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Effect of Combined Folic Acid, Vitamin B_6, and Vitamin B_{12} on Cancer Risk: Results from a Randomized Trial

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Abstract

Context—Folate, vitamin B_6, and vitamin B_{12} are thought to play an important role in cancer prevention.

Objective—To evaluate the effect of combined folic acid, vitamin B_6, and vitamin B_{12} treatment on cancer risk in women at high risk for cardiovascular disease.

Design, Setting, and Participants—In the Women's Antioxidant and Folic Acid Cardiovascular Study, 5442 US female health professionals aged 42 years or older with preexisting cardiovascular disease or 3 or more coronary risk factors were randomly assigned to receive either a daily combination of folic acid, vitamin B_6, and vitamin B_{12} or placebo in April 1998, and treated through July 31, 2005 for 7.3 years.

Intervention—Daily supplementation of a combination of 2.5 mg of folic acid, 50 mg of vitamin B_6, and 1 mg of vitamin B_{12} (n=2721) or placebo (n=2721).

Main Outcome Measures—Confirmed newly diagnosed total invasive cancer.

Results—A total of 379 women developed invasive cancer (187 in the active group and 192 in the placebo group). Compared with placebo, women receiving combined folic acid, vitamin B_6, and vitamin B_{12} had similar risk of developing total invasive cancer (101.1/10000 person-years vs 104.3/10000 person-years for the active vs placebo group; hazard ratio, 0.97; 95% confidence interval, 0.79–1.18; *P*=.75), breast cancer (37.8/10000 person-years vs 45.6/10000 person-years; hazard ratio, 0.83; 95% confidence interval, 0.60–1.14; *P*=.24), and any cancer death (24.6/10000 person-years vs 30.1/10000 person-years; hazard ratio, 0.82; 95% confidence interval, 0.56–1.21; *P*=.32).

Conclusions—Combined folic acid, vitamin B_6, and vitamin B_{12} treatment had no significant effect on overall risk of total invasive cancer or breast cancer among women during folic acid fortification era.

Trial Registration—clinicaltrials.gov Identifier: NCT00000541

Folate, vitamin B_6, and vitamin B_{12} (water-soluble, essential B-vitamins) are important for DNA synthesis and methylation, two critical processes for upholding DNA integrity and

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regulating gene expression, respectively,[1, 2] and are thus thought to play an important role in cancer prevention. Background fortification of the food supply with folic acid (a synthetic form of folate), a policy that began in the US in 1998 to reduce risk of neural tube defects, has improved folate status in the general population.[3] Approximately one third of US adults currently take multivitamin supplements containing folic acid, vitamin B_6, and vitamin B_{12}.[4] Observational studies, which were conducted mostly prior to folic acid fortification, in general support an inverse association between high intake or blood level of folate, vitamin B_6, and vitamin B_{12} and risk of cancer, particularly colorectal neoplasia and breast cancer, and primarily among those consuming alcohol, a known antagonist for these B-vitamins.[5–9] Data from randomized trials of folic acid alone or combined B vitamins and cancer risk are limited, not entirely consistent,[10–14] and one trial has even raised concerns about deleterious effects.[13] Women have been underrepresented in previous B vitamin trials. We thus conducted a detailed analysis of cancer endpoints in the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), a largest randomized trial designed to test the effect of combined folic acid, vitamin B_6, and vitamin B_{12} on prevention of cardiovascular disease (CVD) among high-risk women during the folic acid fortification era.[15–18]

METHODS

Study Design

The WAFACS is a randomized, double-blind, placebo-controlled trial of combined folic acid/vitamin B_6/vitamin B_{12} in the prevention of CVD events among women at high risk for CVD (Clinicaltrials.gov identifier: NCT00000541).[15–18] This report describes the results of cancer endpoints. Details of the design have been reported previously.[15–18] Briefly, from June 1995 to October 1996, 8171 female health professionals were randomized into the vitamin C, vitamin E, and β-carotene arm of the Women's Antioxidant Cardiovascular Study, the parent trial of the WAFACS (Figure 1). Women were eligible for the trial if they were 40 years of age or older, postmenopausal or had no intention of becoming pregnant, and who had a reported history of CVD or at least three coronary risk factors. Women were excluded from participation if they had a previous history of cancer (excluding nonmelanoma skin cancer) within the past ten years, any major serious non-CVD illness, or were currently using warfarin or other anticoagulants. All participants completed a baseline questionnaire inquiring about their medical history and lifestyle factors. At baseline, 98.8% also completed a comprehensive food frequency questionnaire inquiring about the average intake of foods and beverages during the past year and the dose and duration of use of vitamin and mineral supplements. Total intakes of folate, vitamin B_6, and vitamin B_{12} included sources from both foods and supplements. Of the 8171 randomized participants, 5442 women who were additionally willing to forgo the use of individual folic acid, vitamin B_6, and vitamin B_{12} supplements or multivitamin with greater than the Recommended Daily Allowances (RDAs) of folic acid, vitamin B_6, and vitamin B_{12} were further randomized in a retained factorial design to a daily combination of folic acid (2.5 mg), vitamin B_6 (50 mg), and vitamin B_{12} (1 mg), or to placebo in April 1998 completely. The doses used in the WAFACS trial were higher than the adult RDAs for folate (400 μg/day), vitamin B_6 (1.5 mg/day) and vitamin B_{12} (2.4 μg/day) in women.[19]

After randomization, the women were sent annual supplies of monthly calendar packs containing study medications and questionnaires on compliance, side effects, relevant disease endpoints, and risk factors. Study medications and disease ascertainment were continued in a blinded fashion until the scheduled end of the trial (July 31, 2005) for 7.3 years. Follow-up and validation of reported endpoints were completed in July 2006. Using the information at the end of the trial (July 31, 2005), mortality and morbidity follow-ups were calculated to be 92.6% and 90.5% complete, respectively. In terms of percent of potential person-years of follow-up, a calculation that takes into account all information during follow-up, mortality and

morbidity were 98.9% and 98.0% complete, respectively.[18] The average compliance, defined as taking at least two-thirds of the study pills over the course of follow-up, was 83% for both the active and placebo groups.[18] In a random sample of 300 participants, there were no differences in median plasma folate (8.8 vs 8.9 ng/mL; *P*=.94) and homocysteine (12.1 vs 12.5 hmol/L; *P*=.96) between the active and placebo groups at the beginning of the trial, whereas median plasma folate was significantly higher (38.9 vs 15.4 ng/mL; *P*<.001) and homocysteine was significantly lower (9.8 vs 11.8 hmol/L; *P*<.001) in the active group than in the placebo group at the end of the trial.[18] The trial was approved by the institutional review board of the Brigham and Women's Hospital, and was monitored by an external data and safety monitoring board. All patients provided written informed consent.

Women reported the occurrence of cancer through questionnaire, letter, or phone call. Deaths were reported by family members, postal authorities or through a search of the National Death Index (through December 2003). Following the report of a relevant medical event, written permission to obtain medical records was sought from the participant, or next of kin in case of a death. Medical records were obtained from hospitals and treating physicians and reviewed by an endpoints committee of physicians blinded to randomized treatment assignment to confirm final diagnosis. Additional details of breast tumor characteristics were also extracted from medical records. Death from cancer cause was confirmed by examination of autopsy reports, death certificates, medical records, and information obtained from the next of kin or other family members. Death from any cause was confirmed by the endpoints committee or on the basis of a death certificate. Only confirmed invasive cancers were included in this report.

Statistical power for the effect of combined folic acid, vitamin B_6 and vitamin B_{12} treatment on cancer risk reduction was calculated for a two-sided alpha error of 0.05. Based on a total of 379 observed cancer cases at the end of the trial in the 5442 women who were randomized, we estimated that there would be 82.2% power to detect a 25% reduction and 61.1% power to detect a 20% reduction.

Statistical Analysis

Primary analyses were conducted as the intent-to-treat. Baseline characteristics between the active group and the placebo group were compared using t-tests or Wilcoxon signed-rank tests for continuous variables and chi-square tests for categorical variables. Kaplan-Meier survival curves were used to estimate event rates over time, and curves of the active group and the placebo group were compared by the log-rank test. Only those events with large numbers of occurrences in each treatment group (total invasive cancer and breast cancer) were analyzed via Kaplan-Meier survival curves. The hazard ratios (HRs) and 95% confidence intervals (CIs) of cancer comparing women randomized to combined B vitamin treatment vs placebo were computed by Cox proportional hazards regression models with adjustments for age (in years) and randomized treatment assignments of vitamin E (vitamin E vs placebo), vitamin C (vitamin C vs placebo), and β-carotene (β-carotene vs placebo). The proportionality assumption was tested by including an interaction term of combined B vitamin treatment with the logarithm of time in the Cox proportional hazards regression models, and was not violated for total invasive cancer (*P*=.26), breast cancer (*P*=.74), or any cancer death (*P*=.13). To examine the influence of pre-clinical cases, we conducted analyses excluding the first 2 years of follow-up. Those with missing information on mortality and morbidity were treated as non-events throughout follow-up until the end of trial in the intention-to-treat analysis. All *P* values were two-sided with a significance level of α=0.05 (*P* value $\leq$.05).

Total invasive cancer and breast cancer, two events with sufficient numbers of occurrences in each treatment group, were further analyzed across subgroups of baseline characteristics. The cut-points for age chosen were consistent with the analysis of cardiovascular events in the WAFACS trial.[18] Pre-specified subgroup analyses included those according to the baseline

intakes of total folate, vitamin B_6, vitamin B_{12}, and alcohol, and the baseline multivitamin supplement use and history of cancer. Tests for multiplicative interaction between randomized combined B vitamin treatment assignment and categories of baseline characteristics in relation to cancer risk were performed by the Wald test for variables with two categories or log likelihood ratio tests comparing the models with or without interaction terms for variables with more than two categories. We also evaluated the effect of randomized combined B vitamin treatment on risk for invasive breast cancer according to tumor characteristics at diagnosis. To examine the influence of lack of compliance, in a sensitivity analysis, women were censored if and when they stopped taking at least two-thirds of their study medication. The 16 subgroup analyses by participant characteristics were conducted for each outcome, and one statistically significant test would be expected on the basis of chance alone. SAS version 9.1 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Of the 5442 women, 64.2% had a prior CVD event and 35.8% had 3 or more coronary risk factors. In addition, 7.7% had a prior cancer event at baseline (diagnosed at least 10 years before enrollment), 5.6% consumed 15 or more g/day of alcohol, and 22.9% were current users of multivitamin supplements. The mean (±standard deviation) values at baseline were 62.8 (±8.8) years for age and 30.6 (±6.7) kg/m^2 for body mass index. There were no significant differences in baseline characteristics between the active group and the placebo group (Table 1).

A total of 379 women developed invasive cancer (187 in the active group and 192 in the placebo group). Treatment with combined B vitamins had no significant effect on risk of total invasive cancer (101.1/10000 person-years vs 104.3/10000 person-years for the active vs placebo group; HR, 0.97; 95% CI, 0.79–1.18; *P*=.75) or individual cancer endpoints, including breast cancer (37.8/10000 person-years vs 45.6/10000 person-years; HR, 0.83; 95% CI, 0.60–1.14; *P*=.24) and colorectal cancer (9.7/10000 person-years vs 12.0/10000 person-years; HR, 0.81; 95% CI, 0.43–1.50; *P*=.50). There was no difference in any cancer death (24.6/10000 person-years vs 30.1/10000 person-years; HR, 0.82; 95% CI, 0.56–1.21; *P*=.32) or death from any cause (Table 2). Cumulative incidence curves indicated no variation over time for total invasive cancer, whereas a decrease in breast cancer risk appeared to emerge after approximately 3 years (Figure 2). After excluding the first 2 years of follow-up as pre-specified, the HRs were 0.94 (95% CI, 0.73–1.20) for total invasive cancer, 0.73 (95% CI, 0.50–1.06) for breast cancer, and 0.92 (95% CI, 0.53–1.60) for any cancer death.

Subgroup analyses

In analyses censoring women at the time they stopped taking at least two thirds of their study pills, the null results remained for total invasive cancer (148 vs 140 cases; HR, 1.04; 95% CI, 0.83–1.32), breast cancer (59 vs 64 cases; HR, 0.91; 95% CI, 0.64–1.30), and any cancer death (29 vs 36 cases; HR, 0.80; 95% CI, 0.49–1.30).

Age significantly modified the effect of combined B vitamin treatment on risk of total invasive cancer and breast cancer (*P* for interaction = .02 and .05, respectively) (Table 3). A significantly reduced risk was observed for total invasive cancer (HR, 0.75; 95% CI, 0.57–0.99) and breast cancer (HR, 0.62; 95% CI, 0.40–0.98) among women aged ≥ 65 years at study entry, but no reductions in risk were observed among younger women. There were no effect modifications by use of multivitamin supplements, intakes of total folate, vitamin B_6, and vitamin B_{12}, history of cancer, alcohol intake, or other risk factors at baseline. Finally, no significant effects were observed according to hormone receptor status or other characteristics of breast tumors, except that a borderline significant reduction was found for estrogen receptor (ER) positive and progesterone receptor (PR) negative tumors (Table 4).

COMMENT

In the WAFACS trial, up to 7.3 years of treatment with combined folic acid/vitamin B_6/vitamin B_{12} had no significant effect on overall risk of total invasive cancer, breast cancer, other individual cancers, or cancer death among women at high risk for CVD. There were no differences according to current use of multivitamin supplements, intakes of total folate, vitamin B_6, and vitamin B_{12}, or history of cancer at baseline. Lack of effect for total invasive cancer did not vary over time. We found a significant interaction by age group and a possible benefit among women aged ≥ 65 years at study entry.

There is a concern that increasing folate status resulting from mandatory folic acid fortification and supplementation may increase cancer risk in some people because folate may play a dual role in carcinogenesis -- prevent tumor initiation when it is administrated early in carcinogenesis and in individuals with suboptimal folate status, but promote tumor development when it is administrated later in carcinogenesis (i.e., once premalignant lesions are established) and in individuals with high folate intake.[20–23] In the WAFACS trial, consistent with the implementation of US folic acid fortification policy, plasma folate levels were increased in the placebo group during 7.3 years of follow-up (8.9 vs 15.4 ng/mL). With the longest treatment and follow-up, the WAFACS trial showed no effect on cancer risk even among those taking multivitamin supplements or with higher intake of total folate, vitamin B_6, and vitamin B_{12}, or with a history of cancer at baseline. Two other large randomized trials assessing folic acid, vitamin B_6, and vitamin B_{12} in relation to CVD risk have reported cancer outcomes. In the Heart Outcomes Protection Evaluation (HOPE)-2 trial, which included 5522 patients aged ≥ 55 years who had vascular disease or diabetes, an average of 5 years of treatment with the same daily combination of folic acid (2.5 mg), vitamin B_6 (50 mg), and vitamin B_{12} (1 mg) had no significant effect on risk of total cancer (relative risk, 1.06; 95% CI, 0.91–1.23), cancers of breast, colon, lung, or prostate, or cancer death.[12] In the HOPE-2 trial, 71.1 percent of participants were from countries with folic acid fortification. The NORVIT trial, which included 3749 men and women aged 30 to 85 years who recently had acute myocardial infarction in Norway, a country which has no mandatory folic acid fortification in foods, also reported no effect on cancer outcomes.[11] Compared to the placebo group, there was no difference in cancer risk in those treated with combined folic acid (0.8 mg), vitamin B_6 (40 mg) and vitamin B_{12} (0.4 mg) (40 vs 40 cases; relative risk, 1.02; 95% CI, 0.65–1.58) or combined folic acid (0.8 mg) and vitamin B_{12} (0.4 mg) (39 vs 40 cases; no relative risk was given) over an average of 3.3 years of treatment and follow-up.[11] There was a suggestive beneficial effect on cancer risk in those treated with vitamin B_6 (40 mg) alone as compared to those assigned to placebo (25 vs 40 cases) in the NORVIT trial.[11] Taken together, data from randomized trials of combined B vitamins provide reassurance that folic acid supplementation up to a dose of 2.5 mg/day when combined with vitamin B_6 and vitamin B_{12} does not appear to increase cancer occurrences and deaths among individuals at high risk for CVD during the folic acid fortification era. Because cancer has a long latency period, we cannot exclude the possibility that there might be beneficial or harmful effects of combined B vitamins on cancer that were not detectable within 7.3 years of treatment.

The results from randomized trials of folic acid alone and colorectal adenomas and of combined B vitamins and colorectal cancer have been mixed. In a trial that involved 60 men and women and was conducted prior to folic acid fortification, 24 months of treatment with 1 mg/day of folic acid nonsignficantly reduced the recurrence of colon adenomas.[10] In another trial, which involved 1021 men and women and was conducted during folic acid fortification, 3 years of treatment with 1 mg/day of folic acid had no benefit on the recurrence of colorectal adenomas at 3 years of follow-up and at another 3 or 5 years of follow-up.[13] Instead, there was an increased risk for advanced lesions at 3 years (relative risk, 1.32; 95% CI, 0.90–1.92) and at the second follow-up (relative risk, 1.67; 95% CI, 1.00–2.80). Those results did not differ

significantly by sex, age, alcohol use, body mass index, or baseline plasma folate.[13] In the third trial, which involved 945 men and women in UK and was conducted before folic acid fortification, treatment with 0.5 mg/day of folic acid for 3 years had no effect on the recurrence of colorectal adenomas (relative risk, 1.07; 95% CI, 0.85–1.34) or advanced adenomas (relative risk, 0.98; 95% CI, 0.68–1.40).[14] There was no increase in risk of colon cancer among women treated with combined B vitamins in the WAFACS trial, but a slightly increased risk was observed in the HOPE-2 trial.[12] However, the number of colon cancer cases was limited in these randomized trials. The evidence for a beneficial effect of folate, vitamin B_6, and vitamin B_{12} on colorectal cancer is strong in observational studies.[5] Emerging data also suggest that vitamin B_6 may be more important than folate for colon cancer prevention in postmenopausal women.[8] Thus, the effect of folic acid or combined B vitamins on colon cancer risk remains uncertain.

Lack of an overall effect for breast cancer in the WAFACS trial is generally consistent with observational studies that suggest no overall association between intake or blood level of folate, vitamin B_6, and vitamin B_{12} and breast cancer risk.[6] Data from several large cohort and case-control studies suggest that adequate folate may reduce breast cancer risk associated with alcohol intake[6] or decrease risk of developing ER- breast cancer.[24] However, we observed no clear pattern of an effect according to alcohol intake, and a borderline significant reduction was found for ER+PR- breast cancer. Of note, the number of women consuming 15 or more g/day of alcohol or developing ER+PR- or ER−PR- breast cancers was limited in the WAFACS trial. In one randomized trial of folic acid supplementation in pregnancy followed for 36 years, women who received 0.2 or 5 mg of folic acid supplements during pregnancy had increased deaths from total cancer (n = 112) and from breast cancer (n = 31); those who received the higher dose of 5 mg had the highest risk.[25] However, that study had short (several months in pregnancy) and remote (36 years ago) treatment and small number of breast cancer events. The possibility that the findings may be a result of chance and confounding cannot be excluded. The results from experimental studies have been mixed. Folate deficiency suppressed the N-methyl-N-nitrosourea (MNU)-induced mammary tumors,[26–28] while folate supplementation enhanced the initiation or early promotion of the MNU-induced mammary tumors in rats in one study,[26] but had no effect in two other studies.[27, 28]

In the WAFACS trial, a significant benefit of combined folic acid/vitamin B_6/vitamin B_{12} treatment was observed among women aged ≥ 65 years. If the finding is real and substantiated, the results may have public health significance because the incidence rates of cancer are high in the elderly.[29] The finding is biologically plausible because elderly people have increased requirements for these B vitamins.[19] In addition, two large prospective investigations have shown that an inverse association between plasma vitamin B_6 and breast cancer risk is primarily present in postmenopausal women.[7, 9] In the HOPE-2[12] and NORVIT[11] trials, there was no presentation by age for cancer endpoints, which were secondary outcomes. Because many subgroups were evaluated, we cannot exclude the possibility that the results from the subgroup analyses in the WAFACS trial are due to chance.

The strengths of the WAFACS trial include a randomized, double-blind, and placebo controlled design, which minimizes confounding and bias that potentially affect the results from observational studies. To our knowledge, the WAFACS trial also had the longest duration of treatment with combined B vitamins of any trial to date, high follow-up rates, and relatively high compliance to treatment. Women have been underrepresented in other B vitamin trials, and the WAFACS trial is the largest trial of women. However, we cannot distinguish the effect attributable to any single B vitamin supplement as compared to their combination. In addition, statistical power was insufficient to examine site-specific cancers. The WAFACS participants were female health professionals who tended to be health conscious and tended to have well-balanced diets and greater access to health care and screening, which may have led to lower

occurrences of cancer. However, more than two-thirds of participants also were overweight or obese, and thus were at elevated risk for CVD and cancer. Therefore, the findings may not be directly generalizable to the entire US population. However, it seems unlikely that the exposure-disease relationships observed among women in the WAFACS differ from women in general.

In conclusion, treatment with combined folic acid/vitamin B_6/vitamin B_{12} provided neither beneficial nor harmful effects on overall risk of total cancer, breast cancer, or deaths from cancer among women at high risk for CVD.

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Author Contributions: Dr Zhang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Zhang, Cook, Albert, Gaziano, Manson.

Acquisition of data: Gaziano, Manson.

Analysis and interpretation of data: Zhang, Cook, Buring, Manson.

Drafting of the manuscript: Zhang.

Critical revision of the manuscript for important intellectual content: Zhang, Cook, Albert, Gaziano, Buring, Manson.

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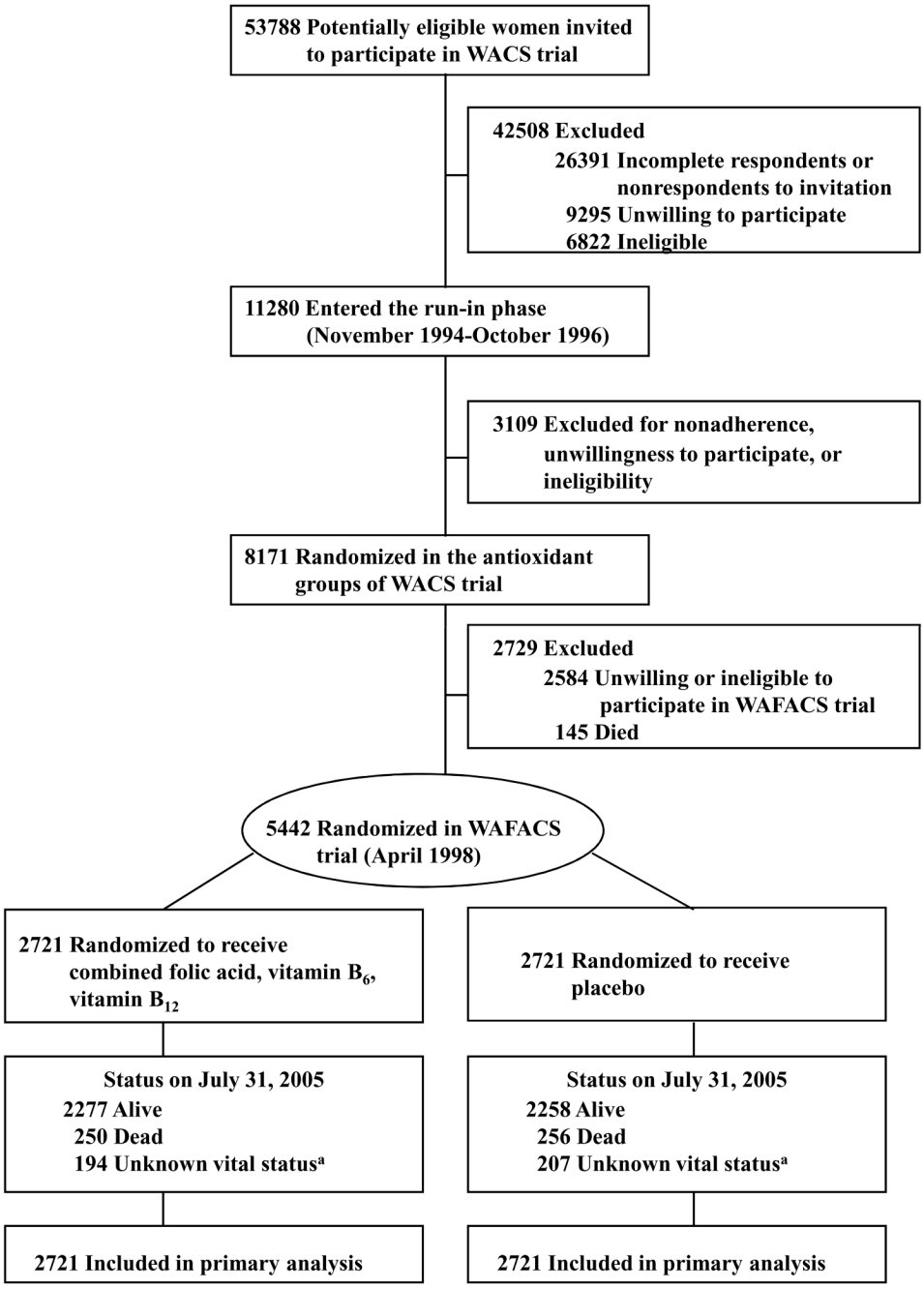

Figure 1. Flow Diagram of the Folic Acid, Vitamin B_6, and Vitamin B_{12} Component of WAFACS Trial

Abbreviations: WACS: Women's Antioxidant Cardiovascular Study; WAFACS: Women's Antioxidant and Folic Acid Cardiovascular Study.

[a] Mortality and morbidity information was complete for 98.9% and 98.0% of person-years of follow-up, respectively.

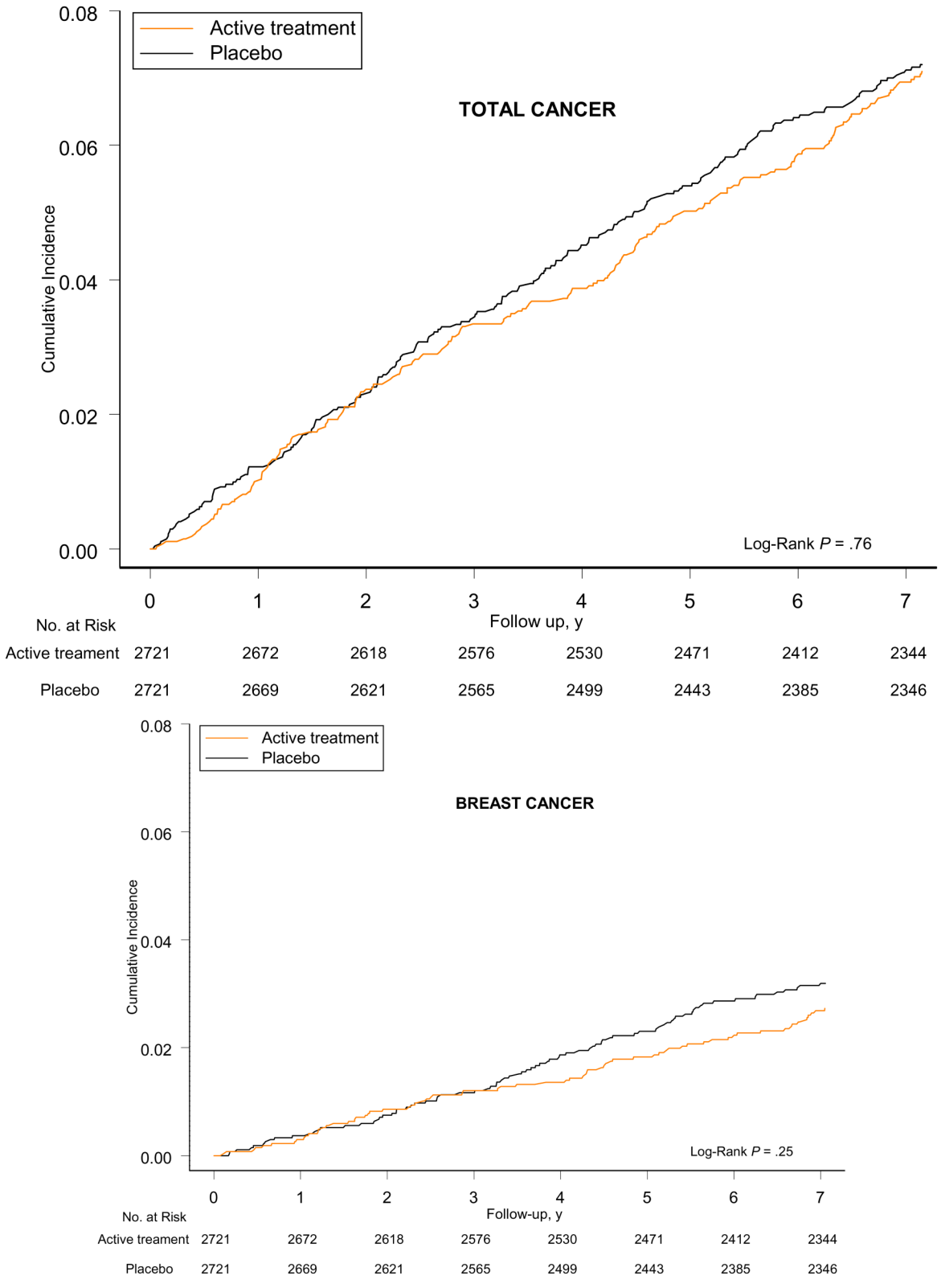

Figure 2.

Cumulative Incidence Estimates of total invasive cancer (panel A) and breast cancer (panel B) (adjusted for age and randomized vitamin E, vitamin C, and beta-carotene assignments)

Table 1

Comparison of Baseline Characteristics by Randomized Folic Acid/Vitamin B_6/Vitamin B_{12} Treatment

	Active Group (N = 2,721)	Placebo Group (N = 2,721)	*P* Value[a]
Age, mean (SD), y	62.8 (8.8)	62.8 (8.8)	.94
Age, No. (%), y			.84
40–54	582 (21.4)	584 (21.5)	
55–64	990 (36.4)	970 (35.7)	
≥ 65	1149 (42.2)	1167 (42.9)	
CVD health history, No. (%)			.31
Prior CVD[b]	1764 (64.8)	1728 (63.5)	
≥ 3 risk factors[c]	957 (35.2)	993 (36.5)	
History of hypertension[d], No. (%)			.32
Yes	2360 (86.7)	2335 (85.8)	
No	361 (13.3)	386 (14.2)	
History of high cholesterol[e], No. (%)			.29
Yes	2118 (77.8)	2150 (79.0)	
No	603 (22.2)	571 (21.0)	
History of diabetes, No. (%)			.89
Yes	570 (21.0)	574 (21.1)	
No	2151 (79.0)	2147 (78.9)	
History of cancer within the past ten years[f], No. (%)			.42
Yes	201 (7.4)	217 (8.0)	
No	2520 (92.6)	2504 (92.0)	
Smoking status, No. (%)			.25
Current	311 (11.4)	334 (12.3)	
Past	1189 (43.7)	1224 (45.0)	
Never	1221 (44.9)	1163 (42.7)	
Alcohol intake in past year, No. (%), g/d			.12
0	1494 (54.9)	1499 (55.1)	
>0 – < 15	1090 (40.1)	1052 (38.7)	
≥ 15	137 (5.0)	170 (6.3)	
Body mass index[g], mean (SD)	30.5 (6.7)	30.6 (6.7)	.71
Body mass index[g], No. (%)			.20
<25	616 (22.6)	566 (20.8)	
25-<30	764 (28.1)	806 (29.6)	
≥ 30	1341 (49.3)	1349 (49.6)	
Menopause status, No. (%)			.32
Premenopausal	164 (6.0)	171 (6.3)	
Uncertain	69 (2.5)	53 (2.0)	
Postmenopausal	2488 (91.4)	2497 (91.8)	
Postmenopausal hormone use[h], No. (%)			.72
Current	1370 (55.1)	1370 (54.9)	
Past	489 (19.7)	512 (20.5)	
Never	629 (25.3)	615 (24.6)	
Current multivitamin use, No. (%)			.62
Yes	616 (22.6)	631 (23.2)	
No	2105 (77.4)	2088 (76.8)	
Physical activity, No. (%), kcal/wk			.10
<1000	1732 (63.7)	1790 (65.8)	
≥ 1000	989 (36.4)	931 (34.2)	
Randomized to receive vitamin E, No. (%)			.83
Yes	1364 (50.1)	1356 (49.8)	
No	1357 (49.9)	1365 (50.2)	
Randomized to receive vitamin C, No. (%)			.85
Yes	1349 (49.6)	1356 (49.8)	
No	1372 (50.4)	1365 (50.2)	
Randomized to receive β-carotene, No. (%)			.81
Yes	1358 (49.9)	1349 (49.6)	
No	1363 (50.1)	1372 (50.4)	
Median nutrient intake[i], range (5^{th}–95^{th} percentiles)			
Total folate, μg/day	424.4 (192.6–924.9)	438.7 (180.5–942.8)	.47
Total vitamin B_6, mg/day	2.5 (1.2–7.0)	2.5 (1.2–7.7)	.38
Total vitamin B_{12}, μg/day	7.1 (2.6–20.4)	7.0 (2.6–20.9)	.70

Abbreviations: CVD: cardiovascular disease

[a] T-test or Wilcoxon signed-rank test for continuous variables and chi-sq test for categorical variables, comparing active and placebo group.

[b] Reported history of myocardial infarction, stroke, coronary revascularization, angina pectoris, transient ischemic attack, carotid endarterectomy, or peripheral artery surgery.

[c] Denotes women with no prior CVD but with at least 3 of the following: hypertension, high cholesterol level, diabetes mellitus, parental history of premature myocardial infarction (before age 60 years), obesity (body mass index ≥ 30 kg/m^2), current cigarette smoking, and inconsistent report of prior CVD.

[d] Denotes self-reported systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, self-reported physician-diagnosed hypertension, or reported treatment with medication for hypertension.

[e] Denotes self-reported high cholesterol level of 240 mg/L or greater, self-reported physician-diagnosed high cholesterol levels, or reported treatment with cholesterol-lowering medication.

[f] Except for non-melanoma skin cancers.

[g] Body mass index is calculated as weight in kilograms divided by height in meters squared.

[h] Among postmenopausal women only.

[i] Calculated from the food frequency questionnaire.

Table 2

Hazard Ratio of Confirmed Invasive Cancer[a] by Randomized Folic acid/Vitamin B_6/Vitamin B_{12} Treatment

Cancer Site	Active Group (N = 2,721)	Placebo Group (N = 2,721)	Hazard Ratio (95% Confidence Interval)	*P* Value
	no. of Cases			
Total	187	192	0.97 (0.79–1.18)	.75
Breast	70	84	0.83 (0.60–1.14)	.24
Colon and rectum	18	22	0.81 (0.43–1.50)	.50
Colon	13	18	0.71 (0.35–1.46)	.35
Rectum	5	4	1.23 (0.33–4.59)	.75
Lung	23	22	1.04 (0.58–1.87)	.89
Uterine	14	13	1.07 (0.50–2.28)	.86
Ovary	5	6	0.83 (0.25–2.72)	.76
Lymphoma	16	8	1.99 (0.85–4.65)	.11
Leukemia	3	5	0.59 (0.14–2.49)	.48
Multiple myeloma	5	2	2.46 (0.48–12.7)	.28
Pancreas	6	4	1.49 (0.42–5.30)	.53
Kidney	5	5	1.00 (0.29–3.45)	>.99
Urinary bladder	3	5	0.60 (0.14–2.50)	.48
Thyroid	4	3	1.33 (0.30–5.95)	.71
Melanoma of the skin	3	3	1.00 (0.20–4.96)	>.99
Other cancers	21	15	1.40 (0.72–2.71)	.32
Any cancer death	47	57	0.82 (0.56–1.21)	.32
Any death	147	152	0.96 (0.77–1.20)	.72

[a] Excluding non-melanoma skin cancer

Table 3

Hazard Ratio of Total Invasive Cancer and Breast Cancer according to Randomized Folic Acid/Vitamin B_6/Vitamin B_{12} Treatment, by Baseline Characteristics of Participants

	Total Invasive Cancer[a]					Breast Cancer				
	Active Group	Placebo Group	Hazard Ratio (95% Confidence Interval)	*P* Value	*P* for Interaction	Active Group	Placebo Group	Hazard Ratio (95% Confidence Interval)	*P* Value	*P* for Interaction
	no. of cases					no. of cases				
Overall	187	192	0.97 (0.79–1.18)	.75		70	84	0.83 (0.60–1.14)	.24	
Age, y					.02					.05
40–54	22	16	1.42 (0.75–2.71)	.28		12	8	1.56 (0.64–3.81)	.33	
55–64	77	59	1.26 (0.90–1.76)	.19		27	26	1.00 (0.58–1.72)	>.99	
≥65	88	117	0.75 (0.57–0.99)	.05		31	50	0.62 (0.40–0.98)	.04	
CVD health history					.79					.26
Prior CVD[b]	123	123	0.99 (0.77–1.27)	.91		49	51	0.95 (0.64–1.40)	.78	
≥3 risk factors[c]	64	69	0.93 (0.66–1.31)	.68		21	33	0.65 (0.37–1.12)	.12	
History of cancer within the past ten years[d]				.76					.91	
Yes	18	18	1.09 (0.57–2.10)	.80		5	6	0.87 (0.27–2.87)	.83	
No	169	174	0.96 (0.78–1.19)	.70		65	78	0.82 (0.59–1.14)	.24	
Smoking status					.50					.82
Current	27	28	1.11 (0.66–1.89)	.69		7	7	1.18 (0.41–3.38)	.76	
Past	86	99	0.86 (0.65–1.15)	.31		35	42	0.83 (0.53–1.30)	.41	
Never	74	65	1.10 (0.79–1.53)	.59		28	35	0.77 (0.47–1.27)	.30	
Alcohol use in past year, g/d				>.99					.51	
0	99	114	0.87 (0.66–1.13)	.29		35	52	0.67 (0.44–1.03)	.07	
>0 – < 15	77	63	1.19 (0.85–1.66)	.31		32	24	1.30 (0.76–2.20)	.34	
≥ 15	11	15	0.88 (0.40–1.92)	.75		3	8	0.44 (0.12–1.67)	.23	
Body mass index[e]					.18					.88
<25	39	44	0.80 (0.52–1.24)	.32		12	13	0.82 (0.37–1.79)	.61	
25–<30	51	62	0.88 (0.61–1.28)	.51		25	30	0.89 (0.52–1.51)	.66	
≥ 30	97	86	1.11 (0.83–1.48)	.48		33	41	0.79 (0.50–1.25)	.32	
Menopausal status					.85					.20
Premenopausal	5	4	1.28 (0.34–4.86)	.71		3	2	1.32 (0.22–8.02)	.76	
Uncertain	6	4	1.31 (0.37–4.70)	.68		2	0	--		
Postmenopausal	176	184	0.95 (0.77–1.17)	.64		65	82	0.79 (0.57–1.09)	.15	
Postmenopausal hormone use[f]				.64					.69	
Current	82	95	0.87 (0.65–1.17)	.35		32	46	0.69 (0.44–1.09)	.11	
Past	35	33	1.08 (0.67–1.74)	.74		15	16	0.97 (0.48–1.96)	.92	
Never	59	56	1.06 (0.74–1.54)	.74		18	20	0.91 (0.48–1.73)	.77	
Physical activity, kcal/wk					.60					.97
<1000	130	131	1.01 (0.79–1.28)	.95		45	55	0.83 (0.56–1.24)	.37	
≥ 1000	57	61	0.90 (0.63–1.29)	.57		25	29	0.83 (0.48–1.41)	.49	
Current multivitamin use				.71					.96	
Yes	43	48	0.90 (0.60–1.36)	.62		15	18	0.84 (0.42–1.67)	.62	
No	144	144	0.99 (0.78–1.24)	.91		55	66	0.82 (0.58–1.18)	.28	
Randomized to receive vitamin E				.65					.90	
Yes	87	93	0.92 (0.69–1.23)	.58		36	42	0.84 (0.54–1.32)	.45	
No	100	99	1.01 (0.77–1.34)	.93		34	42	0.81 (0.52–1.27)	.36	
Randomized to receive vitamin C				.06					.36	
Yes	87	108	0.81 (0.61–1.07)	.13		33	46	0.72 (0.46–1.12)	.14	

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	Total Invasive Cancer[a]					Breast Cancer				
	Active Group	Placebo Group	Hazard Ratio (95% Confidence Interval)	*P* Value	*P* for Interaction	Active Group	Placebo Group	Hazard Ratio (95% Confidence Interval)	*P* Value	*P* for Interaction
No	100	84	1.18 (0.88–1.58)	.27		37	38	0.97 (0.61–1.52)	.88	
Randomized to receive β-carotene				.12					.15	
Yes	91	108	0.83 (0.63–1.10)	.19		33	49	0.66 (0.43–1.03)	.07	
No	96	84	1.15 (0.86–1.54)	.36		37	35	1.06 (0.67–1.69)	.80	
Total folate intake[g], median value				.78					.77	
≤ 431.5 μg/day	94	88	1.01 (0.75–1.35)	.97		36	38	0.90 (0.57–1.41)	.63	
> 431.5 μg/day	87	96	0.95 (0.71–1.27)	.74		32	41	0.82 (0.51–1.30)	.39	
Total vitamin B_6 intake[g], median value				.73					.63	
≤ 2.5 mg/day	91	92	0.94 (0.71–1.26)	.68		33	40	0.79 (0.50–1.25)	.32	
> 2.5 mg/day	90	92	1.01 (0.76–1.36)	.93		35	39	0.92 (0.59–1.46)	.74	
Total vitamin B_{12} intake[g], median value				.10					.39	
≤ 7.0 μg/day	88	106	0.83 (0.62–1.10)	.19		32	43	0.74 (0.47–1.18)	.20	
> 7.0 μg/day	93	78	1.18 (0.87–1.59)	.29		36	36	0.99 (0.62–1.57)	.96	

Abbreviations: CVD: cardiovascular disease

[a] Excluding non-melanoma skin cancer.

[b] Reported history of myocardial infarction, stroke, coronary revascularization, angina pectoris, transient ischemic attack, carotid endarterectomy, or peripheral artery surgery.

[c] Denotes women with no prior CVD but with at least 3 of the following: hypertension, high cholesterol level, diabetes mellitus, parental history of premature myocardial infarction (before age 60 years), obesity (body mass index ≥30 kg/m^2), current cigarette smoking, and inconsistent report of prior CVD.

[d] Except for non-melanoma skin cancers.

[e] Body mass index is calculated as weight in kilograms divided by height in meters squared.

[f] Among postmenopausal women only.

[g] Calculated from the food frequency questionnaire.

Table 4

Hazard Ratio of Invasive Breast Cancer according to Randomized Folic Acid/Vitamin B_6/Vitamin B_{12} Treatment, by Tumor Characteristics at Diagnosis

Variable	Active Group (N = 2,721)	Placebo Group (N = 2,721)	Hazard Ratio (95% Confidence Interval)	*P* Value
Invasive breast cancer — no. of cases	70	84	0.83 (0.60–1.14)	.24
Hormone receptor status				
ER+/PR+	48	52	0.92 (0.62–1.36)	.66
ER+/PR−	6	15	0.40 (0.15–1.02)	.06
ER−/PR+	0	1	--	
ER−/PR−	7	8	0.87 (0.32–2.40)	.79
Tumor size				
≤ 2 cm	55	62	0.88 (0.61–1.27)	.49
>2 cm	8	16	0.50 (0.21–1.16)	.11
Missing	7	6	1.15 (0.39–3.43)	.80
Lymph nodes				
No metastasis	46	53	0.86 (0.58–1.28)	.46
Metastasis to lymph nodes	14	17	0.82 (0.40–1.66)	.57
Missing	10	14	0.70 (0.31–1.58)	.39
Histology				
Ductal carcinoma	52	57	0.91 (0.62–1.32)	.60
Lobular carcinoma	6	10	0.60 (0.22–1.65)	.32
Duct and lobular carcinoma	3	8	0.38 (0.10–1.41)	.15
Adenocarcinoma	2	0	--	
Tubular adenocarcinoma	3	1	2.99 (0.31–28.7)	.34
Mucinous adenocarcinoma	2	3	0.66 (0.11–3.94)	.65
Other	2	5	0.40 (0.08–2.04)	.27
Histologic grading and differentiation				
Well differentiated	19	23	0.82 (0.45–1.51)	.52
Moderately differentiated	26	34	0.76 (0.46–1.27)	.29
Poorly differentiated/anaplastic	16	15	1.06 (0.52–2.14)	.87
Missing	9	12	0.74 (0.31–1.76)	.50

Abbreviations: ER: estrogen receptor; PR, progesterone receptor