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Article

Mammographic Density Decline, Tamoxifen Response, and Prognosis by Molecular Characteristics of Estrogen Receptor–Positive Breast Cancer

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Abstract

Background: Mammographic breast density (MBD) decline post-tamoxifen initiation is a favorable prognostic factor in estrogen receptor (ER)–positive breast cancer (BC) and has potential utility as a biomarker of tamoxifen response. However, the prognostic value of MBD decline may vary by molecular characteristics among ER–positive patients. **Methods:** We investigated associations between MBD decline (≥10% vs <10%) and breast cancer–specific mortality (BCSM) among ER–positive breast cancer patients aged 36-87 years at diagnosis treated with tamoxifen at Kaiser Permanente Northwest (1990-2008). Patients who died of BC (case patients; n = 62) were compared with those who did not (control patients; n = 215) overall and by tumor molecular characteristics (immunohistochemistry [IHC]–based subtype [luminal A–like: ER–positive/progesterone receptor [PR]–positive/HER2–negative/low Ki67; luminal B–like: ER–positive and 1 or more of PR–negative, HER2–positive, high Ki67] and modified IHC [mIHC]–based recurrence score of ER/PR/Ki67). Percent MBD was measured in the unaffected breast at baseline mammogram (mean = 6 months before tamoxifen initiation) and follow-up (mean = 12 months post-tamoxifen initiation). Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were computed from logistic regression models. All statistical tests were 2-sided. **Results:** MBD decline was statistically significantly associated with reduced risk of BCSM overall (OR = 0.38, 95% CI = 0.15 to 0.92). This association was, however, stronger among women with aggressive tumor characteristics including luminal B–like (OR = 0.17, 95% CI = 0.04 to 0.73) vs A–like (OR = 0.74, 95% CI = 0.19 to 2.92); large (OR = 0.26, 95% CI = 0.08 to 0.78) vs small (OR = 0.41, 95% CI = 0.04 to 3.79) tumors; PR–negative (OR = 0.02, 95% CI = 0.001 to 0.37) vs PR–positive (OR = 0.50, 95% CI = 0.18 to 1.40) disease; and high (OR = 0.25, 95% CI = 0.07 to 0.93) vs low (OR = 0.44, 95% CI = 0.10 to 2.09) mIHC3 score. **Conclusion:** The findings support MBD decline as a prognostic marker of tamoxifen response among patients with aggressive ER–positive BC phenotypes, for whom understanding treatment effectiveness is critical.

Decline in mammographic breast density (MBD) following tamoxifen initiation is an independent prognostic marker in hormone receptor–positive (luminal) breast cancer (1-4). Patients who experience large (≥10%) reductions in MBD following tamoxifen initiation tend to have better clinical outcomes than those who do not (5). Accordingly, MBD decline has been proposed as a dynamic biomarker for monitoring treatment response in luminal breast cancer patients (6).

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Although endocrine therapy is the mainstay treatment for luminal breast cancer (7,8), residual molecular and clinical heterogeneity persist within each subtype (9–12). Incorporating MBD decline in clinical decision making will, therefore, require a better understanding of its relationship with prognosis within the context of other clinically and therapeutically relevant tumor characteristics. In a previous analysis within this population (4), we showed reductions in MBD following tamoxifen treatment to be associated with lower risk of breast cancer–specific death among estrogen receptor (ER)–positive breast cancer patients. For the current analysis, we retrieved archival diagnostic tissue blocks for these patients and conducted molecular assays to evaluate associations between MBD decline, as a biomarker of tamoxifen response, and breast cancer–specific mortality (BCSM) according to clinically relevant tumor molecular characteristics.

Methods

Study Population

The study population is comprised of a subset of women with breast cancer (see Figure 1; n = 277) who were included in a case-control study (4) sampled from a retrospective cohort of breast cancer patients within the Kaiser Permanente Northwest (KPNW) integrated health plan (Portland, OR, USA) (13). Cohort members were women who were diagnosed with ER–positive breast cancer and treated with adjuvant tamoxifen therapy (1990-2008) and followed through December 31, 2010. Women with distant metastasis (stage IV) were excluded. Case patients were women who died of breast cancer during follow-up. Information on vital status was obtained from the KPNW tumor registry. Two control patients (women who had not died from breast cancer during follow-up) were matched to each case patient on diagnosis age (50 years or younger, 51-60 years, 61-70 years, 70 years or older), tumor stage (localized, regional spread), and diagnosis year (1990-1993, 1994-1998, 1999-2002, 2003-2008) and were required to have at least as much follow-up time as their matched case patient. Patient characteristics, including diagnosis age, body mass index (BMI) at diagnosis, race, smoking status, tumor stage, and calendar year of diagnosis, were obtained from medical records (4). Treatment and prescription records, including tamoxifen therapy duration, were obtained from KPNW databases. The current analysis included data on women for whom we were able to retrieve archival diagnostic formalin-fixed paraffin-embedded (FFPE) tumor blocks for molecular analysis (62 of the 97 [64%] case patients and 215 of the 281 [76%] control patients, regardless of their matching status). Although the frequency of BCSM tended to be higher among those with unavailable (n = 35 of 101 [35%]) vs available (n = 62 of 277 [22%]) tissue blocks, the distribution of MBD change, our main exposure of interest, and subtype distribution did not differ between the 2 groups. This study was approved by the institutional review boards of the National Institutes of Health and KPNW.

Assessment of MBD

Mammograms were collected at baseline and follow-up as previously described (4). As shown in Figure 1, baseline (mean = 6 months prior to tamoxifen initiation) and follow-up (mean = 12 months post-tamoxifen initiation) craniocaudal mammographic films from the contralateral breast were obtained. All mammograms were digitized using an Array Corporation 2095 Laser Film Digitizer (Roden, the Netherlands; optical density = 4.0). Assessment of MBD, including absolute dense area (cm^2) and total breast area (cm^2), was performed by a single expert reader (EAB) using Cumulus software (14). Percent MBD was calculated by dividing absolute dense area (cm^2) by total tissue area (cm^2) and multiplying by 100. As reported previously (4), reevaluation of 50 randomly selected films yielded intraclass correlation coefficients and coefficients of variation of 0.95 and 8.5% for dense area, 0.99 and 0.5% for total breast area, and 0.96 and 8.5% for percent density. Percent MBD change was estimated by subtracting baseline MBD from follow-up MBD (4).

Assessment of Tumor Molecular Characteristics

Information on tumor characteristics, including progesterone receptor (PR) status (negative or positive), HER2 status (negative or positive), tumor stage (localized, regional spread), tumor size (<2 or ≥2 cm), and histologic grade (1 = low, 2 = intermediate, and 3 = high) was obtained from medical records. Tissue microarrays (TMAs) were constructed from diagnostic FFPE blocks. Two TMAs with 2 cores on each TMA were constructed from the same FFPE block per patient. Immunohistochemistry (IHC) staining of TMAs was performed using standard protocols. ER and PR staining used Dako M7047 and M3569 antibodies at 1:40 and 1:500 concentrations, respectively; antigen retrieval used Tris-EDTA pH 9.0 (Dako S2367). Ki67 staining used Abcam ab16667 at 1:200 dilution. HER2 staining was conducted using the Dako K5204 kit (HercepTest kit, Agilent). Digitized TMA sections for ER, PR, HER2, and Ki67 were visually scored by an expert with semiquantitative (0-10) percent positive staining cells as follows: 0 = 0%, 1 = 1%-10%, 2 = 11%-20%, 3 = 21%-30%, 4 = 31%-40%, and so on, and qualitative intensity scores (0 = none, 1 = weak, 2 = moderate, or 3 = strong). Average scores for each individual marker across all available cores on duplicate TMAs were calculated. HER2 data were obtained from clinical records, as follows: HER2–negative (IHC staining 0, 1+, and 2+ with no amplification on fluorescent in situ hybridization) and HER2–positive (3+ on IHC staining or fluorescent in situ hybridization amplification for IHC 2+).

Breast cancer subtypes were defined based on the St Gallen criteria (15) by using clinically determined ER, PR, and HER2 data (ie, obtained from medical records), in conjunction with Ki67 IHC data obtained from TMAs as follows: luminal A–like (ER–positive and PR–positive and HER2–negative) and luminal B–like (ER–positive and 1 or more of the following: PR–negative [PR–negative], HER2–positive, high Ki67, or high histologic grade, ie, grade 2 or 3). High and low Ki67 categories were defined based on the recommended cut point of 20% positively staining malignant cells (15). In addition to molecular subtype, we leveraged the TMA-based semiquantitative IHC data to define IHC3 and IHC4 scores which are IHC-surrogate recurrence scores that combine data on ER, PR, and Ki67 (IHC3) and ER, PR, Ki67, and HER2 (IHC4) using published equations by Cuzick et al. (16):

$$IHC3\ score = 93.1 \times (-0.086 \times \mathrm{ER10} - 0.081 \times \mathrm{PR10} + 0.281 \times \ln(1 + 10 \times \mathrm{Ki67})).$$

$$IHC4\ score = 94.7 \times \{(-0.100 \times \mathrm{ER10}) + (-0.079 \times \mathrm{PR10}) + (0.586 \times \mathrm{HER2}) + [0.240 \times \ln(1 + 10 \times \mathrm{Ki67})]\}$$

As described above, our semiquantitative scores for ER and PR had a range from 0 to 10, which we used as surrogates for the ER10 and PR10 variables, respectively. In addition, we used

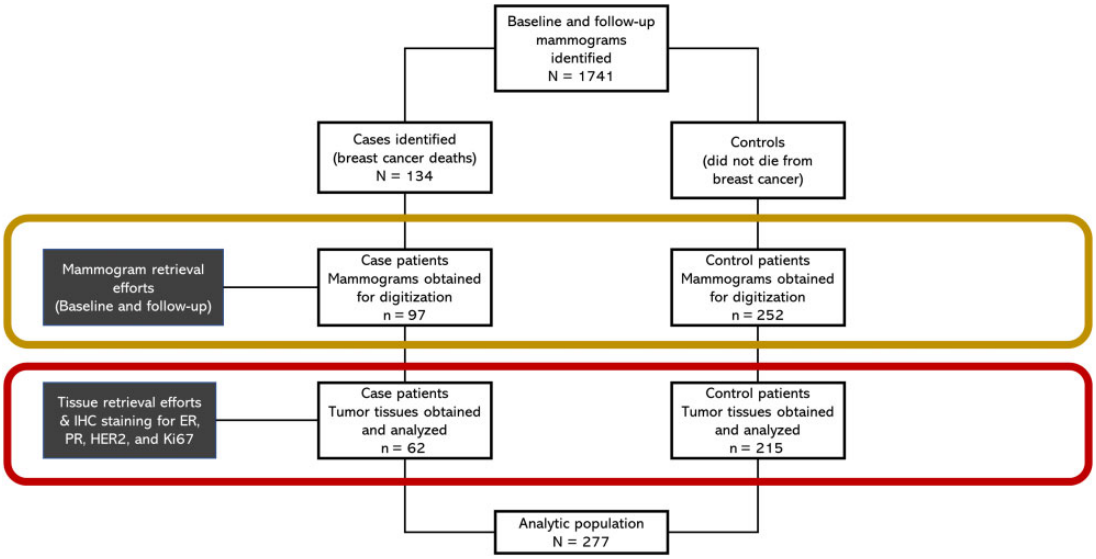

Figure 1. Sampling scheme for study population. Women with estrogen receptor (ER)–positive breast cancer who died of breast cancer (ie, case patients) and those who were alive or died of other causes during the follow-up period (ie, control patients) were selected as part of a previous case-control study that recruited participants with ER–positive breast cancer from the Kaiser Permanente Northwest health-care plan. Case patients (n = 97) and control patients (n = 252) in the original case-control study were matched on age at diagnosis, stage at diagnosis, and year of diagnosis, and film mammograms were retrieved. The current analysis is comprised of 62 case patients and 215 control patients (ignoring the matching) from the previous case-control study with baseline and follow-up mammograms for whom we successfully retrieved archival diagnostic tumor tissues and conducted molecular assays. IHC = immunohistochemistry; PR = progesterone receptor.

semiquantitative (0-10) scores of Ki67 as opposed to the quantitative scores that were used by Cuzick et al (16). As a result, we refer to both scores as "modified" (m) IHC3 and IHC4 score throughout this article.

Statistical Analysis

Participants' ages were categorized as 50 years or younger, 51-60 years, and 60 years and older. Differences in distributions of baseline patient and tumor characteristics between case patients and control patients, or by calendar year of diagnosis, were assessed using χ^2 test (categorical variables) and Kruskal-Wallis test (continuous variables).

Associations between tumor molecular characteristics and MBD change were assessed in logistic regression models fitted to control patients, with tumor characteristics as predictors and MBD decline (≥10% vs <10%) as the outcome. The 10% cut point for MBD decline was selected based on previous publications demonstrating consistent associations with improved breast cancer outcomes at this threshold (6). Partially adjusted models included age (50 years or younger, 51-60 years, 60 years and older), tumor stage (localized, regional spread), and diagnosis year (1990-1996, 1997-2000, 2001-2008) [all matching factors in the original study (4)]. The fully adjusted primary model additionally included BMI, histologic grade, tumor size, nodal status, PR, HER2, Ki67, and baseline MBD. In separate secondary models, PR, Ki67, and HER2 were substituted with tumor subtype (luminal B–like vs A–like) and mIHC3 score (above median vs no more than median).

Associations between tumor characteristics and BCSM were assessed in unconditional logistic regression models. Partially adjusted models included diagnosis age, tumor stage, diagnosis year, and tamoxifen duration. The fully adjusted model additionally included PR, Ki67, HER2, grade, size, nodal involvement, BMI, and follow-up duration in years. Stage was excluded from models adjusted for tumor size and nodal status. In secondary models, PR, HER2, and Ki67 were substituted for subtype and then for mIHC3 score.

Associations between MBD decline (≥10% vs <10%) and BCSM were assessed overall and in analyses stratified by individual tumor molecular characteristics that were statistically significantly associated with BCSM (at a $P < .05$). Multivariable models were adjusted for diagnosis age, tumor stage, diagnosis year, tamoxifen duration, baseline MBD, and follow-up duration. We included interaction terms to test for heterogeneity in associations between MBD decline and BCSM by tumor characteristics. Missing covariate values were addressed using the multiple imputation plus outcome approach (17,18), performing 5 imputations. Imputed datasets were analyzed individually, and results were combined using Rubin rules (19). In sensitivity analyses, model parameters were similar for imputed HER2 as for unimputed HER2. Both mIHC3 and mIHC4 score parameters demonstrated statistically significant prognostic associations among individuals with and without complete data to compute both measures. Given that mIHC3 score was available for most patients, analyses were based on mIHC3 rather than mIHC4 score. Statistical tests were 2-sided, and analyses were performed using Stata statistical software version 16.1 (StataCorp, College Station, TX, USA).

Results

Distribution of Baseline Patient and Tumor Molecular Characteristics Overall and by Case-Control Status

Characteristics of the 62 ER–positive patients who died of breast cancer (case patients) and 215 ER–positive patients who did not (control patients) are shown in Table 1. The average (median [standard deviation]) age at diagnosis was 60 (62 [11.3]) years and 57 (56 [11.1]) years among case patients and control patients, respectively. In general, case patients and control patients were similar with respect to diagnosis age, tumor

Table 1. Distribution of baseline patient and tumor clinicopathological characteristics among women with estrogen receptor–positive breast cancer treated with tamoxifen who died (case patients) and did not die (control patients) from breast cancer at Kaiser Permanente Northwest (Portland, OR, USA)[a]

Characteristics	Case patients (n = 62) No. (%)	Control patients (n = 215) No. (%)	*P*
Age at diagnosis, y			
≤50	15 (24.2)	60 (27.9)	
51-60	9 (14.5)	58 (27.0)	
>60	38 (61.3)	97 (45.1)	.05
Median follow-up time, y	6.0	6.0	.77
Year of diagnosis			
1990-1996	29 (46.8)	80 (37.2)	
1997-2000	20 (32.2)	63 (29.3)	
2001-2008	13 (21.0)	72 (33.5)	.16
Race			
Non-White	1 (1.6)	4 (1.9)	.89
White	61 (98.4)	210 (98.1)	
Missing	0 (0.0)	1 (0.5)	
BMI at baseline, kg/m^2			
<25	13 (23.6)	67 (34.5)	
25 to <30	19 (34.6)	66 (34.0)	
≥30	23 (41.8)	61 (31.5)	
Missing	7 (11.3)	21 (9.8)	.23
Baseline MBD, %, tertiles			
T1 (0.8-20)	26 (41.9)	67 (31.2)	
T2 (20-35)	20 (32.3)	72 (33.5)	
T3 (>35)	16 (25.8)	76 (35.3)	.22
Stage			
Localized	28 (45.2)	80 (37.2)	
Regional, distant, unknown	34 (54.8)	135 (62.8)	.26
Histologic grade			
Low	1 (2.0)	50 (26.6)	
Intermediate	33 (64.7)	99 (52.7)	
High	17 (33.3)	39 (20.7)	
Missing	11 (17.7)	27 (12.6)	.001
Tumor size, cm			
<2	15 (25.4)	89 (41.8)	
≥2	44 (74.6)	124 (58.2)	
Missing	3 (4.8)	2 (0.9)	.02
Nodal status			
Negative	27 (45.0)	76 (36.0)	
Positive	33 (55.0)	135 (64.0)	
Missing	2 (3.2)	4 (1.9)	.21
PR			
Negative	19 (30.7)	32 (14.9)	
Positive	43 (69.3)	183 (85.1)	.005
HER2			
Negative	24 (64.9)	90 (81.1)	
Positive	13 (35.1)	21 (18.9)	
Missing	25 (40.3)	104 (48.4)	.04
Ki67			
Low	36 (59.0)	159 (75.7)	
High	25 (41.0)	51 (24.3)	
Missing	1 (1.6)	5 (2.3)	.01
Subtype			
Luminal A–like	25 (41.0)	142 (67.0)	
Luminal B–like	36 (59.0)	70 (33.0)	
Missing	1 (1.6)	3 (1.4)	<.001
mIHC3 score			
Low (≤41.2)	21 (35.0)	116 (55.5)	
High (>41.2)	39 (65.0)	93 (44.5)	

(continued)

Table 1. (continued)

Characteristics	Case patients (n = 62) No. (%)	Control patients (n = 215) No. (%)	P
Missing	2 (3.2)	6 (2.8)	.005
Surgery type			
No surgery	1 (1.6)	0 (0.0)	
Lumpectomy, excision, partial mastectomy	27 (43.6)	111 (51.6)	
Mastectomy (no removal of contralateral breast)	34 (54.8)	104 (48.4)	.10
Received radiotherapy			
No	28 (45.2)	77 (35.8)	
Yes	34 (54.8)	138 (64.2)	.18
Received chemotherapy			
No	12 (19.4)	53 (24.7)	
Yes	50 (80.6)	162 (75.3)	.39
Duration of tamoxifen, mo, tertiles			
T1 (0-42)	29 (46.8)	64 (29.8)	
T2 (42-58)	21 (33.9)	71 (33.0)	
T3 (>58)	12 (19.3)	80 (37.2)	.01

[a]P values were determined using χ^2 (for categorical) and Kruskal-Wallis (for continuous) tests. Categories (low and high) of mIHC3 score were defined using the median of the distribution in this population. BMI = body mass index; mIHC3 = modified immunohistochemical 3; MBD = mammographic density. For covariates with missing values, the missing categories were excluded from the sum, percentages, and corresponding tests for case-control differences reported in the table.

stage, and diagnosis year (Table 1). The frequencies of poor prognostic tumor characteristics were higher among case patients than control patients (Table 1) and among younger than older patients (Supplementary Table 1, available online). In particular, case patients had higher frequencies of high grade, PR–negative, and HER2–positive tumors than control patients. Among case patients, tumors were more frequently highly proliferative, high mIHC3 score, and to be of the luminal B–like phenotype. The distributions of BMI and baseline MBD did not differ between case patients and control patients (Table 1).

Associations Between Tumor Molecular Characteristics and Mammographic Density Change

There was an average of 18 months between baseline and follow-up mammograms and 12 months between tamoxifen initiation and follow-up mammogram; distributions of both were similar between case patients and control patients. The average MBD decline was 4.9% overall, and this decline was slightly greater among controls (5.4%) than case patients (2.9%) (Supplementary Table 2, available online). Compared with PR–negative patients, those with PR–positive tumors were statistically significantly less likely to experience MBD decline. However, no statistically significant associations were observed between MBD decline and other tumor molecular characteristics, including grade, tumor size, nodal status, Ki67, mIHC3 score, or molecular tumor subtype (Table 2).

Associations Between Tumor Molecular Characteristics and Breast Cancer-Specific Mortality

Luminal B–like subtype was associated with statistically significantly worse BCSM as compared with luminal A–like subtype (odds ratio [OR]$_{\text{luminal B-like vs A-like}}$ = 3.36, 95% confidence interval [CI] = 1.67 to 6.75) (Table 3). Furthermore, larger tumors (OR$_{2\text{ vs }<2\text{ cm}}$ = 3.03, 95% CI = 1.40 to 6.59), PR–negative disease (OR$_{\text{PR–negative vs PR–positive}}$ = 2.34, 95% CI = 1.11 to 4.95), and high Ki67 (OR$_{\text{high vs low}}$ = 2.46, 95% CI = 1.13 to 5.33) were associated with worse BCSM. Compared with patients with low mIHC3 score, those with high mIHC3 score had worse BCSM (OR$_{\text{high vs low}}$ = 2.75, 95% CI = 1.32 to 5.75).

Mammographic Density Decline in Relation to BCSM by Tumor Molecular Characteristics

Overall, patients who experienced reductions in MBD (ie, ≥10% decline) following tamoxifen initiation were statistically significantly less likely to die from breast cancer than those who did not (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.38, 95% CI = 0.15 to 0.92) (Table 4). In analyses stratified by tumor characteristics, MBD decline was strongly associated with lower risk of BCSM in women with luminal B–like (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.17, 95% CI = 0.04 to 0.73) but not luminal A–like (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.74, 95% CI = 0.19 to 2.92) disease (P_{het} = .63). In general, patients with luminal B–like disease who experienced tamoxifen-related reduction in MBD had equivalent BCSM to those with luminal A–like disease (OR = 0.85, 95% CI = 0.22 to 3.29; P = .82), whereas those who did not experience MBD reduction had a statistically significantly worse prognosis (OR = 3.33, 95% CI = 1.60 to 6.89; P = .001). MBD decline was more strongly associated with survival in patients with large (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.26, 95% CI = 0.08 to 0.78) than small (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.41, 95% CI = 0.04 to 3.79) tumors (P_{het} = .53); PR–negative (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.02, 95% CI = 0.001 to 0.37) than PR–positive (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.50, 95% CI = 0.18 to 1.40) disease (P_{het} = .30); and high (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.25, 95% CI = 0.07 to 0.93) than low (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.44, 95% CI = 0.10 to 2.09) mIHC3 score (P_{het} = .89). Similarly, for women with complete information on mIHC4, MBD decline was more strongly associated with lower risk of BCSM among those with high (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.24, 95% CI = 0.04 to 1.43) than low (OR$_{\geq 10\%\text{ vs }<10\%}$ = 0.63, 95% CI = 0.05 to 8.08) mIHC4 (P_{het} = .48). Results were similar following sensitivity analyses adjusting for chemotherapy.

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between patient and tumor characteristics and absolute change (<10% vs ≥10%) in percent mammographic breast density (MBD) among n = 215 ER–positive patients treated with tamoxifen who did not die from breast cancer (control patients)

	Logistic regression of tumor characteristics in relation to MBD decline (<10% vs ≥10%) following tamoxifen therapy			
	Partially adjusted[a]		Multivariable adjusted[b]	
Characteristics	OR (95% CI)	*P*	OR (95% CI)	*P*
Age at diagnosis, y				
<50	1.00 (referent)		1.00 (referent)	
50-60	0.63 (0.30 to 1.35)	.24	0.56 (0.22 to 1.41)	.22
>60	0.22 (0.10 to 0.47)	<.001	0.28 (0.10 to 0.73)	.01
BMI				
Normal	1.00 (referent)		1.00 (referent)	
Overweight	0.59 (0.27 to 1.31)	.19	0.74 (0.40 to 2.36)	.95
Obese	0.30 (0.13 to 0.71)	.006	0.94 (0.34 to 2.58)	.91
Histologic grade				
Low and intermediate	1.00 (referent)		1.00 (referent)	
High	0.94 (0.42 to 2.08)	.88	0.78 (0.26 to 2.37)	.66
Tumor size, cm				
<2	1.00 (referent)		1.00 (referent)	
≥2	1.54 (0.79 to 3.00)	.21	1.27 (0.58 to 2.77)	.55
Nodal status				
Negative	1.00 (referent)		1.00 (referent)	
Positive	0.66 (0.35 to 1.27)	.21	0.77 (0.35 to 1.73)	.53
PR				
Negative	1.00 (referent)		1.00 (referent)	
Positive	0.29 (0.12 to 0.68)	.005	0.21 (0.07 to 0.62)	.005
HER2				
Negative	1.00 (referent)		1.00 (referent)	
Positive	0.61 (0.20 to 1.84)	.37	1.29 (0.19 to 8.93)	.77
Ki67				
Low	1.00 (referent)		1.00 (referent)	
High	0.82 (0.48 to 2.01)	.96	0.72 (0.29 to 1.80)	.48
Subtype				
Luminal A–like	1.00 (referent)		1.00 (referent)	
Luminal B–like	1.75 (0.92 to 3.35)	.09	2.08 (0.90 to 4.77)	.08
mIHC3 score				
Low	1.00 (referent)		1.00 (referent)	
High	1.03 (0.54 to 1.94)	.93	0.92 (0.40 to 2.15)	.85
Baseline MBD, %, tertiles				
T1 (0.8-20)	1.00 (referent)		1.00 (referent)	
T2 (20-35)	9.11 (1.94 to 42.52)	.005	9.69 (1.89 to 49.90)	.007
T3 (>35)	33.30 (7.47 to 148.37)	<.001	37.01 (7.28 to 188.67)	<.001

[a]Partially adjusted models were adjusted for matching factors (age at diagnosis, stage, and year at diagnosis). Stage was omitted in models for tumor size and nodal status because these are contained within the stage variable. ER = estrogen receptor; mIHC3 = modified immunohistochemical 3; PR = progesterone receptor.
[b]Fully adjusted multivariable models were mutually adjusted for tumor characteristics as well as age, baseline MBD and body mass index (BMI). The primary multivariable model comprised of PR, HER2, Ki67, grade, tumor size, nodal status, age, BMI, and baseline MBD. In secondary models, PR, HER2, and Ki67 were replaced by subtype and then by mIHC3 score. Estimates and corresponding *P* values were obtained from logistic regression models. All statistical tests were 2-sided.

Discussion

We investigated the prognostic value of MBD decline by tumor characteristics, including molecular subtypes, among ER–positive breast cancer patients who were treated with tamoxifen. Overall, MBD decline was associated with reduced risk of BCSM in luminal patients, as we have previously reported (4,20). Herein, we extended prior findings by conducting molecular assays and showing for the first time that the prognostic value of MBD decline was most apparent in women with more aggressive ER–positive phenotypes, including luminal B–like disease, PR–negative, larger tumor size, and high mIHC3 score, all of which portended worse BCSM. Accordingly, MBD decline following tamoxifen initiation may be most useful as a biomarker of response among ER–positive patients with more aggressive disease, for whom understanding treatment effectiveness is critical.

For the majority of luminal B–like patients, endocrine therapy failure remains a major cause of fatal relapse, with the greatest relapse risk during the first 5 years postdiagnosis (21). Identification of luminal B–like patients who are most likely to suffer relapse because of poor endocrine therapy response remains an important clinical challenge. Compared with the luminal A–like subtype, luminal B–like tumors tend to be less sensitive to endocrine therapy and are typically considered for adjuvant chemotherapy plus endocrine therapy (15,22). We, and others, have previously shown that most of the tamoxifen-related reduction in MBD occurs within approximately 12 months of starting tamoxifen (5,23,24). Thus, an important implication of the present report is that not only may MBD decline be used to

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between tumor clinicopathological characteristics and breast cancer–specific mortality among ER–positive patients treated with tamoxifen (n = 62 BCSM case patients, 215 control patients)

Tumor characteristic	No. Control patients/Case patients	Partially adjusted[a]		Multivariable[b]	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Subtype					
Luminal A–like	142/25	1.00 (referent)		1.00 (referent)	
Luminal B–like	70/36	2.67 (1.40 to 5.08)	.003	3.36 (1.67 to 6.75)	.001
Histologic grade					
Low/intermediate	149/34	1.00 (referent)		1.00 (referent)	
High	39/17	1.90 (0.89 to 4.03)	.09	0.96 (0.40 to 2.29)	.92
Tumor size, cm					
<2	89/15	1.00 (referent)		1.00 (referent)	
≥2	124/44	2.49 (1.23 to 5.06)	.01	3.03 (1.40 to 6.59)	.005
Nodal status					
Negative	76/27	1.00 (referent)		1.00 (referent)	
Positive	135/33	0.75 (0.40 to 1.41)	.37	0.48 (0.23 to 0.97)	.046
PR					
Positive	32/19	1.00 (referent)		1.00 (referent)	
Negative	183/43	2.21 (1.09 to 4.51)	.02	2.34 (1.11 to 4.95)	.02
HER2					
Negative	90/24	1.00 (referent)		1.00 (referent)	
Positive	21/13	1.75 (0.64 to 4.82)	.28	1.77 (0.59 to 5.28)	.29
Ki67					
Low	159/36	1.00 (referent)		1.00 (referent)	
High	51/25	2.26 (1.16 to 4.42)	.01	2.46 (1.13 to 5.33)	.02
mIHC3 score					
Low	116/21	1.00 (referent)		1.00 (referent)	
High	93/39	2.64 (1.36 to 5.11)	.004	2.75 (1.32 to 5.75)	.007

[a]Unconditional logistic regression models were adjusted for age at diagnosis, stage, year of diagnosis, duration of tamoxifen, and follow-up. Stage was omitted in models for tumor size and nodal status because these are contained within the stage variable. BCSM = breast cancer–specific mortality; ER = estrogen receptor; mIHC3 = modified immunohistochemical 3; PR = progesterone receptor.

[b]Unconditional logistic regression models were mutually adjusted for tumor characteristics in addition to age at diagnosis, stage, year of diagnosis (stage was omitted to allow for tumor size and nodal status to be included in the model), duration of tamoxifen, and follow-up time. The primary model consisted of PR, HER2, Ki67, histologic grade, tumor size, nodal status, duration of tamoxifen, and follow-up duration. In secondary models, PR, HER2, and Ki67 were first replaced with subtype and then with mIHC3 score. Missing variables on covariates were addressed through multiple imputation. All statistical tests were 2-sided.

Table 4. Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between mammographic density decline and breast cancer–specific mortality (BCSM) among ER–positive patients treated with tamoxifen, overall and by tumor clinicopathological characteristics[a]

Characteristic	No. <10%/≥10%	Mammographic density decline (≥10% vs <10%)		P_{het}
		OR (95% CI)	*P*	
Overall	202/75	0.38 (0.15 to 0.92)	.03	
Subtype				
Luminal A–like	124/43	0.74 (0.19 to 2.92)	.67	
Luminal B–like	74/32	0.17 (0.04 to 0.73)	.01	.63
Tumor size, cm				
<2	81/23	0.41 (0.04 to 3.79)	.43	
≥2	117/51	0.26 (0.08 to 0.78)	.02	.53
PR				
Positive	168/58	0.50 (0.18 to 1.40)	.19	
Negative	34/17	0.02 (0.001 to 0.37)	.009	.30
Ki67				
Low	141/54	0.41 (0.13 to 1.36)	.15	
High	55/21	0.36 (0.06 to 2.18)	.27	.55
mIHC3 score				
Low	99/38	0.44 (0.10 to 2.09)	.31	
High	96/36	0.25 (0.07 to 0.93)	.03	.89

[a]All models were adjusted for age at diagnosis, body mass index, baseline mammographic density, tamoxifen duration, stage at diagnosis, diagnosis year, and follow-up time. P_{het} (*P* values for heterogeneity) were obtained by including multiplicative interaction terms between mammographic density decline categories (≥10% vs <10%) and relevant tumor characteristic in full models. All statistical tests were 2-sided. ER = estrogen receptor; mIHC3 score = modified immunohistochemical 3 ; PR = progesterone receptor.

monitor tamoxifen response but it could also have utility as an early indicator of patients with luminal B–like disease at heightened risk of relapse. Conceivably, patients who are identified as responding to tamoxifen through MBD decline could be encouraged to adhere to therapy, whereas those potentially at higher relapse risk because of lack of MBD decline could be monitored more closely or offered additional treatment options.

A strength of the current analysis is inclusion of multiple prognostic indicators, including ER, PR, HER2, Ki67, grade, size, and mIHC3 score, to contextualize the value of MBD decline as a prognostic biomarker for tamoxifen response. In addition to subtype-related differences, we found the association between MBD decline and BCSM to be more apparent among patients with poor prognostic tumor characteristics, including larger, PR–negative tumors, and those with high mIHC3 score. Although patients with luminal B–like breast cancer tend to have a worse prognosis than those with luminal A–like disease, our findings further indicated a disproportionately worse prognosis among patients with luminal B–like disease who did not, but not those who did, experience MBD decline on tamoxifen. These observations support prognostic heterogeneity among patients with luminal breast cancer according to the degree of MBD decline on tamoxifen. In light of accumulating evidence to support the role of the tumor microenvironment in breast cancer progression (25–27), it is conceivable that response to tamoxifen, reflected by density decline, may be influenced by tumor microenvironment features that may differ within, and between, luminal breast cancer subtypes (27). Further studies are required to provide mechanistic insights.

Despite the small sample size, this study was conducted within a retrospective cohort of breast cancer patients from a general community health-care plan, which facilitated linkage of electronic health and prescription records with serial mammograms and archival tissues. As a result, we were able to assemble data on several IHC and tumor clinicopathological characteristics and to define luminal breast cancer phenotypes according to published recommendations. We also measured semiquantitative expression of ER, PR, and Ki67 and defined mIHC3 score for most patients. About half of our study population lacked data on clinical HER2 status because their diagnosis occurred prior to the approval of trastuzumab for treating HER2–positive patients. Owing to weak concordance between clinical and TMA-based HER2, we computed the modified versions of IHC3 score, which does not require HER2, for all patients and IHC4 score for those with complete HER2 data.

The relatively small sample size may have precluded our ability to make definitive conclusions regarding heterogeneity of associations between MBD decline and BCSM by tumor characteristics. We were also unable to assess associations between MBD decline and recurrence because of lack of data on this endpoint. Although we lacked data on menopausal status, this study population comprised of patients aged 36-87 years, approximately 42% of whom were aged younger than 55 years at diagnosis. Given the higher prevalence of aggressive tumor characteristics among younger patients observed in this and other patient populations (28,29), our data suggest the importance of evaluating MBD decline as a biomarker of tamoxifen response among younger patients, for whom tamoxifen has emerged as the mainstay endocrine treatment. Owing to the sampling design and temporal changes in treatment strategies, this study was unable to evaluate the impact of aromatase inhibitors, transition from tamoxifen to an aromatase inhibitor, HER2-targeted therapy, or ovarian suppression therapy. Accordingly, additional studies of the impact of treatment-related factors on the prognostic value of MBD change for breast cancer subtypes defined by gene expression profiling data will be important. Additional studies in racially and ethnically diverse populations are also needed.

In conclusion, we found MBD decline to be more strongly associated with lower BCSM among women with luminal B–like than A–like breast cancer. In addition, the prognostic effect of MBD decline was most apparent among patients with more aggressive ER–positive phenotypes. Findings suggest that MBD decline may be most useful as a biosensor of tamoxifen effectiveness among women with luminal B–like breast cancer, a relatively more aggressive phenotype of ER–positive breast cancer that is characterized by endocrine therapy insensitivity and early relapse.

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Data Availability

The data that support these findings are not publicly available because they contain information that could compromise research participant privacy and confidentiality. The authors will make the data available upon reasonable request and with the permission of the Kaiser Permanente Center for Health Research in Portland, Oregon.

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