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Standardized measures of lobular involution and subsequent breast cancer risk among women with benign breast disease: a nested case–control study

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Abstract

Lesser degrees of terminal duct-lobular unit (TDLU) involution predict higher breast cancer risk; however, standardized measures to quantitate levels of TDLU involution have only recently been developed. We assessed whether three standardized measures of TDLU involution, with high intra/inter pathologist reproducibility in normal breast tissue, predict subsequent breast cancer risk among women in the Mayo benign breast disease (BBD) cohort. We performed a masked evaluation of biopsies from 99 women with BBD who subsequently developed breast cancer (cases) after a median of 16.9 years and 145 age-matched controls. We assessed three metrics inversely related to TDLU involution: TDLU count/mm², median TDLU span (microns, which approximates acini content), and median category of acini counts/TDLU (0–10; 11–20; 21–30; 31–50; >50). Associations with subsequent breast cancer risk for quartiles (or categories of acini counts) of each of these measures were assessed with multivariable conditional logistic regression to estimate odds ratios (ORs) and 95 % confidence intervals (CI). In multivariable models, women in the highest quartile compared to the lowest quartiles of TDLU counts and TDLU span measures were significantly associated with subsequent breast cancer diagnoses; TDLU counts quartile4 versus quartile1, OR = 2.44, 95 % CI 0.96–6.19, p-trend = 0.02; and TDLU spans, quartile4 versus quartile1, OR = 2.83, 95 % CI = 1.13–7.06, p-trend = 0.03. Significant associations with categorical measures of acini counts/TDLU were also observed: compared to women with median category of <10 acini/TDLU, women with >25 acini counts/TDLU were at significantly higher risk, OR = 3.40, 95 % CI 1.03–11.17, p-trend = 0.032. Women with TDLU spans and TDLU count measures above the median were at further increased risk, OR = 3.75 (95 % CI 1.40–10.00, p-trend = 0.008), compared with women below the median for both of these metrics. Similar results were observed for combinatorial metrics of TDLU acini counts/TDLU, and TDLU count. Standardized quantitative measures of TDLU counts and acini counts approximated by TDLU span measures or visually assessed in categories are independently associated with breast cancer risk. Visual assessment of TDLU numbers and acini content, which are highly reproducible between pathologists, could help identify women at high risk for subsequent breast cancer among the million women diagnosed annually with BBD in the US.

Keywords

Lobular involution; Breast cancer risk

Introduction

Analysis of the U.S. mammography registry data from 1996 to 2008 demonstrates that 1.2 % of screening mammograms prompt a breast biopsy, of which nearly 75 % are diagnosed as benign breast disease (BBD), including approximately 70 % with nonatypical lesions and

3.4 % with atypia [1]. A recent meta-analysis demonstrated that women with proliferative BBD without atypia experience a relative risk (RR) of developing breast cancer of 1.76, which increases to 3.93 if atypia is present [2]. Thus, women with biopsy-proven BBD represent a large number of future breast cancer cases. As proliferative BBD with atypia is a factor that is included in some models to predict breast cancer risk and used to counsel patients about chemoprevention [3, 4], developing risk stratification tools for women diagnosed with BBD using breast tissue markers may improve identification of women at highest risk for breast cancer [5, 6].

Data from retrospective studies suggest that analysis of “normal-appearing” terminal duct-lobular units (TDLUs) adjacent to BBD may have value in predicting risk of breast cancer [7–9]. TDLUs are normal structures within the breast that produce milk during lactation and also represent the primary source of breast cancer precursors (Fig. 1) [10]. With physiological aging and completion of child bearing, TDLUs of most women involute, reflected in a reduction in TDLU size, acini counts/TDLU (epithelial substructures) and total TDLU counts per standard unit area [7, 11–14]. In a cohort of women with BBD within the Mayo Clinic, qualitative assessment of degrees of TDLU involution showed that women with lesser degrees of TDLU involution had an approximately two-fold increased risk of developing breast cancer compared with expected population-based rates, irrespective of the severity of BBD [7], and use of these measures in risk prediction models showed improved discrimination of breast cancer cases and noncases [4]. Similarly, in a cohort within the Nurses’ Health Study II, women with BBD whose breast tissue contained TDLUs with the lowest acini content were also at about 30 % lower risk of developing breast cancer [8]. Preliminary efforts to use quantitative measures of TDLU involution, which could further discriminate women at highest risk, have focused on acini content within TDLUs. Using image analysis to evaluate a subset of biopsies from the Mayo BBD cohort, investigators showed women with higher acini counts/TDLU were at increased risk, independent of Gail model risk prediction [15], supporting quantitative measures of involution to further discriminate women at highest risk for breast cancer.

Using normal breast tissues donated by volunteer participants in the Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center (KTB), we developed standardized, reproducible metrics for evaluating TDLU involution using digital images and demonstrated associations of these measures with multiple breast cancer risk factors [11, 16–20]. Acini counts are difficult to quantitate visually and image analysis tools have not yet been validated; therefore, to approximate the size and acini content within TDLUs, we recently developed TDLU span measures which are highly correlated with acini counts [4]. In addition to the acini content within TDLUs, higher TDLU counts per unit area of tissue irrespective of their acini content have been hypothesized to represent a marker of increased breast cancer risk [11, 13, 14, 21], especially after menopause [14]; yet, whether such measures are associated with subsequent breast cancer risk has not been evaluated. Here we assessed whether standardized measures of TDLU involution, specifically, TDLU counts/mm², acini content, and TDLU span measures are associated with risk of a future diagnosis of ductal carcinoma in situ (DCIS) or invasive breast cancer in a nested comparison within the Mayo BBD cohort [15].

Methods

Study population

We utilized a previously designed nested case–control study within the Mayo BBD cohort, which includes women ($N = 9376$) who had an open breast biopsy between January 1, 1967 and December 31, 1991 with benign findings: medical record review for future breast cancer diagnoses was obtained through January 2014, for a median of 16.9 years [9]. Risk factor information and subsequent breast pathology diagnoses were obtained from a study-specific questionnaire which was sent after benign biopsy diagnosis and medical records [9]. All necessary consent from any patients involved in the study, including consent to participate in the study, was obtained. All patient contact materials and procedures were reviewed and approved by the Mayo Clinic Institutional Review Board.

As previously described, cases were randomly selected from 823 cases stratified by 5-year categories of year of benign biopsy to represent the entire spectrum of the cohort. One hundred cases were matched on age and year of benign biopsy with two controls [15]. Adequate tissue for assessment was available for 99 cases and 145 controls.

Risk factor information

Risk factors were captured on the questionnaire or from medical records, as previously described [9]. Family history of breast cancer was categorized as (1) strong, if at least one first-degree relative was diagnosed with breast cancer before the age of 50 years or if two or more relatives had breast cancer, with at least one being a first-degree relative; (2) weak, if any lesser degree of family history of breast cancer; or (3) none. To clarify, the family history would not be considered strong if only a first-degree relative was above age 50 years, unless there was a second affected relative. Other exposures examined were body mass index (BMI, kg/m^2) at biopsy (< 25 , 25–29, 30+); age at menarche (< 12 , 13, 14, 15 years); age at first live birth (< 20 , 20–24, 25–29, 30+ years); parity (yes/no); breastfeeding (ever/never); use of menopausal hormones (MH) (ever/never); and type of BBD (nonproliferative, proliferative without atypia, and proliferative with atypia).

Histologic assessment of TDLU involution

Qualitative measures of TDLU involution were assessed microscopically by a pathologist (DWV), as previously described [7]. Briefly, samples containing one or more normal TDLUs were assessed for the degree of involution classified as none/mildly involuted (0–24 %), partially involuted (25–74 %), or completely involuted (75 %).

For standardized measures of TDLU involution, stained H&E tissue sections were digitized at 20X using a Hamamatsu NanoZoomer 2.0HT (Hamamatsu, Bridgewater NJ), and managed for web-based viewing and annotation with Digital Image Hub software (Slidepath/Leica, Dublin, Ireland), as previously described [11]. Only TDLUs that appeared entirely normal or showed focal benign changes (duct dilatation, metaplasia, and hyperplasia) were assessed. TDLUs were not considered normal if more than half the acini were dilated 2–3 times the normal diameter or if there were metaplastic changes involving more than half the acini. TDLUs showing ductal hyperplasia, defined as ducts or acini lined

by more than a single epithelial cell layer, were not considered normal and therefore not assessed for involution analysis. In addition, the numbers of TDLUs with proliferative changes, and therefore not considered normal or suitable for assessment of involution, were recorded.

Images of sections were reviewed masked to other data to estimate percentage of fat (0–25, 26–50, 51–75, 76–100 %) and to enumerate the total number of normal TDLUs (as defined above). Up to ten normal TDLUs were reviewed sequentially to assess (1) TDLU span, measured with an electronic ruler (microns) and (2) number of acini per TDLU (1: 10; 2: 11–20; 3: 21–30; 4: 31–50; 5: 51+, Fig. 1). To determine standardized number of TDLUs, total tissue area was measured using the lasso drawing function in Slidepath to outline the perimeter of the tissue (in mm²). Prior reports have found that assessment of at least six TDLUs per section per patient provides stable representative measures of TDLU characteristics [15, 22, 23]. For samples with multiple tissue pieces, the area was summed across the number of tissues represented on the H&E image. For acini counts/TDLU and TDLU span measures, we used the median of the values obtained across the multiple TDLUs measured for each woman. More than 75 % of cases and controls had ten TDLUs reviewed.

Statistical analysis

Spearman correlations between measures of involution, such as TDLU counts/100 mm² (referred to as TDLU counts), median TDLU span, median category of acini counts/TDLU, as well as qualitative TDLU involution assessment (none, partial, and complete involution), were compared. Quartile levels of standardized TDLU involution measures were created based on the distribution in the controls to determine associations with the outcome of breast cancer risk. To identify potential confounders associated with TDLU measures, we fitted ordinal logistic regression models using quartile levels of TDLU involution measures as the outcome and risk factor variables as the independent predictors ($p < 0.05$). Multivariable conditional logistic regression models were used to estimate odd ratios (ORs) and 95 % confidence intervals (CI) to determine associations between TDLU metrics and breast cancer risk, adjusted for significantly ($p < 0.05$) associated risk factors for breast cancer, which were family history of breast cancer, MH use, and BBD severity. Subjects missing data on covariates were considered a separate group and retained in all models. Analyses were performed using SAS V9.3.

Results

Patient characteristics

The median age of BBD diagnosis for cases and controls was 51 years of age. Although not statistically significant, cases were more likely to have younger ages at menarche (12 (55 vs. 37 %, $p = 0.095$), be current MH users (31 vs. 21 %, $p = 0.098$), have a strong family history of breast cancer (14 vs. 8 % $p = 0.064$), and have proliferative disease with atypia (11 vs. 6 %, $p = 0.159$).

Relationships between standardized TDLU involution measures and demographic data

TDLU counts were significantly and positively correlated with TDLU span (Spearman $\rho = 0.27$, $p < 0.0001$) and with acini/TDLU (Spearman $\rho = 0.37$, $p < 0.0001$); however, TDLU span and acini counts/TDLU were more strongly correlated (Spearman $\rho = 0.66$, $p < 0.0001$). All standardized TDLU measures were significantly and inversely correlated with qualitative TDLU involution assessment: TDLU counts (Spearman $\rho = -0.39$, $p < .0001$), TDLU span (Spearman $\rho = -0.32$, $p < 0.0001$), and acini/TDLU (Spearman $\rho = -0.31$, $p < 0.0001$), consistent with studies using normal breast tissues donated by volunteers [11].

Younger age at biopsy diagnosis and lower percentage of fat on digital images of biopsies diagnosed as BBD were significantly associated with higher TDLU counts and larger TDLU spans (Supplemental Table 1). In addition, having given birth, younger age at first birth and lower BMI were significantly associated with higher TDLU counts (Supplemental Table 1). Although based on only 14 in situ cases (DCIS), analysis by case status showed significantly higher median number of TDLU counts in DCIS compared to invasive cases (53 vs. 24 $p = 0.005$, Supplemental Table 2). Risk factors, including age at BBD, age at DCIS/invasive diagnosis, did not differ significantly between DCIS vs. invasive cancer, and these were combined in a single endpoint (Supplemental Table 3).

Measures of TDLU involution by case status are presented in Table 1. No statistically significant difference could be seen between cases and controls for qualitative measures of involution. In contrast, compared to controls, women who subsequently developed breast cancer had statistically significant higher TDLU counts per 100 mm² area (median of 28 vs. 20, $p = 0.029$), a nonstatistically significant increase in TDLU spans (median of 300 vs. 267 microns, $p = 0.136$), and categorical measures of acini/TDLU (median acini category 2.5 (approximately 25 acini/TDLU), 11 vs. 5 %, $p = 0.134$). Median and IQR of quantitative measures of TDLU involution by qualitative measures (none, partial, and complete) are presented in Supplemental Table 4 and showed, as expected, that those classified as completely involuted had many fewer TDLUs, smaller TDLU spans, and fewer acini counts/TDLU as compared with those classified as partial or complete.

TDLU involution assessment and breast cancer risk

Unadjusted and multivariable associations of TDLU involution assessment and breast cancer are shown in Table 2. Compared with women in the lowest quartile of TDLU counts, women in the highest quartile had significantly elevated risk (OR = 2.41, 95 % CI 1.01–5.76, p -trend = 0.026), and estimates were similar when further adjusted for potential confounding factors. Compared to women in the lowest quartile of median TDLU span, women in the highest quartile were at a nonsignificantly 65 % increased risk (OR = 1.65, 95 % CI 0.75–3.62, p -trend = 0.212); however, increasing quartile levels of median TDLU span measures were significantly associated with risk when further adjusted for covariates (OR = 2.83, 95 % CI 1.13–7.06, p -trend = 0.032). Findings for median acini counts/TDLU were similar to TDLU span and statistically significantly associated with the risk of subsequent breast cancer diagnosis (Table 2).

Breast cancer risk associations for participants cross-classified by several TDLU metrics (above or below medians) are shown in Table 3. Women above the median for both TDLU span and TDLU counts were at increased risk compared with women below the median for all of these characteristics (OR = 3.75; 95 % CI 1.40–10.00, p-trend = 0.008). Associations with risk based on cross-classification by TDLU counts and acini counts were similar to associations seen with TDLU span measures. There were too few cases to reliably estimate the risk for those subjects with atypical hyperplasia (ADH) or subjects with proliferative diseases without atypia (Supplemental Table 1). We did however remove those with a diagnosis of ADH, which is presented in Table 3 and show that even among lower risk BBD lesions, TDLU involution measures show a significant association with risk.

To determine if the number of TDLUs evaluated influences associations with risk, we performed sensitivity analysis restricted to only those subjects with five or more TDLUs evaluated. Associations with subsequent breast cancer risk remained significant, where those above the median of TDLU counts and span measures had OR = 7.06 (95 % CI 1.75–28.46) compared to those below the median for TDLU counts and span measures. Similarly, results for combinatorial metrics for median acini and TDLU counts, where those above the median of TDLU counts and acini measures, had OR = 12.89 (95 % CI 2.58–64.41) compared to those below the median for TDLU counts and span measures. Restricting analyses to invasive cases or stratification of data by time interval between BBD and cancer diagnoses (0–5, 6–10, or >10 years) yielded similar results. Tests for interactions between TDLU measures and time since diagnosis, MH use, family history of breast cancer, and BBD histology were not significant (*p*values > 0.20).

Discussion

Studies employing visual qualitative assessment of TDLU involution among women biopsied for BBD have demonstrated associations between decreased involution and increased risk of developing breast cancer [7, 8, 15]. Using standardized measures of TDLU involution, we found that women with the lowest levels of TDLU involution, as reflected in higher TDLU counts, and measures of acini content noted by larger TDLU span (which approximate acini content of TDLUs) or visually assessed acini counts/TDLU, had approximately a doubling of risk of developing breast cancer. Further, cross-classification using TDLU counts and either TDLU span or acini number per TDLU showed even higher risk. Thus, we propose that further evaluation of these metrics in clinical studies assessing risk of breast cancer development in additional cohorts is warranted.

Using quantitative as compared with qualitative measures of TDLU involution could enable the development of potentially clinically applicable markers of breast cancer risk among women undergoing benign biopsies [7]. In this analysis, higher TDLU counts/mm², acini counts per TDLU, and TDLU span, were associated with risk of developing breast cancer, and a combinatorial metric combining TDLU counts and acini content seemed to demonstrate a stronger association. We hypothesize that the latter combination may reflect the at-risk epithelial content of the breast. As found previously, TDLU counts and specific features of TDLUs were only weakly correlated [11]. This could suggest that separate mechanisms control these two features of involution-reduced size of individual TDLUs and

decrease in numbers of TDLUs; for example, TDLU counts may reflect differences in baseline breast development. Alternatively, involution may occur in a dynamic continuum in which individual TDLUs undergo simplification and obsolescence at differing rates.

Prior analyses demonstrating associations between breast cancer risk factors and the TDLU involution metrics evaluated here support that these are biologically plausible markers of risk [11, 16, 17]. Levels of TDLU involution increase with age and menopause, paralleling the slower rise in breast cancer incidence rates around age 50 years, a surrogate for menopause [24]. Further, mammographic density, a strong breast cancer risk factor, is also associated with levels of TDLU involution [12, 25–27], and data suggest that IGF signaling may be interrelated with both of these characteristics [16], [28]. These data support further studies using standardized measures of TDLU involution and mammographic density, which might enhance risk assessment among women with BBD.

Degrees of TDLU involution have been consistently shown to be lower among parous women and those with short intervals since childbirth [7, 11], suggesting that pregnancy-associated proliferation may possibly relate to the possible transient increase in cancer risk after a delivery. However, TDLU involution has not informed questions about the protective effect of pregnancy and breast cancer diagnosed at older ages, supporting a role for other mechanisms, including molecular changes, which may be related to the protective effect of parity [29, 30]. Additionally, data suggest that involution is inversely related to circulating concentrations of factors associated with elevated breast cancer risk, such as prolactin, testosterone, and postmenopausal estrogens, as well as use of menopausal hormone therapy [11, 16, 17]. Exposures such as smoking, which may reduce estrogenic effects, have also shown an association with increased TDLU involution [4].

The biologic mechanisms underlying age-related TDLU involution remain unknown. Studies of postpartum involution in animal models have been related to complex signaling pathways, including cytokine signaling (such as FAS-L, interleukins 6 and 10, and TGF-beta 3), apoptosis (including BAX and BCL-2), inflammatory process (including COX-2), and STAT proteins [31]. Ultimately, understanding the mechanisms that govern the distinctive processes of postweaning involution and age-related involution may have value for breast cancer risk reduction [32], if TDLU measurement can be shown to represent both a marker of cancer risk and a biological intermediate. Further, biologic processes related to postpartum involution may be distinct from those involved in age-related involution, and understanding these mechanisms could help identify potentially relevant pharmacological targets for chemoprevention.

In our analysis, higher TDLU counts were associated with increased breast cancer risk independent of family history of breast cancer, parity, and MH use, possibly suggesting that involution may be downstream of these factors on a common etiological pathway. The magnitude of the associations for number of acini per TDLU and TDLU span showed more variable relationships with breast cancer risk in multivariate models; however, the reasons for these findings are unclear.

Strengths of this study are the use of a nested case–control study design to determine associations with subsequent breast cancer risk and standardized measures of TDLU involution that have been shown to have good intraand interobserver reproducibility. Previous data suggest involution takes place at a relatively uniform rate throughout the breast tissue [22]; therefore, associations are likely robust despite review of only a portion of tissue from the entire breast; sampling error would likely bias results to the null. The generalizability of our findings is limited to women with BBD who have undergone an excisional biopsy. Sampling for histopathology of tissues was based on pathologists' gross visual inspection of tissue, suggesting that oversampling of nonfatty areas is a potential issue. We did evaluate models adjusted for percentage fat in the tissue sections, and this did not substantially change associations observed for breast cancer risk suggesting associations are robust. Additional limitations include a relatively small sample size and lack of tumor marker data, particularly estrogen receptor (ER) expression on subsequently diagnosed tumors. A prior case–case analysis found that among women less than 55 years, levels of TDLU involution are lower for ER-negative breast cancers compared with tumors that express hormone receptors [23].

In summary, these data show that the lack of TDLU involution, as measured by increased standardized number of TDLU counts, larger median TDLU spans, higher categories of acini counts/TDLU, and cross-classification using these measures, is associated with increased breast cancer risk among women with BBD. This work extends prior research in this cohort linking decreased TDLU involution to increased risk of developing breast cancer [7, 15]. We note that several groups have developed automated methods to assess TDLU histology and to estimate levels of involution [15, 17, 18, 33, 34] that agree well with visual assessment. Hence, we view visual assessment of involution levels as a temporary approach to advance this area as automated algorithms are developed. Future studies are warranted to identify determinants of TDLU involution and to assess its role as a risk marker or potential intermediate endpoint in different clinical contexts. Given that there are approximately one million women diagnosed annually with BBD in the US, visual assessment of TDLU numbers and acini content, which are highly reproducible between pathologists, could help identify women at high risk for subsequent breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CI 95 % confidence intervals

BBD	Benign breast disease
BMI	Body mass index
ER	Estrogen receptor
MH	Menopausal hormones
ORs	Odds ratios
RR	Relative risk
KTB	Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center
TDLU	Terminal duct-lobular unit
DCIS	Ductal carcinoma in situ

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Fig. 1.

Standardized terminal duct-lobular unit (TDLU) involution assessment in the Mayo Benign Breast Disease Cohort. Greater TDLU counts, TDLU span, and number of acini per TDLU associated with reduced levels of TDLU involution were assessed from digitized images of H&E-stained tissue sections. *Panel A* shows a digital H&E section with multiple TDLUs (TDLU counts), as well as the outline of the tissue area captured in mm² using the lasso tool, to calculate the TDLU/mm² measures. For up to ten normal TDLUs per section, the longest TDLU span was measured in microns and the counts of acini/TDLU in categories (1 = 1–10, 2 = 11–20, 3 = 21–30, 4 = 31–50, and 5 > 50) were recorded. *Panel B* shows three representative TDLUs for which the longest TDLU span was measured in microns using a digital ruler. The *black arrow* indicates a representative acinus

Table 1

Standardized TDLU involution assessment by outcome status

TDLU involution measures	Cases <i>N</i> = 99	Controls <i>N</i> = 145	<i>p</i>
Qualitative TDLU involution, <i>N</i> (%)			
Complete (>75 %)	12 (11)	17 (13)	
Partial (1–74 %)	67 (68)	91 (67)	
None	20 (21)	28 (20)	0.972 ^{**}
Quantitative measures of TDLU involution, Median (IQR)			
Standardized number of TDLUs/100 mm ²	28 (12–51)	20 (7–35)	0.029 [*]
Median TDLU span (microns)	300 (221–387)	267 (201–362)	0.136 [*]
Median Acini/TDLU in categories for up to 10 sequential TDLUs <i>N</i> (%)			0.134 ^{**}
1	50 (51)	90 (64)	
1.5	10 (10)	14 (10)	
2	27 (23)	30 (21)	
2.5	11 (11)	7 (5)	

The categories of acini counts/TDLU were recorded as: 1 = 1–10, 2 = 11–20, 3 = 21–30, 4 = 31–50, and 5 > 50

* Kruskal-Wallis test

** χ^2 test

*** Exact test

Table 2

Standardized TDLU involution assessment and subsequent breast cancer risk (invasive or in situ) among women with benign breast disease

	Cases		Controls		Unadjusted Model			Multivariable Model		
	N		N		OR	95 %CI	P-trend	OR	95 %CI	P-trend
#TDLUs/100 mm ²										
Q1	19		34	1.00				1.00		
Q2	17		36	0.87	0.38	1.99		0.71	0.28	1.76
Q3	23		36	1.52	0.64	3.57		1.50	0.57	3.93
Q4	40		36	2.41	1.01	5.76	0.026	2.44	0.96	6.19 0.023
Median TDLU span microns										
Q1	18		35	1.00				1.00		
Q2	23		36	1.11	0.49	2.48		1.56	0.62	3.91
Q3	23		36	1.19	0.52	2.75		1.59	0.62	4.06
Q4	34		35	1.65	0.75	3.62	0.212	2.83	1.13	7.06 0.032
Median category of acini/TDLU										
1	50		90	1.00				1.00		
1.5	10		14	0.97	0.37	2.56		1.40	0.48	4.04
2	27		30	1.54	0.77	3.08		1.91	0.87	4.19
2.5	11		7	2.50	0.88	7.13	0.081	3.40	1.03	11.17 0.032

The categories of acini counts/TDLU were recorded as: 1 10, 2 = 11–20, 3 = 21–30, 4 = 31–50, and 5 > 50. The median category of acini count was 2.5 (approximately 25 acini/TDLU)

The cutoffs for TDLU count quartiles were: 1: 7.11, 2: 19.35, 3: 35.46, 4: >35.46

The cutoffs for TDLU span quartiles were: 1: 199.5, 2: 266.5, 3: 363.5, 4: >363.5

ORs and 95 % CI estimated using conditional logistic regression model. Multivariable models adjusted for MH use, family history of breast cancer, and BBD histology

Table 3

Cross classification of TDLU counts and TDLU span or acini counts/TDLU and breast cancer risk among women with benign breast disease

		Cases		Controls		Unadjusted			Multivariable		
		N		N		OR	95 %CI	P-trend	OR	95 %CI	P-trend
In situ and invasive cases											
TDLUs	Span										
	Median	19		43	1.00				1.00		
	Median	16		27	1.37	0.58	3.25		1.71	0.68	4.36
	>Median	22		28	2.18	0.89	5.36		2.38	0.90	6.30
	>Median	41		44	2.50	1.07	5.83	0.026	3.75	1.40	10.00
	Acini category										0.008
TDLUs	Median	28		58	1.00				1.00		
	Median	7		11	1.54	0.49	4.91		1.56	0.12	5.74
	>Median	32		46	1.89	0.90	4.01		2.05	0.92	4.59
	>Median	31		26	3.07	1.27	7.45	0.014	3.75	1.41	10.01
Excluding subjects with BBD classified as proliferative disease with atypia											
TDLUs	Span										
	Median	19		40	1.00				1.00		
	Median	14		24	1.44	0.53	3.86		1.74	0.61	4.90
	>Median	16		26	1.67	0.62	4.57		2.22	0.78	6.34
	>Median	38		44	2.44	0.98	6.05	0.054	3.11	1.14	8.53
	Acini										0.027
TDLUs	Median	27		54	1.00				1.00		
	Median	6		9	2.07	0.50	8.51		1.92	0.40	9.20
	>Median	25		45	1.61	0.70	3.74		1.81	0.76	4.31
	>Median	29		25	3.46	1.28	9.31	0.028	3.73	1.35	10.30
Restricted to cases with a subsequent diagnosis of invasive cancer only											
TDLUs	Span										
	Median	16		37	1.00				1.00		
	Median	15		22	1.51	0.61	3.76		1.66	0.62	4.47
	>Median	18		24	2.12	0.82	5.46		1.96	0.69	5.63

	Cases N	Controls N	Unadjusted			Multivariable		
			OR	95 %CI	P-trend	OR	95 %CI	P-trend
>Median	30	40	1.93	0.76 4.89	0.15	2.20	0.76 6.38	0.15
TDLUs								
Acini category								
Median	24	51	1.00			1.00		
Median	7	8	2.00	0.59 6.78		1.76	0.46 6.77	
>2								
Median	25	42	1.63	0.74 3.62		1.48	0.62 3.53	
>Median								
Median	23	22	2.73	1.05 7.08	0.05	2.70	0.94 7.70	0.10
>2								

The cutoff for TDLU count median was: 19.35
The cutoff for TDLU span median was: 266.5
Multivariable model adjusted for family history of breast cancer, MH use and BBD histology