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Ki-67 Expression in Sclerosing Adenosis and Adjacent Normal Breast Terminal Ductal Lobular Units: A Nested Case-Control Study from the Mayo Benign Breast Disease Cohort

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Abstract

Purpose—Sclerosing adenosis (SA) increases risk for invasive breast cancer (BC) 2.1 times relative to that in the general population. Here, we sought to evaluate whether the proliferation marker Ki-67 stratifies risk among women with SA.

Methods—A nested case-control sample of patients with SA was obtained from the Mayo Clinic Benign Breast Disease Cohort. Ki-67 expression was evaluated in SA lesions and in the adjacent normal terminal duct lobular units (TDLU) in women who did (cases, $n=133$) or did not (controls, $n=239$) develop BC. Ki67 was scored by intensity and number of positively stained cells per one high-power field (magnification, $\times 40$) ($40\times$ HPF) for both SA and normal TDLU. Associations of Ki-67 expression with case-control status were assessed using conditional logistic regression.

Results—Higher Ki-67 expression was significantly associated with case-control status in both SA ($P=.03$) and normal background TDLU ($P=.006$). For the SA lesion, >2 average positively stained cells/ $40\times$ HPF showed an adjusted odds ratio (OR) of 1.9 (95% CI, 1.1–3.4) compared to samples with an average of ≤ 2 positively stained cells. For background TDLU, lobules with >2 but ≤ 6 average positively stained cells showed an adjusted OR of 1.3 to 1.5, whereas those with an average of >6 positively stained cells had an OR of 2.4 (95% CI, 1.1–5.3) compared to those with an average of ≤ 2 positively stained cells.

Conclusions—Among women with SA, increased Ki-67 expression in either the SA lesion or the normal background TDLU carried an approximately 2-fold increased odds of subsequent BC as compared to lower Ki-67 expression.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors

This research has been approved by the institutional review board of the Mayo Clinic for conducting research under ethical standards

All authors declare that they have no conflict of interest.

Keywords

benign breast disease; breast cancer; Ki-67; sclerosing adenosis; terminal duct lobular unit

Introduction

Sclerosing adenosis (SA) is a benign lobulocentric lesion of disordered acinar, myoepithelial, and connective tissue elements that can mimic infiltrating carcinoma both grossly and microscopically [1]. SA is a form of fibrocystic change with unknown etiology that is a combination of adenosis (epithelial and myoepithelial proliferation of lobules) and stromal sclerosis [2]. The incidence of SA has been found to decline after menopause, perhaps as an abnormal pattern of age-related regression or an abnormal involutional process after lactation [3–5]. SA is strongly associated with a complex of various lesions, including calcification, moderate to florid epithelial hyperplasia, apocrine change, intraductal papillomas, and atypical lobular hyperplasia [1]. It is found in association with atypical lobular hyperplasia almost 3 times as often as in otherwise benign breast specimens [1,3]. SA commonly occurs in perimenopausal women and has a weak association with a family history of invasive breast carcinoma [1].

A diagnosis of SA is associated with a 1.5 to 2.1 times relative risk for development of breast cancer (BC), independent of its association with other proliferative lesions; thus, it is included with histologic lesions designated as proliferative disease without atypia [1,6,7]. In one study, the risk for patients with both SA and atypical hyperplasia was 6.7 compared to only 1.7 for patients with SA alone [1].

The proliferation marker Ki-67 is expressed in normal breast tissue, atypical hyperplasia, and carcinoma [8–10]. Higher Ki-67 expression correlated with poorer clinical outcome in patients with invasive BC [10] and with increased risk of subsequent BC in women with atypia [9].

We hypothesize that measures of proliferative index might identify women with increased risk of subsequent BC. Using a monoclonal antibody that recognizes Ki-67, the nuclear antigen present in proliferating cells, we evaluated the proliferation status of SA and neighboring normal terminal duct lobular units (TDLU) in women who developed BC (cases) compared with controls matched by year of biopsy and age [11]. Additionally, we assessed the association of Ki-67 expression in SA and adjacent normal TDLU with age, histologic impression, family history of BC, and lobular involution status.

Materials and Methods

Study Population

Patients with SA on a benign biopsy were randomly selected for this nested case-control study from an institutional cohort with benign breast disease (BBD) known as the Mayo BBD Cohort. Earlier reports have described cohort inclusion and exclusion criteria, characteristics, and available pathologic specimens [6,12]. The Mayo Clinic Institutional Review Board approved this study.

The study population included 9,076 women aged 18 to 85 years who had surgical excision of a benign breast lesion at Mayo Clinic, Rochester, Minnesota, between January 1, 1967, and December 31, 1991. The histologic slides of these women were reviewed by an expert breast pathologist (DWV) for characterization of the different histologic lesions including SA, as part of the initial cohort development, and subsequently reviewed by another breast pathologist (AN) for confirmation of SA lesions in the study sections. BC events and family history (negative, weak, or strong) were obtained from medical records and questionnaires. A strong family history was defined as at least 1 first-degree relative with BC before age 50 years or as 2 or more relatives (1 first-degree) with BC.

SA was defined as a tumor-like lobulocentric proliferation of epithelium and myoepithelium classified as a proliferative lesion (Figure 1). SA was included in the “proliferative fibrocystic changes” category if present in at least 2 foci, with at least 1 having a diameter greater than 1 normal lobule (Figure 1); otherwise, it was considered nonproliferative. These criteria distinguished adenosis from lobular involution with sclerosis.

Study Samples

This study was designed as a nested case-control study among the Mayo BBD cohort subset of women with pathologic findings of SA. Cases were women who developed BC during follow-up, whereas controls remained cancer free longer than the time to cancer in matched cases. Cases and controls were additionally matched by age and year at biopsy. For inclusion, a sample required at least 1 SA lesion for scoring. The planned sample size was 300 controls matched 2:1 to 150 cases to provide 80% power to detect odds ratios (ORs) of 2.0, assuming exposure frequencies as low as 20% and a correlation of exposure between cases and controls of 0.2 for a 2-sided test with $\alpha=.05$.

Immunohistochemistry Staining

Immunohistochemistry staining was performed using the Leica Bond III Stainer (Leica Microsystems Inc, Buffalo Grove, Illinois). An ethylenediaminetetraacetic acid-based solution (Epitope Retrieval Solution 2; Leica Microsystems Inc) was used for Ki-67 for 20 minutes at 100°C. Samples were treated with 3% H₂O₂ for 5 minutes to inactivate endogenous peroxides. The primary antibody was incubated for 15 minutes at room temperature. Slides were incubated with a reference mouse monoclonal primary antibody against Ki-67 (Clone Mib-1; 1:300 dilution; Dako North America Inc, Carpinteria, California; Agilent Technologies Inc). The detection system was a 2-step polymer detection system (Bond Polymer Refine Detection; Leica Microsystems Inc), with each step incubated for 8 minutes.

Measurements

Ki-67 staining was scored using manual analysis based on 1) the number of positively stained cells per one high-power field (HPF) (magnification, $\times 40$) (40 $\times$ HPF) and 2) intensity (no staining, weak, moderate, or strong). For each sample, up to 3 SA lesions and 3 normal TDLUs were scored individually, as available. For samples with more than 3 SA lesions or normal TDLUs, those with highest Ki-67 expression were scored. Intensity scores were assigned using numeric values: 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3

(strong staining). A product score was created by multiplying the number of positively stained cells/40×HPF by the intensity score.

Aperio image analysis (IA) quantitation was performed in a subsample for comparison to manual scores. Ki-67–stained sections from a random sample of 40 patients with proliferative SA were analyzed manually (using randomly selected lobules rather than ones with highest Ki-67 expression as described above) and digitally using digital microscopy hardware and software (Aperio Technologies Inc, Vista, California). In each section, 3 SA lesions and 3 normal TDLUs were selected (using an adjacent hematoxylin-eosin–stained section as a reference), and Ki-67 positive nuclei were measured manually and digitally. For manual counts, the number of positively stained cells per randomly chosen 40×HPF field was recorded. For digital counts, all slides were scanned at 20×, producing high-resolution images annotated using ImageScope analysis software (Aperio Technologies Inc). The number of positively stained cells in the entire lobule or lesion was calculated using a custom-designed nuclear ImageScope algorithm that identified nuclei and detected optical density of the chromogen of the Ki-67 immunostain. In each case, the number of positive cells was divided by the area to create a cell/area density; these values were multiplied by 1,000² to convert from cells/micrometer² scale to cells/mm².

Statistical Methods

For analysis, scores from the 1 to 3 measured lesions of each type (SA and normal TDLU) were summarized using the mean per lesion type. Analysis of the maximum value was performed but showed results similar to the average scores, so data are presented only for average scores. The association of Ki-67 staining with case-control status was assessed using conditional logistic regression to account for the matched case-control design. Restricted cubic splines with 3 knots placed at the 10th, 50th, and 90th percentiles were used to examine the functional form of the relationship between Ki-67 positively stained cells/40×HPF and case-control status [13]. Due to a strong right skew for both the number of signals and the product score, rank transformations were used in fitting statistical models. Odds ratios (ORs) were also estimated in quartile categories derived from the empirical distribution among controls. The association of Ki-67 staining with other variables, such as degree of lobular involution and age at BBD, was examined with generalized linear models to account for correlation within matched sets in estimating *P* values. The relationship between scores for SA lesion and normal TDLU within a sample was analyzed using the Wilcoxon signed-rank test and Spearman rank correlations. Samples with sufficient tissue for inclusion were compared to samples excluded for unavailable or insufficient tissue using the χ^2 and 2-sample *t* tests. Agreement between manual and digital Ki-67 measures was assessed using the intraclass correlation coefficient (ICC) and the Wilcoxon signed-rank test to test for bias between the 2 measures. Descriptive statistics for continuous variables were generally reported using the median and interquartile range (IQR) except where otherwise noted. *P* < .05 was considered statistically significant. Statistical analysis was performed using SAS (version 9.3; SAS Institute Inc, Cary, North Carolina) and R (version 3.0.2; <http://www.r-project.org/>), including the rms package [14].

Results

Patients

The original matched case-control set included 150 cases and 300 controls. Upon review, not all samples were usable, most frequently because of insufficient sample (e.g., no SA lesion remaining in the block available for sectioning). If 1 control remained usable, a second replacement control was not identified. An attempt was made to replace case-control pairs that had either the case or both controls excluded. Seventeen originally matched cases and 61 originally matched controls were not usable and not replaceable. Thus, the sample size for final analysis included 133 cases matched to 239 controls (106 cases with 2 controls; 27 cases with 1 control). Excluded samples showed a significantly lower percentage with a palpable lump and both a higher degree of involution and older age (mean age, 59 years vs 52 years; $P<.001$), differences suggesting that excluded samples had somewhat smaller SA lesions and more involuted lobules than included samples. Characteristics of the included samples are summarized in Table 1. Mean [73%]), whereas few had a strong family history of BC. A palpable lump was the indication for biopsy in most (214/358 [60%]) when this information was available. In addition to SA (present in all samples), nearly 10% (37/372) overall had atypical hyperplasia (22/133 [17%] cases; 15/239 [6%] controls). Median time to cancer among cases was 12 years (range, 1–33 years), and median length of cancer-free follow-up among controls was 22 years (range, 3–40 years).

Ki-67 Expression in SA Lesions

SA was scored in 372 subjects (133 cases; 239 controls). At least 3 SA lesions were available for scoring in most samples (297/372 [80%]), whereas 17% (62/372) had 2 SA lesions scored and 3% (13/372) had 1 SA lesion scored; the number of SA lesions scored did not differ significantly between cases and controls. Nearly all SA lesions (959/1,028 [93%]) showed some degree of Ki-67 immunostaining (expression in 1 nuclei/field) (Figure 2). Generally, the number of positively stained cells/40×HPF showed more discrimination between cases and controls than did the intensity or product scores. The Ki-67 expression median score was 4.67 and 4.00 positively stained cells/40×HPF in cases and controls, respectively ($P=.03$) (Table 2). However, examination of a restricted cubic spline fit (Figure 3A) suggested a nonlinear functional form, with risk increasing initially but then flattening out and remaining stable. This relationship is also evident in Table 3, where the 2 highest quartiles have similar odds of BC, with ORs of approximately 2 relative to the first quartile. After adjustment for key factors known to affect BC risk (age, histologic impression, degree of lobular involution, family history of BC), all categories with >2 positively stained cells/40×HPF each had approximately a 2-fold increase in the odds of BC relative to those with 2 positively stained cells (adjusted OR, 1.9 [95% CI, 1.1–3.4]; $P=.03$).

Ki-67 Expression in Normal TDLU

At least 1 normal TDLU was available for scoring in 343 subjects (119 cases; 224 controls) (Figure 4). Generally, the normal TDLUs showed fewer positively stained cells/40×HPF than the SA lesions, but the difference was small (median paired difference, −0.5; interquartile range [IQR], −3 to 1; $P<.001$). Comparing the SA lesion average to the normal TDLU average within sample, the SA lesion showed a higher Ki-67 count in 57%, while the

normal was higher in 36%, and they were the same in 7%. Ki-67 expression in normal TDLUs showed moderate correlation with expression in SA lesions ($r=0.59$). Median Ki-67 expression was 3.67 and 3.00 positively stained cells/40×HPF for cases and controls, respectively ($P=.006$) (Table 2). The number of Ki-67 signals in normal TDLUs remained significant ($P=.03$) after adjustment for standard variables associated with BC risk (age, histologic impression, degree of lobular involution, family history of BC). As with Ki-67 in the SA lesion from each sample, Ki-67 in normal TDLU from each sample demonstrated nonlinear functional form, with odds of BC increasing but then leveling off across higher values (Figure 3B). ORs by quartile (Table 3) showed an unadjusted OR of 1.6 for women with Ki-67 expression in the normal TDLU of >2 to 6 positively stained cells; the OR was 2.6 (95% CI, 1.4–5.0) in women with >6 positively stained cells. After adjustment, the odds of BC remained significantly increased in patients with >6 average signals in normal TDLU relative to those with 0 to 2 signals (OR, 2.4 [95% CI, 1.1–5.3]; $P=.03$). Again, the intensity score did not differ significantly between cases and controls.

Association of Ki-67 with Other Characteristics

The number of Ki-67 positively stained cells in normal TDLUs was significantly associated with degree of lobular involution ($P=.001$), with the highest expression in women with no lobular involution (median, 4.7 signals), followed by partial involution and then complete involution, with a median of 3.3 and 2.7 positively stained cells/40×HPF, respectively. Similarly, normal TDLU Ki-67 expression decreased significantly with increasing patient age ($r=0.21$, $P<0.0001$). Ki-67 expression in normal TDLUs did not differ significantly from that in SA samples with or without atypical hyperplasia (median, 3.3 positively stained cells/40×HPF per group; $P=.33$); however, Ki-67 expression in the SA lesion differed significantly between that in the SA samples with and without atypia (median, 3.3 vs 4.3 positively stained cells/40×HPF, respectively; $P=.02$). Women with any family history of BC also showed significantly higher Ki-67 expression in both the SA lesion ($P<.001$) and the normal TDLU ($P=.02$). We found no significant association between Ki-67 expression in SA and time to cancer in cases with a median of 12.3 years vs 11.3 years in those with high (>2 average signals) vs low (≤ 2 average signals) Ki-67 expression ($P=.18$); the same was true for Ki-67 in the normal TDLU.

Manual and Digital Ki-67 Quantitation Using Aperio IA

Manual and digital Ki-67 cell counts were collected for 102 SA lesions and 108 normal TDLUs from a randomly selected subsample of 40 patients to assess concordance. Aperio digital IA showed at least 1 positive Ki-67 cell in most (93/102 [91%]) SA lesions and (81/108 [75%]) normal TDLUs. The median digital count of Ki-67 cell density was 24.9 cells/mm² for SA lesions (IQR, 6.4 to 64) and 25.3 for adjacent normal TDLUs (IQR, 1.5 to 75.1). No statistically significant difference was found between manual and digital density for SA lesions ($P=.94$) or normal lobules ($P=.26$), with median paired differences of 1.9 (IQR, −12.9 to 10.7) and 0 (IQR, −11.2 to 19.0) cells/mm², respectively, suggesting no significant bias between manual and digital assessments. Digital and manual density measures showed excellent agreement (ICC, 0.86 for SA lesions and 0.94 for normal TDLUs, Figures 5A and 5B).

Discussion

This study is the first to address Ki-67 as a biomarker specific to SA, a benign proliferative lesion associated with an approximate doubling of risk for BC relative to the general population [7]. This study characterized Ki-67 expression as the number of positively stained cells/40×HPF, due to the relatively low frequency of these cells in benign breast tissues compared to cancers.

In this nested case-control study of BBD patients with 1 SA lesions, higher Ki-67 expression in either the SA lesion or the adjacent normal TDLU was associated with increased odds of BC during follow-up. The increased risk did not appear to increase linearly with increasing numbers of Ki-67 positively stained cells but rather increased to a threshold level and stayed relatively stable. For SA lesions, the expression of Ki-67 at a rate of >2 average positively stained cells/40×HPF was associated with an increased risk for future development of BC. Whereas for normal TDLUs, the expression of Ki-67 at a rate of >6 average positively stained cells/40×HPF increases a woman's risk for developing BC. The findings with respect to normal TDLUs are particularly compelling in that histologically normal lobules from the biopsy slide show differential Ki-67 associated with future BC risk.

These results are generally consistent with the literature on Ki-67 as it relates to BC in finding that higher Ki-67 expression is associated with increased risk for BC. In this study, Ki-67 expression was characterized as the number of positively stained cells/40×HPF, which is different from the percentage of cells positive measure used by researchers and clinicians in BC, thus precluding direct comparisons to results from other studies. We chose to take this approach because Ki-67 expression in normal TDLU and SA is low with few signals in the whole section, so it is much easier and simpler to count the number of positively stained cells rather than percentage of cells expressing the biomarker. Yet, these results suggest that, regardless of differences in scale of measurement, Ki-67 has a role in proliferation and is associated with BC development in patients with SA. The excellent agreement observed between manual and digital counts collected in a subsample suggests that measurement of Ki-67 in SA is reproducible and helps validate our results.

Although Ki-67 was significantly associated with case-control status, the distributions showed substantial overlap, with many controls also demonstrating relatively high Ki-67 expression. Thus, the values of >2 positively stained cells in SA lesions or >6 positively stained cells in normal TDLUs represent statistically significant but still modest results that correspond to approximately a 2-fold increase in the odds of cancer and would not alone provide effective discrimination between the 2 outcome categories. Ki-67 was also significantly associated with other variables known to affect BC risk in this cohort, including age, degree of lobular involution, presence of atypical hyperplasia, and family history of BC.

Our group has previously studied Ki-67 immunoreactivity in Mayo BBD subjects with atypical hyperplasia (N=192 women), using a cutoff of 2% positive cells to separate the group into high and low expressers (9). Women with high expression (>2%) in the atypia lesion had a shorter time to BC development in the first 10 years of follow-up [9]. Whereas

women with low Ki-67 expression (<2%) showed increased risk of BC after 10 years of follow-up [9]. In the current study, we found no significant association between Ki-67 expression and time to cancer in cases.

Previous studies have found that Ki-67 expression shows a progressive increase across the continuum from BBD, to atypical ductal hyperplasia, to ductal carcinoma in situ (DCIS) and invasive carcinoma [8,15]. Its high expression (>11%) has been correlated with worse prognosis in invasive BC [10]. In a study comparing BC with adjacent nonmalignant tissue, higher Ki-67 cell expression was noted in breast carcinoma cells (25%) and lower Ki-67 expression was noted in fibrocystic changes with atypia and fibroadenoma (3%–4%) [16]. In another study examining BC, DCIS, and adjacent nonmalignant tissue, the mean value of Ki-67 proliferation in cases with DCIS showed a threshold increase of up to 16.8%; with invasive carcinoma, it was 23.3% [17]. Similarly, in a comparison of benign and malignant breast cancer tissue, the mean Ki-67 proliferating index was 4.4% and 16.1% for benign and malignant breast groups, respectively ($P<.001$) [18].

Strengths of our study include a well-characterized BBD cohort with long-term follow-up, plus review by a breast pathologist to capture Ki-67 immunoreactivity in both SA and normal TDLU. A limitation was that not all of the selected case-control pairs could be analyzed due to insufficient tissue, possibly introducing a slight bias in this retrospective case-control study.

In conclusion, patients with SA showed variable Ki-67 expression in both the SA lesion and the adjacent normal TDLU. Ki-67 expression was associated with other BC risk features and future BC case status. Specifically, higher Ki-67 expression in either the SA lesion or the normal TDLU had an OR approximately 2-fold compared to lower Ki-67 expression. Furthermore, manual and digital scoring using Aperio IA quantitation showed excellent agreement.

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Abbreviations

BBD	benign breast disease
BC	breast cancer
DAB	diaminobenzidine substrate
DCIS	ductal carcinoma in situ
HPF	high-power field

40×HPF	high-power field (magnification, ×40)
IA	image analysis
ICC	intraclass correlation coefficient
IQR	interquartile range
OR	odds ratio
SA	sclerosing adenosis
SIR	standardized incidence ratio
TDLU	terminal duct lobular unit

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Figure 1.
Sclerosing Adenosis. (Hematoxylin-eosin, original magnification $\times 20$.)

Figure 2.
Ki-67 Immunostain in Sclerosing Adenosis Lesion. The average Ki-67 signals per 40×HPF was high (9.0) in this case. Arrows indicate nuclei that have immunopositivity for Ki-67 antibody. (Original magnification ×20.)

Figure 3.

Functional Form of Relationship between Ki-67 Expression in a Sclerosing Adenosis (SA) Lesion (Figure 3A) and in a Normal Terminal Duct Lobular Unit (TDLU) [Figure 3B] with Estimated Log (Odds) of Breast Cancer. Estimated log (odds) values were derived from conditional logistic regression using restricted cubic splines, with knots at the 10th, 50th, and 90th percentiles. For improved visualization of the form, a smoothing curve was overlaid using a locally estimated scatterplot smoother (loess). HPF indicates high-power field.

Figure 4.
Ki-67 Immunostain in Normal Terminal Duct Lobular Unit. The average Ki-67 signals per 40×HPF was high (9.6) in this case. Arrows indicate nuclei that show positive immunoexpression to Ki-67 antibody. (Original magnification, ×20.)

Figure 5.

Agreement between manual assessment of Ki-67 density and digital quantitation of Ki-67 density using Aperio image analysis for the SA lesion (Figure 5A) and Normal TDLU (Figure 5B).

Table 1Clinicopathologic Characteristics of Nested Case-Control Sample of Patients with Sclerosing Adenosis^a

Variable	Breast Cancer (n=133)	Unaffected (n=239)	Total (N=372)
Age at BBD, y			
<45	30 (22.6)	61 (25.5)	91 (24.5)
45–55	51 (38.3)	93 (38.9)	144 (38.7)
>55	52 (39.1)	85 (35.6)	137 (36.8)
Year of BBD			
1967–1981	62 (46.6)	115 (48.1)	177 (47.6)
1982–1991	71 (53.4)	124 (51.9)	195 (52.4)
Histologic impression			
SA without atypia	111 (83.5)	224 (93.7)	335 (90.1)
SA with atypia	22 (16.5)	15 (6.3)	37 (9.9)
Atrophy			
None	32 (24.1)	27 (11.3)	59 (15.9)
1%–74% TDLU	86 (64.7)	187 (78.2)	273 (73.4)
>75% TDLU	8 (6.0)	18 (7.5)	26 (7.0)
NA	7 (5.3)	7 (2.9)	14 (3.8)
Family history of BC ^b			
None	73 (54.9)	153/238 (64.3)	226/371 (60.9)
Weak	40 (30.1)	65/238 (27.3)	105/371 (28.3)
Strong	20 (15.0)	20/238 (8.4)	40/371 (10.8)

Abbreviations: BC, breast cancer; BBD, benign breast disease; NA, not available; SA, sclerosing adenosis; TDLU, terminal duct lobular unit.

^aValues are number (percentage) or number/total (percentage).^bInformation about family history of BC was missing for 1 patient in the unaffected group.

Table 2
Distribution of Ki-67 Immunoeexpression in Patients with Sclerosing Adenosis Overall and Separately by Case-Control Status with Tests for Association Between Cases and Controls

Variable	Breast Cancer (n=133)	Unaffected (n=239)	Total (N=372)	Unadjusted P Value ^a	Adjusted P Value ^{a,b}
Sclerosing adenosis					
Average no. of signals/40× HPF				.03	.11
No.	133	239	372		
Mean (SD)	8.02 (8.96)	6.66 (7.92)	7.15 (8.32)		
Median	4.67	4.00	4.33		
IQR	2.67–9.67	1.67–8.33	2.00–8.67		
Average intensity				.79	.99
No.	133	239	372		
Mean (SD)	1.76 (0.55)	1.78 (0.53)	1.78 (0.54)		
Median	2.00	2.00	2.00		
IQR	2.00–2.00	2.00–2.00	2.00–2.00		
Average product score				.04	.16
No.	133	239	372		
Mean (SD)	15.80 (18.05)	13.51 (17.48)	14.33 (17.69)		
Median	9.33	8.00	8.33		
IQR	5.00–19.33	3.33–16.00	3.33–17.33		
Normal TDLU					
Average no. signals/40× HPF				.006	.03
No.	119	224	343		
Mean (SD)	7.15 (9.56)	5.22 (7.06)	5.89 (8.06)		
Median	3.67	3.00	3.33		
IQR	2.33–8.67	1.67–5.67	2.00–6.33		
Average intensity				.73	.43
No.	119	224	343		
Mean (SD)	1.79 (0.45)	1.79 (0.50)	1.79 (0.48)		
Median	2.00	2.00	2.00		
IQR	2.00–2.00	2.00–2.00	2.00–2.00		

Variable	Breast Cancer (n=133)	Unaffected (n=239)	Total (N=372)	Unadjusted P Value ^a	Adjusted P Value ^{a,b}
Average product score				.01	.03
No.	119	224	343		
Mean (SD)	13.89 (19.08)	10.53 (16.58)	11.70 (17.54)		
Median	7.33	6.00	6.67		
IQR	4.00–16.67	3.00–11.17	3.33–12.00		

Abbreviations: IQR, interquartile range; TDLU, terminal duct lobular unit; 40×HPF, high-power field (magnification, ×40).

^a P values were calculated from the conditional logistic regression model. The number of signals and product score variables were rank transformed prior to this analysis.

^b P values were adjusted for presence of atypia, degree of lobular involution, indication for biopsy (lump or mammogram), and family history of breast cancer (none, weak, or strong).

Table 3

Unadjusted and Adjusted Odds Ratios by Quartiles for Average Number of Signals of Ki-67 per 40×HPF in Sclerosing Adenosis Lesions and Normal Terminal Duct Lobular Units

Variable	Breast Cancer, No. (%)	Unaffected, No. (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI) ^a
SA lesion				
Average no. of signals per 40× HPF	(n=133)	(n=239)		
0 to 2 signals	28 (21.1)	77 (32.2)	1.0 (Reference)	1.0 (Reference)
>2 to 4 signals	27 (20.3)	52 (21.8)	1.5 (0.8, 2.8)	1.9 (0.9, 4.0)
>4 to 8 signals	39 (29.3)	50 (20.9)	2.1 (1.2, 3.9)	1.8 (0.9, 3.6)
>8 signals	29 (29.3)	60 (25.1)	2.0 (1.1, 3.6)	1.9 (0.9, 3.9)
			$P=.01^b$	$P=.09^b$
Normal TDLU				
Average no. of signals per 40× HPF	(n=119)	(n=224)		
0 to 2 signals	27 (22.7)	72 (32.1)	1.0 (Reference)	1.0 (Reference)
>2 to 3 signals	21 (17.6)	43 (19.2)	1.6 (0.7, 3.3)	1.3 (0.5, 3.1)
>3 and 6 signals	33 (27.7)	60 (26.8)	1.6 (0.9, 3.0)	1.5 (0.7, 3.1)
>6 signals	38 (31.9)	49 (21.9)	2.6 (1.4, 5.0)	2.4 (1.1, 5.3)
			$P=.005^b$	$P=.03^b$

Abbreviations: 40×HPF, high-power field (magnification ×40); SA, sclerosing adenosis; TDLU, terminal duct lobular unit.

^aOdds ratios were adjusted for presence of atypia, degree of lobular involution, indication for biopsy (lump or mammogram), and family history of breast cancer (none, weak, or strong).

^bP values were calculated from conditional logistic regression model as a linear trend across quartiles.