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EPIDEMIOLOGY

Inflammation markers on benign breast biopsy are associated with risk of invasive breast cancer in African American women

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Abstract

Purpose Markers of inflammation, including crown-like structures of the breast (CLS-B) and infiltrating lymphocytes (IL), have been identified in breast tissue and associated with increased risk of breast cancer (BrCa), however most of this work has been performed in primarily non-Hispanic white women. Here, we examined whether CLS-B and IL are associated with invasive BrCa in African American (AA) women.

Methods We assessed breast biopsies from three 5-year age-matched groups: BrCa-free AA women (50 Volunteer) from the Komen Normal Tissue Bank (KTB) and AA women with a clinically-indicated biopsy diagnosed with benign breast disease (BBD) from our Detroit cohort who developed BrCa (55 BBD-cancer) or did not develop BrCa (47 BBD only, year of biopsy matched to BBD-cancer). Mean adipocyte diameter and total adipose area were estimated from digital images using the Adiposoft plugin from ImageJ. Associations between CLS-B, IL, and BrCa among KTB and Detroit biopsies were assessed using multivariable multinomial and conditional logistic regression models.

Results Among all biopsies, Volunteer and BBD only biopsies did not harbor CLS-B or IL at significantly different rates after adjusting for logarithm of adipocyte area, adipocyte diameter, and BMI. Among clinically-indicated BBD biopsies, BBD-cancer biopsies were more likely to exhibit CLS-B (odds ratio (OR) = 3.36, 95% Confidence Interval (CI): 1.33–8.48) or IL (OR = 4.95, 95% CI 1.76–13.9) than BBD only biopsies after adjusting for total adipocyte area, adipocyte diameter, proliferative disease, and BMI.

Conclusions CLS-B and IL may serve as histological markers of BrCa risk in benign breast biopsies from AA women.

Keywords Breast cancer risk · Inflammation · Benign breast disease · Obesity · African american

Abbreviations

AA	African American
BBD	Benign breast disease
BMI	Body mass index
BCRAT	Breast cancer risk assessment tool
BrCa	Breast cancer
CI	Confidence interval
CLS-B	Crown-like structures of the breast
EA	European American
H&E	Hematoxylin & eosin
IL	Infiltrating lymphocytes
KTB	Susan G. Komen Normal Tissue Bank
OR	Odds ratio

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Introduction

Though breast cancer (BrCa) incidence has remained stable for non-Hispanic and Hispanic White women over the last decade, incidence rose for African American women, who suffer a 40% higher BrCa-specific mortality than Non-Hispanic White women [1, 2]. Despite this excess burden, African American (AA) women are poorly represented in most cohorts examining BrCa risk. Resulting breast cancer

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risk models underestimate risk in AA women [3, 4]. Among women of all races, commonly used risk models cannot discriminate between women who will develop BrCa from women who will not with a high degree of accuracy at the individual level [5]. Novel factors that can be reliably measured and improve risk prediction are necessary to best identify women at highest risk.

Tissue-based markers are ideal candidates for inclusion in risk models as they reflect the cumulative impact of genetic, behavioral, and environmental exposures. Cohort studies of women who underwent a breast biopsy have shown that women who require a biopsy are at increased risk of breast cancer [6–8]. Breast biopsies that reveal benign histologic abnormalities such as proliferative breast disease and cellular atypia are at further increased risk [7–9]. Identifying additional markers that can distinguish lower risk from higher risk biopsy findings could impact the clinical management of more than 1 million women a year who receive a benign biopsy diagnosis in the United States [10].

Here, we seek to examine two histologic measures of inflammation, crown-like structures of the breast (CLS-B) and infiltrating lymphocytes (ILs) in the breast, which may be associated with breast cancer risk. CLS-B, or adipocytes surrounded by macrophages on light microscopy, were originally described in mouse models [11]. CLS-B have been subsequently linked to measures of obesity including BMI and adipocyte diameter in studies of mastectomy tissue from BrCa patients [11–13]. However, these inflammation markers have not been well described in normal tissue (tissue from volunteers not undergoing mastectomies or reduction mammoplasty), particularly from AA women. Given that AA women have high obesity rates, as well as the poorest survival after a BrCa diagnosis, we examined whether two previously described markers of inflammation, crown-like structures of the breast (CLS-B) or inflammatory lymphocytes (ILs) are associated with benign breast disease (BBD) and BrCa risk in AA women.

Materials and methods

Study design and population

We conducted a study utilizing a case-control design nested in our Detroit BBD Cohort [8] and additional 5-year age group matched AA women controls from the Komen Normal Tissue Bank (Volunteer) [14]. The subset from the Detroit BBD cohort included AA women with BBD who developed invasive breast carcinoma (BBD-cancer) and women with BBD who did not develop in situ or invasive breast carcinoma (BBD only), individually matched on 5-year age groups and year of BBD biopsy.

Study participants included three cohorts of AA women. The BBD-cancer and BBD only cohorts were selected from the larger Detroit BBD cohort, a retrospective cohort of almost 4000 AA women diagnosed with BBD in the Detroit Metropolitan area between 1997 and 2010 who were followed over time for subsequent in situ or invasive breast carcinoma development. Further details on the Detroit BBD cohort are located in a previous report and in Supplemental Table 1 [8]. The BBD-cancer cohort consisted of AA women from the Detroit BBD cohort without fibroadenoma or cellular atypia on benign biopsy who developed a subsequent invasive breast cancer and had both BBD and BrCa tissue available ($n = 84$). 55 of these women were randomly selected and tissue from their initial benign biopsy was used for analysis. Median time to cancer was 8.00 years (range 0.93–17.15 years) for the BBD-cancer cohort, similar to in situ and invasive carcinomas in the entire Detroit BBD cohort. The BBD only cohort was comprised of AA women from the Detroit BBD cohort who had not developed invasive or in situ BrCa as of December 2016 ($n = 3{,}655$) and were propensity-score matched to the BBD-cancer subjects on 5-year-age groups and year of biopsy. Ultimately, 47 women comprised the BBD only cohort, as adequate tissue was unavailable for 8 women. Median follow up for the BBD only cohort was 13.20 years (range 3.02–18.92 years), similar to the controls in the entire Detroit BBD cohort. Lastly, the Volunteer cohort was comprised of women from the Komen Normal Tissue Bank (KTB). The KTB collects percutaneous needle biopsy breast tissue, blood, and questionnaire data including BMI from healthy volunteers at collection events around the country; 13% of KTB donors are AA [15]. An additional 50 AA women from the KTB with no self-reported history of BBD or BrCa were matched to BBD-cancer subjects on 5-year-age group and self-reported race.

Volunteer biopsies were reviewed for BBD features by our study pathologist (RAF), who previously reviewed all biopsies in the Detroit BBD cohort, using Dupont and Page criteria to identify biopsies including proliferative disease with and without atypia [9]. We excluded any Volunteer or BBD only subjects that showed cellular atypia on biopsy from analysis (1 biopsy from each group), as these were excluded from the BBD-cancer subjects. Body mass index (BMI) recorded closest to the BBD date and prior to BrCa diagnosis was ascertained via medical record review for the Detroit BBD Cohort and measured at the time of tissue donation for Volunteer participants. This study was approved by the Institutional Review Board of Wayne State University (WSU IRB# 073717M1E).

Laboratory methods

A single block of formalin-fixed paraffin embedded biopsy tissue from each study participant was serially sectioned

Table 1 Clinicopathologic characteristics of African American women with breast biopsy tissue from the Komen Normal Tissue Bank and the Detroit BBD Cohort (1997-2010)

N (%)	Volunteer	BBD only	BBD-cancer	BBD only to Volunteer *p* value[d]	BBD-cancer to Volunteer *p* value[d]	BBD-cancer to BBD only *p* value[d]
	n = 50	*n* = 47	*n* = 55			
Age (mean, range)	53.5 (25–76)	54.8 (29–75)	53.9 (29–73)	0.54	0.85	0.65
Age (group)						
< 45	7 (14.0%)	7 (14.9%)	8 (14.5%)	0.94	0.98	0.87
45–55	22 (44.0%)	19 (40.4%)	25 (45.5%)			
> 55	21 (42.0%)	21 (44.7%)	22 (40.0%)			
BMI[a,b] (mean, range)	33.1 (20.4–54.8)	31.5 (18.0–53.9)	31.5 (21.5–54.4)	0.32	0.28	0.98
BMI (group)						
< 25	9 (18.0%)	12 (26.1%)	11 (21.6%)	0.22	0.26	0.49
25–29	11 (22.0%)	8 (17.4%)	16 (31.4%)			
30–34	8 (16.0%)	13 (28.3%)	9 (17.6%)			
35–39	15 (30.0%)	6 (13.0%)	6 (11.8%)			
40 +	7 (14.0%)	7 (15.2%)	9 (17.6%)			
BBD[a,c]						
No histologic abnormalities	27 (55.1%)	0	0	N/A	N/A	1
Non-proliferative disease	16 (32.7%)	24 (52.2%)	28 (50.9%)			
Proliferative disease without atypia	6 (12.2%)	22 (47.8%)	27 (49.1%)			

[a]*BMI* body mass index, *BBD* benign breast disease

[b]BMIs could not be ascertained from women with unavailable height and weight. These included 5 biopsies: 1 BBD only and 4 BBD-cancer

[c]Biopsies containing proliferative disease with atypia were excluded from further analyses. These included 2 biopsies: 1 Volunteer and 1 BBD only

[d]*p* value from *t* tests or Chi-square tests for continuous or categorical variables, respectively

and de-paraffinized in a xylene-ethanol series. Endogenous peroxides were removed with a methanol/1.2% hydrogen peroxide incubation at room temperature for 30 min. HIER antigen removal was completed with a pH 6 citrate buffer and the BIOCARE Decloaking Chamber. A 40-min blocking step with Super Block Blocking buffer (Thermo Scientific) was performed prior to adding the primary antibody for CD68, DAKO #M0876, 1:100 dilution overnight. Detection was obtained using GBI Labs DAB chromagen kit (#D41-18) and counterstained with Mayer's Hematoxylin. Sections were then de-hydrated through a series of ethanol to xylene washes and cover slipped with Permount.

Slides were assessed by a pathologist (KK) for presence of CLS-B on CD68 stained slides as described in Morris et al and the presence of at least 1% of IL in stromal or epithelial tissue on hematoxylin and eosin (H&E) slides [11]. One slide was assessed for each subject, and the pathologist was blinded as to group categorization. CLS-B number was assessed on digital images of slides scanned using 3DHISTECH Pannoramic Desk from Perkin Elmer. Ten percent of slides read for CLS-B, IL, and BBD features were reviewed again with the pathologist blinded to prior read to assess internal validity. Volunteer slides reviewed for histologic abnormalities showed 80% agreement. Volunteer, BBD only and BBD-cancer slides reviewed for CLS-B presence showed 73.9% agreement; study slides reviewed for IL presence showed 80.0% agreement. Slides with discrepant calls were discussed until reviewers came to consensus.

As adipose inflammation was more likely to be detected on slides containing more adipose tissue, total adipose area (μ^2) and mean adipocyte diameter (μ) were calculated from digital images of H&E slides using the Adiposoft plugin from ImageJ (version 1.15, source: https://imagej.net/Adiposoft) according to Galarraga's methodology in batch mode [16, 17]. Adipocyte number and diameter have been linked to obesity and decreased insulin resistance [18–20] and mean adipocyte diameter has been linked to BMI in breast adipose tissue [11–13, 21, 22]. CLS-B density was calculated as number per millimeter squared, as previously described [11, 23].

Statistical methods

Differences in distribution of clinicopathologic characteristics were examined with Pearson chi-square or, where appropriate, Fisher Exact tests. We reported the

distribution of biopsy populations among normal, overweight, and obesity classes I to III as defined by the World Health Organization [24]. Kruskal–Wallis or Wilcoxon Signed-Rank Tests were used to test differences in distribution.

We assessed whether adipocyte diameter was associated with clinicopathologic characteristics among all of the women including BMI, CLS-B presence, five or more CLS-B, age, cohort, and IL presence [23]. These associations were evaluated using linear regression models. A two-sided p value of < 0.05 was considered statistically significant.

Multinomial logistic models were used to estimate the odds of being in a specific cohort by the presence of CLS-B, five or more CLS-B, or IL adjusting for the BMI, adipocyte diameter, and the logarithm of adipocyte area on biopsy (as more tissue is examined, the likelihood of identifying CLS-B or IL increases). A similar analysis was performed examining the presence of either or both CLS-B and IL. The referent group for this model was the Volunteer biopsies; comparison groups were the BBD only and BBD-cancer biopsies.

We also examined the association between BrCa risk and inflammation markers in clinically indicated biopsies (BBD only and BBD-cancer groups from the Detroit cohort). To evaluate this association, we estimated the odds of having BrCa by the presence of CLS-B, five or more CLS-B, or IL in the Detroit cohort using multivariable conditional logistic regression models with age as a stratification variable and adjusting for adipocyte diameter, proliferative disease, BMI, and the logarithm of adipocyte area on biopsy. A similar analysis was performed examining the presence of either or both CLS-B and IL. The referent group for these models was the BBD only biopsies and comparison group the BBD-cancer biopsies. R version 3.4.4 was used to perform all analyses [25].

Results

Clinicopathologic characteristics

Cohorts did not differ by age or BMI (Table 1). Distribution of women among the WHO BMI classes also did not differ significantly by cohort; overall, 21.8% were normal weight, 23.8% overweight, 20.4% obese class I, 18.4% obese class II, and 15.6% obese class III. Histological review of the Volunteer biopsies revealed that 55% of the biopsies did not show histologic abnormalities or any BBD features. BBD only and BBD-cancer biopsies from the Detroit BBD cohort did not significantly differ in distribution of Dupont and Page categorization; for both groups, 51.5% of the BBD tissue were classified as non-proliferative.

Adipocyte diameter and BMI

Mean adipocyte diameter increased with BMI (Fig. 1a) and the presence of IL (Fig. 1f), although it was not statistically significant. Mean adipocyte diameter was positively associated with the presence of CLS-B (Fig. 1b), five or more CLS-B (Fig. 1c), and study cohort (Fig. 1e). Mean adipocyte diameter did not vary by age group (Fig. 1d). BMI was not associated CLS-B, five or more CLS-B, or IL on biopsy (data not shown, $p > 0.1$ for all).

Adipose inflammation in breast tissue of varying risk

CLS-B were found in 60 (42.5%), five or more CLS-B were found in 25 (17.7%), and IL were found in 44 (31.7%) of all tissue assessed (Table 2). After adjusting for logarithm of total adipocyte area, adipocyte diameter and BMI in a multinomial logistic model, BBD only and Volunteer biopsies were similar with respect to the proportion with CLS-B, five or more CLS-B, and IL. In a similarly adjusted model, BBD-cancer biopsies were more likely to independently exhibit CLS-B (odds ratio (OR) = 5.09, 95% Confidence Interval (CI): 1.25–20.7), five or more CLS-B (OR = 10.1, 95% CI 0.94–109), or IL (OR = 4.47, 95% CI 0.83–24.0) compared to Volunteer biopsies.

Among the Detroit BBD subsets, after adjusting for logarithm of adipocyte area, adipocyte diameter, proliferative disease and BMI in a conditional logistic model, BBD-cancer biopsies were more likely to independently exhibit CLS-B (OR = 3.36, 95% CI 1.33–8.48), five or more CLS-B (OR = 5.18, 95% CI 1.65–16.3), or IL compared to BBD only biopsies (OR = 4.95, 95% CI 1.76–13.9; Table 2). In similarly adjusted models with CLS-B and IL on biopsy, both inflammation measures were associated with BrCa risk ($OR_{CLS\text{-}B}$ = 3.98, 95% CI 1.40–11.3; OR_{IL} = 4.73, 95% CI 1.61–13.9; Table 3). The effect size for five or more CLS-B and IL on biopsy and BrCa risk, was even greater ($OR_{CLS\text{-}B\geq5}$ = 4.99, 95% CI 1.32–18.9; OR_{IL} = 4.44, 95% CI 1.51–13.1). Lastly, models considering an inflammation indicator (neither IL nor CLS-B, either IL or CLS-B, or both IL and CLS-B) showed that increased inflammation was associated with increased BrCa risk (data not shown; all $p < 0.01$).

Discussion

Our findings demonstrate that CLS-B and IL on biopsy are common in benign breast tissue and are associated with subsequent risk of BrCa in AA women. Our data suggests that these measures are histologic biomarkers for BrCa risk among women with BBD and may provide additional

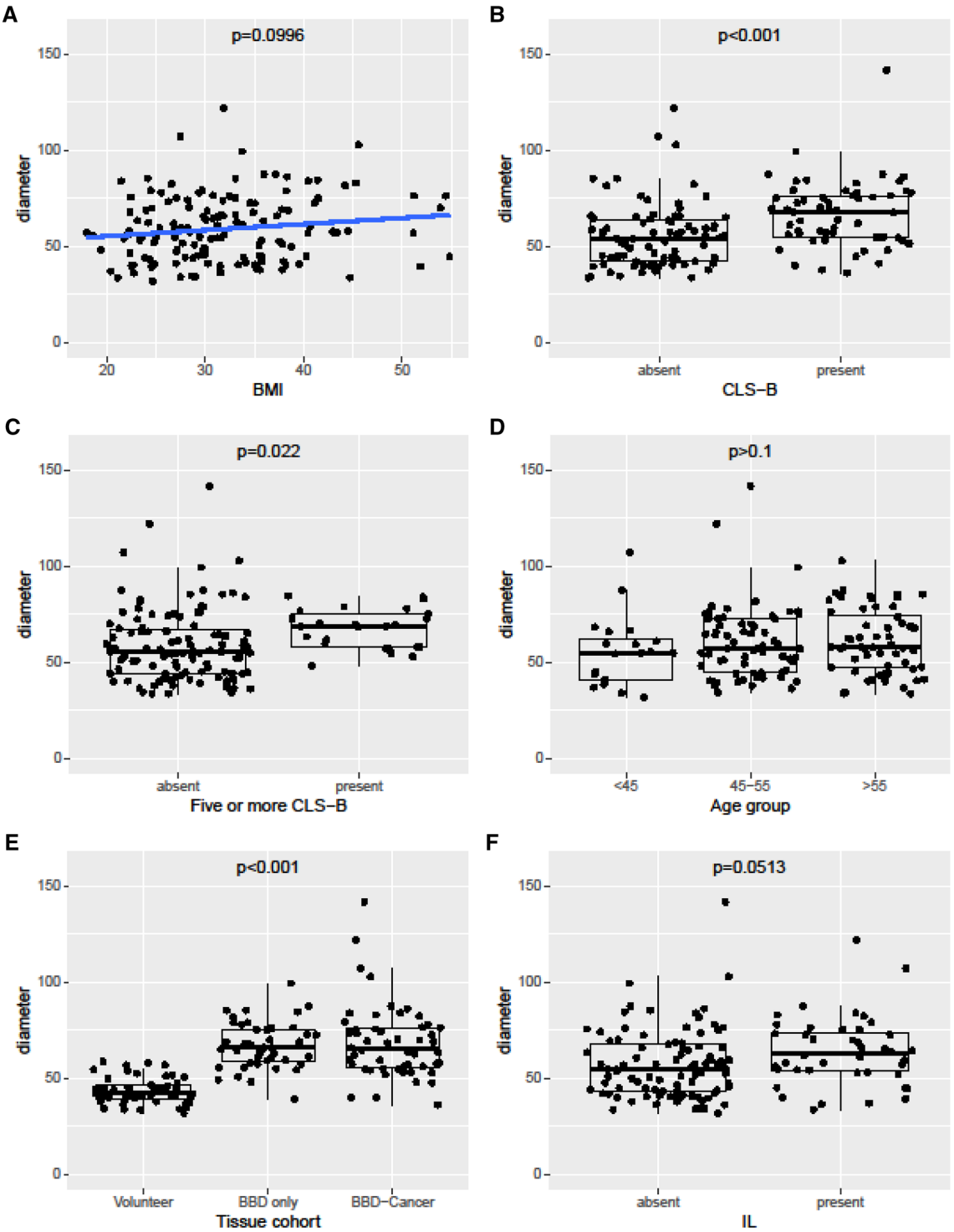

Fig. 1 Associations between adipocyte diameter (μ) and **a** BMI, **b** CLS-B presence, **c** presence of five or more CLS-B, **d** age group, **e** tissue cohort, and **f** IL presence on biopsy among African-American women in the Komen Normal Tissue Bank and the Detroit BBD Cohort (1997–2010)

insight into early events in carcinogenesis. Additionally, these markers could be candidates to better estimate risk in cohorts of women with benign breast disease, who are already at increased risk compared to women who have never undergone a clinically indicated breast biopsy.

Prior investigations of BrCa mastectomy tissue revealed that presence of CLS-B is associated with increased BMI,

Table 2 Inflammation and breast cancer risk among all breast biopsy tissue from African American women in the Komen Normal Tissue Bank and the Detroit BBD Cohort (1997–2010)

N (%)	Volunteer	BBD only	BBD-cancer	BBD only to volunteer OR (95% CI)[a,d]	BBD-cancer to volunteer OR (95% CI) [a,d]	BBD-cancer to BBD only OR (95% CI) [a,e]
	$n = 49$	$n = 46$	$n = 55$			
CLS-B[a,b]						
None	36 (81.8%)	27 (60.0%)	17 (32.7%)	Ref	Ref	Ref
Any	8 (18.2%)	18 (40.0%)	35 (67.3%)	1.22 (0.27–5.49)	5.09 (1.25–20.7)	3.36 (1.33–8.48)
< 5	43 (97.7%)	40 (88.9%)	33 (63.5%)	Ref	Ref	Ref
≥ 5	1 (2.3%)	5 (11.1%)	19 (36.5%)	1.45 (0.11–18.6)	10.1 (0.94–109)	5.18 (1.65–16.3)
Median (range)						
CLS-B number	2 (1–5)	2 (1–33)	5 (1–109)			
CLS-B/cm^2	7.2 (2.0–14.2)	0.8 (0.5–12.4)	3.7 (0.6–44.5)			
IL[a,c]						
None	41 (83.7%)	30 (75.0%)	25 (49.0%)	Ref	Ref	Ref
Any	8 (16.3%)	10 (25.0%)	26 (51.0%)	0.85 (0.13–5.54)	4.47 (0.83–24.0)	4.95 (1.76–13.9)

[a]*CLS* crown-like structures of the breast, *IL* infiltrating lymphocytes, *OR* odds ratio, *CI* confidence interval

[b]CLS-B presence could not be ascertained in slides with insufficient adipose tissue, missing tissue, or staining difficulties. These included 9 biopsies: 5 Volunteer, 1 BBD only, and 3 BBD-cancer

[c]IL presence could not be ascertained in slides with insufficient epithelial or adipose tissue. These included 10 biopsies: 6 BBD only and 4 BBD-cancer

[d]Odds ratio estimated from multinomial logistic regression (outcome variables: BBD only vs Volunteer; BBD-cancer vs Volunteer) adjusted for the logarithm of adipocyte area, adipocyte diameter and BMI (continuous)

[e]Odds ratio estimated from conditional logistic regression (outcome variable: BBD-cancer vs BBD only) stratified by 5-year age groups and adjusted for the logarithm of adipocyte area, adipocyte diameter, proliferative disease, and BMI (continuous).

Table 3 Inflammation markers and breast cancer risk in fully-adjusted regression models from African American women from the Detroit BBD Cohort, 1997-2010

Model	Variable	Odds ratio[b] (95% confidence interval)
All (CLS-B[a])		
	No CLS-B or IL	Ref
	CLS-B	3.98 (1.40–11.3)
	IL[a]	4.73 (1.61–13.9)
All (CLS-B ≥ 5)		
	No CLS-B or IL	Ref
	CLS-B ≥ 5	4.99 (1.32–18.9)
	IL	4.44 (1.51–13.1)

[a]Abbreviations: CLS, crown-like structures of the breast. IL, infiltrating lymphocytes

[b]Odds ratio estimated from conditional logistic regression (outcome variable: BBD-cancer vs BBD only) stratified by 5-year age groups and adjusted for the logarithm of adipocyte area and displayed variables. BMI was a continuous variable

post-menopausal status, and mechanisms that increase estrogen in the breast microenvironment including increased aromatase expression, activity, and elevated local estrogen to androgen ratio [11, 13, 21, 26]. These estrogen-increasing mechanisms illustrate how adipose inflammation may increase estrogen receptor-positive BrCa risk; and, with obesity and post-menopausal status, may prove important to future estrogen receptor-positive BrCa risk models. CLS-B may also serve as a general marker of inflammation in breast tissue as its presence increases with smoking exposure [13]. IL have been associated with BrCa prognosis, tumor subtype, and therapy response in mastectomy tissue from women [27, 28]. Tissue inflammation elicits wound healing responses that lead to tissue remodeling [29]; excess inflammation is associated with cancer risk in several tissues [30, 31]. These inflammation markers may be differentially associated with hormonal-dependent and hormonal-independent BrCas.

To our knowledge, only BBD cohorts from Detroit and the Mayo Clinic have been utilized to study inflammation in breast tissue prior to the development of BrCa [23, 32]. Previously suggested by Carter et al [23], our study found an association between presence of CLS-B and BrCa risk among clinically indicated biopsies. Similarly, in both studies, a more specific metric of five or more CLS-B on biopsy was associated with subsequent BrCa. Contrary to our positive associations between IL presence in the epithelial or stromal compartments and BrCa risk, Degnim et al. reported that absence of B-lymphocytes in breast lobules was associated with BrCa risk [32]. This comparison is complicated as ILs in our study were assessed on H&E stained slides

and comprise of several cell types including natural killer cells, CD20+ B cells, CD4+ and CD8+ T cells. Differences between studies may stem from differences between study cohorts. The Mayo BBD cohort primarily consists of EA women with biopsies between 1967 and 2010 [23, 32], while the Detroit BBD cohort consists of AA women taken between 1997 and 2010. Prevalence of obesity is higher in AA women compared to EA women [33], and a recent study of CLS-B in mastectomy tissue from BrCa patients indicates that CLS-B presence is also elevated in AA women compared to Hispanic and EA women [34]. Obesity prevalence has increased since the 1960s [35] and other changes in reproductive or hormonal exposures and mammographic screening over time may further contribute to potential differences between studies. As the BrCa cases of both the Detroit and Mayo BBD cohorts were predominantly estrogen receptor-positive (data not shown), these data may be more useful for estrogen receptor-positive BrCa risk models.

Contrary to other investigations [11, 12, 21–23, 26, 36], CLS-B was not associated with BMI in our study. Only one other CLS-B study had a sample size large enough for race-specific estimates in AA patients, but Koru-Sengul et al was unable to test for an association with BMI as patient heights were not collected [34]. This discrepancy may arise because of the reduced utility of BMI as a measure of adiposity in this population: AA women have lower body fat compared to EA women at the same BMI [37, 38]. Our study and several investigations of mastectomy tissue from BrCa patients or reduction mammoplasty tissue found that CLS-B is associated with adipocyte diameter [11, 13, 21, 39, 40]. Iyengar et al found CLS-B was not only present in normal weight women, but also associated with adipocyte diameter, leptin and aromatase expression [39]. Subcutaneous and visceral adipocyte diameter and number are associated with obesity and type II diabetes mellitus [18–20], thus breast adipocyte diameter may serve as a superior proxy for adipose that elicits metabolic dysfunction. Our findings suggest that adipocyte diameter and CLS-B presence may serve as better markers of a metabolically obese state than BMI in AA women. Our adipocyte diameter estimates were smaller than estimates from studies manually or digitally measuring diameters on light microscopy [11–13, 21], but were equivalent to the estimates described by Vaysse et al who also utilized the Adiposoft plugin [22]. Studies should consider this protocol to ease comparisons between studies.

Strengths of our study include the use of a contemporary cohort of AA women diagnosed with BBD and subsequently followed for BrCa to assess the relationship between CLS-B and BrCa risk. Additionally, our study included donated tissue controls from KTB, i.e. tissue from women without a clinical indication for biopsy who donated breast tissue for research purposes [14]. A majority of CLS-B studies [11, 12, 21, 22, 26, 34, 36] utilize mastectomy or prophylactic mastectomy tissue, which reflects extremely high-risk tissue that may be enriched for CLS-B compared to our study tissue of biopsies from normal and BBD tissue from the KTB and Detroit BBD Cohort. Other CLS-B studies were able to utilize reduction mammoplasty tissue [39, 41], however this tissue is also likely higher risk as it is more likely to exhibit BBD and less likely to exhibit lobular involution on microscopy than KTB normal population-level risk tissue [42]. Another strength of the study is the ease of translating this approach to the clinic. Our results suggest that reading H&E slides for IL presence and staining only one additional slide for CD68 to assess CLS-B presence can, if replicated, provide valuable information on BrCa risk without a prohibitive cost. Standardized measures that are reproducible and cost-effective are better candidate biomarkers for future risk models.

This was a pilot study, with a limited sample size, but was adequately powered to detect an association between CLS-B and BrCa risk independent of age and BMI. There is currently no standard metric to evaluate CLS-B and IL presence among breast biopsy tissue and studies vary in biomarker assessment, complicating comparisons between studies. We examined one CD68-stained slide per patient to determine CLS-B status as BBD tissue is limited, while several other CLS studies [11, 12, 21, 36] using tumor-adjacent or mammoplasty tissue examined five slides per patient. Increases in adipose tissue area assessed likely increases the likelihood of finding a CLS-B. Another potential limitation is no standardly available clinical information on other BrCa risk factors including age at menarche, parity, BRCA status, and family history that may confound the relationship between CLS-B and BrCa risk was available due to the retrospective nature of the BBD cohort. Examination of the tissue may compensate somewhat for this limitation, as the tissue represents the totality of all exposures. We were unable to examine BrCa survival in this study, however Koru-Sengul et al found that CD206+ macrophage density was associated with poorer progression-free survival in a diverse cohort [34]. This group also found that AA BrCa patients had higher numbers of CD163 + macrophages and CLS-B than non-black Latina and Caucasian BrCa patients. Cha et al found that CD163 + macrophages were associated with shorter disease-free survival in node-negative patients and that CLS-B were associated with shorter overall survival in node-positive BrCas [43]. Given that the AA women in this study had undergone a breast biopsy, they were more likely to have access to care than other women in this population. AA women in Detroit are also at higher risk of BrCa compared to their counterparts in the United States [44]. Thus, risk estimates derived from this population may overestimate the association between tissue-based markers of risk and subsequent BrCa.

Our study findings are applicable to women with an indication for breast biopsy, approximately 1.6 million women a year in the US [10]. Approximately 95% of women with BBD who develop BrCa have non-proliferative or proliferative disease without atypia on biopsy, and unlike those with atypical hyperplasia, there is currently no evidence-based standard of care protocols for follow up [7]. Refining our understanding of BrCa risk can allow us to personalize surveillance and prevention efforts, especially against the rising incidence of estrogen receptor-positive cancers. CLS-B could be used to better identify patients exhibiting metabolic obesity who are poised to benefit from behavioral changes or more frequent BrCa screening. Additional studies must be completed before CLS-B or IL can be used as a histological marker of BrCa risk. Risks associated with these markers need to be validated in a larger cohort and, while quite distinct, formal studies of pathologic reproducibility are also warranted. Our study and a few others in mastectomy tissue [12, 34] suggest race-specific nuances to the relationship between CLS-B and risk of a subsequent BrCa, and further point to the importance of diverse and contemporary cohorts to characterize BrCa risk factors.

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Author contributions ANS, JJR, GD, and MLC were involved in the conception and design of the study. ANS, KK, KS, JLB, JJR, HD, SB, and RAF were involved in the acquisition of data. ANS, JJR, and MLC were involved in study supervision. ANS, JJR, GD, and MLC were involved in the analysis and interpretation of data. All authors were involved in the writing, review, and/or revision of this manuscript and approved the final manuscript.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Compliance with ethical standards

Conflicts of interest The study sponsors had no role in the design of the study; the collection, analysis or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication. All authors declare they have no conflicts of interest.

Ethical approval This study was approved by Institutional Review Board of Wayne State University for human subject protection. The study complies with all current laws of the USA.

Informed consent This retrospective study received a waiver for informed consent due to the minimal risk and feasibility of re-contacting patients from the Detroit BBD cohort, many who no longer receive care at Barbara Ann Karmanos Cancer Center, moved, or are deceased. Informed consent was obtained from participants donating tissue to the Susan G. Komen Normal Tissue Bank at Indiana University Simon Cancer Center; tissue was de-identified before inclusion in this study.

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