	1325	67	42	41	31
Age at first breast cancer	20-34	22 (3.7)	76 (5.7)	22 (32.8)	9 (21.4)	4 (9.8)	5 (16.1)
	35-44	204 (34.2)	484 (36.5)	35 (52.2)	23 (54.8)	22 (53.7)	10 (32.3)
	45-55	371 (62.1)	765 (57.7)	10 (14.9)	10 (23.8)	15 (36.6)	16 (51.6)
	Mean Age[2]	46.5 (5.8)	45.7 (6.1)	38.3 (6.0)	40.1 (6.8)	42.8 (6.3)	43.8 (7.2)
Age at reference date[3]	22-34	8 (1.4)	27 (2.1)	7 (10.4)	7 (16.7)	1 (2.4)	2 (6.5)
	35-44	73 (12.2)	217 (16.3)	35 (52.2)	17 (40.5)	12 (29.3)	7 (22.6)
	≥ 45	516 (86.5)	1081 (81.7)	25 (37.3)	18 (42.9)	28 (68.3)	22 (71.0)
	Mean Age[2]	51.6 (6.6)	50.8 (6.8)	42.8 (7.1)	43.8 (7.7)	47.3 (6.9)	47.7 (8.0)
At risk period (years)	Mean time interval[2]	5.1 (3.2)	5.1 (3.1)	4.9 (2.8)	4.2 (2.7)	5.0 (3.0)	4.5 (2.5)
Geographic Location	Denmark	151 (25.2)	339 (25.5)	13 (19.4)	8 (19.0)	12 (29.3)	10 (32.3)
	USA	446 (74.8)	986 (74.5)	54 (80.6)	34 (81.0)	29 (70.7)	21 (67.7)
Race	White	552 (92.5)	1223 (92.3)	59 (88.1)	38 (90.5)	35 (85.4)	26 (83.9)
	Hispanic White	17 (2.8)	44 (3.3)	4 (6.0)	2 (4.8)	3 (7.3)	2 (6.5)
	Black	18 (3.0)	35 (2.6)	2 (3.0)	1 (2.4)	1 (2.4)	3 (9.7)
	Asian or other	10 (1.7)	23 (1.7)	2 (3.0)	1 (2.4)	2 (4.9)	0 (0.0)

[1] Non-carrier group includes wildtype and unclassified variants

[2] Mean (std dev)

[3] Reference date is the date of second diagnosis for cases

Table 2

Risk of asynchronous contralateral breast cancer in relation to treatment for first primary breast cancer according to *BRCA1/2* mutation carrier status

	Non-carriers				***BRCA1***				***BRCA2***				
Treatment for first Breast Cancer	**Bilateral**	**Unilateral**			**Bilateral**	**Unilateral**			**Bilateral**	**Unilateral**			
	n (%)	**n (%)[1]**	**RR[2]**	**95% CI**	**n (%)**	**n (%)[1]**	**RR[2]**	**95%CI**	**n (%)**	**n (%)[1]**	**RR[2]**	**95% CI**	**p-value[3]**
Chemotherapy													
No	343 (57.5)	611 (43.6)	1.0	(ref)	20 (29.9)	5 (21.4)	1.0	(ref)	21 (51.2)	12 (31.3)	1.0	(ref)	0.34
Yes	254 (42.5)	714 (56.4)	0.6	0.5-0.7	47 (70.1)	37 (78.6)	0.5	0.1-1.6	20 (48.8)	19 (68.7)	0.3	0.1-1.0*	
Tamoxifen													
No	434 (72.7)	899 (68.7)	1.0	(ref)	65 (97.0)	35 (90.7)	1.0	(ref)	29 (70.7)	25 (82.2)	1.0	(ref)	0.72
Yes	163 (27.3)	424 (31.0)	0.7	0.6-1.0*	2 (3.0)	7 (9.3)	0.2	0.0-1.3	12 (29.3)	6 (17.8)	0.9	0.5-6.9	

[1] Weighted percentages

[2] Rate Ratios (RR) and 95% confidence intervals (CI) are adjusted for age at first diagnosis.

[3] Testing for heterogeneity between non-carriers, *BRCA1*, and *BRCA2* mutation carriers

* Due to rounding, p-value < 0.05

Table 3

Risk of asynchronous contralateral primary breast cancer associated with chemotherapy regimen stratified by *BRCA1/2* mutation carrier status

	Non-carriers				*Carriers*				
Treatment	**Bilateral**	**Unilateral**			**Bilateral**	**Unilateral**			
	n (%)	**n (%)[1]**	**RR[2]**	**95% CI**	**n (%)**	**n (%)[1]**	**RR[2]**	**95% CI**	**p-value[3]**
No Chemotherapy	343 (57.4)	611 (43.6)	1.0	(ref)	41 (38.0)	17 (23.2)	1.0	(ref)	
Chemotherapy Type[4,5]									
CMF	123 (20.6)	421 (29.5)	0.6	0.4-0.7	30 (27.8)	26 (36.7)	0.4	0.2-0.9	0.24
CEF/CAF	48 (8.0)	95 (8.9)	0.8	0.5-1.2	9 (8.3)	14 (17.7)	0.3	0.1-0.8	0.10
Other anthracycline-based regimen	45 (7.5)	102 (8.7)	0.7	0.5-1.2	18 (16.7)	10 (14.3)	0.9	0.3-2.6	0.27
Multiple/other regimens	14 (2.3)	46 (4.2)	0.4	0.2-0.8	7 (6.5)	5 (7.2)	0.7	0.2-3.3	0.19

[1] Weighted percentages

[2] Rate Ratios (RR) and 95% CIs are adjusted for age at first diagnosis.

[3] Testing heterogeneity between carriers and non-carriers

[4] A: adriamycin; C: cyclophosphamide; E: epirubicin; F: 5-fluorouracil; M: methotrexate

[5] Numbers may not sum to total because of missing information