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Histologic changes in the breast with menopausal hormone therapy use: correlation with breast density, estrogen receptor, progesterone receptor, and proliferation indices

Jennifer A. Harvey, MD[1], **Richard J. Santen, MD**[2], **Gina R. Petroni, PhD**[3], **Viktor E. Bovbjerg, PhD**[3], **Mark E. Smolkin, MS**[3], **Fathima S. Sheriff, MD**[4], and **Jose Russo, PhD**[4]

[1]Department of Radiology, University of Virginia, Charlottesville, VA

[2]Department of Endocrinology, University of Virginia, Charlottesville, VA

[3]Public Health, University of Virginia, Charlottesville, VA

[4]Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA

Abstract

Objective—This retrospective study systematically compared mammographic density with histology in women receiving or not receiving menopausal hormone therapy (HT).

Design—This study was approved by the institutional review board. Twenty-eight postmenopausal women using HT were matched with 28 postmenopausal women not using HT at the time of breast cancer diagnosis. Noncancerous tissue from mastectomy specimens was examined histologically to quantitate the content of fibrous stroma, ducts, and lobule types 1, 2, and 3. Tissue samples were also evaluated for estrogen receptor, progesterone receptor, and Ki67 activity in the ducts and lobules. Breast density was quantified by digitizing the contralateral mammogram and computer-assisted interactive thresholding.

Results—High breast density in women using HT was correlated with greater fibrous stroma ($P = 0.020$) and lobule type 1 ($P = 0.016$). Breast density also correlated with Ki67 activity in the ducts ($P = 0.031$) and lobules ($P = 0.023$) for both groups combined. Estrogen and progesterone receptors did not correlate with either breast density or HT use.

Conclusions—Increased fibrous stroma and lobule type 1 are associated with increasing mammographic density in women using HT, independent of estrogen and progesterone receptor up-regulation. These findings suggest that increased breast density may be mediated through a paracrine effect. The increase in breast cancer risk with HT use may be due to an increase in target lobule type 1 cells.

Keywords

Breast; Mammography; Histology; Breast density; Hormone therapy

Address correspondence to: Jennifer A. Harvey, MD, Department of Radiology, University of Virginia, Charlottesville, VA 22908. jah7w@virginia.edu.

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Use of hormone therapy (HT) during menopause and mammographic breast density are both associated with an increased risk of breast cancer.[1-4] Specifically, the prospective, randomized Women's Health Initiative trial found a 26% increase in the relative risk of breast cancer for women using continuous combined estrogen plus progestogen therapy (EPT) over a 5-year period.[5] A large observational study, the Million Women Study, also found a twofold increased risk with EPT.[4] The risk from estrogen alone is not certain based on existing data. The Million Women Study reported a 30% increased relative risk of breast cancer with use of estrogen alone, whereas the prospective Women's Health Initiative trial did not confirm this finding.[6,7] The Nurses' Health Study has reported an increased risk of breast cancer, particularly estrogen receptor– and progesterone receptor–positive invasive carcinomas, with use of estrogen alone for more than 20 years.[8]

Mammographic breast density seems to be a biomarker for breast cancer risk.[9-11] Mammographic breast density is a strong predictor of risk of breast cancer developing over the ensuing 10 to 15 years of follow-up in postmenopausal women.[11] Menopausal HT increases mammographic density as assessed by quantitative methods, although this finding varies by regimen.[12,13] Similar to breast cancer risk, a higher percentage of women experience increased mammographic breast density when using EPT than those who use only estrogen therapy (ET).[13] Likewise, women using therapies that reduce breast cancer risk, such as tamoxifen[14,15] and raloxifene,[16-18] experience a decrease in mammographic breast density.

Prior studies have examined the histologic changes resulting from HT in a systematic fashion, and others have evaluated changes in breast density. Hofseth et al[19] have shown increased proliferation in the terminal ductal lobular unit as measured by proliferating cell nuclear antigen in women using HT as compared with nonusers. The increment was greater in response to EPT than to ET in the terminal ductal lobular unit. Greendale et al[20,21] and others[13] have shown that HT and particularly EPT increase mammographic density substantially over that with use of placebo or no therapy. However, no studies have correlated breast density and histologic changes in women using HT.

An increase in mammographic breast density might result from an absolute increase in fibrous stroma, ductal tissue, or terminal ductal lobular units or from a decrease in fat content with only relative increases in the other elements. Such changes would reflect alterations in proliferation, apoptosis, or differentiated function. The biochemical regulation of breast tissue proliferation and differentiated function in postmenopausal women is poorly understood at present. Inferences from studies in isolated breast cancer cells or cocultures of fibroblasts and cancer cells allow a focus on several concepts. For example, an increase in fibrous stroma could directly result in increased aromatase, an enzyme known by immunohistochemical analysis and real-time polymerase chain reaction to be present in fibroblasts.[22-24] The resulting increase in production of estradiol could then stimulate epithelial cells to proliferate. Alternatively, the stromal cells produce growth factors such as insulinlike growth factor 1 or proteoglycans, which could act in a paracrine fashion to stimulate epithelial cell proliferation directly.[25] Increased growth factor production, stimulated by the effects of exogenous estrogen, could directly enhance the effects of estradiol.

Precise quantitation of breast density has allowed the conclusion from at least 14 studies that mammographic breast density provides a powerful means to predict the risk of breast cancer for a 10- to 15-year period after the mammogram is taken.[26-28] Women older than 55 years whose breast density exceeds 75% have a greater than sixfold increased risk of breast cancer.[9,11] However, little is known about the histology of the dense tissue seen on mammography in postmenopausal women and particularly in those receiving HT. Although fibrous stroma has been reported as the primary difference between dense and nondense breast tissue,[29] no previous reports have correlated differences in breast epithelium, mammographic breast density, and use of HT.

Our review of the literature revealed a paucity of data correlating mammographic density, HT, and histologic findings in postmenopausal women. Thus, in this study, we sought to systematically compare mammographic density with histology in women receiving or not receiving HT. To obtain sufficient tissues, we used a strategy to examine the benign areas of mastectomy specimens in women for whom recent mammograms were available. In this study, we specifically evaluated mammographic breast density with a computerized method and evaluated correlations with histology as well as estrogen receptor (ER), progesterone receptor (PR), and proliferation indices.

Methods

This study was approved by our institutional review board. To obtain tissue for histologic examination, we identified all women who underwent mastectomy for breast cancer between January 1991 and December 1998 at our institution. We then initially selected HT users who met strict criteria and matched them to women who were nonusers. The criteria for selecting the HT case group were that the women were postmenopausal at the time of mastectomy and had used HT for at least 1 year before diagnosis. Criteria for establishing menopause were no menses for 1 year before mastectomy or a history of hysterectomy and age older than 55 years. Case patients were identified starting with women operated on in January 1991 and continuing sequentially through December 1998.

Study patients were matched to postmenopausal women not using HT at the time of diagnosis (control subjects). One control subject was matched to each case patient by age (within 5 y) and year of mammogram that resulted in the diagnosis of breast cancer (within 1 y).

Women were excluded if they had bilateral breast cancer, if they had had previous contralateral breast cancer, if they had undergone neoadjuvant chemotherapy, if they were premenopausal or perimenopausal or if menopause status was unknown, or if a mammogram or clinical history was not available. Control women were also excluded if they had used HT within the last year or were using tamoxifen for prophylaxis.

Clinical history obtained included age, number of pregnancies, number of live births, and age at time of menopause. If age at menopause was not available, the duration of menopause was estimated by subtracting 51 years from the participant's age, which is the average age at menopause in the United States.[30] Age at menopause was known for all postmenopausal

women younger than 50 years. The use, type, and duration of HT were obtained from the mammographic clinical history questionnaire and clinic notes.

Mammograms from the contralateral breast were digitized using a high-resolution Lumisys 75 scanner (Eastman-Kodak, Rochester, NY) at 8-bit depth, 2K by 2.8K, optical density range 0.0 to 3.8, and 50-μ resolution. Images were reduced in size (12-inch height) to view the entire image on the monitor. Computer-assisted measurement of percent breast density[31] was performed by one radiologist (J.A.H.), who was blinded to the use or nonuse of HT. Interobserver variability with this technique is less than 5%.[31] The contralateral breast was chosen because breast cancer can elevate percent breast density in the affected breast, so the density of this breast may not accurately reflect the underlying breast density.

Tissue blocks from the mastectomy specimen made at the time of mastectomy were retrieved. Standard practice at the University of Virginia is to sample each quadrant of the breast at the time of accessioning. Paraffin blocks found to contain cancer on routine histologic sectioning were excluded. Five-micron sections of the selected blocks, free of cancer, were used for conventional staining with hematoxylin and eosin and immunocytochemical reactions against ERs (monoclonal, clone 185, Dako, Carpinteria, CA) and PRs (monoclonal, clone PgR636, Dako). Cell proliferation was determined with Ki67, a mouse monoclonal antibody raised against a human recombinant peptide corresponding to a 1,002-base pair Ki67 cDNA fragment (Oncogene Science, Cambridge, MA).

The slides stained with hematoxylin and eosin were used for determining the type of lobular structures (ductal structures, lobule type 1 [Lob1], lobule type 2 [Lob2], and lobule type 3 [Lob3]) using a classification described previously.[32] The amounts of fibrous stroma and fat were visually estimated using the percentage of area covered in the 10× field of the microscope.

The immunocytochemical reactions were performed in tissues fixed in formalin, dehydrated, and embedded in paraffin cut at 5-μm thickness. Tissue sections were mounted on aminoalkylsilane-coated or positively charged slides, deparaffinized, rehydrated, and incubated in 2% hydrogen peroxide at room temperature for 15 minutes for quenching endogenous peroxidase activity. The sections were sequentially incubated in two changes of target retrieval solution at 98°C for 5 minutes each. All the tissue sections were incubated in diluted normal blocking serum for 20 minutes. Excess serum was blotted from the slides, and the sections were incubated with mouse monoclonal anti-specific protein at a dilution of 1:400. This step was followed by incubation in a humidity chamber at 4°C overnight and washing in buffer. The sections were then incubated with horse anti-mouse biotinylated secondary antibody (Vector Laboratories, Inc, Burlingame, CA) at room temperature for 30 minutes. After a buffer rinse, the slides were incubated for 30 minutes with Vectastain Elite ABC kit for mouse (Vector Laboratories), washed in phosphate-buffered saline buffer, and incubated in peroxidase substrate solution containing hydrogen peroxide and 3,3-diaminobenzidine HCl for 2 minutes. Sections incubated with nonimmune serum were used as negative controls. All sections were lightly counterstained with hematoxylin. Immunostaining was evaluated by examination of slides under a bright-field microscope and graded according to the intensity of the brown staining as negative (−) or positive (+). The

numbers of cells expressing the nuclear antigen Ki67 were counted and tabulated according to their location in ducts, Lob1, Lob2, and Lob3. Results were expressed as the percentage of positive cells over the total number of cells counted in each lobular structure. All the observations were done by one author (J.R.), who was blinded to the source of the tissue examined.

Statistical methods

Case patients were matched to postmenopausal women not using HT at the time of diagnosis (control subjects). This was a 1 to 1 case-control matched study with matching occurring by age (within 5 y) and year of mammogram that resulted in the diagnosis of breast cancer (within 1 y). In situations in which more than one eligible control subject could be matched to a case patient, a random control subject was chosen from among the eligible matches. Random numbers were generated using the RANUNI function in SAS, which returns a random variate that is generated from the uniform distribution on the interval (0,1), given an initial seed.

Sign and signed-rank tests were used to assess significant differences between the HT and non-HT groups for clinical and demographic parameters. Mixed linear effect models were used to assess relationships between the outcome variable, breast density, and histologic variables. To account for correlation between matched patients, a compound symmetric correlation structure was assumed. Using this modeling approach, the relationship between each potential predictor and breast density was assessed, adjusting for group and age. Interaction effects between each predictor and group on the outcome variable breast density were explored. All comparisons are reported at the two-sided 5% level of significance without adjustments for multiple comparisons. Spearman partial correlations were calculated between breast density and each available parameter separately within the HT and non-HT groups while adjusting for age and overall adjusting for both HT status and age.

Results

Between January 1991 and December 1998, 327 postmenopausal women underwent mastectomy at our institution. One hundred eighty women were excluded because of unavailable mammograms and/or clinical history (n = 128), prior mastectomy or bilateral breast cancer (n = 50), tamoxifen use for prophylaxis (n = 1), or use of neoadjuvant chemotherapy (n = 1). Of the remaining 147 women, 32 were using menopausal HT at the time of mastectomy. Four women were subsequently excluded for the following reasons: the breast was diffusely involved with carcinoma, and no tissue blocks containing uninvolved tissue could be located (n = 2); no blocks could be found (n = 1); or a match could not be identified (n = 1). The remaining 28 women were evaluated as the HT users. Thirteen women in this group used ET, 10 used EPT, and type of HT was unknown for 5 women. Duration of HT use was at least 1 year in 15 women, at least 2 years in 6 women, at least 3 years in 2 women, at least 4 years in 2 women, and longer than 5 years in 3 women. One hundred fifteen postmenopausal women not using HT for at least 1 year before diagnosis were identified as possible control subjects. Twenty-eight of these women were randomly selected as the control sample on the basis of the criteria listed in “Methods”.

The overall median age of the HT users and nonusers was 60 years and did not differ between groups (Table 1). The overall mean difference in age between HT users and nonusers was 0.5 years, and within matched pairs the subjects differed by an average of 2.3 years. Numbers of pregnancies, number of childbirths, and duration of menopause were not significantly different between the HT users and nonusers.

Breast density by HT use

There was a statistically significant difference in breast density noted between the HT and non-HT groups ($P < 0.001$) with a median difference in breast density between matched pairs of 23% (54% for HT users and 31% for non-HT users). By using mixed linear effect models, significant clinical predictors of breast density adjusting for HT usage and age included nongravid compared with gravid ($P = 0.002$) and nulliparous compared with parous ($P = 0.034$).

Histology by HT use

Percent fibrous stroma was 7% higher for HT users compared with nonusers (Table 2). However, this difference was not found to be statistically significant ($P = 0.31$). Neither the number of lobules nor the number of ducts differed significantly between the groups ($P > 0.30$) (Table 2). The type of lobules identified were all Lob1 except for three HT users and one nonuser who had mixed Lob1 and Lob2. No patients exhibited Lob3.

Differences between ER, PR, and Ki67 activity associated with HT usage were examined in both ducts and Lob1 (Table 3). Greater PR and Ki67 activities were found in the ducts of those not treated with HT ($P = 0.019$ and $P < 0.001$). No significant differences were seen in ERs in ducts or Lob1 or in PR and Ki67 activities in Lob1 between groups.

Histology by breast density

Using Spearman partial correlations, increased breast density in women using HT was associated with increased fibrous stroma ($P = 0.020$) and Lob1 ($P = 0.016$) (Table 4) but not ducts. Conversely, in nonusers of HT, increased breast density was associated with greater numbers of ducts ($P = 0.022$) but not fibrous stroma or lobules. Overall, increased breast density was significantly associated with greater fibrous stroma ($P = 0.005$) and a greater number of Lob1 ($P = 0.021$), adjusting for HT use and age. With mixed linear effect models, no significant differences in ERs or PRs were observed with increased breast density. Increased Ki67 activities in the ducts ($P = 0.031$) and Lob1 ($P = 0.023$) were also seen in association with increasing breast density for both groups combined.

Discussion

In the current study we sought to determine what histologic changes are present to explain the increase in breast density that occurs in postmenopausal women using HT. Our prior expectation was that ductal tissue, epithelial tissue, or terminal duct lobular units would be increased in quantity in women taking HT. This concept was based on the fact that ductal and lobular tissues in breast respond to HT during the pubertal period in girls and in various animal species. Unexpectedly, however, we found a statistically significant correlation

between an increase in breast density and fibrous stroma ($r = 0.44$, $P = 0.020$) in hormone users but not in nonusers ($r = 0.28$, $P = 0.16$). Although not statistically significant, the hormone users also had a higher percentage of stroma (42% vs 35%). These findings are surprising because human breast stroma is not thought to be an endocrine-responsive tissue. Stromal tissue in the human breast seems to contain no ERα. Although ERβ are present, their function is thought to be antiproliferative in breast.[33] Based on these considerations, our findings in this study on stroma are remarkable and require explanation.

An increasingly important focus in human breast physiology has been the study of stromal-epithelial interactions. A recently developed model has, for the first time, enabled examination of these interactions with human breast elements. Parmar and Cunha[34] implanted mammary fibroblasts together with mammary epithelial cells as xenografts under the renal capsules of female nude mice. These studies demonstrated the ability of stromal elements to interact with epithelial cells to enhance stimulation of mammary cell proliferation with estrogens. When our study is interpreted in light of the role of stroma in mammary gland physiology, it is not totally surprising that HT might be associated with increased stromal tissue in breast.

We consider our findings about HT and stroma to be highly novel and to raise several physiologic questions about how HT could increase the amount of breast stroma. Recent studies have shown that estrogens can increase the levels of fibroblast growth factors, particularly of fibroblast growth factor 2.[35] The role of fibroblast growth factor 2 differs depending on the system tested but can affect wound healing and fibrosis as well as arterial restenosis and blood vessel remodeling. Other mitogens for stromal cells could also be stimulated by exogenous hormones given to postmenopausal women. Based on these considerations, we raise the hypothesis that HT could stimulate growth factors that would enhance the degree of stroma in the human breast. This intriguing hypothesis could explain our findings and will now need to be studied in more detail.

A second important finding in our study is that increased breast density was significantly associated with Lob1 ($P = 0.016$) as well as Ki67 activity in the ducts ($P = 0.031$) and Lob1 ($P = 0.023$) for both groups. This finding may explain why HT increases the susceptibility of the breast to cancer: it increases the number of target cells or structures to be affected, given that ductal carcinoma in situ starts in the ductules of Lob1.[36,37] Dual effects of the hormones are observed here. First, activation of cell proliferation in the breast epithelium of the postmenopausal breast results in formation of more branching from the ducts, leading to the formation of Lob1, which therefore explains the higher number of Lob1 in the HT group in association with increased breast density. Second, the formation of Lob1 from the ductal structures is associated with the increase in the fibrous stroma because both processes go together.[37]

Known concepts about receptor physiology in the normal breast require comment. The content of ERs and PRs in the normal breast tissue, as detected by immunocytochemistry, varies with the degree of lobular development in a linear relationship with the rate of cell proliferation of the same structures.[38] The use of a double-labeling immunocytochemical technique for staining in the same tissue section of steroid hormone receptors and

proliferating cells, ie, Ki67 positive, allowed us to determine that the expression of the receptors occurs in cells other than the proliferating cells. The findings that proliferating cells are different from those that are ER and PR positive support data indicating that estrogen controls cell proliferation by a paracrine mechanism, and the results presented in this article clearly demonstrate that this cell proliferation is not related to the expression of ERs or PRs. This phenomenon has been demonstrated using supernatants of estrogen-treated ER-positive cells that stimulate the growth of ER-negative cell lines in culture. The same phenomenon has been shown in vivo in nude mice bearing ER-negative breast tumor xenografts.[38]

Neither greater mammographic breast density nor HT use was associated with increased ER or PR activity in our study. This finding implies that the mechanism for the observed increase in fibrous stroma is not up-regulation of endocrine receptors. The increase in proliferation of stroma may therefore be due to increased levels of growth factors or alternative methods of up-regulation of endocrine pathways, such as aromatase activity or increasing the metabolic pathway of catechol formation. The Nurses' Health Study showed higher serum levels of insulinlike growth factor 1 and lower levels of insulinlike growth factor–binding protein 3 in premenopausal women with greater mammographic breast density, although the effect was not observed in postmenopausal women.[39] We are not aware of any studies evaluating aromatase activity in women using HT or correlating the association with breast density. We plan to evaluate aromatase activity by immunohistochemical staining using this same population set. Whereas this postulate requires further investigation, the data strongly support the concept that HT, in modifying the structure of the breast by increasing the number of target cells, creates an adequate milieu for cancer formation.

The results of our study differ from those of Hofseth et al,[19] who found a significant increase in proliferation in the breast lobules of women using HT compared with those not using HT as observed by proliferating cell nuclear antigen activity and the trend observed with Ki67 activity. These differences may be due to the use of Ki67 to assess proliferation rather than proliferating cell nuclear antigen. Alternatively, our study may have insufficient statistical power to observe a significant difference.

Our findings of increased breast density in association with HT use confirm the results of several other prospective and retrospective studies.[13],[20],[21] However, the degree of difference in mammographic density in the hormone users was more robust than previously reported. The median difference between matched pairs of hormone users and nonusers was 23% (54% for HT users and 31% for nonusers). This degree of difference was unexpected since the Postmenopausal Estrogen/Progestin Interventions Trial, using similar methodology to quantify breast density, showed only modest increases in breast density.[21] For example, a 1.17% increase in breast density occurred in response to conjugated equine estrogens alone, a 4.76% increase with conjugated equine estrogens plus cyclic medroxyprogesterone, a 4.58% increase with conjugated equine estrogens and continuous medroxyprogesterone, and a 3.089% increase with conjugated equine estrogens plus micronized progesterone.

The Postmenopausal Estrogen/Progestin Interventions Trial involved changes in mammographic density during exposure to HT for only 1 year. In our study 18 of the 28 women used HT for 2 or more years. It is possible, then, that the greater increase in breast density in HT users in this study reflects the prolonged nature of use. On the other hand, all women in this study had a diagnosis of breast cancer. These subjects could be more sensitive to the effects of hormones on breast density than average-risk women, as were those in the Postmenopausal Estrogen/Progestin Interventions trial. Further studies will be needed to test this hypothesis.

Our finding of an association of increasing mammographic breast density with greater numbers of ducts in women not using HT is also novel. This result may be due to the increased incidence of duct ectasia in postmenopausal women. The primary dense areas in postmenopausal women not using HT may represent ectatic ducts with involution of the majority of the lobular tissue.

A significant limitation of our study is the retrospective use of archived breast tissue from prior mastectomies. When mastectomy specimens are accessioned, the densest area in each quadrant of the breast is sampled to evaluate for additional foci of carcinoma. These samples are made into tissue blocks. This process may explain some lack of correlation of breast density and fibrous stroma in our study. The results of this study could be best validated by prospectively obtaining representative breast tissue from each quadrant of the mastectomy specimen, which may show better correlation between percent breast density and percent fibrous stroma. In addition, our study had a small number of women. We were also unable to obtain body mass information to correlate with breast density changes. Only one reader was used to measure breast density (J.A.H.) and histology (J.R.), which is a confounding factor in our study. A larger study would be helpful to better characterize histologic changes associated with HT use and greater mammographic breast density and might allow evaluation of changes by type of HT.

Conclusions

In summary, we report the novel finding that greater mammographic breast density is associated with increased fibrous stroma. We also found an increase in Lob1 as well as increased lobular proliferation with HT use. The lack of increase in ER or PR activity with increasing breast density or HT use implies that the process of increasing breast density is not directly receptor mediated but could involve the paracrine effects of growth factors.

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TABLE 1

Demographic characteristics of study participants

	Nonusers	HT users
Age at surgery, y		
Median	60	60
Mean	62.2	61.7
Range	45-84	49-85
Nulliparity, n (%)	3 (11)	3 (11)
No. of pregnancies		
Median	3	3
Mean	3.9	3.4
Range	1-12	1-8
No. of live births		
Median	3	2
Mean	3.6	2.8
Range	1-12	1-7
Duration of menopause, y, median (range)	10 (2-34)	10 (1-34)

HT, hormone therapy.

TABLE 2

Histologic components by HT use or nonuse

	HT use	No HT
Mean fibrous stroma, %	42	35
Degree of lobules seen		
0	13	11
1+	8	11
2+	5	3
3+	2	3
Degree of ducts seen		
0	9	4
1+	13	18
2+	4	2
3+	2	4

HT, hormone therapy.

TABLE 3

Overall mean and median values for estrogen receptor, progesterone receptor, and Ki67 activity in lobule type 1, lobule type 2, and ducts, as well as the distribution of matched pair (for women using and not using HT) differences for each variable

	Overall		Differences matched			
Variable	**Mean**	**Median (range)**	**10%**	**Median**	**90%**	***P***
ER Lob1	5.4	0.1 (0-39.3)	−7.9	0.7	17.2	0.21
ER Lob2	0.2	0.0 (0-3.6)	0.0	0.0	2.9	ND
ER ducts	11.9	6.8 (0-55.4)	−30.3	−2.6	10.1	0.18
PR Lob1	6.4	0.6 (0-51.0)	−25.5	0.2	21.2	0.62
PR Lob2	0.3	0.0 (0-9.3)	0.0	0.0	0.0	ND
PR duct	13.7	11.4 (0-57.6)	−35.6	−10.7	19.9	**0.019**
Ki67 Lob1	0.5	0.0 (0-7.0)	−2.7	0.0	0.5	0.11
Ki67 Lob2	0.0	0.0 (0-0.9)	0.0	0.0	0.0	ND
Ki67 ducts	0.7	0.2 (0-6.1)	−3.1	−0.6	0.1	**<0.001**

ER, estrogen receptor; Lob1, lobule type 1; Lob2, lobule type 2; ND, not done; PR, progesterone receptor. *P* values in bold indicate statistical significance.

TABLE 4

Partial correlations (Spearman) between increasing mammographic density and histology findings

	HT	No HT	Overall
Fibrous stroma	$r = 0.44$ **($P = 0.020$)**	$r = 0.28$ ($P = 0.16$)	$r = 0.38$ **($P = 0.005$)**
Lobules	$r = 0.46$ **($P = 0.016$)**	$r = 0.13$ ($P = 0.51$)	$r = 0.31$ **($P = 0.021$)**
Ducts	$r = 0.04$ ($P = 0.86$)	$r = 0.44$ **($P = 0.022$)**	$r = 0.20$ ($P = 0.14$)

HT, hormone therapy. *P* values in bold indicate statistical significance.