Association between Mammographic Breast Density and Breast Cancer Tumor Characteristics

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Abstract

Objectives: **Few studies have examined the association between breast density and breast cancer tumor characteristics. We examined the association between hormonal, proliferative, and histologic tumor characteristics and mammographic breast density in women with breast cancer.**

Methods: **We conducted a cross-sectional analysis in 546 women diagnosed with invasive breast cancer to evaluate the associations between breast density and tumor size, lymph node status, lymphatic or vascular invasion, histologic grade, nuclear grade, tumor differentiation, mitotic index, tumor necrosis, Ki-67 proliferation, estrogen receptor, progesterone receptor, p53, p27, cyclin E, Bcl-2, and C-erb-B2 invasion. Breast density was classified as fatty (Breast Imaging Reporting and Data System code 1 or 2; $n = 373$) or dense (Breast Imaging Reporting and Data System code 3 or 4; $n = 173$) for the cancer-free breast. A single pathologist measured all tumor markers. We examined whether the relationships were modified by interval cancer or screen-detected cancer.**

Results: **Women with a tumor size >1.0 cm were more likely to have dense breasts compared with women with a tumor size ≤1.0 cm after adjusting for confounders (odds ratio, 2.0; 95% confidence interval, 1.2-3.4 for tumor sizes 1.1-2.0 cm; odds ratio, 2.3; 95% confidence interval, 1.3-4.4 for tumor sizes 2.1-10 cm). Tumor size, lymph node status, and lymphatic or vascular invasion were positively associated with breast density among screen-detected cancers. Histologic grade and mitotic index were negatively associated with breast density in women diagnosed with an interval cancer.**

Conclusions: **These results suggest that breast density is related to tumor size, lymph node status, and lymphatic or vascular invasion in screen-detected cancers. Additional studies are needed to address whether these associations are due to density masking the detection of some tumors, a biological relationship, or both.** (Cancer Epidemiol Biomarkers Prev 2005;14(3):662–8)

Introduction

Breast density has been associated with a 1.8- to 6.0-fold increase in breast cancer risk (1, 2). There are several hypotheses that may help explain this positive association (3). Breast density is positively associated with stromal and epithelial cell proliferation (4) as well as with growth factors, such as insulin-like growth factor-1, in premenopausal women (5-8). Therefore, increased cell growth may be one of the contributing factors to the association between breast density and breast cancer. Both breast density (9-11) and breast cancer risk (12) are associated with hormone therapy use. Therefore, endogenous sex hormones, especially progestagens, may also be involved in the relationship between breast density and breast cancer risk (9-11).

It is not known whether tumor markers, such as mitotic index, cell cycle characteristics, or hormone receptor status, are also associated with breast density and if these associations differ between screen-detected and interval cancers. Breast cancers diagnosed after a negative screening mammogram but before the next screen (interval cancers) are more likely to be diagnosed among women with greater density (13, 14) and with worse prognostic factors than screen-detected cancers (15). Interval cancers may arise because they are truly faster-growing tumors (15). However, they may also be masked at initial screening examinations by breast density (16); increased mammographic density has been associated with decreased mammogram sensitivity in several studies (17-19). We may be able to learn more about how density is related to faster-growing and slower-growing tumors by separately evaluating the associations between tumor characteristics and breast density among screen-detected and interval cancers.

The purpose of this study was to examine the associations between tumor characteristics and mammographic density in a cross-sectional analysis of women enrolled in a mammography screening program who were diagnosed with invasive breast cancer. We examined whether these associations differed between screen-detected and interval cancers. We believe that this exploratory analysis is the first report of the relationships between multiple known tumor characteristics and breast density. Results from this study may allude to biological mechanisms that help explain the association between breast density and increased breast cancer risk.

Materials and Methods

Population Characteristics. Subjects were selected from women enrolled in Group Health Cooperative, a nonprofit integrated health system with >500,000 members serving western Washington State. For this study, we identified women enrolled in the Breast Cancer Screening Program, which has been described in detail elsewhere (20-22). As part of the Breast Cancer Screening Program, women ages ≥50 years received reminders for a screening mammogram every 1 to 3 years until 1992 and 1 to 2 years thereafter based on their risk for breast cancer (determined through a self-administered questionnaire at each mammogram). Women ages 40 to 49 years received screening reminders every 1 to 3 years until 1992 and 1 to 2 years thereafter only if they were at increased risk for breast cancer. To be eligible

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for the study, women had to have at least one screening mammogram during the period January 1, 1988 to December 31, 1993 and be diagnosed with a first primary invasive breast cancer within 24 months after their index mammogram (the last screening mammogram before their diagnosis; ref. 15). We restricted study eligibility to women who did not have a history of breast cancer before their index mammogram. We identified breast cancers by linking the Breast Cancer Screening Program database with the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. We identified 576 women with invasive breast cancer as potential subjects (15). We excluded 26 women whose index mammogram films were unavailable for review for breast density and 4 women with breast implants, leaving a total of 546 women in the final analyses. If specific tumor characteristic data were missing, we excluded women from those individual analyses. The Group Health Cooperative Institutional Review Board approved all study procedures.

Breast Density Data. Index mammograms were reviewed for breast density by a single expert radiologist who was unaware of the screen-detected/interval cancer status or laterality of breast cancer. Density was classified into four groups defined by the Breast Imaging Reporting and Data System (BIRADS) of the American College of Radiology (23): (*1*) almost entirely fat (n = 124), (*2*) scattered fibroglandular tissue (n = 249), (*3*) heterogeneously dense (n = 150), and (*4*) extremely dense (n = 23). We collapsed the categories so that density codes 1 and 2 were classified as ''fatty breasts'' (n = 373) and codes 3 and 4 were classified as ''dense breasts'' (n = 173). We used density in the cancer-free breast in all analyses.

Classification of Interval and Screen-Detected Cancers. Classification of interval and screen-detected cancers has been described in detail elsewhere (15). The index mammogram final assessment was interpreted in accordance with the American College of Radiology BIRADS (23) by Group Health Cooperative radiologists. The definitions for interval and screening cancers have varied across previous studies (13, 24). The length of the follow-up interval, the definition of a negative mammogram, and whether interval cancers were detectable on review all affect the classification of interval and screening cancers; therefore, we evaluated several definitions of interval cancers to account for these differences. We followed women for 24 months or until their next screening mammogram for a diagnosis of breast cancer, whichever came first. Women were classified as interval cancer cases if their breast cancer occurred after receiving a BIRADS assessment of 1 or 2 (negative or benign) on the index mammogram. Women were classified as screen detected if their breast cancer occurred after receiving a BIRADS assessment of 3, 4, or 5 (probably benign with short interval follow-up, suspicious, or highly suggestive of malignancy; ref. 23). This resulted in the classification of 151 interval cancers and 395 screen-detected cancers within 24 months; 66 interval cancers were diagnosed within 12 months. A single radiologist reviewed the index mammograms for all interval cancers (mixed in a set with 50 screen-detected cancers and 50 cancer-free women) to determine if the cancer was visible on the screening examination in retrospect (15). This secondary classification resulted in 100 ''true interval cancers.''

Laboratory Data. All laboratory measures have been described elsewhere (15, 25). Pathology data were obtained by pathology report abstraction and examination of H&E-stained slides made from the tissue blocks. Information concerning tumor size, extent of disease, and age at diagnosis was abstracted from the pathology report and from Surveillance, Epidemiology, and End Results to minimize missing values. We used available data on tumor size, extension of tumor to neighboring organs, and lymph node involvement to generate American Joint Committee on Cancer (AJCC) staging.

We examined paraffin-embedded primary breast tumor tissue samples, collected before any adjuvant treatment, for tumor characteristics, diagnosis, and immunocytochemistry. The study pathologist made all scoring and interpretations without knowledge of screen-detected/interval cancer status or other clinical variables. Histologic grade was assigned according to the modified Bloom and Richardson grading scheme for invasive ductal carcinoma (26, 27). Individual scores for differentiation, nuclear grade, and mitotic index were recorded along with the presence of lymphatic or vascular invasion, levels of tumor necrosis, and stromal and lymphocyte response. We completed immunoperoxidase assays for estrogen receptor, progesterone receptor, p53 tumor suppressor gene protein, Ki-67 proliferation-related antigen, C-erb-B2 oncogene protein, apoptosis regulatory protein Bcl-2, and cell cycle regulatory proteins p27 and cyclin E. About 80 women were missing various biomarker data because they did not have enough tumor sample to complete all of the laboratory assays.

The study pathologist scored all antibodies by subjective interpretation of staining intensity and/or the percentage of tumor cells that were positive (15). Categories of intensity and/or the percentage of positive cells were collapsed into positive/high or negative/low categories according to the assay. For estrogen receptor and progesterone receptor, any nuclear staining above negative was considered positive. The percentage of Ki-67-positive tumor cells, averaged over four high-power fields, was converted to the lowest quartile (≤5.7% positive) versus the upper three quartiles. Nuclear staining of >10% tumor cells for p53 was considered positive. A membranous staining pattern was considered positive for C-erb-B2. The negative and low-intensity Bcl-2 stains were categorized as ''low,'' whereas intermediate and high stains were categorized as ''high.'' Immunostaining for cyclin E and p27 was given a value from 1 (negative) to 7 (highest intensity); low intensity included values 1 to 4 and high intensity included values 5 to 7 (28). All immunohistochemical runs for all antibodies were conducted with a standard positive control that was compared with controls for previous runs before the data were considered acceptable for interpretation. After the primary interpretation was complete, 5% of cases were randomly selected and read a second time by the study pathologist; discrepancies were arbitrated by group review.

Mammographic Quality Data. A single expert mammography reader reviewed mammographic quality blinded to age, year of mammogram, interval or screen-detected status, and cancer laterality. Details of the definitions have been reported elsewhere (19, 29). Briefly, image quality was read on two mediolateral and two craniocaudal views; the worst quality rating of the four views was used. We used a grading scale that was developed specifically for this study to rate breast position, exposure, noise, contrast, compression, sharpness, artifact, and overall quality. The overall quality rating was a subjective rating delivered after rating the other seven categories.

Demographic Data. We obtained data from the Breast Cancer Screening Program Risk Factor Questionnaire on age, reproductive factors, hormone use, self-reported height and weight, and family history of breast cancer (13). Body mass index (BMI) was calculated as weight/height squared. Race and marital status were obtained from the Surveillance, Epidemiology, and End Results registry. Women were categorized as premenopausal, perimenopausal, or postmenopausal based on

their self-reported menopausal status and medical records at the time of the index mammogram (13). We categorized women as postmenopausal if they reported a natural menopause, hysterectomy with bilateral oophorectomy, or hysterectomy without bilateral oophorectomy and age ≥50 years at the time of the mammogram. Premenopausal and perimenopausal women had similar levels of breast density and were classified together in this analysis. Age at menopause was recorded in 5-year intervals.

Statistical Analysis. We used unconditional multivariate logistic regression to compute odds ratios (OR) with 95% confidence intervals (95% CI) for dense breasts compared with fatty breasts for various tumor characteristics. We identified age at diagnosis, marital status, ethnicity, menopausal status, age at menopause, age at menarche, family history of breast cancer, prior breast cancer diagnosis, breast biopsy history, hormone use, parity, age at first birth, BMI, stage of disease, and mammographic quality variables as potential confounders. We included each covariate individually in the crude regression model to see if the regression coefficient changed by ≥10%. We included BMI (as octiles), age at index examination (in 10-year intervals), menopausal status, age at menopause, age at first birth (nulliparous and 5-year age intervals), and AJCC stage (I, II, and III/IV) in the final multivariate models. We evaluated the associations separately for screen-detected and interval cancers detected within 24 months of the index examination. We used a likelihood ratio test to determine if the differences in the ORs for interval and screen-detected cancers were statistically significant. All analyses were conducted using Stata SE 7.0 (StataCorp, College Station, TX).

Subanalyses. For the first subanalysis, we used multinomial logistic regression to compute the OR and 95% CI using three categories of breast density (BIRADS 3 + 4 versus 1 and BIRADS 2 versus 1). Only a few women (n = 23) were classified as having extremely dense breasts; therefore, we could not analyze these women separately and categorized them with the women with heterogeneously dense breasts. We adjusted the ORs for the same covariates as the main analysis. Second, we excluded premenopausal and perimenopausal women from the analyses because menopausal status is an important determinant of breast density. Third, we excluded screen-detected cancers for women with no screening mammograms before their index mammogram. These cancers may have had different biomarker profiles than screen-detected cancers among women with prior screening examinations because they had a longer lead time before they were detected. Last, we evaluated the associations between tumor characteristics and breast density among ''true interval cancers'' that were not visible on the screening mammogram in retrospect.

Results

On average, women with dense breasts were younger; more likely to be nulliparous, premenopausal, or perimenopausal; and have a lower BMI compared with women with fatty breasts (Table 1). Women with dense breast tissue were also more likely to be diagnosed at a higher AJCC stage and have interval breast cancer.

Tumor size, lymph node status, and presence of lymphatic or vascular invasion were positively associated with breast density before and after adjustment for confounders (Table 2). Women with a tumor size >1.0 cm were more likely to have dense breasts compared with women with a tumor size ≤1.0 cm after adjusting for confounders (OR, 2.0; 95% CI, 1.2-3.4 for tumor sizes 1.1-2.0 cm; OR, 2.3; 95% CI, 1.3-4.4 for tumor sizes 2.1-10 cm). Having a progesterone receptor–negative tumor was modestly associated with breast density (P = 0.10) after adjusting for confounders. No other tumor characteristics were significantly associated with breast density.

When we examined the associations separately for interval and screen-detected cancers, the association between density and tumor size was only noted among women with screen-detected cancers (OR, 2.2; 95% CI, 1.1-4.1 for tumor sizes 1.1-2.0 cm; OR, 2.4; 95% CI, 1.0-5.9 for tumor sizes 2.0-10 versus ≤1.0 cm; Table 3). The presence of vascular or lymphatic invasion was modestly associated with density among screen-detected cancers (P = 0.09). Density was negatively associated with histologic grade (P for trend = 0.05), differentiation (P for trend = 0.04), and mitotic index (P for trend = 0.04) among women with interval cancers. None of the ORs were statistically significantly different between interval and screen-detected cancers; therefore, these results should be interpreted with caution.

Overall, the results did not change when we evaluated three categories of breast density for most tumor characteristics. However, the relationship strengthened with three categories of breast density for tumor size, lymph node status, and lymphatic or vascular invasion (data not shown). The results did not change when we excluded premenopausal and perimenopausal women from the analyses or when we limited the results to women with a prior screening mammogram (removing prevalent cancers; data not shown). When we restricted the analyses to ''true interval cancers,'' the results were similar to those for all interval cancers with negative associations between density and histologic grade (P for trend = 0.02), differentiation (P for trend = 0.04), mitotic index (P for trend = 0.001), and nuclear grade (P for trend = 0.03; data not shown).

Table 1. Characteristics of 546 women with invasive breast cancer stratified by breast density

Characteristics	Fatty breasts (n = 373), n (%)	Dense breasts (n = 173), n (%)
Age at diagnosis (y)		
<50	31 (8.3)	37 (21.4)
50-59	72 (19.3)	51 (29.5)
60-69	120 (32.2)	51 (29.5)
≥70	150 (40.2)	34 (19.7)
Age at first birth (y)		
Nulliparous	42 (11.3)	42 (24.3)
<20	48 (12.9)	25 (14.5)
20-24	148 (39.7)	38 (22.0)
25-29	93 (24.9)	39 (22.5)
≥30	42 (11.3)	29 (16.8)
Age at menopause (y)		
Premenopausal/ perimenopausal	36 (10.0)	41 (23.8)
<45	80 (22.2)	30 (17.4)
45-49	119 (33.1)	55 (32.0)
50-54	92 (25.6)	34 (19.8)
≥55	33 (9.2)	12 (7.0)
BMI (kg/m^2)		
<18.5	2 (0.5)	5 (2.9)
18.5-24.9	149 (39.9)	117 (67.6)
25.0-29.9	132 (35.4)	31 (17.9)
≥30	90 (24.1)	20 (11.6)
AJCC stage at diagnosis		
I	256 (71.7)	99 (60.0)
IIA/IIB	73 (20.4)	47 (28.5)
III/IV	28 (7.8)	19 (11.5)
Interval cancer*		
Interval cancer	73 (19.6)	78 (45.1)
Screen detected	300 (80.4)	95 (54.9)

*Interval cancer defined as a cancer diagnosed within 24 months of a negative mammogram (BIRADS codes 1 and 2) and before the next screening examination.

Discussion

Breast density was positively associated with tumor size, lymph node status, and lymphatic or vascular invasion among women with screen-detected cancers but not with interval cancers. Among women with interval cancers, we observed negative associations between breast density and histologic grade and mitotic index. We did not note any statistically significant associations between steroid receptor status or any other prognostic factors and breast density.

Two previous studies have examined the association between tumor characteristics and breast density. Similar to

Table 2. Crude and adjusted associations between tumor characteristics and breast density

	Fatty (n = 373), %	Dense (n = 173), %	Crude analyses, OR* (95% CI)	Adjusted analyses, OR† (95% CI)
Tumor size (cm)‡				
0.1-1.0	39.7	28.0	1 (reference)	1 (reference)
1.1-2.0	43.0	47.6	1.6 (1.0-2.4)§	2.0 (1.2-3.4)§
2.1-10	17.3	24.4	2.0 (1.2-3.3)§	2.3 (1.3-4.4)§
Missing (n)	15	9		
P for trend			0.007	0.003
Lymph node status‡				
Negative	78.1	67.5	1 (reference)	1 (reference)
Positive	21.9	32.5	1.7 (1.1-2.6)§	1.7 (1.0-2.8)§
Missing (n)	63	19		
Lymphatic or vascular invasion				
No	90.0	80.5	1 (reference)	1 (reference)
Yes	10.0	19.5	2.2 (1.3-3.8)§	2.1 (1.0-4.2)§
Missing (n)	62	19		
Histologic grade				
Low	37.8	44.5	1 (reference)	1 (reference)
Intermediate	42.0	32.9	0.7 (0.4-1.0)	0.9 (0.5-1.4)
High	20.2	22.6	1.0 (0.6-1.6)	0.8 (0.4-1.5)
Missing (n)	61	18		
P for trend			0.56	0.47
Nuclear grade				
Low	22.7	23.2	1 (reference)	1 (reference)
Intermediate	51.1	50.3	1.0 (0.6-1.6)	0.9 (0.5-1.7)
High	26.2	26.5	1.0 (0.6-1.7)	0.7 (0.4-1.4)
Missing (n)	60	18		
P for trend			0.97	0.33
Differentiation				
Low	15.1	16.8	1 (reference)	1 (reference)
Intermediate	26.3	28.4	1.0 (0.5-1.8)	0.8 (0.4-1.6)
High	58.7	54.8	0.8 (0.5-1.4)	0.9 (0.4-1.8)
Missing (n)	61	18		
P for trend			0.45	0.93
Mitoses				
Low	67.0	61.3	1 (reference)	1 (reference)
Intermediate	18.9	22.6	1.3 (0.8-2.1)	1.1 (0.6-1.9)
High	14.1	16.1	1.3 (0.7-2.2)	0.8 (0.4-1.7)
Missing (n)	61	18		
P for trend			0.29	0.69
Ki-67 (%)				
<25	31.1	29.2	1 (reference)	1 (reference)
25-100	68.9	70.8	1.1 (0.7-1.7)	1.0 (0.6-1.7)
Missing (n)	68	19		
Tumor necrosis				
None	63.6	62.6	1 (reference)	1 (reference)
Low	27.5	29.0	1.1 (0.7-1.7)	1.0 (0.6-1.7)
Intermediate/high	8.9	8.4	1.0 (0.5-1.9)	0.9 (0.4-2.2)
Missing (n)	60	18		
P for trend			0.95	0.88
Estrogen receptor				
Positive	84.1	81.3	1 (reference)	1 (reference)
Negative	15.9	18.7	1.2 (0.7-2.0)	1.1 (0.6-2.0)
Missing (n)	64	18		
Progesterone receptor				
Positive	76.3	71.0	1 (reference)	1 (reference)
Negative	23.7	29.0	1.3 (0.9-2.0)	1.5 (0.9-2.6)
Missing (n)	65	18		
p53				
≤10% Positive	87.4	89.0	1 (reference)	1 (reference)
>10% Positive	12.6	11.0	0.9 (0.5-1.6)	0.7 (0.3-1.4)
Missing (n)	71	18		
p27				
Medium/high	49.4	49.7	1 (reference)	1 (reference)
Negative/low	50.6	50.3	1.0 (0.7-1.5)	0.8 (0.5-1.3)
Missing (n)	65	18		

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Table 2. Crude and adjusted associations between tumor characteristics and breast density (Cont'd)

	Fatty (n = 373), %	Dense (n = 173), %	Crude analyses, OR* (95% CI)	Adjusted analyses, OR† (95% CI)
Cyclin E				
Low/medium	93.2	94.2	1 (reference)	1 (reference)
High	6.8	5.8	0.8 (0.4-1.9)	0.6 (0.2-1.6)
Missing (n)	65	19		
Bcl-2				
Medium/high	49.7	54.8	1 (reference)	1 (reference)
Negative/low	50.3	45.2	0.8 (0.6-1.2)	0.9 (0.5-1.4)
Missing (n)	65	18		
C-erb-B2				
Negative/low	83.3	81.9	1 (reference)	1 (reference)
Medium/high	16.7	18.1	1.1 (0.7-1.8)	1.2 (0.6-2.2)
Missing (n)	67	18		

*For dense breasts compared with fatty breasts.
†Adjusted for BMI, age at diagnosis, menopausal status/age at menopause, age at first birth, and AJCC stage.
‡Tumor size and lymph node status were not adjusted for stage.
§$P < 0.05$.

our results, Roubidoux et al. (30) and Sala et al. (31) showed that density was positively associated with tumor size and positive lymph nodes. Roubidoux et al. also found positive associations with tumor grade and negative estrogen receptor status in crude analyses (30); however, these associations were not independent of age. Stage was not analyzed as a confounding variable and the relationships were not explored by whether cancers were interval versus screen detected. We are unaware of any studies that have examined tumor characteristics, such as histologic grade, mitotic index, or other

Table 3. Association between tumor characteristics and high mammogram density compared with low stratified by interval and screen-detected cancers

	Screen detected (BIRADS 3-5 with cancer in 24 mo)			Interval cancer (BIRADS 1 and 2 with cancer in 24 mo)		
	Fatty (n = 300), %	Dense (n = 95), %	OR* (95% CI)	Fatty (n = 73), %	Dense (n = 78), %	OR* (95% CI)
Tumor size (cm)†						
0.1-1.0	44.5	32.2	1 (reference)	19.1	23.0	1 (reference)
1.1-2.0	42.4	48.9	2.2 (1.1-4.1)‡	45.6	45.9	1.1 (0.4-3.3)
2.1-10	13.1	18.9	2.4 (1.0-5.9)‡	35.3	31.1	0.8 (0.3-2.6)
Missing	10	5		5	4	
P for trend			0.02			0.69
Lymph node status†						
Negative	80.3	71.3	1 (reference)	68.9	63.5	1 (reference)
Positive	19.7	28.8	1.7 (0.9-3.5)	31.1	36.5	1.6 (0.6-4.0)
Missing	51	15		12	4	
Lymphatic or vascular invasion						
No	90.8	78.5	1 (reference)	87.1	82.7	1 (reference)
Yes	9.2	21.5	2.2 (0.9-5.4)	12.9	17.3	1.6 (0.4-5.8)
Missing	51	16		11	3	
Histologic grade						
Low	41.4	48.8	1 (reference)	23.8	40.0	1 (reference)
Intermediate	42.2	32.5	0.8 (0.4-1.5)	41.3	33.3	0.6 (0.2-1.8)
High	16.5	18.8	0.8 (0.3-2.0)	34.9	26.7	0.3 (0.1-1.0)
Missing	51	15		10	3	
P for trend			0.43			0.05
Nuclear grade						
Low	24.8	26.3	1 (reference)	14.3	20.0	1 (reference)
Intermediate	51.6	52.5	1.0 (0.5-2.1)	49.2	48.0	0.3 (0.1-1.3)
High	23.6	21.3	0.6 (0.2-1.4)	36.5	32.0	0.3 (0.1-1.2)
Missing	50	15		10	3	
P for trend			0.24			0.14
Differentiation						
Low	17.3	18.8	1 (reference)	6.3	14.7	1 (reference)
Intermediate	27.3	27.5	0.9 (0.4-2.4)	22.2	29.3	0.2 (0.0-1.3)
High	55.4	53.8	1.0 (0.4-2.4)	71.4	56.0	0.1 (0.0-0.8)‡
Missing	51	15		10	3	
P for trend			0.96			0.04
Mitoses						
Low	71.5	68.8	1 (reference)	49.2	53.3	1 (reference)
Intermediate	18.5	20.0	0.8 (0.4-1.9)	20.6	25.3	0.8 (0.3-2.5)
High	10.0	11.3	0.8 (0.3-2.3)	30.2	21.3	0.3 (0.1-0.9)‡
Missing	51	15		10	3	
P for trend			0.59			0.04

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Table 3. Association between tumor characteristics and high mammogram density compared with low stratified by interval and screen-detected cancers (Cont'd)

	Screen detected (BIRADS 3-5 with cancer in 24 mo)			Interval cancer (BIRADS 1 and 2 with cancer in 24 mo)		
	Fatty (n = 300), %	Dense (n = 95), %	OR* (95% CI)	Fatty (n = 73), %	Dense (n = 78), %	OR* (95% CI)
Ki-67 (%)						
<25	35.7	40.5	1 (reference)	14.1	17.3	1 (reference)
25-100	64.3	59.5	0.7 (0.4-1.4)	85.9	82.7	0.7 (0.2-2.4)
Missing	59	16				
Tumor necrosis						
None	63.1	66.3	1 (reference)	65.6	58.7	1 (reference)
Low	29.3	28.8	0.7 (0.3-1.4)	20.3	29.3	2.3 (0.8-6.6)
Intermediate/high	7.6	5.0	0.3 (0.1-1.4)	14.1	12.0	1.1 (0.3-3.8)
Missing	51	15		9	3	
P for trend			0.10			0.49
Estrogen receptor						
Positive	86.1	88.8	1 (reference)	76.6	73.3	1 (reference)
Negative	13.9	11.3	0.6 (0.2-1.6)	23.4	26.7	1.0 (0.4-2.8)
Missing	55	15		9	3	
Progesterone receptor						
Positive	76.2	73.8	1 (reference)	76.6	68.0	1 (reference)
Negative	23.8	26.3	1.2 (0.6-2.5)	23.4	32.0	2.0 (0.7-5.2)
Missing	56	15		9	3	
p53						
≤10% Positive	88.8	95.0	(reference)	82.3	82.7	1 (reference)
>10% Positive	11.3	5.0	0.3 (0.1-0.9)‡	17.7	17.3	0.7 (0.2-2.1)
Missing	60	15		9	3	
p27						
High	52.5	53.8	1 (reference)	37.5	45.3	1 (reference)
Medium/low	47.5	46.3	0.7 (0.4-1.3)	62.5	54.7	0.7 (0.3-1.7)
Missing	56	15		9	3	
Cyclin E						
Low/medium	93.9	97.5	1 (reference)	90.6	90.5	1 (reference)
High	6.1	2.5	0.1 (0.0-1.2)	9.4	9.5	0.8 (0.2-3.4)
Missing	56	15		9	4	
Bcl-2						
Medium/high	48.8	46.3	1 (reference)	53.1	64.0	1 (reference)
Negative/low	51.2	53.8	0.9 (0.5-1.7)	46.9	36.0	0.8 (0.3-1.9)
Missing	56	15		9	3	
C-erb-B2						
Negative/low	84.0	81.3	1 (reference)	80.6	82.7	1 (reference)
Medium/high	16.0	18.8	1.2 (0.5-2.8)	19.4	17.3	1.1 (0.3-3.3)
Missing	56	15		11	3	

*For dense breasts compared with fatty breasts. ORs adjusted for BMI, age at diagnosis, menopausal status/age at menopause, age at first birth, and AJCC stage.
†Tumor size and lymph node status were not adjusted for stage.
‡$P < 0.05$.

cell growth, apoptotic, or cell cycle characteristics and their associations with breast density, and whether the associations are modified by interval versus screen-detected cancers. One small study (n = 100) examined the associations among mammographic density, atypia/hyperplasia, and DNA S-phase percentages but found no associations (32).

There are two possible explanations for the positive associations that we observed among density, tumor size, lymph node status, and lymphatic or vascular invasion. It is well known that denser breasts are associated with decreased mammogram sensitivity (17-19). Therefore, it is possible that breast tumors in denser breasts go undetected longer than tumors in fatty breasts, giving them more time to grow and metastasize. Breast density has also been positively associated with cell growth factors, such as insulin-like growth factor-1, in premenopausal women (5-7). Although we evaluated both premenopausal and postmenopausal women, it is possible that tumors in dense breasts grow faster than tumors in fatty breasts. These two hypotheses may not be mutually exclusive, as suggested by Harrison et al. (33). Using deterministic models, Harrison et al. (33) showed that a positive association between density and tumor grade was more likely to be the result of a biological relationship; however, they could not entirely rule out a relationship based on decreased mammogram sensitivity and a longer time to diagnosis. The fact that the relationship between tumor size and breast density was only present in screen-detected cancers provides further evidence of a biological relationship rather than a relationship due to mammographic masking.

We are unaware of any studies that have evaluated the associations between density and tumor characteristics among screen-detected and interval cancers separately. We hypothesized that breast density would be positively associated with histologic grade and mitotic index because we showed previously that interval cancers were more likely to occur among women with dense breast tissue (13) and have a worse prognosis based on tumor characteristics (15). However, we found that density was negatively associated with histologic grade, differentiation, and mitotic index. It is possible that the interval cancers found in women with dense breasts were actually present at the time of the screening mammogram (but invisible to the radiologist), whereas interval cancers that developed in women with fatty breasts were not present at screening and thus developed very quickly in the interval between screening examination and diagnosis. Therefore, interval cancers in women with fatty breasts may have a worse prognosis if they grew more quickly than interval cancers in women with dense breasts, which might explain the negative associations that we observed. When we restricted the analyses for interval cancers to those that were not visible on

the index examination in retrospect, we noted similar results, including an additional inverse association between breast density and nuclear grade. These results support the hypothesis that tumors in fatty breasts may develop more quickly; however, this needs to be evaluated in additional studies with more statistical power.

Two major strengths of our study are its sample size and the comprehensive assessment of histologic and protein expression characteristics. A single pathologist evaluated all tumor characteristic data and a single radiologist evaluated mammogram density. However, this study also has limitations. Sample sizes for certain tumor characteristics among women with interval cancers were small, resulting in some large 95% CIs. The differences between the ORs for interval and screen-detected cancers should be interpreted with caution because of wide 95% CIs. In addition, we used a categorical measure for breast density, which is less precise than methods that use a continuous measure for breast density (34, 35). At the time of the study, we did not determine biopsy laterality; therefore, we do not know who had a biopsy on the cancer-free breast, which could have affected breast density. We were missing biomarker data for ~80 women who did not have enough tumor sample to complete all of the laboratory assays. We compared tumor size, AJCC stage, and breast density between women with and without biomarker data and found that women without biomarker data, on average, had smaller tumors, were diagnosed at an earlier stage of disease, and had fattier breasts. Therefore, our results for fatty breasts and smaller tumors may have greater variability and less generalizability than the results for dense breasts and larger tumors. However, we adjusted for stage in the analyses, of which tumor size is a component.

In summary, we found that breast density was positively associated with tumor size, lymph node status, and lymphatic or vascular invasion among women with screen-detected invasive breast cancer. The positive association we observed between density and tumor size could be due to a delayed diagnosis of tumors in women with dense breasts or due to an association between breast density and increased cell proliferation. Additional studies should be done that can address these two issues separately.

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