

Hormone Receptor Status of a Contralateral Breast Cancer Is Independent of the Receptor Status of the First Primary in Patients Not Receiving Adjuvant Tamoxifen

G. Arpino, H.L. Weiss, G.M. Clark, S.G. Hilsenbeck, and C.K. Osborne

From the Breast Center, Baylor College of Medicine and The Methodist Hospital, Houston, TX.

Submitted April 15, 2004; accepted November 9, 2004.

Supported by grant Nos. P01 CA30195 and P50 CA58183 (SPORE) from the National Institutes of Health (Bethesda, MD) and Association Italian of Medical Oncology.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to C. Kent Osborne, MD, Breast Center, Baylor College of Medicine, One Baylor Plaza, BCM 600, Houston, TX 77030; e-mail: kosborne@breastcenter.tmc.edu.

© 2005 by American Society of Clinical Oncology

0732-183X/05/2321-4687/\$20.00

DOI: 10.1200/JCO.2005.04.076

A B S T R A C T

Purpose

To determine whether the hormone receptor status of the primary breast cancer (PBC) is predictive of the hormone receptor status of the subsequent contralateral breast cancer (CBC).

Patients and Methods

We identified patients in our database with known estrogen receptor (ER; $n = 193$) and/or progesterone receptor (PgR; $n = 178$) status in their PBC and in their subsequent CBC. One hundred twenty-six of these patients had received no adjuvant therapy, 34 had received adjuvant tamoxifen, and 33 had received adjuvant chemotherapy alone. The median interval between the first diagnosis of PBC and the development of the subsequent CBC was 3 years. ER and PgR assays were assessed biochemically in two central reference laboratories using identical quality-controlled ligand-binding methods.

Results

Among systemically untreated patients ($n = 126$), 88% of patients with ER-positive PBC and 75% of patients with ER-negative PBC developed an ER-positive CBC ($P = .11$). Among the tamoxifen-treated patients, those with an ER-positive PBC were almost equally likely to develop an ER-positive (47%) or ER-negative (53%) CBC ($P = .99$). PgR status was similar. In the untreated group ($n = 112$), 59% of patients with a PgR-positive PBC and 66% with a PgR-negative PBC developed a PgR-positive CBC ($P = .48$). Among tamoxifen-treated patients ($n = 33$), 50% of patients with a PgR-positive PBC versus 27% of patients with a PgR-negative PBC developed a PgR-positive CBC ($P = .28$).

Conclusion

ER and PgR status of the primary tumor does not predict the hormone receptor status of the subsequent CBC in the absence of selective pressure of adjuvant therapy. Thus, other reasons should be considered to clarify the failure of tamoxifen to reduce the incidence of CBC in patients with a receptor-negative PBC.

J Clin Oncol 23:4687-4694. © 2005 by American Society of Clinical Oncology

INTRODUCTION

The increasing incidence of breast cancer coupled with improved long-term survival after effective treatment of the primary tumor have placed an increased number of women at risk for development of another cancer in the opposite breast. Five years of adjuvant tamoxifen treatment is associated with a 45% reduction in the risk of contralateral breast cancer

(CBC).¹ Although the cumulative database is still relatively small, this benefit seems limited largely to the subset of patients whose initial breast cancer was estrogen receptor (ER) positive (R. Peto, personal communication).^{2,3}

Why adjuvant hormonal therapy may reduce the incidence of CBC mainly in patients with receptor-positive primary tumors is still unclear. Data addressing this question are sparse, especially in patients

not receiving adjuvant tamoxifen or other forms of endocrine therapy, a therapy that could confound the results by selecting relatively more ER-negative tumors as shown in the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 Study prevention trial.⁴ As a possible explanation, we hypothesized that the receptor status of the CBC may be related to that of the primary breast cancer (PBC), so that patients with ER-negative PBC would be more likely to develop an ER-negative CBC that would not be suppressed by tamoxifen.

To determine whether the hormone receptor status of the PBC is predictive of the hormone receptor status of the CBC, we examined the ER and progesterone receptor (PgR) status and other tumor characteristics of the PBC and of subsequent CBCs in the same patients with synchronous or metachronous bilateral breast cancer. We performed separate analyses for patients who had or had not received adjuvant tamoxifen for their PBC.

PATIENTS AND METHODS

Study Population

The Breast Center at Baylor College of Medicine (Houston, TX) maintains databases of breast cancer patients whose biopsy or mastectomy specimens were sent to central laboratories for steroid receptor assays. These patients were diagnosed and treated at more than 370 academic and community institutions throughout the United States. Histologic diagnoses were made by pathologists at community hospitals and were not reviewed centrally. Follow-up information was obtained from tumor registries, by direct review of medical records performed by data managers, or by data collection forms completed by the office of the referring physicians. These databases contain 50,538 patients who were diagnosed between 1970 and 1998. Breast cancer was defined as invasive or in situ lesions. Overall, a total of 2,855 patients with CBC were identified in our databases. However, the number of patients for whom reliable clinical and biologic information could be obtained was limited to 1,158 patients. Among those, only patients whose ER and/or PgR status were performed in the two central laboratories on both the PBC and the subsequent CBC were included in this study (n = 193). Among the 193 PBCs, 77% were invasive ductal carcinoma (IDC); 7% were invasive lobular carcinoma (ILC); 14% were less frequent invasive histotypes such as tubular, mucinous, mixed ILC and IDC, and adenocystic; and 2% were in situ lesions. Information on tumor histotype was available for 117 CBCs. The vast majority of CBCs (75%) were IDC, 12% were ILC, 10% were less frequent invasive histotypes, and 3% were in situ lesions. The low incidence of in situ lesions may be a result of the fact that, until recently, receptor assays were not required for their management and, thus, the tissue was not submitted for testing. The following clinical data were examined: age, date of diagnosis, stage, interval between PBC and CBC, and adjuvant treatment for the PBC. The median follow-up time for the patients included onto the study was 111 months (range, 15 to 301 months), and these patients were observed in a manner consistent with community standards at the time.

Steroid Receptor Determination

ER levels were measured by the dextran-coated charcoal method as previously described.⁵ From 1970 to 1984, [³H]estradiol was used as labeled ligand. During the same time period, PgR levels were measured by sucrose density gradient.⁶ In 1985, the standard multipoint dextran-coated charcoal assay was modified to incorporate ¹²⁵I-labeled estradiol and [³H]R5020 in a single assay, allowing the simultaneous determination of both ER and PgR.⁷ Levels ≥ 3 fmol/mg protein were considered positive for ER, and levels ≥ 5 fmol/mg protein were considered positive for PgR. These cutoffs represent the lower level of assay sensitivity.

S-Phase Fraction and DNA Ploidy Determination

DNA ploidy and S-phase fraction were evaluated by DNA flow cytometry as previously described.^{8,9} S-phase fractions less than 6% were considered low, S-phase fraction of 6% to 10% were considered intermediate, and S-phase fractions more than 10% were considered high.

Statistical Analysis

Analysis was performed in the overall patient group and separately for the untreated, tamoxifen-treated (tamoxifen only and chemotherapy plus tamoxifen), and chemotherapy only-treated patients. The proportion of CBC tumors that were ER or PgR positive was compared with the proportion of PBC tumors that were ER or PgR positive using χ^2 or Fisher's exact tests. Because chemotherapy may cause amenorrhea in premenopausal women, thus acting like an endocrine therapy, we included only the subset of untreated and tamoxifen-treated premenopausal patients in addition to all postmenopausal patients in the logistic regression models. The univariate association of the characteristics of the PBC with ER and PgR status of the PBC was evaluated using the logistic regression model. In addition, the association of each of the PBC variables with ER and PgR status of the CBC was also assessed using logistic regression models with adjustment for treatment group (untreated *v* tamoxifen treated). The simultaneous modeling of all the variables in a multivariate model was not performed because of smaller patient numbers resulting from missing data. Odds ratios (ORs) and 95% CIs were calculated for each variable with adjustment for the effect of treatment group (untreated *v* tamoxifen treated) in the model.

Because age may influence the hormone receptor status of breast cancer and because patients would be older when they were diagnosed with a CBC, we determined whether we needed to adjust for patient age in assessing ER positivity rates for the CBC. Using our entire database, we estimated age-specific ER positivity rates according to 5-year age groups. These probabilities were then applied to our study set, and logistic regression modeling was used to determine whether the probability of ER positivity according to age at diagnosis of the CBC was associated with CBC ER positivity rates.

RESULTS

Patients and Tumor Characteristics

To test whether the population of patients included in the present study (n = 193) was representative of the whole CBC patient population available in our database (n = 965), we compared clinical and biologic tumor characteristics of PBCs between the two patient populations stratified by decades of diagnosis (1970 to 1979, 1980 to

1989, and 1990 to 1999). None of the clinical and tumor characteristics analyzed (ER status, PgR status, tumor size, axillary node status, S phase, and ploidy), except the number of metastatic lymph nodes among patients diagnosed from 1980 to 1989, was statistically significantly different.

Among the 193 patients included in this study, 126 (65%) received no adjuvant treatment, allowing assessment of the natural development and progression of CBC uninfluenced by the selective pressure of systemic treatment. Thirty four patients (18%) received tamoxifen (27 received tamoxifen only and seven received chemotherapy plus tamoxifen), and 33 patients (17%) received chemotherapy only as adjuvant therapy for their PBC.

Women at PBC diagnosis were younger, by definition, than at CBC diagnosis. The median age at the PBC diagnosis was 62 years (range, 28 to 96 years), and the median age at diagnosis of the CBC was 65 years (range, 30 to 99 years; Table 1). Overall, CBCs were smaller in size compared with PBCs (73% v 52% were < 2 cm in diameter, respectively; $P = .013$). The slightly higher incidence of metastases in the axillary lymph nodes (33% in CBC v 27% in PBC, $P = .08$) was not significant. Among the PBCs, 153 (79%) of 193 were ER positive, and 106 (59%) of 178 were PgR positive. Fifty-seven (48%) of 120 PBCs were diploid, and 73 (71%) of 103 PBCs were considered to have a low or intermediate

S-phase fraction. In the CBCs, 141 (73%) of 193 and 97 (52%) of 187 were ER positive and PgR positive, respectively; 49 (41%) of 120 were diploid, and 68 (63%) of 108 had low or intermediate S-phase fractions. With exception of tumor size, none of these characteristics of the CBCs was statistically significantly different from the PBCs (Table 1).

The median interval between the PBC and the CBC was 3 years (range, 5 months to 16 years). To evaluate the potential drift (time-dependent change) on tumor receptor status, we evaluated the impact of the time interval between PBC and CBC diagnosis for both ER and PgR status (Table 2). Whether patients were initially ER positive or ER negative, the median interval between diagnosis was not significantly different for concordant versus discordant ER status on the subsequent CBC. Median interval between diagnosis of PBC and CBC did not affect the ER status of CBC in either the untreated or the tamoxifen only-treated patients (data not shown). A similar result was found for PgR status (Table 2), suggesting that a longer time interval between diagnoses is not a contributing factor in the evolution of tumors from hormone receptor positivity to hormone receptor negativity or vice versa.

Hormone Receptor Status in PBC and CBC

ER status was available for 193 sequential pairs of assays, and PgR status was available for 178 assays. Because intervening therapy might be expected to have a significant impact on both ER and PgR status, we further analyzed our study population according to the adjuvant therapy that was administered for the PBC.

Overall, 75% of women with ER-positive PBC and 65% of women with ER-negative PBC developed an ER-positive CBC ($P = .20$; Table 3). Among the patients who received no systemic adjuvant therapy ($n = 126$), 88% who had an ER-positive PBC and 75% who had an ER-negative PBC developed an ER-positive CBC ($P = .11$). In this group of 126 patients, the median ER value in the PBCs was similar to that in the CBCs (62 fmol/mg protein v 82 fmol/mg protein, respectively; $P = .48$). In the tamoxifen-treated patients ($n = 34$), those with an ER-positive PBC were equally likely to develop an ER-positive or ER-negative CBC. Only two patients in this subset had an ER-negative PBC (Table 3). It should be noted that the ER positivity rate of the CBCs in the tamoxifen-treated patients was only 47%. Furthermore, the median ER values in the CBCs were markedly lower than in the PBCs in this subset (0 fmol/mg protein v 55.5 fmol/mg protein, respectively), and this difference was statistically significant ($P = .0030$). The difference between the CBC ER positivity rates between untreated and tamoxifen-treated patients with ER-positive PBCs was statistically significant ($P < .0001$). This finding is consistent with results from the NSABP P-1 prevention study.^{4,10,11} However, an alternative explanation might be that tamoxifen, by occupying ER, may interfere with the ligand-binding assay that was used to

Table 1. Patients and Tumor Characteristics of PBC and CBC

Characteristic	PBC (n = 193)	CBC (n = 193)	P*
Tumor size			
No. with data	193	120	
≤ 2 cm, %	52	73	
2-5 cm, %	37	23	.013
> 5 cm, %	11	4	
Age			
No. with data	193	193	—
Median age, years	62	65	
Positive lymph nodes			
No. with data	184	110	
≥ 1, %	27	33	.08
Estrogen receptor			
No. tested	193	193	
Positive, %	79	73	.13
Progesterone receptor			
No. tested	182	187	
Positive, %	59	52	.13
DNA ploidy			
No. tested	120	120	
Diploid, %	48	41	.27
S-phase fraction			
No. tested	103	108	
Low, %	51	45	
Intermediate, %	20	18	.56
High, %	29	37	

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer.
*P value based on paired PBC and CBC data using the McNemar's test.

Table 2. Median Intervals Between Diagnosis of the PBC and the CBC

PBC	CBC								P
	ER Positive		ER Negative		PgR Positive		PgR Negative		
	Interval (years)	No.*	Interval (years)	No.*	Interval (years)	No.*	Interval (years)	No.*	
ER positive	2.49	115	2.86	38	—	—	—	—	.70
ER negative	3.62	26	3.74	14	—	—	—	—	.81
PgR positive	—	—	—	—	2.11	57	2.94	49	.43
PgR negative	—	—	—	—	3.01	35	2.94	37	.69

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer; ER, estrogen receptor; PgR, progesterone receptor.
*No. of patients demonstrating given assay response.

determine ER status in the subsequent CBC.¹² The majority of CBCs in the tamoxifen-treated subgroup occurred within 5 years from the diagnosis of the first invasive breast cancer (n = 28, 82%). During the time period when these patients were treated, tamoxifen was usually administered for 2 to 5 years. Thus, we cannot exclude the possibility that some of the apparent discordance of ER positivity rates might be a result of false-negative CBC assay results in the subset of patients with ER-positive PBC caused by receptor occupancy by the drug.

Overall, 54% of patients with PgR-positive PBCs compared with 49% of patients with PgR-negative PBCs developed a PgR-positive CBC (P = .50; Table 4). In the untreated group of patients whose PgR status was known for both PBC and CBC (n = 112), 59% of patients with PgR-positive PBCs and 66% of patients with PgR-negative PBCs developed a PgR-positive CBC (P = .48). Among tamoxifen-treated patients (n = 33), 50% of patients with PgR-positive PBCs compared with 27% of patients with PgR-negative PBCs developed a PgR-positive CBC (P = .28; Table 4). If only PgR-positive PBCs were considered, the incidence of a PgR-positive CBC was similar for the un-

treated patients (59%) compared with tamoxifen-treated patients (50%; P = .45).

Interestingly, patients treated only with adjuvant chemotherapy seemed to behave like patients administered tamoxifen (Tables 3 and 4). Only 53% of the patients with ER-positive PBCs and 50% of patients with ER-negative PBCs administered adjuvant chemotherapy developed ER-positive CBCs, which is in striking contrast to the 88% and 75% ER-positive rate in the untreated subset. However, 26 (79%) of the 33 patients administered chemotherapy were ≤ 50 years of age and likely to be premenopausal. Thus, chemotherapy might be expected to induce ovarian ablation in many of these women, resulting in an antiestrogenic effect on the receptor profiles similar to that with tamoxifen.

It is known that there is an increasing number of ER-positive cancers with increasing age, whereas the number of ER-negative cancers remains constant.^{13,14} Thus, older women are more likely to be ER positive. To investigate whether the rate of ER-positive CBCs, which were observed especially in the untreated group, was affected by the older

Table 3. ER Status of the PBC and CBC

PBC ER Status	CBC ER Negative		CBC ER Positive		Total		P
	No.	%	No.	%	No.	%	
All patients, n = 193							
Negative	14	35	26	65	40	100	.20
Positive	38	25	115	75	153	100	
Untreated patients, n = 126							
Negative	6	25	18	75	24	100	.11
Positive	12	12	90	88	102	100	
Tamoxifen-treated patients, n = 34							
Negative	1	50	1	50	2	100	.99
Positive	17	53	15	47	32	100	
Chemotherapy treated patients, n = 33							
Negative	7	50	7	50	14	100	.99
Positive	9	47	10	53	19	100	
Unknown treatment, n = 0	—	—	—	—	—	—	

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer; ER, estrogen receptor.

Table 4. PgR Status of the PBC and CBC

PBC PgR Status	CBC PgR Negative		CBC PgR Positive		Total		P
	No.	%	No.	%	No.	%	
All patients, n = 178							
Negative	37	51	35	49	72	100	.54
Positive	49	46	57	54	106	100	
Untreated patients, n = 112							
Negative	14	34	27	66	41	100	.54
Positive	29	41	42	59	71	100	
Tamoxifen-treated patients, n = 33							
Negative	8	73	3	27	11	100	.28
Positive	11	50	11	50	22	100	
Chemotherapy-treated patients, n = 33							
Negative	15	75	5	25	20	100	.99
Positive	9	69	4	31	13	100	
Unknown treatment, n = 0	—	—	—	—	—	—	

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer; PgR, progesterone receptor.

age of the patients at the time of the CBC diagnosis, we calculated the age-specific incidence of ER-positive breast cancer in our entire database (n = 50,538). There was no statistically significant association between the ER status of the CBC and the probability of ER positivity at the age of diagnosis of the CBC (data not shown). Therefore, the ER status of the CBC was completely independent of patient age at diagnosis. Indeed, the estimated ER positivity rates from the logistic model were similar to the observed ER positivity rates in this study set.

Logistic Regression Analyses

We next performed logistic regression analyses to identify factors potentially influencing ER and/or PgR status. We first evaluated tumor and patient characteristics at PBC diagnosis and the ER status of the PBC. We found a significant association between ER-positive status and older patient age, PgR-positive status, tumor size, and low S-phase fraction. The incidence of ER-positive breast cancer increased with the age of patients in a linear manner (OR = 1.08 per year, P < .0001). Not unexpectedly, ER status was strongly and directly correlated with PgR status (OR = 9.5, P < .0001). Larger tumors and tumors with high S-phase fractions were more likely to be ER negative (OR = 1.69, P = .04; and OR = 2.27, P = .012, respectively). There was no consistent relationship between ER status, DNA ploidy, and nodal status.

None of the patient or tumor characteristics measured in the PBC was predictive of the hormone receptor status of the CBC, with the exceptions of tamoxifen adjuvant therapy and S-phase fraction (Tables 5 and 6). As shown earlier, the relative proportion of ER-positive CBCs was significantly reduced among women treated with tamoxifen (OR = 0.15, P < .0001). Interestingly, patients with a high S-phase frac-

tion in their PBC were not only more likely to have an ER-negative PBC, but were also more likely to develop an ER-negative CBC (OR for ER positivity = 0.5, P = .031; Table 5).

Similarly, the PgR-positive status of the PBC was statistically significantly associated with low S-phase fraction and older patient age at diagnosis, ER-positive status, and aneuploidy (data not shown). Furthermore, the same patterns of association observed between ER status and tumor characteristics of the PBC and the CBC were found with PgR. High S-phase fraction was inversely associated with

Table 5. Association Between PBC Characteristics and ER Positivity of the CBC

Variable	OR	95% CI	P
Endocrine treatment, n = 160			
Tam	0.15	0.06 to 0.34	< .0001
ER status, n = 160*			
ER positivity	2.20	0.77 to 6.28	.143
Age at CBC, n = 160*			
Older age	1.01	0.98 to 1.04	.412
S-phase fraction, n = 82*			
High S-phase fraction	0.51	0.27 to 0.94	.031
PgR status, n = 149*			
PgR positivity	0.69	0.28 to 1.70	.424
DNA ploidy, n = 95*			
Aneuploidy	0.49	0.18 to 1.34	.424
Positive lymph nodes, n = 153*			
≥ 1	0.56	0.22 to 1.43	.226
Tumor size, n = 160*			
≥ 2 cm	0.67	0.37 to 1.21	.182

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer; ER, estrogen receptor; PgR, progesterone receptor; OR, odds ratio; Tam, tamoxifen.
*Adjusted for treatment group (tamoxifen-treated v not treated).

Table 6. Association Between PBC Characteristics and PgR Positivity of the CBC

Variable	OR	95% CI	P
Endocrine treatment, n = 154			
Tam	0.47	0.21 to 1.0	.057
ER status, n = 154*			
ER positivity	1.11	0.46 to 2.65	.814
Age at CBC, n = 154*			
Older age	1.01	0.99 to 1.04	.321
S-phase fraction, n = 82*			
High S-phase fraction	0.55	0.32 to 0.95	.032
PgR status, n = 145*			
PgR positivity	0.99	0.49 to 1.99	.979
DNA ploidy, n = 95*			
Aneuploidy	0.61	0.26 to 1.39	.238
Positive lymph nodes, n = 147*			
≥ 1	0.78	0.33 to 1.85	.575
Tumor size, n = 154*			
≥ 2 cm	0.93	0.56 to 1.54	.771

Abbreviations: PBC, primary breast cancer; CBC, contralateral breast cancer; ER, estrogen receptor; PgR, progesterone receptor; OR, odds ratio; Tam, tamoxifen.
*Adjusted for treatment group (tamoxifen-treated v not treated).

the PgR-positive status of the CBC (OR = 0.55, $P = .032$), and a trend for tamoxifen treatment to be inversely associated with the PgR-positive status of the CBC was also observed (OR = 0.47, $P = .057$; Table 6).

DISCUSSION

Results from this study suggest that the ER and PgR status of the PBC are not related to the hormone receptor status of the subsequent CBC and that they are independent events. In the absence of adjuvant tamoxifen, patients with an ER-negative PBC are just as likely to develop an ER-positive CBC as patients with an ER-positive PBC. Conversely, we found a greater proportion of ER-negative CBCs among tamoxifen-treated patients than among patients without tamoxifen treatment, which is consistent with previous evidence that tamoxifen treatment reduces the incidence of ER-positive CBC.^{1,3} The same trends were true for the PgR status. Among other tumor characteristics of the PBC, high S-phase fraction was associated with hormonal receptor negativity in the CBC. Thus, the data do not support the hypothesis that the failure of tamoxifen to reduce the incidence of CBC when the PBC is ER negative is a result of the more likely development of an ER-negative CBC.

One possible explanation for these discrepant observations is that the interpretation of the clinical data suggesting that the beneficial effect of tamoxifen in reducing the incidence of CBC is restricted to ER-positive PBC is incorrect. It is well known that tamoxifen reduces the incidence of CBC.^{1,15-17} A recent update of the Early Breast Cancer Tri-

alists' Collaborative Group overview analysis of all adjuvant tamoxifen trials does suggest that only patients with an ER-positive PBC have a significant reduction in the risk of CBC when treated with tamoxifen (R. Peto, personal communication). However, even in this overview of all the studies, there are relatively few events. In the group with ER-positive or ER-unknown PBCs, there were 172 of 5,137 CBCs with tamoxifen and 260 of 5,092 CBCs without tamoxifen. In the ER-negative subset, there were 67 of 2,209 CBCs with tamoxifen and 70 of 2,259 CBCs without tamoxifen. Two large trials prospectively addressed the value of tamoxifen in patients with ER-negative tumors.^{2,3} Preliminary results presented in abstract form only from one of these trials showed that, among tamoxifen-treated patients, the subgroup of women with ER-negative tumors had no improvement in disease-free survival and no apparent reduction in the risk of CBC. In the ER-positive cohort, there were 34 CBCs without tamoxifen and 11 CBCs with tamoxifen; and in the ER-negative cohort, there were 17 CBCs without tamoxifen and 20 CBCs with tamoxifen.³ The results of the other trial, NSABP B-23, also suggest that women with ER-negative tumors do not benefit from tamoxifen in terms of either disease-free survival or the development of a CBC. Only ER-negative patients were included in this study, and 19 CBCs occurred in patients not treated with tamoxifen, and 18 CBCs occurred in patients administered tamoxifen.² Thus, although the reduction in the incidence of CBC observed in women taking tamoxifen seems to be restricted only to the subset of patients whose initial breast cancer is ER positive, the available data are limited and may not be able to exclude a benefit in the ER-negative subset.

Other studies have addressed the relationship between hormone receptors in PBC and CBC. Coradini et al¹⁸ reported an analysis on 399 patients with known ER and PgR levels in PBC and CBC. In this report, the mean steroid hormone receptor level did not differ markedly between PBC and CBC. This observation was true especially in synchronous tumors, and the author concluded that PBC and CBC, although independent, tend to be characterized by a similar receptor profile. Unfortunately, none of the analyses in study by Coradini et al¹⁸ were performed in patients not receiving endocrine therapy. Thus, the comparison with our study in this subgroup is not possible. In the tamoxifen-treated population of patients, however, they observed lower ER levels in CBCs compared with PBCs, which is consistent with our data that also demonstrated a higher proportion of ER-negative CBCs in this group.

Our data do not support a correlation between the time interval between the diagnoses and the discordance or concordance rate of hormone receptor status on PBC and CBC. No association between the hormone receptor status of the PBC and CBC was found in synchronous breast cancers when we analyzed these tumors separately from the

metachronous tumors (data not shown), although the number of patients in this subgroup ($n = 6$) was small.

A recent NSABP analysis¹⁹ evaluated the ER status of PBC and CBC in patients included in several NSABP studies (B-18, B-22, and B-25).²⁰⁻²² Tamoxifen therapy in these studies was administered based on the patient's age rather than ER status. Women older than 50 years received tamoxifen, regardless of ER and PgR status; whereas younger patients did not receive tamoxifen. Results from this study showed that, among the young patients not treated with tamoxifen, the hormone receptor status of the CBC correlated with the status of the PBC.¹⁹ According to this study, women whose PBCs were ER negative were more likely to develop an ER-negative CBC, providing a potential reason why tamoxifen is not effective in reducing the incidence of CBC in this subset. However, the NSABP studies B-18, B-22, and B-25 were designed to test the effectiveness of high and standard doses of chemotherapy in breast cancer patients at a high risk of relapse. The untreated women included in the reported analyses were mostly premenopausal (patients 49 years old or less), and they had all been treated with intensive chemotherapeutic regimens that were likely to induce ovarian ablation in the majority. Therefore, although the patients in these trials were not administered tamoxifen, the chemotherapy-induced ovarian ablation through its similar antiestrogenic effects could suppress the development of ER-positive tumors just like treatment with tamoxifen. In addition, women younger than 49 years of age who develop an aggressive breast cancer are more likely to be carriers of a hereditary genetic mutation or susceptibility gene, such as *BRCA1*, that is associated with the development of ER-negative breast cancer.^{23,24} In the present study, to avoid these confounding factors for premenopausal patients, we included only the subsets of totally untreated patients (endocrine or chemotherapy) and tamoxifen-treated patients in the logistic regression models.

Not surprisingly, adjuvant tamoxifen therapy in the present study significantly impacted the hormone receptor status of the CBC. Indeed, we found a statistically significantly greater proportion of ER-negative CBCs in tamoxifen-treated patients than in patients without tamoxifen treatment. This finding is consistent with the concept that tamoxifen therapy selectively inhibits the growth of tumor cells expressing ER. Several other studies have previously reported that CBCs evolving in patients receiving adjuvant tamoxifen are more frequently ER negative than the respective PBCs or CBCs evolving in untreated patients.²⁵⁻²⁷ However, we have previously shown that measuring ER by ligand-binding assay during tamoxifen therapy or within 2 months after its discontinuation results in a high rate of false-negative ER assays caused by receptor occupancy by the drug.¹² In our study,

the exact date of stopping tamoxifen therapy was not uniformly recorded for all patients, and therefore, it is not possible to estimate the false ER-negative rate in CBCs. However, because the majority of CBCs in the treated patients occurred within the 5 years after the PBC diagnosis, we cannot exclude the possibility that some of the ER-negative CBCs are falsely negative as a result of the presence tamoxifen. Many of the preceding studies in which patients received tamoxifen have this same concern. Studies using an immunohistochemical antireceptor antibody method for measuring ER that can detect ligand-occupied receptors are necessary to address this question.

Interestingly, many patients develop an ER-positive CBC despite receiving adjuvant tamoxifen.^{25,27,28} Thus, the emergence of ER-negative breast cancer is only one mechanism of tumor escape of CBCs evolving in tamoxifen-treated patients, and alternative mechanisms, such as altered ER coactivator or corepressor expression and cross talk between growth factor and ER pathways,^{29,30} may be equally relevant.

High S-phase fraction is a marker of more aggressive tumor behavior,³¹ and it has been associated with tumor ER negativity in several studies.³²⁻³⁴ Interestingly, our study also found that the S-phase fraction of the PBC was associated with the ER status of not only the PBC, but also the CBC. However, the association between S-phase fraction and ER status of the CBC was not strong, and because the analyses included only half of the patients enrolled onto the study, it could have been a result of the play of chance given the multiple analyses performed. Future studies are needed to confirm or reject this finding and to clarify the biologic phenomena underlying it.

In conclusion, data from the present study indicate that the hormonal receptor status of the CBC is independent of the receptor status of the PBC in patients who have not received adjuvant tamoxifen therapy. Further studies are needed to confirm these findings. To date, evidence, although not definitive, suggests that patients with ER-negative PBCs may not experience a risk reduction in CBCs when treated with tamoxifen. The explanation for this observation, if true, remains unclear, but our data do not support the idea that CBCs occurring in patients with ER-negative PBCs are more likely to be ER negative.

Acknowledgment

We thank Richard M. Elledge, MD, for review of this manuscript.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group: Tamoxifen for early breast cancer: An overview of the randomised trials. *Lancet* 351: 1451-1467, 1998
2. Fisher B, Anderson S, Tan-Chiu E, et al: Tamoxifen and chemotherapy for axillary node-negative, estrogen receptor-negative breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-23. *J Clin Oncol* 19:931-942, 2001
3. Hutchins L, Green S, Ravdin P, et al: CMF versus CAF +/- tamoxifen in high-risk node-negative breast cancer patients and a natural history follow-up study in low-risk node-negative patients: Update of tamoxifen results. *Breast Cancer Res Treat* 57:25, 1999 (abstr 1)
4. Fisher B, Costantino JP, Wickerham DL, et al: Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 90:1371-1388, 1998
5. McGuire WL, De La Garza M, Chamness GC: Evaluation of estrogen receptor assays in human breast cancer tissue. *Cancer Res* 37:637-639, 1977
6. Powell B, Garola RE, Chamness GC, et al: Measurement of progesterone receptor in human breast cancer biopsies. *Cancer Res* 39: 1678-1682, 1979
7. Dressler LG, Seamer LC, Owens MA, et al: DNA flow cytometry and prognostic factors in 1331 frozen breast cancer specimens. *Cancer* 61:420-427, 1988
8. Clark GM, Dressler LG, Owens MA, et al: Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. *N Engl J Med* 320:627-633, 1989
9. Clark GM, Wenger CR, Beardslee S, et al: How to integrate steroid hormone receptor, flow cytometric, and other prognostic information in regard to primary breast cancer. *Cancer* 71:2157-2162, 1993
10. Cuzick J: Aromatase inhibitors in prevention: Data from the ATAC (arimidex, tamoxifen alone or in combination) trial and the design of IBIS-II (the second International Breast Cancer Intervention Study). *Recent Results Cancer Res* 163:96-103; discussion 264-266, 2003
11. Hutchings O, Evans G, Fallowfield L, et al: Effect of early American results on patients in a tamoxifen prevention trial (IBIS): International Breast Cancer Intervention Study. *Lancet* 352: 1222, 1998
12. Hull DF III, Clark GM, Osborne CK, et al: Multiple estrogen receptor assays in human breast cancer. *Cancer Res* 43:413-416, 1983
13. Tarone RE, Chu KC: The greater impact of menopause on ER- than ER+ breast cancer incidence: A possible explanation (United States). *Cancer Causes Control* 13:7-14, 2002
14. Clark GM, Osborne CK, McGuire WL: Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. *J Clin Oncol* 2:1102-1109, 1984
15. Rutqvist LE, Johansson H, Signomkiao T, et al: Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies: Stockholm Breast Cancer Study Group. *J Natl Cancer Inst* 87:645-651, 1995
16. Fisher B, Costantino J, Redmond C, et al: A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. *N Engl J Med* 320:479-484, 1989
17. Fisher B, Dignam J, Bryant J, et al: Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *J Natl Cancer Inst* 88:1529-1542, 1996
18. Coradini D, Oriana S, Mariani L, et al: Is steroid receptor profile in contralateral breast cancer a marker of independence of the corresponding primary tumour? *Eur J Cancer* 34:825-830, 1998
19. Swain SM, Wilson J, Eleftherios J, et al: Estrogen receptor (ER) status of primary breast cancer is predictive of ER status of contralateral breast cancer (CBC). *J Natl Cancer Inst* 96:516-523, 2004
20. Fisher B, Anderson S, DeCillis A, et al: Further evaluation of intensified and increased total dose of cyclophosphamide for the treatment of primary breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-25. *J Clin Oncol* 17:3374-3388, 1999
21. Fisher B, Anderson S, Wickerham DL, et al: Increased intensification and total dose of cyclophosphamide in a doxorubicin-cyclophosphamide regimen for the treatment of primary breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-22. *J Clin Oncol* 15: 1858-1869, 1997
22. Fisher B, Brown A, Mamounas E, et al: Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* 15:2483-2493, 1997
23. Robson M, Gilewski T, Haas B, et al: BRCA-associated breast cancer in young women. *J Clin Oncol* 16:1642-1649, 1998
24. Lancaster JM, Carney ME, Futreal PA: BRCA 1 and 2: A genetic link to familial breast and ovarian cancer. *Medscape Womens Health* 2:7, 1997
25. Rutqvist LE, Cedermark B, Glas U, et al: Contralateral primary tumors in breast cancer patients in a randomized trial of adjuvant tamoxifen therapy. *J Natl Cancer Inst* 83:1299-1306, 1991
26. Kaas R, Peterse JL, Hart AA, et al: The influence of tamoxifen treatment on the oestrogen receptor in metachronous contralateral breast cancer. *Br J Cancer* 88:707-710, 2003
27. Li CI, Malone KE, Weiss NS, et al: Tamoxifen therapy for primary breast cancer and risk of contralateral breast cancer. *J Natl Cancer Inst* 93:1008-1013, 2001
28. Bachleitner-Hofmann T, Pichler-Gebhard B, Rudas M, et al: Pattern of hormone receptor status of secondary contralateral breast cancers in patients receiving adjuvant tamoxifen. *Clin Cancer Res* 8:3427-3432, 2002
29. Osborne CK, Bardou V, Hopp TA, et al: Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J Natl Cancer Inst* 95:353-361, 2003
30. Shou J, Massarweh S, Osborne CK, et al: Mechanisms of tamoxifen resistance: Increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst* 96:926-935, 2004
31. Chang J, Clark GM, Allred DC, et al: Survival of patients with metastatic breast carcinoma: Importance of prognostic markers of the primary tumor. *Cancer* 97:545-553, 2003
32. Wenger CR, Beardslee S, Owens MA, et al: DNA ploidy, S-phase, and steroid receptors in more than 127,000 breast cancer patients. *Breast Cancer Res Treat* 28:9-20, 1993
33. Wenger CR, Clark GM: S-phase fraction and breast cancer: A decade of experience. *Breast Cancer Res Treat* 51:255-265, 1998
34. Hedley DW, Clark GM, Cornelisse CJ, et al: Consensus review of the clinical utility of DNA cytometry in carcinoma of the breast: Report of the DNA Cytometry Consensus Conference. *Cytometry* 14:482-485, 1993