Association of Mammographic Density with the Pathology of Subsequent Breast Cancer among Postmenopausal Women

Karthik Ghosh,[1] Kathleen R. Brandt,[1] Thomas A. Sellers,[2] Carol Reynolds,[1] Christopher G. Scott,[1] Shaun D. Maloney,[1] Michael J. Carston,[1] V. Shane Pankratz,[1] and Celine M. Vachon[1]

[1]Mayo Clinic College of Medicine, Rochester, Minnesota and [2]H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Abstract

Background: **Limited studies have examined the associations between mammographic density and subsequent breast tumor characteristics.**
Methods: **Eligible women were part of a case-control study of postmenopausal breast cancer, were 40 years or older and had a routine mammogram 4 years or more before their diagnosis. Mammographic density (percent density, dense area, and nondense area) was estimated using a computer-assisted thresholding program. At the time of cancer diagnosis, cases were classified as asymptomatic or symptomatic based on medical record review and breast imaging workup. Pathologic review was done blinded to the density status. Linear regression models and tests for trend examined the association between pathologic characteristics of the breast tumor and the components of density for all participants, and stratified by symptom status at diagnosis.**
Results: **Of the 286 eligible cases, 77% were 60 years or older and mean percent density was 29.5% (SD, 14.6%). Density was not significantly associated with tumor size (P = 0.22), histologic type (P = 0.77), estrogen receptor (P = 0.11) or progesterone receptor (P = 0.37) status, mitotic activity (P = 0.12), or nuclear pleomorphism (P = 0.09; P values for percent density). An inverse association was suggested between tumor grade and percent density (32.0%, 30.3%, 26.7% for grades 1-3; P = 0.06 for trend). The inverse association with tumor grade and its components (nuclear pleomorphism and tubular differentiation) was only evident among the 97 symptomatic women; positive associations of estrogen receptor (P = 0.009) and progesterone receptor (P = 0.04) were also seen with percent density only in this subgroup.**
Conclusions: **The inverse association between tumor grade and percent density in the symptomatic population could inform the biology of the association between mammographic density and breast cancer risk.** (Cancer Epidemiol Biomarkers Prev 2008;17(4):872–9)

Introduction

Mammographic density has been consistently associated with increased breast cancer risk. Studies show that women with >75% breast density have a four to six times greater risk of breast cancer than women with minimal density (1-3). Despite this evidence, the underlying biological mechanism by which mammographic density affects breast cancer risk remains unclear. Histologic studies of mammographic density suggest an association between density and epithelial and stromal proliferation (4, 5). Li et al. (6) obtained noncancerous breast tissue from forensic autopsy of 519 women and studied quantitative microscopy of the breast tissue in relation to the percent density in the Faxitron image of the tissue slice. Results indicated that percent density was positively associated with total nuclear area (epithelial and nonepithelial nuclear area), the proportion of collagen, and the area of glandular structures.

Only a few studies have examined mammographic density in relation to breast tumor characteristics, and most of these studies assessed mammographic density of the contralateral breast at the time of cancer diagnosis (7-10). These illustrated positive associations between mammographic density and tumor size, lymphatic invasion, later stage, and lymph node status but were inconsistent on associations with tumor grade and estrogen receptor status of the tumor. One interpretation of these findings is that dense breasts decrease the sensitivity of the mammogram, resulting in delayed detection and corresponding larger and more advanced tumors (11). Alternatively, tumors may grow faster in an environment of increased density, which suggests that density may influence the microenvironment in which these tumors develop. To add to the few studies on the topic, we examined mammographic density at least 4 years before the diagnosis of breast cancer and subsequent breast cancer tumor characteristics.

Materials and Methods

Study Population. Subjects were selected from a case-control study (n = 372 cases, n = 713 controls), which is described in detail elsewhere (12). Briefly, breast cancer cases for the case-control study were women who were

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older than 40 years, had been diagnosed with primary invasive breast cancer between 1997 and 2001, had at least two prior screening mammograms done 2 years or more before the breast cancer diagnosis, and lived within a 193.12-km (120-mile) radius of the Mayo Clinic in Rochester, Minnesota. Multiple mammograms ensured a population undergoing routine screening, and the residency requirement improved the representativeness of the study population. For the current study, we used a subset of postmenopausal women with invasive breast cancer (n = 286) who had a screening mammogram available 4 or more years before the cancer diagnosis. This reduced the possibility of an occult tumor being present at the time of the density measurement on the study mammogram (13). When multiple mammograms were available for the study period, the mammogram closest to and at least 4 years before the date of cancer diagnosis was used for density estimation for the study.

Pathologic Review. An experienced breast pathologist (C.R.) blinded to the mammographic density status systematically reviewed the tumor pathology of all cases. Original H&E slides were reviewed to obtain the diagnosis, histologic subtype of the tumor, tumor grade (Nottingham grade 1, 2, or 3), and the three components of tumor grade—mitotic activity, nuclear pleomorphism, and tubule differentiation—with a value of 1 being favorable and 3 being unfavorable for each component of grade (14). The estrogen and progesterone receptor status of the tumors was also assessed and classified as negative (0%) or positive. Positive status was graded by the pathologist as 1-25%, 26-50%, or 51-100% based on the proportion of the slide that stained positive for the receptor. Tumor size was obtained from the original pathology report. All categories were predefined by the study pathologist before review.

Medical Record Review. Medical records provided weight and height for the clinical visit closest to the study mammogram date. Height and weight were used to calculate the body mass index (BMI). Current hormone replacement therapy use at the time of the study mammogram and in the interval between this mammogram and breast cancer diagnosis was ascertained at medical exams associated with mammography visits, recorded in the medical record, and subsequently abstracted for the current study. Data on hormone replacement therapy were available for 84% of participants and recorded as ever versus never use during the study interval. All remaining patient information, including menopausal status at mammogram, were obtained from a clinical database of self-reported information recorded during the study mammogram visit.

Review of medical records and the breast imaging workup at the time of cancer diagnosis classified cancer symptom status as asymptomatic or symptomatic. A tumor was classified asymptomatic if it was detected at the time of screening mammogram and was not associated with presenting breast symptoms such as lump, pain, or nipple discharge. Hence, asymptomatic cancers were mammographically screen-detected cancers. On the other hand, symptomatic cancers were diagnosed when the patient presented with breast symptoms and the imaging workup, including mammography, revealed breast cancer. In the literature, breast cancer has been described as an ''interval'' cancer if a woman is diagnosed with breast cancer in the 12- to 24-month interval following a negative screening mammogram report (10, 15). However, in our study, we were unable to assess for interval cancers because we did not have the information on the interval from last negative screening mammogram to cancer diagnosis for these women.

The Mayo Clinic Institutional Review Board approved this study.

Mammographic Density Estimation. Multiple prediagnostic mammograms with craniocaudal and mediolateral oblique views were available on all study subjects. The analyses used the mammogram closest to but at least 4 years or more preceding the diagnosis of cancer (mean $\pm$ SD, 4.87 $\pm$ 0.91 years; Table 1).

All four mammogram views were digitized on a Lumiscan 75 scanner with 12-bit grayscale depth. The pixel size was 0.130×0.130 mm^2 for both the 18×24-cm^2 and 24×30-cm^2 films.

Percent mammographic density (dense area / total area $\times$ 100), dense area, and nondense area were estimated using a computer-assisted thresholding program that has routinely been used in several mammographic density studies (16-19). Briefly, two thresholds are set by a trained programmer: One separates the breast from the background, and the second separates dense from nondense tissue. Values are calculated for overall breast area and dense area in pixels; the ratio provides the percent density. Nondense area is calculated as the total breast area minus the dense area. Area estimates were converted to square centimeters. Given the similarity in density estimates from craniocaudal or medio-lateral oblique views (16, 20, 21), only craniocaudal images from the ipsilateral breast (breast with subsequent cancer) were used for analysis. A 5% repeat set of images from the overall case-control study was assessed for reliability. We consistently showed high reliability ($r > 0.90$) for all measures.

Statistical Analyses. The distribution of baseline characteristics and mammographic density were summarized as means and SDs or counts and percentages. Percent density, dense area, and nondense area were approximately normally distributed. Linear regression models were fit to the data to analyze associations between density measures and the tumor characteristics of interest. Analyses were adjusted for age alone and for age, BMI, hormone replacement therapy use within the interval, family history, and a combined age at first birth and number of births variable (Table 1). Because results were similar, we present only the fully adjusted model. Adjusted means and 95% confidence intervals for each level of tumor characteristics were estimated from the linear models. When appropriate, tests for trends in mammographic density across ordered categories of tumor characteristics were done by analyzing the levels of the characteristic as an ordinal variable. For the tumor characteristic without a natural ordering, that is, histology, we obtained the P value for the t test that compared mean differences between ductal cancer (infiltrating ductal and low-grade ductal cancer) and lobular cancer (infiltrating lobular cancer). Analyses were conducted on all women combined and stratified according to symptom status at the time of diagnosis (asymptomatic versus symptomatic). In addition, we repeated analyses on

Table 1. Characteristics of breast cancer cases (n = 286) forming the study sample

Characteristics	Levels	Categorical: n (%); continuous: mean (SD)/range
Age at cancer diagnosis (y)	41-50	4 (1.4)
	51-60	61 (21.3)
	61-70	87 (30.4)
	>70	134 (46.9)
Age at mammogram (y)	41-50	30 (10.5)
	51-60	81 (28.3)
	61-70	86 (30.1)
	>70	89 (31.1)
Parity and age at first birth combined	No children	35 (12.2)
	Age at first birth <20 and children 1 or 2	15 (5.2)
	Age at first birth >20 and children 1 or 2	90 (31.5)
	Age at first birth <20 and children 3+	51 (17.8)
	Age at first birth >20 and children 3+	95 (33.2)
Menopausal status	Postmenopausal	286 (100.0)
First-degree family history of breast cancer	No	236 (82.5)
	Yes	50 (17.5)
Hormone replacement therapy during interval from mammogram to cancer diagnosis	Ever	168 (58.7)
	Never	118 (41.3)
BMI (kg/m^2)	Mean (SD)	27.92 (5.1)
	Median	27.3
	Lower quartile	24.1
	Upper quartile	30.4
Time from study mammogram to cancer diagnosis (y)	4-5	207 (72)
	5-6	50 (17)
	6-7	16 (6)
	7-8	9 (3)
	8-9	4 (1)
	Mean (SD)	4.87 (0.91)
	Median	4.6
	Lower quartile	4.2
	Upper quartile	5.1
Mammographic density ipsilateral side		
Craniocaudal percent density (%)	Mean (SD)	29.5 (14.6)
	Median	28.3
	Lower quartile	19.2
	Upper quartile	37.8
Medio-lateral oblique percent density (%)	Mean (SD)	27.5 (14.7)
	Median	26.4
	Lower quartile	17.4
	Upper quartile	35.3
Craniocaudal dense area (cm^2)	Mean (SD)	37.2 (20.2)
	Median	34.0
	Lower quartile	24.0
	Upper quartile	46.4
Medio-lateral oblique dense area (cm^2)	Mean (SD)	39.9 (22.2)
	Median	36.0
	Lower quartile	24.7
	Upper quartile	52.4

mammograms ascertained 4 to 6 years before the breast cancer diagnosis to determine whether the timing of mammogram influenced our results. All analyses were done using the SAS statistical software.

Results

The 286 cases included for analyses were women with breast cancer who were postmenopausal at the time of the study mammogram, and 77% of the cases were older than 60 years (Table 1). Most of the women (83%) did not have a family history of breast cancer, and 41% had never used hormone replacement therapy during the interval from study mammogram to breast cancer diagnosis. The mean BMI for the cohort was 27.9 (SD, 5.1), and 12% were nulliparous. In this population of women, 66% of the tumors were asymptomatic and detected by screening mammography, and the remainder was symptomatic at

the time of diagnosis. Most of the tumors were <2 cm at diagnosis (81% of overall, 60% of symptomatic, and 90% of asymptomatic). The mean percent density for the craniocaudal view on the cancer side was 29.5 ± 14.6%, and the mean dense area was 37.2 ± 20.2 cm^2. The study used mammograms 4 or more years before cancer diagnosis, with most of the mammograms (89%) being within 4 to 6 years of the diagnosis.

In the combined analyses of all cases, assessment of tumor histology revealed no associations with mean mammographic density 4 years or more before cancer diagnosis, assessed as percent density or its components, dense area or nondense area. There was a positive trend of tumor size with percent density and dense area and an inverse trend with nondense area, but none of these were statistically significant. No significant association was found between estrogen and progesterone receptor status of the cancer and percent density, dense area, or nondense area (Table 2). There was a borderline inverse association between tumor grade and percent density, with mean adjusted percent density for low-grade tumors being 32% and that of high-grade tumors being 27% ($P = 0.06$). Of the components of tumor grade (mitotic activity, nuclear pleomorphism, and tubule differentiation) and density, we noted an inverse association between tubular differentiation and percent density ($P = 0.05$), and a positive association between tubular differentiation and nondense area ($P = 0.04$) but not with dense area ($P = 0.78$). The other two components of grade, mitotic activity and nuclear pleomorphism, also suggested inverse associations with percent density ($P = 0.12$ and $P = 0.09$, respectively) but not with dense area ($P = 0.58$ and $P = 0.15$, respectively). However, similar to what was seen with tubular differentiation, there was a borderline positive association between mitotic activity and nondense area ($P = 0.06$). We also did the above analyses, restricting mammograms to 4 to 6 years before the cancer diagnosis, and found similar results to those outlined above (data not shown).

We were unable to stratify screen-detected and interval cancers as in previous reports but stratified cancers based on symptom status at the time of cancer diagnosis (asymptomatic or symptomatic; Tables 3 and 4). Assessment of the asymptomatic breast cancers revealed no associations of density with any pathologic findings (Table 3). In fact, all significant associations were noted only among the symptomatic breast cancers (Table 4). These included a significant positive association between estrogen and progesterone receptor status of the tumors with percent density ($P = 0.009$ and

Table 2. Association between mammographic density (percent density, dense area, and nondense area from craniocaudal mammogram view) and pathology of subsequent tumor characteristics

Tumor characteristics	Variable levels	*n*	Adjusted mean percent density (95% confidence interval)*	Percent density *P*	Adjusted mean dense area (95% confidence interval)*	Dense area *P*	Adjusted mean nondense area (95% confidence interval)*	Nondense area *P*
Histology †	Infiltrating ductal	181	30.26 (27.41-33.12)	0.77	37.08 (32.49-41.67)	0.17	90.71 (82.00-99.41)	0.50
	Infiltrating lobular	44	30.92 (26.66-35.19)		41.93 (35.00-48.86)		95.49 (82.34-108.64)	
	Low-grade ductal	19	29.42 (23.05-35.79)		36.77 (26.55-47.00)		93.96 (74.57-113.36)	
	Mixed ductal and lobular	17	31.09 (24.57-37.61)		41.04 (30.57-51.50)		90.88 (71.03-110.73)	
	Other	8	30.97 (22.07-39.87)		45.21 (30.93-59.49)		105.65 (78.56-132.73)	
Estrogen receptor (%)	0 (negative)	26	26.91 (21.11-32.72)	0.11	31.31 (21.77-40.85)	0.41	101.40 (83.42-119.38)	0.07
	1-25	11	26.92 (19.11-34.74)		48.16 (34.45-61.87)		108.70 (82.86-134.54)	
	26-50	9	36.10 (27.30-44.90)		47.64 (33.46-61.82)		93.82 (67.08-120.55)	
	51-100	205	31.32 (28.50-34.14)		38.69 (34.11-43.27)		89.04 (80.41-97.68)	
Progesterone receptor (%)	0 (negative)	33	29.47 (24.31-34.63)	0.37	42.60 (34.28-50.92)	0.77	103.64 (88.24-119.04)	0.25
	1-25	33	30.42 (25.50-35.33)		37.20 (29.03-45.37)		82.54 (67.42-97.67)	
	26-50	34	30.78 (25.83-35.72)		36.74 (28.69-44.79)		96.15 (81.24-111.05)	
	51-100	153	31.36 (28.28-34.45)		39.20 (34.19-44.20)		89.38 (80.12-98.65)	
Tumor size (cm)	<1	69	29.56 (25.99-33.13)	0.22	36.25 (30.67-41.83)	0.06	94.84 (83.73-105.94)	0.47
	1-2	154	30.07 (27.08-33.06)		38.52 (33.90-43.14)		93.04 (83.85-102.23)	
	>2	54	32.58 (28.46-36.69)		43.09 (36.77-49.41)		89.56 (76.98-102.13)	
Tumor grade	1	118	31.95 (28.81-35.08)	0.06	39.82 (34.86-44.78)	0.26	90.62 (81.03-100.21)	0.43
	2	120	30.29 (27.09-33.50)		38.10 (33.06-43.15)		89.86 (80.10-99.61)	
	3	28	26.73 (21.51-31.95)		35.13 (26.95-43.31)		100.25 (84.43-116.06)	
Mitotic activity	1	228	31.09 (28.35-33.83)	0.12	39.34 (35.02-43.65)	0.58	90.52 (82.18-98.85)	0.06
	2	27	27.03 (21.86-32.20)		31.58 (23.45-39.70)		96.80 (81.11-112.50)	
	3	8	27.43 (17.49-37.37)		47.31 (31.70-62.91)		119.17 (89.04-149.30)	
Nuclear pleomorphism	1	88	31.41 (28.07-34.74)	0.09	39.94 (34.66-45.22)	0.15	91.12 (80.84-101.39)	0.90
	2	90	32.42 (28.86-35.98)		40.28 (34.68-45.88)		92.25 (81.36-103.14)	
	3	87	28.09 (24.62-31.55)		35.42 (29.95-40.90)		91.85 (81.20-102.51)	
Tubule differentiation	1	33	33.79 (29.01-38.57)	0.05	39.10 (31.51-46.68)	0.78	83.20 (68.64-97.77)	0.04
	2	58	32.04 (27.74-36.35)		36.07 (29.25-42.88)		84.40 (71.31-97.48)	
	3	173	29.49 (26.65-32.33)		38.82 (34.34-43.29)		95.24 (86.64-103.83)	

*Adjusted for age at mammogram, combined variable of age at first birth and number of births, BMI at date closest to mammogram, family history of breast cancer, and hormone replacement therapy use.
†*P* value for contrast testing the difference between ductal (infiltrating ductal and low-grade ductal) and lobular (infiltrating lobular).

Table 3. Association between mammographic density (percent density, dense area, and nondense area from craniocaudal mammogram view) and pathology of subsequent tumor characteristics for symptomatic cancers

Tumor characteristics	Variable levels	*n*	Adjusted mean percent density (95% confidence interval)*	Percent density *P*	Adjusted mean dense area (95% confidence interval)*	Dense area *P*	Adjusted mean nondense area (95% confidence interval)*	Nondense area *P*
Histology †	Infiltrating ductal	129	29.08 (25.79-32.38)	0.86	36.85 (31.23-42.48)	0.27	97.31 (86.21-108.41)	0.85
	Infiltrating lobular	26	29.49 (24.36-34.61)		41.61 (32.67-50.56)		99.17 (81.52-116.83)	
	Low-grade ductal	15	26.92 (19.73-34.10)		33.90 (21.66-46.15)		101.44 (77.27-125.60)	
	Mixed ductal and lobular	7	30.40 (20.90-39.90)		48.51 (32.29-64.73)		111.49 (79.48-143.50)	
	Other	4	27.62 (15.53-39.71)		49.41 (28.81-70.01)		129.49 (88.83-170.15)	
Estrogen receptor (%)	0 (negative)	17	28.27 (21.31-35.22)	0.90	35.36 (22.93-47.79)	0.84	106.39 (81.86-130.93)	0.41
	1-25	5	30.46 (19.31-41.62)		45.03 (25.69-64.36)		106.90 (68.74-145.06)	
	26-50	5	40.89 (29.67-52.12)		54.49 (35.04-73.94)		92.88 (54.49-131.26)	
	51-100	138	29.58 (26.27-32.90)		38.85 (33.05-44.66)		97.32 (85.86-108.77)	
Progesterone receptor (%)	0 (negative)	19	29.94 (23.48-36.39)	0.83	45.61 (34.50-56.71)	0.43	110.92 (89.42-132.42)	0.53
	1-25	18	30.44 (23.96-36.92)		37.77 (26.14-49.41)		85.72 (63.20-108.24)	
	26-50	30	31.55 (26.25-36.85)		38.37 (29.17-47.57)		98.88 (81.08-116.69)	
	51-100	100	29.23 (25.62-32.85)		38.67 (32.37-44.97)		97.39 (85.19-109.58)	
Tumor size (cm)	<1	62	28.67 (24.95-32.39)	0.66	36.49 (29.98-43.00)	0.15	100.85 (87.68-114.01)	0.99
	1-2	106	27.92 (24.51-31.32)		38.54 (32.63-44.45)		103.53 (91.58-115.48)	
	>2	18	31.49 (25.26-37.73)		45.43 (34.73-56.12)		97.81 (76.20-119.43)	
Tumor grade	1	82	29.64 (26.10-33.19)	0.35	38.82 (32.78-44.86)	0.60	98.29 (85.94-110.63)	0.52
	2	78	29.43 (25.77-33.09)		38.36 (32.21-44.50)		96.22 (83.65-108.79)	
	3	17	25.56 (19.14-31.98)		35.35 (24.63-46.06)		111.05 (89.14-132.95)	
Mitotic activity	1	155	29.25 (26.13-32.37)	0.20	38.69 (33.43-43.95)	0.34	98.09 (87.36-108.82)	0.15
	2	14	27.99 (21.31-34.68)		34.36 (23.18-45.54)		103.76 (80.95-126.57)	
	3	6	21.53 (10.17-32.89)		33.02 (14.03-52.01)		126.23 (87.46-164.99)	
Nuclear pleomorphism	1	62	29.86 (26.10-33.62)	0.40	39.49 (33.12-45.87)	0.38	96.54 (83.45-109.63)	0.98
	2	62	29.50 (25.53-33.47)		38.84 (32.20-45.48)		102.77 (89.14-116.40)	
	3	53	27.84 (23.61-32.07)		35.94 (28.84-43.03)		96.23 (81.65-110.80)	
Tubule differentiation	1	24	29.16 (23.77-34.54)	0.56	35.99 (26.84-45.14)	0.48	94.83 (76.17-113.50)	0.24
	2	37	31.46 (26.54-36.37)		37.55 (29.28-45.83)		89.06 (72.17-105.94)	
	3	116	28.70 (25.41-31.99)		39.09 (33.54-44.65)		101.68 (90.35-113.01)	

NOTE: $n = 189$.
*Adjusted for age at mammogram, combined variable of age at first birth and number of births, BMI at date closest to mammogram, family history of breast cancer, and hormone replacement therapy use.
†*P* value for contrast testing the difference between ductal (infiltrating ductal and low-grade ductal) and lobular (infiltrating lobular).

$P = 0.04$, respectively), as well as an inverse association between tumor grade ($P = 0.03$) and its components, tubular differentiation and nuclear pleomorphism ($P = 0.01$ and $P = 0.03$, respectively), and percent density. Repeating the estrogen and progesterone receptor analyses in the symptomatic population after adjusting for tumor grade, we found a decreased significance of the associations (*P* value changed from 0.009 to 0.05 for estrogen receptor and from 0.04 to 0.23 for progesterone receptor).

Discussion

Our study adds to the limited data on mammographic density and breast cancer tumor characteristics. Among all women, mammographic density assessed at least 4 years before postmenopausal breast cancer was not associated with the histologic subtype of the tumor or the estrogen or progesterone receptor status. There seemed to be a trend, albeit nonsignificant, toward a positive association with tumor size and an inverse association with tumor grade, the latter perhaps driven by the inverse association between tubular differentiation and percent density, as well as the positive association with nondense area. When cancers were stratified based on symptom status at the time of cancer diagnosis, the asymptomatic cancers showed no association between density and tumor pathology. Among the symptomatic cancers, a positive association was seen between estrogen and progesterone receptor status and percent density, and an inverse association was seen between tumor grade and percent density.

The positive trend of tumor size with percent density and dense area among all women, although not significant, is consistent with two previous studies that examined this question (8, 10). This association may be due to reduced mammogram sensitivity for dense breasts and delayed detection (tumor masking by breast density), resulting in larger tumor size. Differences between our study and previous reports include the methodology used for density measurement. The Breast Imaging Reporting and Data System density measure used in prior reports is a categorical measure assessed clinically, whereas the density measure used in this study was a continuous measure estimated from a computer-assisted thresholding program. Furthermore, our study used the mammogram of the ipsilateral breast at least 4 years before the cancer unlike other studies that analyzed the mammogram of the contralateral breast at

Table 4. Association between mammographic density (percent density, dense area, and nondense area from craniocaudal mammogram view) and pathology of subsequent tumor characteristics for asymptomatic cancers

Tumor characteristics	Variable levels	*n*	Adjusted mean percent density (95% confidence interval)*	Percent density *P*	Adjusted mean dense area (95% confidence interval)*	Dense area *P*	Adjusted mean nondense area (95% confidence interval)*	Nondense area *P*
Histology †	Infiltrating ductal	52	33.31 (27.29-39.33)	0.68	37.96 (29.18-46.74)	0.74	76.04 (60.99-91.08)	0.10
	Infiltrating lobular	18	32.36 (24.23-40.48)		40.87 (29.01-52.74)		91.13 (70.81-111.46)	
	Low-grade ductal	4	40.46 (26.32-54.60)		47.91 (27.31-68.51)		67.81 (32.52-103.10)	
	Mixed ductal and lobular	10	29.70 (19.82-39.57)		30.10 (15.69-44.51)		75.84 (51.15-100.53)	
	Other	4	33.24 (19.70-46.78)		39.72 (19.96-59.48)		83.31 (49.45-117.17)	
Estrogen receptor (%)	0 (negative)	9	25.02 (14.83-35.21)	0.009	23.05 (7.95-38.14)	0.16	91.40 (65.47-117.34)	0.03
	1-25	6	25.06 (14.00-36.12)		50.84 (31.27-70.40)		105.11 (71.50-138.73)	
	26-50	4	31.01 (17.23-44.80)		36.84 (16.45-57.23)		91.81 (56.79-126.83)	
	51-100	67	36.05 (30.34-41.76)		38.34 (29.73-46.95)		71.29 (56.50-86.08)	
Progesterone receptor (%)	0 (negative)	14	28.17 (19.42-36.91)	0.04	34.25 (21.19-47.30)	0.23	94.09 (72.00-116.18)	0.13
	1-25	15	31.14 (23.56-38.71)		36.72 (25.25-48.19)		75.89 (56.48-95.30)	
	26-50	4	31.53 (17.44-45.62)		40.45 (19.41-61.50)		87.02 (51.41-122.64)	
	51-100	53	36.24 (29.96-42.52)		41.09 (31.66-50.52)		74.85 (58.89-90.81)	
Tumor size (cm)	<1	7	36.59 (25.70-47.48)	0.61	40.00 (25.51-54.48)	0.96	72.25 (47.2497.26)	0.43
	1-2	48	33.49 (27.25-39.73)		37.87 (29.56-46.18)		74.62 (60.26-88.97)	
	>2	36	33.06 (26.54-39.57)		38.51 (29.85-47.18)		79.82 (64.86-94.78)	
Tumor grade	1	36	38.15 (31.19-45.11)	0.03	43.19 (33.07-53.31)	0.12	75.19 (57.72-92.66)	0.53
	2	42	32.53 (26.01-39.04)		37.78 (28.28-47.28)		78.12 (61.73-94.52)	
	3	11	28.61 (19.85-37.37)		33.37 (20.63-46.10)		82.83 (60.84-104.81)	
Mitotic activity	1	73	36.22 (30.07-42.37)	0.15	41.19 (32.78-49.60)	0.93	72.97 (57.59-88.35)	0.08
	2	13	26.36 (18.36-34.37)		26.62 (15.67-37.56)		84.75 (64.73-104.76)	
	3	2	38.94 (19.33-58.54)		78.98 (52.15-105.82)		114.34 (65.28-163.41)	
Nuclear pleomorphism	1	26	35.85 (28.88-42.82)	0.03	41.57 (31.17-51.97)	0.13	79.25 (61.43-97.08)	0.58
	2	28	40.03 (32.84-47.21)		44.05 (33.32-54.77)		67.45 (49.07-85.82)	
	3	34	28.88 (22.84-34.92)		34.07 (25.04-43.10)		82.93 (67.45-98.40)	
Tubule differentiation	1	9	46.11 (36.08-56.15)	0.01	47.25 (32.39-62.10)	0.43	56.01 (30.83-81.19)	0.04
	2	21	33.14 (24.63-41.65)		33.42 (20.76-46.09)		75.37 (53.90-96.84)	
	3	57	31.49 (26.03-36.95)		37.63 (29.55-45.71)		82.25 (68.56-95.95)	

NOTE: $n = 97$.
*Adjusted for age at mammogram, combined variable of age at first birth and number of births, BMI at date closest to mammogram, family history of breast cancer, and hormone replacement therapy use.
†*P* value for contrast testing the difference between ductal (infiltrating ductal and low-grade ductal) and lobular (infiltrating lobular).

the time of cancer diagnosis. Also, previous studies included premenopausal and postmenopausal women, whereas our study population only included postmenopausal women, who are more likely to have lower breast density and are less likely to have a tumor remain undetected for prolonged periods of time. Moreover, it is possible that using the mammogram 4 years or more before cancer diagnosis reduced, although did not completely remove, the possibility of tumor masking.

Our null findings between estrogen receptor and percent density and dense area among all women in the study are consistent with some of the other reports in literature (7, 10, 22), including the findings by Ziv et al. (22) showing density as a risk factor for estrogen receptor–positive and estrogen receptor–negative breast cancer. This supports the hypothesis that the association between mammographic density and breast cancer may be due to other factors besides estrogen exposure. In fact, in a recent report, Tamimi et al. (23) suggest that endogenous estrogen levels and mammographic density affect breast cancer risk through independent pathways. However, there was a strong positive association between density and estrogen receptor status among symptomatic cancers. This association was attenuated when the analyses were adjusted for tumor grade, suggesting that this may be related to the presence of well-differentiated, low-grade cancers among women with dense breasts (as was seen in the symptomatic cancer group). In the report by Ziv et al. (22), density was also positively associated with progesterone receptor–positive and progesterone receptor–negative breast cancers. In our study, we found no association between density and progesterone receptor status of the tumors among all cases. However, although progesterone receptor was unrelated to percent density among asymptomatic cancers, there was a positive association between progesterone receptor and percent density ($P = 0.04$) among symptomatic cancers, again likely related to the earlier described association of density and tumor grade.

The present study suggested an inverse trend, although nonsignificant, between tumor grade and mammographic density assessed as percent density among all cases combined. This trend seemed to primarily reflect the inverse associations of tubular differentiation with percent density and positive associations between tubular differentiation and nondense area. When stratifying by symptom status, only symptomatic

cancers showed a significant inverse association between tumor grade and its components, tubular differentiation and nuclear pleomorphism, with percent density. This finding is in agreement with the report by Aiello et al. (10), who found that density was inversely associated with grade, differentiation, and mitotic index for interval cancers, using the cancer-free breast at the time of diagnosis.

This is contrary to the other two studies that showed a positive association with tumor grade but did not assess screen-detected and interval cancers separately (7, 8). As described by Aiello et al. (10), a potential explanation for the inverse association between density and tumor grade is that interval cancers in dense breasts may have been present at the screening mammogram, whereas those in fatty breasts may not have been present at screening and later appeared as higher-grade, rapidly growing tumors. Another hypothesis is that fatty breasts indicate a tissue environment that may be conducive to higher-grade tumors. This hypothesis is supported by a report on BMI and prognosis of breast cancer in which Daling et al. (24) found an association between higher BMI and markers of cell proliferation (Ki-67, mitotic count, and S-phase fraction) in breast cancers, suggesting rapid growth rate of tumors in overweight or obese women.

One strength of the current study is the semiautomated method to quantify mammographic density that has consistently been shown to be associated with breast cancer (20) and has high intra-reader reliability (12). In addition, a comprehensive pathology review of the tissue was done by a single expert pathologist blinded to density data. Choosing the ipsilateral breast 4 years before the breast cancer diagnosis reduces, although does not eliminate, the likelihood of the tumor contributing to mammographic density. This study, however, did not address the association between density and tumor characteristics among premenopausal women owing to the composition of our primarily postmenopausal case-control study. Because higher breast density is thought to contribute to the likelihood of a tumor either being missed (masking) or growing larger (owing to biology or causal relation), it may be possible that the lack of significance in our study compared with prior reports is related to the postmenopausal study population with generally lower breast density and reduced variability in the distribution of density, unlike previous studies that included premenopausal and postmenopausal women. Moreover, because the age at menopause was not available, we could not adjust for this variable.

This study could not assess screen-detected and interval cancers specifically owing to the paucity of information on the interval between the last negative screening mammogram and date of cancer diagnosis. However, we attempted to address this by assessing asymptomatic and symptomatic cancers separately. We, unfortunately, had limited power for subgroup analyses, including assessment of effect modification by interval between the mammogram and the breast cancer. This latter analysis could potentially be relevant because it is not possible to determine when the cancer actually developed in the breast, and time to cancer following the mammogram may influence what pathologic associations are present.

Finally, we did comparisons for multiple tumor characteristics and subsets. As a result, it is possible that some of the significant tests arose by random chance, as expected when multiple statistical tests are done. This, coupled with the lack of statistical significance, underscores the need for further study to better understand relationships between mammographic density and subsequent tumor characteristics among women who are ultimately diagnosed with breast cancer.

In summary, this report adds to the limited literature on mammographic density and subsequent breast tumor characteristics by suggesting that, in a population of regularly screened postmenopausal women, density measured in the ipsilateral breast 4 years or more before breast cancer was not associated with the type of tumor. The positive trend of tumor size with percent density and dense area measures, although nonsignificant, is consistent with prior reports and may be due to reduced sensitivity of the mammogram and delayed detection in dense breasts, resulting in larger size of tumors. An inverse association between density and tumor grade that was of borderline significance among all women but significant among symptomatic cancers only may be reflective of the underlying tumor biology. It may reflect high-grade tumors not present at the screening mammogram and presenting as interval cancers among women with fatty breasts. Although density was not associated with estrogen or progesterone receptor status overall, there was a significant association between estrogen and progesterone receptor status with percent density among symptomatic cancers likely related to tumor grade. Further studies assessing larger populations and premenopausal women and studies of screen-detected and interval cancers are warranted to enhance our understanding of this association.

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References

1. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356:227–36.
2. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1159–69.
3. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, Yaffe MJ. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
4. Boyd NF, Rommens JM, Vogt K, et al. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6:798–808.
5. Alowami S, Troup S, Al-Haddad S, Kirkpatrick I, Watson PH. Mammographic density is related to stroma and stromal proteoglycan expression. Breast Cancer Res 2003;5:R129–35.
6. Li T, Sun L, Miller N, et al. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:343–9.
7. Roubidoux MA, Bailey JE, Wray LA, Helvie MA. Invasive cancers detected after breast cancer screening yielded a negative result: relationship of mammographic density to tumor prognostic factors. Radiology 2004;230:42–8.
8. Sala E, Solomon L, Warren R, et al. Size, node status and grade of breast tumours: association with mammographic parenchymal patterns. Eur Radiol 2000;10:157–61.
9. Porter GJ, Evans AJ, Cornford EJ, et al. Influence of mammographic parenchymal pattern in screening-detected and interval invasive breast cancers on pathologic features, mammographic features, and patient survival. Am J Roentgenol 2007;188:676–83.
10. Aiello EJ, Buist DS, White E, Porter PL. Association between

mammographic breast density and breast cancer tumor characteristics. Cancer Epidemiol Biomarkers Prev 2005;14:662–8.
11. Haars G, van Noord PA, van Gils CH, Grobbee DE, Peeters PH. Measurements of breast density: no ratio for a ratio. Cancer Epidemiol Biomarkers Prev 2005;14:2634–40.
12. Vachon CM, Brandt KR, Ghosh K, et al. Mammographic breast density as a general marker of breast cancer risk. Cancer Epidemiol Biomarkers Prev 2007;16:43–9.
13. Moskowitz M. Breast cancer: age-specific growth rates and screening strategies. Radiology 1986;161:37–41.
14. Breast. In Greene FL, Page DL, Fleming ID, et al., editors AJCC: Cancer Staging Manual, Sixth edition. New York: Springer, 2002. p 229.
15. Mandelson MT, Oestreicher N, Porter PL, et al. Breast density as a predictor of mammographic detection: comparison of interval- and screen-detected cancers. J Natl Cancer Inst 2000;92:1081–7.
16. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629–38.
17. Boyd NF, Lockwood GA, Martin LJ, et al. Mammographic densities and risk of breast cancer among subjects with a family history of this disease. J Natl Cancer Inst 1999;91:1404–8.
18. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995; 87:670–5.
19. Ursin G, Ma H, Wu AH, et al. Mammographic density and breast cancer in three ethnic groups. Cancer Epidemiol Biomarkers Prev 2003;12:332–8.
20. Vachon CM, Pankratz VS, Scott CG, et al. Longitudinal trends in mammographic percent density and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2007;16:921–8.
21. Yaffe M, Boyd N. Mammographic breast density and cancer risk: the radiological view. Gynecol Endocrinol 2005;21 Suppl 1:6–11.
22. Ziv E, Tice J, Smith-Bindman R, Shepherd J, Cummings S, Kerlikowske K. Mammographic density and estrogen receptor status of breast cancer. Cancer Epidemiol Biomarkers Prev 2004;13: 2090–5.
23. Tamimi RM, Hankinson SE, Colditz GA, Byrne C. Endogenous sex hormone levels and mammographic density among postmenopausal women. Cancer Epidemiol Biomarkers Prev 2005;14: 2641–7.
24. Daling JR, Malone KE, Doody DR, Johnson LG, Gralow JR, Porter PL. Relation of body mass index to tumor markers and survival among young women with invasive ductal breast carcinoma. Cancer 2001; 92:720–9.