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Non-tumor breast tissue from obese black women exhibit higher prevalence of crown like structures and inflammation compared to Caucasians

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Abstract

Non-tumor breast tissue from obese black women exhibit higher prevalence of crown like structures and inflammation compared to Caucasians

By Aswathy Miriam Cheriyan

Background: BC rates are higher among NHW women, however, there is an increased BC incidence risk and worse prognosis among NHB [1]. These disparities maybe partly due to the higher rates of obesity in NHB population. In this pilot study, we conducted an epidemiologic investigation of obesity, CLS prevalence, and associated outcomes in a retrospective cohort of NHB and NHW women diagnosed with BC, and we hypothesized that the presence and density of CLS-B are more prevalent among NHB than NHW women.

Methods: The study population consisted of 283 NHB and NHW women. Preoperative height and weight were used to calculate BMI measured using cut-points based on World Health Organization (WHO) definitions[2]. CLS-B presence was defined as any CLS-B observed on the tissue section examined. The severity of breast WAT inflammation was quantified as number of CLS-B per square centimeter of breast WAT with the median as the cutoff to differentiate between severe and mild inflammation. To assess whether race and BMI were associated with CLS-B presence and breast WAT inflammation, we evaluated relationships using multivariable logistic regression to determine prevalence odds ratios (PORs) and 95% confidence intervals (CIs) for the presence of CLS-B.

Results: A total of 155 (53%) of the women where NHB and the remaining were NHW. The higher proportion of obese women were NHB, while a higher proportion of normal weight women were NHW. The age-adjusted prevalence odds of having presence of any CLS-B was 1.15 times [95% CI (0.69, 1.92] for NHB compared to NHW women. The adjusted prevalence odds of presence of any CLS-B was highest among obese women when compared to normal weight women [POR = 5.42; 95% CI (2.72, 10.82)]. NHB women were approximately 83% more likely to have severe breast WAT inflammation compared to NHW women [POR=1.83; 95% CI (0.937, 3.59]. The adjusted prevalence odds of severe breast WAT inflammation among women with BMI $\geq 30 kg/m^2$ was 3.60 [95% CI (1.76, 7.37)] compared to women with BMI $\leq 29.9 kg/m^2$.

Conclusion: Our work demonstrates that CLS-B presence and breast WAT inflammation is associated with higher BMI among NHB women with breast cancer compared to NHW women.

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INTRODUCTION

Breast cancer (BC) is the most common type of cancer and also the leading cause of cancer-related deaths in women, worldwide. In the United States (U.S) in 2019, there will be an estimated 268,600 new cases of invasive BC diagnosed in women [3]. Compared with White women, African-American (AA) women are ~6% less likely to develop BC but ~40% more likely to die of the disease[4]. Epidemiological studies have identified numerous non-modifiable risk factors for female BC, which includes reproductive and hormonal factors (like early menarche, late menopause, and nulliparity), older age, high breast density, genetic predisposition and adult attained height [5-7]. Studies have also shown that there is considerable evidence of several modifiable risk factors such as body fatness especially postmenopausal obesity, physical inactivity, tobacco smoking, alcohol consumption and dietary factors to be individually associated with BC risk [8-12].

Worldwide obesity increases the risk of BC by ~25% and is hypothesized to account for ~20% of all BC deaths [13, 14]. Obesity disproportionately affects AA women with an age-adjusted prevalence of 56% compared to ~38% among Whites, and compared to White women, AA women are ~ 2% less likely to develop BC but ~41% more likely to die of the disease[4, 15]. Obesity leads to altered expression of hormones, growth factors, inflammatory cytokines, and adipokines which promote cancer cell survival, angiogenesis and decreased cancer cell apoptosis [16]. Epidemiological and experimental studies show evidence that the endocrine function of adipose tissue, especially white adipose tissue (WAT), is to secrete adipocytokines such as resistin, adiponectin and leptin. Resistin, an adipokine secreted by adipose tissue, has been correlated to inflammation, obesity, and breast cancer mortality through the stimulation of interleukin-6 (IL-6), and studies have indicated higher resistin expression in breast cancer tissue of AA patients[17]. In addition to the systemic effect of

obesity, adipocyte hypertrophy occurring locally in the breast of overweight or obese women can lead to macrophage recruitment and inflamed WAT [18].

Adipose tissue is the primary source for many pro-inflammatory cytokines, and adipose tissue macrophages (ATMs) which are important cellular components of adipose tissue, perform key regulatory functions in inflammation, insulin resistance and adipocyte functions [19]. ATMSs are responsible for all adipose tissue tumor necrosis factor- alpha (TNF- α) expression and significant amounts of nitric oxide and IL-6 expression[20]. Obesity is accompanied by a transformation in the polarized states of macrophages from an anti-inflammatory 'alternatively activated' M2 form to a more pro-inflammatory 'classically activated' M1 form, and the ratio of M1-to-M2 macrophages is increased in obesity [2, 20-22]. Both animal models and human studies of overweight and obesity have shown that macrophages infiltrate visceral and subcutaneous adipose tissues. The mature macrophages interact with the adipocytes and form a vicious cycle that aggravates inflammatory changes in the adipose tissue, forming a characteristic crown -like structure (CLS) around necrotic adipocytes. CLS's exhibits a unique microenvironment for macrophage proliferation and CLS macrophages intensely produce pro-inflammatory cytokines. CLSs in the WAT of the breast (CLS-B) in obese women with BC are responsible for both increase in local aromatase activity and enhanced invasiveness and metastasis capacity of BC[23]. CLS-B in obese women with BC indicates the relationship between inflammation and aromatase activity and also points to the increased BC risk and poor prognosis [20, 24]. Breast inflammation defined by CLS-B is paralleled by increased NFkB- binding activity and elevated levels of aromatase expression, the rate-limiting enzyme for estrogen biosynthesis. Previous studies have shown that among White women, CLS-B is found at higher rates in the adipose tissue of obese individuals and is associated with activation of the NFkB transcription factor in breast tissue and higher

expression of aromatase.[24, 25]. Also, data suggests that CLS-B is associated with worse disease-free survival among White breast cancer patients [26].

Approximately 90% of U.S. women with BMI ≥30 kg/m^2 have breast WAT inflammation which is defined by the presence of CLS-B, and that increased levels of aromatase occurs in association with both obesity and breast WAT inflammation [24, 25, 27]. Also, a recent case-control study showed that higher CLS-B density is associated with elevated risk of BC in women with benign breast disease [28]. In addition, breast WAT inflammation has been shown to be associated with systemic metabolic and proinflammatory abnormalities and a worse clinical course in patients that develop metastatic breast cancer [26].

There are higher rates of obesity among Non-Hispanic black (NHB) women with BC which is also reflective of the general U.S. NHB population [29, 30]. Though BC rates are higher among Non-Hispanic White (NHW) women, there is an increased BC incidence risk and worse prognosis among NHB [1]. These disparities maybe partly due to the higher rates of obesity in NHB population. CLS-B have not been studied in NHBs and in comparison, to NHWs to assess their relative frequency overall and within subgroups defined by age and BMI. In this pilot study, we conducted an epidemiologic investigation of obesity, CLS prevalence, and associated outcomes in a retrospective cohort of African-American and White women diagnosed with BC, and we hypothesized that the presence and density of CLS-B are more prevalent among NHB than NHW women.

METHODS

Study population and biospecimen acquisition

Non-Hispanic Black (NHB) and Non-Hispanic White (NHW) women who underwent mastectomy at Emory University Hospital (EUH) and Emory University Hospital Midtown (EUHM) between January 1, 2007 and December 31, 2012 where identified via the tumor registries of EUH and EUHM, with verification through the Clinical Data Warehouse (CDW) database [Fig 1.]. The study population consisted of 283 self-identified Non-Hispanic Black (NHB) and Non-Hispanic White (NHW) women, aged ≥18 years, diagnosed with a primary invasive stage I-III BC (ICD: C50) between January 1, 2007 and December 31, 2012 who underwent mastectomy for treatment of their BC at EUH or EUHM. Eligible cases had not received neoadjuvant treatment for treatment of the primary breast cancer, or received any systemic therapy for treatment of previous cancer diagnoses. Eligible case women had available archived formalin-fixed, paraffin-embedded (FFPE) sample of normal adjacent breast tissue from a quadrant uninvolved by tumor obtained via mastectomy. Informed consent was provided by the women undergoing mastectomy at EUH and EUHM. This study was approved by the Institutional Review Board of Emory University.

Non-tumor containing breast WAT specimens were obtained under a standard tissue acquisition protocol. For each participant, up to three paraffin blocks were selected from the FFPE tissue blocks prepared from the breast WAT obtained from quadrants not involved by tumor, following mastectomy surgery. Specimens where examined with hematoxylin and eosin (H&E) staining by a pathologist to ensure samples were representative of normal breast tissue and to select the best block for further processing. For each case, non-tumor block with the greatest area of fat and neither evidence of biopsy tract change, fat necrosis and increased

inflammation was selected, to ensure the selection of the one best WAT enriched block was selected per patient.

Demographics and clinical characteristics

Demographic and clinicopathological characteristics included: age, race, obstetric and gynecologic history, family history of breast cancer among first degree relatives, BRCA mutation status, tumor stage, tumor grade, tumor size, hormone receptor status, Ki-67 status, diagnoses of comorbidities (*e*.g., hypertension, diabetes, thyroid disease and dyslipidemia), alcohol use and smoking history, medications used, and administered treatments (*e.g.*, adjuvant systemic treatments and radiation therapy), which were systematically extracted from tumor registries and electronic medical records (EMR) by research staff. Independent data reviews were conducted for quality assurance. BMI was calculated using height and weight recorded prior to surgery (measured continuously as well as using cut-points based on World Health Organization (WHO) definitions: BMI<25kg/m2 [under or ideal weight], BMI 25-29.9 kg/m2 [overweight], or BMI ≥ 30kg/m2 [obese])[2]. Tumors were classified as estrogen receptor (ER) and/or progesterone receptor (PR) positive if >1% staining by immunohistochemistry (IHC) was reported. Additionally, human epidermal growth factor receptor-2 (HER2) was considered positive if IHC 3+ or FISH amplification ≥ 2.2. Subtypes were defined as luminal (ER or PR positive), HER2 overexpressing (ER and PR negative and HER2 positive), and triple negative (ER and PR and HER2 negative). Menopausal status was categorized as premenopausal, perimenopausal or postmenopausal with women in the latter group exhibiting one of the following characteristics at time of diagnosis: (1) having had bilateral oophorectomy; (2) reporting permanent cessation of menses for 12 or more months in the

absence of chemotherapy or endocrine therapy; or (3) age >55 years at diagnosis if data were missing.

CLS-B Analysis

To evaluate the presence of CLS-B, consistent with previously established methods, IHC was performed on deparaffinized, rehydrated sections obtained from representative formalin-fixed, paraffin-embedded tissue blocks (one block/sample), using antibody-specific epitope retrieval techniques with the Dako Envision (Dako) automated system for detection of CD68 (1:200 dilution, monoclonal mouse anti-human CD68 clone KP1, M0814, DAKO, Denmark) at Winship Cancer Institute's Pathology Core Laboratory (Emory University). The unstained tissue sections were sequentially cut from the corresponding tissue blocks and were within an estimated 100 to 200μm of the original H&E-stained section. Whole slide digital images of anti–CD68-immunostained slides were captured with the3DHISTECH Panoramic Scanner 150 and the images were analyzed using Panoramic Viewer 1.15.4 (3DHISTECH Ltd, Budapest, Hungary). Immunostains were scored by pathologist, masked to clinicopathological data at the time of the scoring. CD68 and CLS-B were visually assessed by a single reviewer (AMC) with validation of a subset by two independent pathologist (UK and MS).

CLS-B presence was defined as any CLS-B observed on the tissue section examined. The presence and number of CLS-B were assessed within the observed fat area on the whole tissue slide, and each CLS- B was manually counted and annotated on the digital image [Fig 2.]. Complete CLS-B was defined as-encirclement of adipocytes by CD68-positive macrophages ≥ 90%, and borderline CLS-B was defined as encirclement of adipocytes by CD68-positive macrophages ≥ 50% but < 90% [Fig 2.]. Borderline CLS-B was further divided as encirclement of adipocytes by CD68-positive macrophages ≥ 50% but < 75% and

encirclement of adipocytes by CD68-positive macrophages ≥ 76% but < 89%. We also accounted for clustered CLS-B defined as two or more adipocytes, directly neighboring one another, surrounded by CD68+ macrophages and the degree of encirclement was complete or borderline.

CLS- B density or Breast WAT inflammation

Total breast WAT area (cm^2) was determined as the product of total fat percentage (eyeballed) and the total tissue area on the slide. As previously defined, the severity of breast WAT inflammation or CLS-B density was quantified as number of CLS-B per square centimeter of breast WAT (CLS-B/cm^2) with the median 0.84 CLS-B/cm^2 as the cutoff to differentiate between severe and mild inflammation. To ensure reproducibility, assessment of CLS-B presence and percentage of fat area for the study participants (n = 80) was compared between three independent pathologists.

Statistical Analysis

Median and interquartile range were used to describe selected characteristics of the study population such as age at diagnosis, BMI, age at first pregnancy, number of pregnancies, number of children, duration of breastfeeding and tumor size. Additionally, frequencies and percentages were used to describe categorical characteristics such as BMI (based on WHO classification), hormone receptor status, tumor grade, tumor stage, alcohol use history, smoking history of the study population. Potential confounding factors such as age, BMI and smoking status were selected for inclusion in final multivariable models based on known associations in the literature and causal graphical analyses (directed acyclic graphs, DAGs)[31,

32] . To assess whether race and BMI were associated with CLS-B, we evaluated relationships using multivarible logistic regression to determine prevalence odds ratios (PORs) and 95% confidence intervals (CIs) for the presence of CLS-B. For assessing the association of race and BMI with severity of breast WAT inflammation, we evaluated relationships using multivariate logistic regression and polytomous logistic regression to determine PORs and 95% CIs of severity of breast WAT inflammation.

RESULTS

Clinicopathologic Characteristics

A subset of 283 eligible women with stage I-III breast cancer who underwent mastectomy, with usable breast white adipose tissue samples, were selected from the entire CLS-B cohort. A total of 155 (53%) of the women where NHB and the remaining were NHW. The median age of the 283 patients was 54 years (range: 45.5, 62 years; Table 1). The median BMI was 27.7 kg/m^2 (range: 23.2, 33.4 kg/m^2; Table1). Approximately 61% of the women where postmenopausal and 30% premenopausal. Overall, the subset of participants for this thesis were similar to the entire cohort [Fig 1.].

Clinicopathologic characteristics among NHB and NHW women are compared in Table 1. Age and menopausal status were similar between two races. Median BMI was higher in NHB than in NHW women. The median age of pregnancy was 25 years (range: 21, 30 years) for NHW while it was 21 years (range: 18, 26 years) for NHB women. Among NHW 40% reported breastfeeding and the median duration of breastfeeding was for 10 months, while it was 27% and 6 months respectively for NHB women. The proportion of poorly differentiated tumors were higher among NHB than NHW women. Table 1 also compared the

clinicopathologic characteristics among the different classes of BMI, normal weight, overweight and obesity, based on the WHO classification. The higher proportion of obese women were NHB, while a higher proportion of normal weight women were NHW. Age, menopausal status, tumor grade and hormone receptor status were similar between the different classes of BMI.

Association between CLS-B and Race

Overall CLS-B was present in approximately 33% of the patients with BC of which approximately 18% was among NHB women. There was no evidence of conventional adipose tissue necrosis. The age-adjusted prevalence odds of having presence of any CLS-B was 1.15 times [95% CI (0.69, 1.92); Table 2] in NHB compared to NHW women. Also among NHB women the prevalence odds of presence of any CLS-B ≥ 75% and complete CLS-B were 1.14 [95% CI (0.63,2.09)] and 1.07 [95% CI (0.61, 1.87)] compared to NHW women.

Association between CLS-B and BMI

CLS-B presence was highest among obese category women at approximately 20% and, the overall association of BMI with CLS-B presence was strong. In an unadjusted logistic regression model of BMI as a three level category variable the prevalence odds ratio of any CLS-B was highest among obese women when compared to normal weight [POR= 5.11; 95%CI (2.69, 9.67)] and in a multivariable logistic regression model adjusted for age, race and smoking history, the adjusted prevalence odds of presence of any CLS-B was highest among obese women when compared to normal weight women [POR = 5.42; 95% CI (2.72, 10.82); Table 2]. The prevalence odds of CLS-B across the BMI class overweight and obese, for presence of CLS-B was significant (all $P<0.05$). When BMI was used in the analysis as a

continuous variable the prevalence odds ratio of any CLS-B was 8.5% [POR=1.085; 95% CI (1.05, 1.12)].

Association between Breast WAT inflammation and Race

Using the median as a cutoff, the severity of breast WAT inflammation was defined as mild (<0.84 CLS-B/cm2) and severe (≥0.84 CLS-B/cm2). Overall NHB had a higher prevalence of breast WAT inflammation at 55%. While NHW women had a higher prevalence of mild breast WAT inflammation at 25%, NHB had a higher prevalence of severe breast WAT inflammation at 33%. In an unadjusted logistic regression model. In the age-adjusted multivariable logistic model, NHB women were approximately 83% more likely to have severe breast WAT inflammation compared to NHW women [POR=1.83; 95% CI (0.937, 3.59), Table 3]. Additionally, in the age adjusted multivariable logistic model, the prevalence odds of severity of breast WAT inflammation compared to mild breast WAT inflammation among CLS-B positive NHB women were 2.78, compared to NHW [95% CI (1.15, 6.68) Table 3]. Also, in a multivariate logistic model the prevalence odds of severe breast WAT inflammation among NHB women with CLS-B presence was 3.91 times that of mild breast WAT inflammation compared NHW with CLS-B presence, when adjusted for age, BMI and total fat area [POR= 3.91;95% CI (1.23, 7.74)].

Association between Breast WAT inflammation and BMI

For this association BMI was considered as a binary variable with the cut off value being 29.9 kg/m^2. Overall obese category had a higher prevalence of breast WAT inflammation at 60%. In the unadjusted analysis, overweight women had a higher odds of prevalence of mild inflammation compared to normal weight women [POR=3.67;

95%CI(1.35, 10.0)]. In a fully adjusted multivariable polytomous regression model adjusted for age, race and smoking history, the adjusted prevalence odds of severe breast WAT inflammation among women with BMI ≥30 kg/m^2 was 3.60 [95% CI (1.76, 7.37) Table 3] compared to women with BMI ≤ 29.9 kg/m^2. While in a similar multivariate model when adjusting for age and smoking history only, the adjusted prevalence odds of severe breast WAT inflammation among women with BMI ≥30 kg/m^2 was 3.85 times higher than women with BMI ≤ 29 kg/m^2 [POR= 3.85; 95% CI (1.94, 7.64)].

DISCUSSION

In this pilot study, we investigated CLS status in the adipose tissue of non-tumor areas of mastectomy specimens and its association with race and BMI. Macrophagic CLSs are a hallmark of chronic inflammation within adipose tissue and are frequently present in breast adipose tissue from women with breast cancer and in breast tissue of obese women. Using a well characterized non-tumor breast cancer patient cohort, we assessed the frequency, prevalence and quantity of CLS-B in the stromal breast tissues.

Our findings demonstrate that CLS-B were found to have a higher prevalence among NHB women compared to NHW women. This consistent with a previous study which reported that black breast cancer patients had a larger number of CLSs than the other races (Caucasians and non-black Latinas) [1]. In the present study on BMI comparisons, overweight and obese women had a higher CLS-B prevalence when compared to normal weight women. Also, our findings show that, the prevalence of CLS-B was highest among NHB obese women at approximately 25% while among NHW obese women the prevalence was 14%. Differences in genetic makeups could have impacted these differences.

The prevalence and severity of breast WAT inflammation were strongly associated with NHB women and with higher BMI in this cohort. The prevalence of severe breast WAT inflammation was higher among NHB women, while the prevalence of mild breast WAT inflammation was higher among NHW women. Our findings also demonstrated that among CLS-B positive women, obese category women had a higher prevalence of breast WAT inflammation at 60%. In this cohort, overall among NHB obese women, severe breast WAT inflammation had a prevalence of 42%, while NHW obese women had a higher prevalence of mild breast WAT inflammation at 28%. Collectively, these findings identify higher BMI as a potential risk factor in African American women, and breast WAT inflammation may be a contributing mechanism.

The prevalence of CLS-B and severity of breast WAT inflammation in this cohort of NHB and NHW breast cancer women is comparable to with previous studies of NHW patients, in which 15-59% of patients with breast cancer had CLS-B and breast WAT inflammation [14, 24-26, 28, 33]. Consistent with our hypothesis and previous literature, patients who were overweight or obese were positively associated with severity of breast WAT inflammation. However, breast WAT inflammation was present in approximately 19% of normal weight women in the cohort, and was absent in 35% of women in the overweight and obese category. The fact that some obese patients are not inflamed and a subset of patients with normal BMI is inflamed suggests that BMI alone does not accurately predict breast WAT inflammation. In this cohort of breast cancer patients, the proportion of NHB women in the obese category was approximately equal to the proportion of NHW normal BMI women (~51%). The high number of CLS- and thus, the amount of breast adipocyte inflammation-observed in the non-tumor breast tissue of NHB patients was more likely associated with their obesity status rather than their biological race. However, there are higher rates of obesity

among Non-Hispanic black (NHB) women with breast cancer which is also reflective of the general U.S. NHB population[29, 30].

Strengths of our study include the use adipose tissue from the non-tumor area of the mastectomy specimens and tissue assessment by pathologist experienced in the detection and grading of breast WAT inflammation. The BMI measurements were calculated based on pre-operative measured height and weight. Also, another strength of the study was that each immunostained tissue slides had internal CD68+ positive controls. Though this is a pilot study, to our knowledge this is the largest study of human non-tumor breast WAT to date. Moreover, elucidating the intersections among race and breast WAT inflammation is novel and biologically informed approach to combating the obesity epidemic which has disproportionately affected minority populations.

Our study is limited by its retrospective design. Specifically, detailed information on smoking status, alcohol history, physical activity were not collected prospectively using standard questionnaires, making it difficult to examine the causal relations between lifestyle factors and CLS-B presence and breast WAT inflammation. Prospectively designed studies in ethnically diverse populations are needed. The relatively small sample size limited our power to analyze multiple factors simultaneously. Our analysis relied on visual assessment of CLS-B which introduces the possibility of sampling error due to the subjective nature of the visual assessment. However, we addressed this limitation and to ensure reproducibility, assessment of CLS for the study participants (n=80) was compared between three pathologists. More recent applications of novel imaging technologies, including Raman spectroscopy, have successfully detected CLS in ex vivo fresh frozen non-cancerous tissue from mouse models as well as women without cancer, and may enable future objective high-throughput assessments of CLS. The use of such imaging methods will have important implications for

the translation of CLS assessment into a clinical setting [34]. The cross-sectional association between CLS-B and obesity does not imply that chronic inflammation as measured by CLS-B mediates the obesity-breast cancer association. An additional limitation of our study was that the cohort did not include with normal breast tissue or benign breast disease or women who received neo-adjuvant chemotherapy making the study less generalizable. Nonetheless, this is the first large study to evaluate CLS_B presence and breast WAT inflammation of non-tumor breast tissue in a cohort exclusively of NHB and NHW breast cancer women. Our findings establish rationale for further studies of CLS-B presence and breast WAT inflammation in African American women.

CONCLUSIONS

In summary, our work demonstrates that CLS-B presence and breast WAT inflammation is associated with higher BMI among NHB women with breast cancer compared to NHW women. Understanding the mechanisms contributing to worse breast cancer outcomes among AA women, such as breast WAT inflammation, will provide key insights into selecting intervention strategies that are likely to be effective. As breast WAT inflammation has been associated with increased risk of BC and worse BC prognosis, the findings here support the need to develop noninvasive strategies to identify women with breast WAT inflammation and identify effective interventions to reduce breast WAT inflammation and reduce BC and/or improve BC prognosis. Efforts are underway to develop noninvasive histologic biomarkers to identify women with CLS-B. Likewise, for women with CLS-B presence undergoing mastectomy, anti-inflammatory interventions may improve BC prognosis. Additionally, it is possible that lifestyle interventions (e.g., diet, physical activity, smoking cessation, weight loss) could reduce breast WAT inflammation and reduce the risk

of BC. The findings of our study are stimulating and require future prospectively designed studies with diverse race/ethnicity and larger sample sizes and more extensive tissue sampling to better understand the role of macrophagic CLS-B and the prevalence correlates of breast WAT inflammation in BC risk prediction and breast carcinogenesis.

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TABLES

Table 1: Clinicopathological characteristics

		Race		BMI Who Classification		
Patient Characteristics	**Overall (n=283)**	**Non-Hispanic Black (n= 150)**	**Non-Hispanic White (n=133)**	**<25 kg/m2 (n=107)**	**25-29.9 kg/m2 (n=62)**	**≥30 kg/m2 (n=110)**
Age (median, IQR)	54 (45.5, 62)	54 (45, 63)	54 (47, 62)	52 (43,59)	57 (46, 68)	56 (49, 62)
18-49 years n(%)	90 (31.8)	48 (32.0)	42 (31.6)	46 (43.0)	17 (27.4)	27 (24.6)
50-59 years n(%)	87 (30.7)	41 (27.3)	46 (34.6)	33 (30.8))	19 (30.6)	34 (30.9)
≥ 60 years n(%)	95 (33.6)	50 (33.4)	45 (33.8)	25 (23.4)	24 (38.7)	44 (40)
Missing	11 (3.89)	11 (7.3)	0	3 (2.8)	2 (3.3)	5 (4.5)
Race n(%)						
Non- Hispanic Black	150 (53)	-	-	38 (35.5)	31 (50.8)	77 (70.0)
Non- Hispanic White	133 (47)	-	-	69 (64.5)	30 (49.2)	33 (30.0)
BMI (median, IQR)	27.7 (23.2, 33.4)	30.3 (24.6, 35)	24.7 (22.4, 30.2)	22.5 (21, 23.7)	27.4 (26.2, 29)	34.5 (32.6, 39)
<25kg/m2 n(%)	107 (37.8)	38 (25.4)	69 (51.9)	-	-	-
25-29.9 kg/m2 n(%)	62 (21.9)	31 (21.3)	30 (22.6)	-	-	-
≥30 kg/m2 n(%)	110(38.9)	77 (51.3)	33 (24.8)	-	-	-
Missing n(%)	4 (1.4)	3 (2.0)	1 (0.7)	-	-	-
Age at Menarche (median, IQR)	12 (12, 13)	12 (11.5, 13.5)	12 (12,13)	13 (12, 14)	13 (12, 14)	12 (11, 13)
Missing	44	30	14	11	12	18
Age at First Pregnancy (median, IQR)	23 (19, 28)	21 (18, 26)	25 (21, 30)	24.5 (20.5, 31)	24 (19, 27)	22 (18, 26)
Missing	82	52	30	35	17	27
Total Number of Pregnancies (median, IQR)	2 (1,3)	2 (1, 4)	2 (1,3)	2 (1,3)	2 (1,3)	2 (2, 4)
Missing	51	39	12	12	9	27
Total Number of Children (median, IQR)	2 (1,3)	2 (1,3)	2 (1,3)	2 (1,2)	2 (1,3)	2 (1,3)
Missing	52	27	25	14	16	19

Breastfeeding n(%)						
Yes	93 (32.9)	40 (26.7)	53 (39.8)	43 (40.1)	16 (25.8)	34 (30.9)
No	113 (39.9)	48 (32.0)	65 (48.9)	42 (39.3)	27 (43.5)	44 (40)
Missing	77 (27.2)	62 (41.3)	15 (11.3)	22 (20.6)	19 (30.7)	32 (29.1)
Duration of Breastfeeding (median, IQR)	8.5 (4, 15)	6 (3,12)	10 (4, 18)	6 (3, 12)	6 (3, 18)	6 (3, 18)
Missing	201	119	82	67	47	83
Menopausal Status n(%)						
Premenopausal	84 (29.7)	45 (30.0)	39 (29.3)	42 (39.3)	16 (25.8)	25 (22.7)
Perimenopausal	9 (3.2)	4 (2.7)	5 (3.7)	4 (3.7)	1 (1.6)	4 (3.6)
Post-Menopausal	173 (61.1)	88 (58.7)	85 (64.0)	58 (54.2)	39 (64.5)	73 (66.4)
Missing	17 (6.0)	13 (8.6)	4 (3.0)	3 (2.8)	5 (8.1)	8 (7.3)
HRT Use n(%)						
Yes	61 (21.6)	21 (14.0)	40 (30.1)	28 (26.2)	16 (25.8)	17 (15.5)
No	186 (65.7)	101 (67.3)	85 (63.9)	69 (64.5)	36 (58.1)	80 (72.7)
Missing	36 (12.7)	28 918.7)	8 (6.0)	10 (9.3)	10 (16.1)	13 (11.8)
Family history of Breast Cancer n(%)						
Yes	73 (25.8)	40 (26.6)	33 (24.8)	25 (23.4)	16 (25.8)	32 (29.1)
No	196 (69.3)	97 (64.7)	99 (74.5)	78 (72.9)	41 (67.7)	74 (67.3)
Missing	14 (4.9)	13 (8.7)	1 (0.7)	4 (3.7)	4 (6.5)	4 (3.6)
BRCA1 n(%)						
Positive	3 (1.0)	2 (1.3)	1 (0.7)	-	2 (3.2)	1 (0.9)
Negative	51 (18.0)	21 (14.0)	30 (22.6)	28 (26.2)	8 (12.9)	15 (13.6)
Missing	229 (81)	127 (84.7)	102 (76.7)	79 (73.8)	52 (83.9)	94 (85.5)
Tumor Size (median, IQR)	1.9 (1, 3)	2.1 (1.1, 3.5)	1.6 (0.9, 2.5)	1.6 (0.9, 2.4)	1.6 (0.8, 3.4)	2.2(1.2, 3.5)
Missing	15	10	5	6	5	4
Tumor Grade n(%)						
Well Differentiated	68 (24.0)	27 (18.0)	41 (30.8)	26 (24.3)	15 (24.2)	26 (23.6)
Moderately Differentiated	112 (39.6)	57 (38.0)	55 (41.4)	44 (41.1)	27 (43.6)	38 (34.6)
Poorly Differentiated	90 (31.8)	58 (38.7)	32 (24.0)	31 (29)	17 (27.4)	42 (38.2)
Missing	13 (4.6)	8 (5.3)	5 (3.8)	6 (5.6)	3 (4.8)	4 (3.6)
Tumor Stage n(%)						

Stage 0	6 (2.1)	5 (3.3)	1 (0.8)	-	3 (4.8)	3 (2.7)
Stage I	1 (0.4)	1 (0.7)	-	-	-	1 (0.9)
Stage I A	116 (41.0)	52 (34.7)	64 (48.1)	50 (46.7)	26 (41.9)	39 (35.4)
Stage I B	3 (1.1)	2 (1.3)	1 (0.8)	1 (0.9)	1 (1.6)	1 (0.9)
Stage II A	73 (25.8)	39 (26.0)	34 (25.6)	29 (27.1)	16 (25.8)	28 (25.5)
Stage II B	39 (13.8)	27 (18.0)	12 (9.0)	10 (9.3)	3 (4.8)	23 (20.9)
Stage III	2 (0.7)	2 (1.3)	-	-	1 (1.6)	1 (0.9)
Stage III A	29 (10.3)	15 (10.0)	14 (10.5)	9(8.4)	8 (12.9)	12 (10.9)
Stage III B	2 (0.7)	1 (0.7)	1 (0.8)	1 (0.9)	-	1 (0.9)
Stage III C	5 (1.8)	2 (1.3)	3 (2.3)	2 (1.8)	3 (4.8)	-
Missing	7 (2.5)	4 (2.7)	3 (2.3)	5 (4.7)	1 (1.6)	1 (0.9)
ER Status n(%)						
Positive	131 (46.3)	65 (43.3))	66 (49.6)	60 (56.1)	27 (43.6)	43 (39.1)
Negative	47 (16.6)	36 (24.0)	11 (8.3)	11 (10.3)	10 (16.1)	26 (23.6)
Missing	105 (37.1)	49 (32.7)	56 (42.1	36 (33.6)	25 (40.3)	41 (37.3)
PR Status n(%)						
Positive	109 (38.5)	54 (36.0)	55 (41.4)	49 (45.8)	23 (37.1)	36 (32.7)
Negative	70 (24.7)	48 (32.0)	22 (16.5)	22 (20.6)	14 (22.6)	34 (30.9)
Missing	104 (36.7)	42 (32.0)	56 (42.1)	36 (33.6)	25 (40.3)	40 (36.4)
HER2 Status n(%)						
Positive	30 (10.6)	20 (13.3))	10 (7.5)	13 (12.1)	6 (9.7)	11 (10)
Negative	136 (48.1)	69 (46.0)	67 (50.4)	54 (50.5)	27 (45.2)	54 (49.1)
Equivocal	21 (7.4)	11 (13.4)	10 (7.5)	8 (7.5)	3(4.8)	9 (8.2)
Missing	96 (33.9)	50 (33.3)	46 (34.6)	32 (29.9)	25 (40.3)	36 (32.7)
Ki-67 Status n(%)						
Low	25 (8.8)	12 (8.0)	13 (9.8)	13 (12.2)	3 (4.8)	9(8.2)
Intermediate	27 (9.5)	16 (10.7)	11 (8.2)	9 (8.4)	8 (12.9)	9(8.2)
High	107 (37.8)	63 (42.0)	44 (33.1)	41 (38.3)	18 (29)	46 (41.8)
Missing	124 (43.8)	59 (39.3)	65 (48.9)	44 (41.1)	33 (53.2)	46 (41.8)
Alcohol history n(%)						
Yes/Current	37 (13.1)	7 (4.7)	30 (22.5)	20 (18.7)	8 (12.9)	8 (7.3)
Yes/ Social or Occasional	107 (37.8)	54 (36.0)	53 (39.9)	46 (43.0)	22 (35.5)	39 (35.5)
No/Previously	9 (3.2)	3 (2.0)	6 (4.5)	4 (3.7)	1 (1.6)	4 (3.6)

No	125 (44.2)	81 (54.0)	44 (33.1)	37 (34.6)	30 (48.4)	56 (50.9)
Missing	5 (1.8)	5 (3.3)		-	1 (1.6)	3 (2.7)
Tobacco Use n(%)						
Yes / Ever	92 (32.5)	51 (34.0)	41 (30.8)	28 (26.2)	26 (42.6)	37 (33.6)
No / Never	190 (67.1)	99 (66.0)	91 (68.4)	78 (72.9)	36 (57.4)	73 (66.4)
Missing	1 (0.4)	0	1 (0.8)	1 (0.9)	0	0
Diabetes Mellitus n(%)						
Yes	40(14.1)	31 (20.7)	9 (6.8)	8 (7.5)	10 (16.4)	21 (19.1)
No	243 (85.9)	119 (79.3)	124 (93.2)	99 (92.5)	51 (83.6)	89 (90.9)
Heart Disease n(%)						
Yes	19 (6.7)	13 (8.7)	6 (4.5)	3 (2.8)	2 (3.3)	13 (11.8)
No	264 (93.3)	137 (91.3)	127 (95.5)	104 (97.2)	59 (96.7)	97 (88.2)
Hypercholesterolemia n(%)						
Yes	47 (16.6)	29 (19.3)	18 (13.5)	12 (11.2)	9 (14.8)	24 (21.8)
No	236 (83.4)	121 (80.7)	115 (86.5)	95 (88.8)	52 (85.2)	86 (78.2)
Thyroid Disease n(%)						
Yes	31 (10.9)	11 (7.3)	20 (15.0)	9 (8.4)	10 (16.4)	12 (10.9)
No	252 (89.1)	139 (92.7)	113 (85.0)	98 (91.6)	51 (83.6)	98 (89.1)

Table 2: Association of CLS-B presence with Race and BMI

Characteristics		Any CLS-B≥50%		Any CLS>75%		Any CLS-B ≥90%	
		POR	**95% CI**	**POR**	**95% CI**	**POR**	**95% CI**
Race [a]	**NHB**	1.151	(0.691, 1.918)	1.144	(0.627,2.088)	1.069	(0.611, 1.869)
	NHW (ref.)	-	-	-	-	-	-
BMI [b]	**<25kg/m²** **(ref.)**	-	-	-	-	-	-
	25-29.9 kg/m² [c]	2.367	(1.092, 5.128)	1.748	(0.740, 4.128)	2.031	(0.854, 4.831)
	≥30 kg/m² [c]	5.422	(2.716, 10.824)	4.648	(2.246, 9.620)	4.639	(2.188, 9.835)

a Race model adjusted for age

b BMI model adjusted for age, race and smoking status

c POR for the two categories of BMI 25-29 kg/m2 and≥30kg m2 are significantly different at alpha <0.05

Table 3: Association of Severity of BWAT inflammation (CLS-B/cm^2) with Race and BMI

Characteristics		Mild inflammation vs no inflammation		Severe inflammation vs no inflammation	
		POR	**95% CI**	**POR**	**95% CI**
Race [b c]	**NHB**	0.723	(0.369, 1.416)	1.825	(0.927, 3.593)
	NHW (ref.)	-	-	-	-
BMI [d e]	**≤29.9 kg/m^2**	-	-	-	-
	≥30 kg/m^2	3.715	(1.792, 7.704)	3.598	(1.757, 7.369)

a Severity of BWAT inflammation defined as presence of CLS-B per total fat area or BWAT (CLS-B/cm^2), 3 categories, 0= no inflammation, <0.84 CLS-B/m^2= mild inflammation and ≥0.84 CLS-B/cm^2 severe inflammation.

b Race model adjusted for age

c Age adjusted race model for association of severity of BWAT inflammation among CLS-B positive women gave POR= 2.777, 95% CI (1.154, 6.683) for Severe inflammation vs mild inflammation.

d BMI model adjusted age, race and smoking status

e association of severity of BWAT inflammation and BMI, categorized as non-obese ≤29.9kg/m^2 and obese ≥30 kg/m^2

FIGURES

Figure 1: Study flow diagram

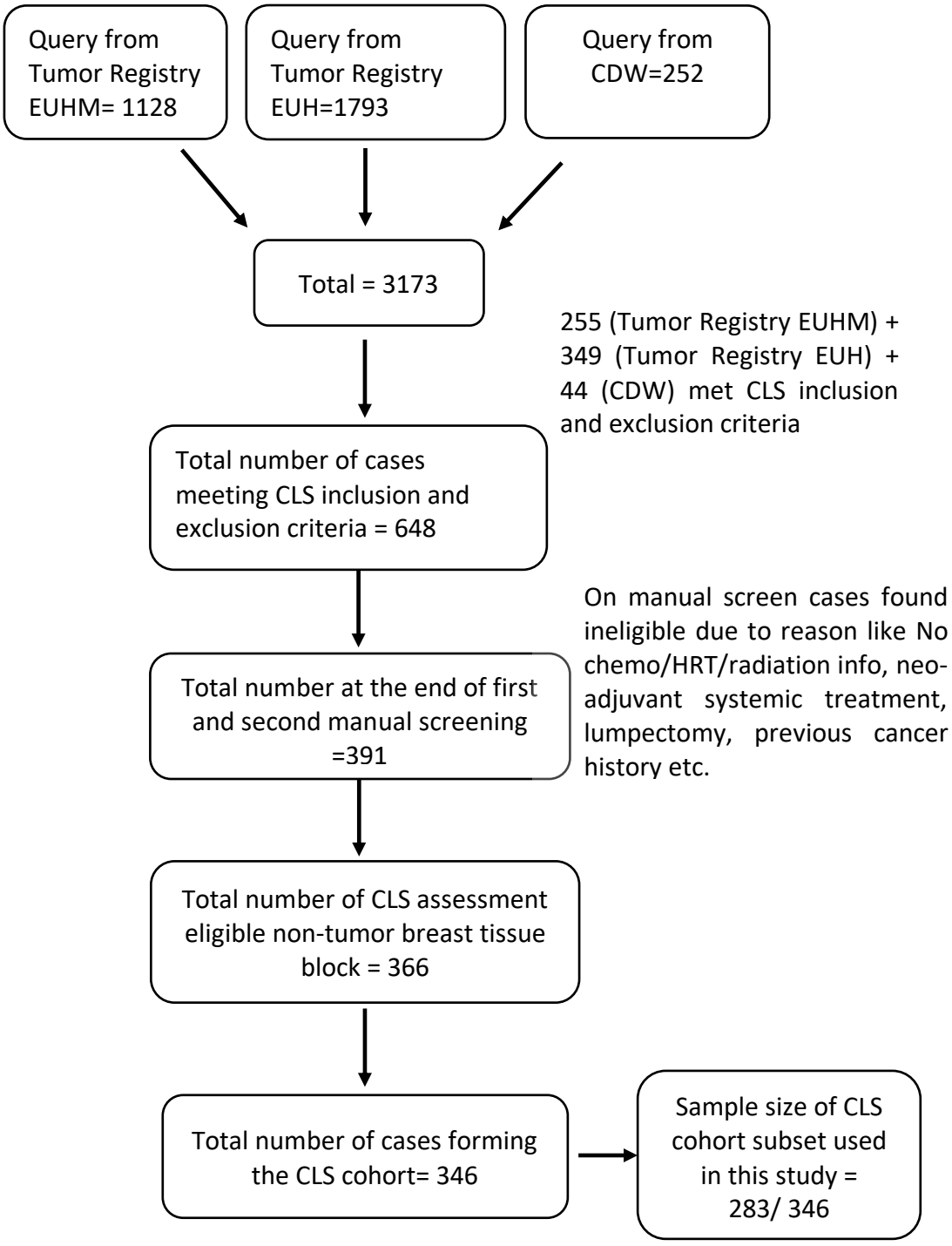

Figure 2: CD68- immunostained breast non-tumor tissue from a CLS-B positive case sample. (A) at low magnification, at high magnification (B) Complete CLS-B, (C) Cluster CLS-B, (D) Borderline CLS: >50% macrophage encirclement and (E) Borderline CLS-B: with >76% CLS-B macrophage encirclement.