

Obesity-associated Breast Inflammation among Hispanic/Latina Breast Cancer Patients

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Abstract

Breast white adipose tissue inflammation (BWATi) is associated with obesity and higher breast cancer risk among non-Hispanic white women. Obesity is prevalent in Hispanic/Latina patients with breast cancer, and the occurrence of BWATi in this population is not well-characterized. The association between BWATi and body mass index (BMI) was evaluated in Hispanic/Latina patients with breast cancer who underwent mastectomy. BWATi was defined as the presence of crown-like structures of the breast (CLS-B), detected by CD68 IHC in nontumor breast tissue. BWATi severity was quantified as number of CLS-B/cm². Adipocyte diameter was measured using hematoxylin and eosin-stained breast tissue sections. Preoperative BMI (within 1 week prior to mastectomy) was categorized as normal (18.5–<25.0 kg/m²), overweight (25.0–<30.0 kg/m²), class I obesity (30.0–<35.0 kg/m²), and class II–III obesity

(35.0 kg/m² or above). Patient charts were abstracted to record clinicopathologic features and liver function tests <90 days before mastectomy. The study included 91 women (mean age 69 years; range 36–96 years). Prevalence of BWATi increased with BMI (24% in normal weight, 34% in overweight, 57% in class I obesity, and 65% in class II–III obesity; $P_{\text{trend}} < 0.01$). Severe BWATi (>0.27 CLS-B/cm²) was associated with higher BMI ($P_{\text{trend}} = 0.046$) and greater adipocyte diameter ($P = 0.04$). Adjusting for BMI, neoadjuvant chemotherapy, and elevated alanine aminotransferase were associated with severe BWATi, and current smoking was associated with mild BWATi (all $P < 0.05$). BWATi was associated with higher BMI in Hispanic/Latina patients with breast cancer, consistent with previously described associations in other populations.

Introduction

Obesity is a risk factor for both hormone receptor-positive breast cancer in postmenopausal women and triple-negative breast cancer (1–4). In addition to its impact on the risk of breast cancer, obesity is associated with poorer prognosis among breast cancer survivors (5, 6). Obesity-related effects on adipokines, hormones, and inflammation have all been suggested to play a role in

breast carcinogenesis (7–10). As obesity is a modifiable risk factor for breast cancer risk and recurrence, biomarkers are needed to elucidate the pathways by which obesity exerts its effects on breast cancer.

Obesity causes subclinical inflammation in adipose tissue (11, 12). Both animal models and human studies of overweight and obesity have shown that macrophages infiltrate visceral and subcutaneous adipose tissue and form characteristic crown-like structures (CLS) around dead or dying adipocytes (12–15). In a population of predominantly non-Hispanic white patients with breast cancer, we previously reported that the presence of CLS in the breast (CLS-B) is associated with activation of the NFκB transcription factor in breast tissue and higher expression of aromatase, the rate-limiting enzyme for estrogen biosynthesis (16). Consistent with these findings, breast white adipose tissue inflammation (BWATi), as defined by the presence of CLS-B, has also been associated with an increased ratio of estrogens to androgens in both breast tissue and blood (17). In addition, BWATi is associated with altered systemic levels of adipokines, proinflammatory mediators, and insulin (18) and the postmenopausal state (19). On the basis of both the local and systemic

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changes that correlate with inflamed breast adipose tissue, BWATi may be involved in both an increased risk of breast cancer and worse prognosis of obese patients with breast cancer. This hypothesis is supported by recent case-control studies showing that higher CLS-B density is associated with elevated risk of breast cancer in women with benign breast disease (20, 21). Moreover, our group has reported that BWATi is associated with worse distant recurrence-free survival and the metabolic syndrome in patients with breast cancer (18). In another recent study, BWATi was associated with reduced overall survival among breast cancer survivors (22).

There are high rates of obesity and overweight among Hispanic/Latina patient populations with breast cancer, which is reflective of the general U.S. Hispanic/Latina population (23). Compared with non-Hispanic white women, Hispanics/Latinas are less likely to be diagnosed with localized breast cancer, and are more likely to be diagnosed with larger, hormone receptor-negative tumors, which have poorer survival rates (24–26). Previous studies have reported conflicting results on whether Hispanic/Latinas have worse breast cancer survival after accounting for differences in age, stage at diagnosis, and tumor characteristics (25, 27). These disparities may be partly due to the high rates of overweight and obesity in this population. Studies are needed to elucidate mechanisms by which obesity may be related to breast cancer risk and recurrence in Hispanics/Latinas. In this pilot study, we extended our prior investigations of the association between BWATi and body mass index (BMI) to examine whether similar patterns previously reported in non-Hispanic white women are also observed in Hispanic/Latina patients with breast cancer.

Methods

Study participants

Hispanic/Latina patients with stage 0–III breast cancer who underwent mastectomy at Columbia University Medical Center (CUMC; New York, NY) between February 2007 and November 2012 were identified via the Herbert Irving Comprehensive Cancer Center (HICCC; New York, NY) breast oncology database and the CUMC Pathology database. All patients had provided written, informed consent for future use of their tissue for research. Patients who met the following eligibility criteria were included: (i) self-identified Hispanic/Latina, (ii) stage 0–III breast cancer, (iii) BMI \geq 18.5 kg/m² at the time of surgery, (iv) previously provided written informed consent to be included in the breast oncology database and that their pathology specimens could be used for future research, and (v) available archived formalin-fixed, paraffin-embedded (FFPE) sample of normal adjacent breast tissue from a quadrant uninvolved by tumor obtained via mastectomy. This study was approved by the Institutional Review Boards at

Columbia University Medical Center and Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY).

Anthropometric measures

BMI was calculated using preoperative body weight and height measured by anesthesiologists and recorded in the patient chart within 1 week prior to mastectomy. BMI was categorized as normal weight (18.5 to <25.0 kg/m²), overweight (25.0 to <30.0 kg/m²), class I obesity (30.0 to <35.0 kg/m²), and class II–III obesity (\geq 35.0 kg/m²).

Adipose tissue collection

Within each BMI group, 18–54 patients with banked tissue samples were selected, which were provided to the CUMC Pathology laboratory. Pathology staff pulled FFPE blocks from stored mastectomy specimens and blocks from nontumorous quadrants were examined. Blocks were excluded if the representative section showed biopsy site change or traumatic fat necrosis. In this manner, one white adipose tissue-enriched block was selected per patient. Useable samples in each BMI category ($n = 29$ for BMI 18.5 to <25.0 kg/m², $n = 51$ for BMI 25.0 to <30.0 kg/m², $n = 33$ for BMI 30.0 to <35.0 kg/m², and $n = 18$ for BMI \geq 35.0 kg/m²) were sent to the HICCC Molecular Pathology Shared Resource for pathology examination and microtomy. For each case, tissue samples were cut into seven sections that were approximately 50 μ m apart. Slides were 5-mm thin and approximately 2 cm in diameter. Sections were cut at 50 μ m intervals to avoid duplicate quantification of CLS-B.

Measurement of BWATi

The MSKCC Pathology Core Facility stained 2 sections from each block with hematoxylin and eosin (H&E) and 5 sections for cluster of differentiation 68 (CD68), a macrophage marker (mouse monoclonal KP1 antibody; Dako; dilution 1:4,000). CD68-stained sections were examined under light microscopy by the study pathologist (D.D. Giri) to detect CLS-B. When present, the number of CLS-B in each section were counted and recorded. BWATi was defined by the presence of CLS-B. Consistent with previously reported methods (16), the density of CLS-B by surface area was determined as follows: each CD68-stained slide was digitally archived by gross photography. Images were stored in the tagged image file format, and the white adipose tissue area on each slide was measured using ImageJ Software (NIH, Bethesda, MD; ref. 28). Epithelial tissue area and areas of fibrosis were excluded. The severity of BWATi was quantified as CLS-B per square centimeter of white adipose tissue (CLS-B/cm²), with the median CLS-B/cm² as the cutoff to differentiate between severe and mild inflammation. Previously using this definition, BWATi severity was shown to be positively associated with aromatase expression in breast tissue (29), providing useful biological information beyond presence or absence of CLS-B.

Measurement of adipocyte size

Following previously reported methods (16), we measured adipocyte diameters using H&E-stained FFPE breast tissue sections. Representative H&E sections with preserved adipocyte architecture were manually selected by a histopathologist (D.J. Falcone) and were photographed at $20\times$ using an Olympus BX50 Microscope and MicroFire Digital Camera (Optronics). Mean adipocyte diameters were calculated using the average diameters of 30 randomly selected individual adipocytes for each patient using the linear dimensional tool in the Canvas 11 Software (ACD Systems International, Inc.).

Demographic and clinical data

Demographic and clinical characteristics were collected from the electronic medical record, including age at date of surgery, and tumor characteristics. The HICCC breast oncology database provided data on chemotherapy and endocrine therapy received as well as menopausal status. Women were defined as postmenopausal if they had undergone bilateral oophorectomy or reported permanent cessation of menses for ≥ 12 months in the absence of chemotherapy or endocrine therapy. For patients whose menopausal status was unknown, women >51 years old were defined as postmenopausal. When available, liver function tests (LFT; i.e., alanine aminotransferase and aspartate aminotransferase) measured up to 90 days prior to date of mastectomy were abstracted from CUMC electronic medical records; values were averaged for each patient if more than one LFT was recorded.

Statistical analysis

We tested the linear trend in BWAT_i from low to high BMI levels using the trend test of proportions. We examined the correlates of BWAT_i using Fisher exact test for categorical variables and F test for continuous variables. For factors associated with the presence or severity of BWAT_i, we further examined their associations by adjusting for BMI in multivariable logistic regression and multinomial logistic regressions. All statistical tests were two-sided with $\alpha = 0.05$. Statistical analyses were performed in R (version 3.4.3).

Results

Participant characteristics

A total of 137 eligible Hispanic/Latina patients with breast cancer with breast white adipose tissue samples were identified, of which 99 patients with useable tissue were selected according to BMI category. Upon examination by the study pathologist, 8 samples with traumatic fat necrosis were excluded, leaving a total of 91 participants in the final analyses (Supplementary Fig. S1). The median^c age of the 91 patients was 69 years (range: 36–96 years; Table 1). The median BMI was 29.9 kg/m^2 (range: 19.9–

50.5 kg/m^2). The majority of participants were of Dominican Republic descent (63%), followed by Puerto Rican (16%), and other Hispanics (16%). Approximately, 79% of women were postmenopausal, and 21% were premenopausal. The majority of patients had tumors that were estrogen receptor–positive (86%) and progesterone receptor–positive (70%), and 16% received neoadjuvant chemotherapy (Table 1).

Association between presence of CLS-B and BMI

CLS-B was present in 45% of Hispanic/Latina patients with breast cancer. The prevalence of CLS-B increased with BMI, with CLS-B present in 24% of normal weight patients, 34% of overweight patients, 57% of patients with class I obesity, and 65% of patients with class II–III obesity ($P_{\text{trend}} < 0.01$; Fig. 1A). Compared with the normal weight group, the presence of CLS-B was statistically significantly more common in patients with class I obesity ($P = 0.03$) and class II–III obesity ($P = 0.02$).

Association between severity of BWAT_i and BMI

Using the median as a cutoff, the severity of BWAT_i was defined as mild ($\leq 0.27 \text{ CLS-B/cm}^2$) in 22 of 91 (24%) patients and severe ($> 0.27 \text{ CLS-B/cm}^2$) in 19 of 91 (21%) patients. The prevalence of severe BWAT_i was positively associated with BMI among patients with CLS-B: 18% in normal weight, 14% in overweight, 21% in class I obesity, and 35% in class II–III obesity ($P_{\text{trend}} = 0.046$; Fig. 1B).

Association between BWAT_i and adipocyte diameter

Patients with CLS-B had a trend toward larger adipocyte diameters (mean = $105.6 \mu\text{m}$, SD = $14.1 \mu\text{m}$) compared with patients without CLS-B (mean = $99.3 \mu\text{m}$, SD = $16.9 \mu\text{m}$; $P = 0.06$; Table 1; Fig. 2A). The adipocyte diameter was significantly larger in patients with severe BWAT_i (mean = $108.3 \mu\text{m}$, SD = $16.6 \mu\text{m}$) compared with those without CLS-B (mean = $99.3 \mu\text{m}$, SD = $16.9 \mu\text{m}$; $P = 0.04$; Table 2; Fig. 2B). Larger adipocyte size was also associated with overweight and obesity (all $P < 0.05$; Figs. 2C and D). In addition, larger adipocyte size was associated with higher serum alanine aminotransferase (ALT; $P < 0.05$; Fig. 2E) but not serum aspartate aminotransferase (AST) (Fig. 2F).

Associations between BWAT_i and clinical and demographic characteristics

Both the incidence ($P = 0.02$, Table 1) and severity ($P = 0.04$, Table 2) of BWAT_i were greater in patients who received neoadjuvant chemotherapy. In multivariable logistic regression models adjusting for BMI, neoadjuvant chemotherapy was associated with higher odds of CLS-B [adjusted OR = 4.3; 95% confidence interval (CI), 1.2–18.3] and higher odds of severe BWAT_i (adjusted OR = 5.2; 95% CI, 1.1–25.7) independent of BMI (Table 3).

In unadjusted analyses, patients with severe BWAT_i were more likely to be Puerto Ricans compared with patients with mild or no inflammation ($P = 0.03$; Table 2).

^cPatients with stage 0 breast cancer were considered not applicable for IHC test.

Table 1. Association of CLS-B with demographic and clinical characteristics

Characteristics		Total N (%)	CLS-B ⁺ (N = 41) N (%)	CLS-B ⁻ (N = 50) N (%)	P ^a		
Age	Median (range)	69 (36–96)	72 (36–91)	65.5 (39–96)	0.19		
BMI (kg/m ²)	Median (range)	29.9 (19.9–50.5)	31.6 (23–50.5)	28 (19.9–43.2)	<0.01		
Country of origin	Dominican Republic	57 (63%)	23 (56%)	34 (68%)	0.10		
	Puerto Rican	15 (16%)	10 (24%)	5 (10%)	—		
	South American	2 (2%)	0 (0%)	2 (4%)	—		
	Mexican	2 (2%)	2 (5%)	0 (0%)	—		
	Cuban	0 (0%)	0 (0%)	0 (0%)	—		
	Other Hispanic	15 (16%)	6 (15%)	9 (18%)	—		
Education	High school	31 (34%)	14 (34%)	17 (34%)	0.48		
	College	22 (24%)	7 (17%)	15 (30%)	—		
	Graduate school	24 (26%)	12 (29%)	12 (24%)	—		
Employment	Employed	18 (20%)	7 (17%)	11 (22%)	0.43		
	Unemployed	25 (27%)	10 (24%)	15 (30%)	—		
	Retired	27 (30%)	16 (39%)	11 (22%)	—		
	Homemaker	8 (9%)	3 (7%)	5 (10%)	—		
Income	0–\$15,000	49 (54%)	27 (66%)	22 (44%)	0.30		
	\$15,000+	21 (23%)	8 (20%)	13 (26%)	—		
Post menopause	Yes	72 (79%)	33 (80%)	39 (78%)	0.80		
	No	19 (21%)	8 (20%)	11 (22%)	—		
Current smoking	Yes	8 (9%)	6 (15%)	2 (4%)	0.13		
	No	71 (78%)	30 (73%)	41 (82%)	—		
History of smoking	Current	7 (8%)	5 (12%)	2 (4%)	0.38		
	Ever	11 (12%)	5 (12%)	6 (12%)	—		
	Never	52 (57%)	22 (54%)	30 (60%)	—		
Current alcohol use	Yes	25 (27%)	12 (29%)	13 (26%)	1.00		
	No	55 (60%)	25 (61%)	30 (60%)	—		
Stage	0	9 (10%)	5 (12%)	4 (8%)	0.48		
	I	36 (40%)	15 (37%)	21 (42%)	—		
	II	35 (38%)	14 (34%)	21 (42%)	—		
	III	11 (12%)	7 (17%)	4 (8%)	—		
ER	Positive	78 (86%)	34 (83%)	44 (88%)	0.53		
	Negative	11 (12%)	6 (15%)	5 (10%)	—		
PR	Positive	64 (70%)	29 (71%)	35 (70%)	1.00		
	Negative	25 (27%)	11 (27%)	14 (28%)	—		
HER2 ^b	Positive	7 (8%)	5 (12%)	2 (4%)	0.53		
	Negative	42 (46%)	17 (41%)	25 (50%)	—		
	Indeterminate	29 (32%)	13 (32%)	16 (32%)	—		
	Not applicable	7 (8%)	3 (7%)	4 (8%)	—		
Neoadjuvant chemotherapy	Yes	15 (16%)	11 (27%)	4 (8%)	0.02		
	No	75 (82%)	29 (71%)	46 (92%)	—		
Biological markers		N	N	Mean (SD)	N	Mean (SD)	P
Liver function tests							
	ALT (U/L)	86	39	20.5 (9.8)	47	17.5 (9.1)	0.14
	AST (U/L)	86	39	20.6 (6.4)	47	19.5 (6.0)	0.42
Adipocyte diameter (µm)							
	Mean (SD)	89	40	105.6 (14.1)	49	99.3 (16.9)	0.06
	Range			59.7–139.8		59.7–135.2	—

NOTE: For all variables, the number of participants who did not respond to the question were not displayed in this table, therefore the percentages may not add up to 100%.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ER, estrogen receptor; PR, progesterone receptor.

^aFisher exact test excluded the "Unknown" category.

^bPatients with stage 0 breast cancer were considered not applicable for IHC test.

However, there was no significant association between country of origin and BWATi after adjusting for BMI. In unadjusted analyses, patients with mild BWATi were also more likely to be current smokers compared with patients with severe or no inflammation ($P = 0.04$; Table 2). The association between smoking and mild inflammation remained after adjusting for BMI (adjusted OR = 6.4; 95% CI, 1.0–39.2; Table 3).

On average, patients had 2.2 LFTs recorded in the electronic medical record in the 90 days before mastectomy,

with a median of 2 measurements and a range of 1–8 measurements. In unadjusted analyses, patients with severe BWATi had higher serum ALT compared with patients with mild ($P = 0.01$) or no BWATi ($P = 0.01$; Table 2; Fig. 3A). Similarly, in unadjusted analyses, patients with severe BWATi had higher AST compared with patients with mild BWATi ($P = 0.03$; Table 2; Fig. 3B). After adjusting for BMI, severe BWATi remained associated with elevated ALT when compared with no BWATi (per interquartile range increase: OR = 2.0; 95% CI, 1.1–3.9; Table 3).

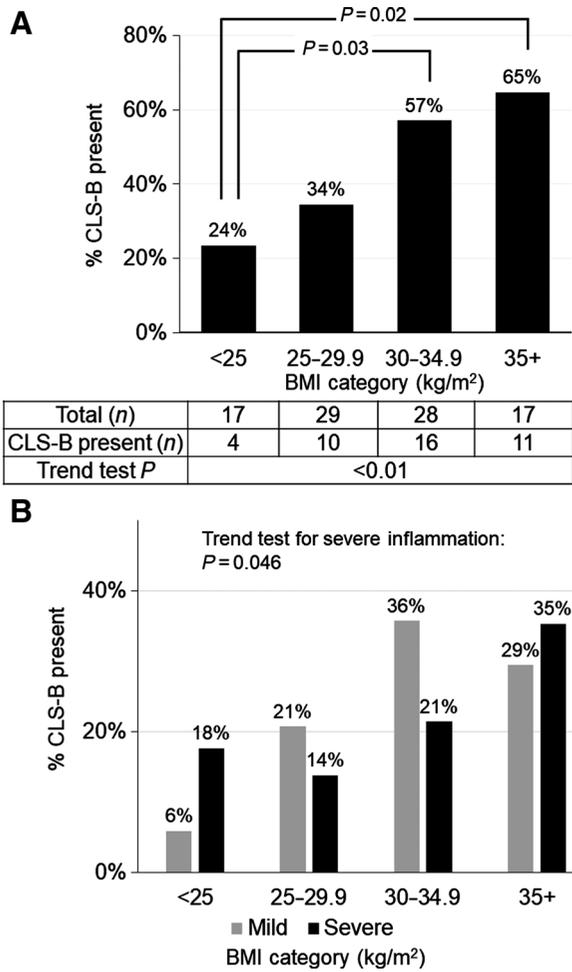


Figure 1. Breast white adipose tissue inflammation by BMI category ($n = 91$). **A**, Shows the prevalence of CLS-B by BMI category. **B**, Shows the distribution of mild and severe inflammation by BMI category. Inflammation severity is defined categorically as follows and using median number of CLS-B/cm² (0.27) as the cutoff: no inflammation (CLS-B absent), mild inflammation (≤ 0.27 CLS-B/cm²), and severe inflammation (> 0.27 CLS-B/cm²).

Discussion

In this pilot study, BWATi was observed in nearly half of Hispanic/Latina breast cancer survivors who underwent mastectomy. The prevalence and severity of BWATi were strongly associated with higher BMI in Hispanic/Latina patients with breast cancer. Patients with severe BWATi had significantly larger adipocytes. These findings are consistent with prior studies in predominantly non-Hispanic white and Asian populations (16, 18, 19, 30).

The prevalence of BWATi in this cohort of Hispanic/Latina patients with breast cancer (45%) is comparable with previous reports in non-Hispanic white patients, in which 36%–59% patients with breast cancer had BWATi (16, 18, 19, 30–32). The positive association between presence and severity of BWATi and BMI category is also

consistent with prior publications (16, 30, 32, 33). However, BWATi was present in 24% of Hispanic/Latina patients with breast cancer who had normal weight (BMI < 25 kg/m²), but was absent in 35% of patients with class II and III obesity. 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 BWATi.

Consistent with our hypothesis and previous studies (16, 19), patients who were overweight or obese had larger adipocytes, and adipocyte diameter was positively associated with BWATi severity. These findings support the hypothesis that obesity-associated adipocyte hypertrophy leads to immune cell recruitment and BWATi (34).

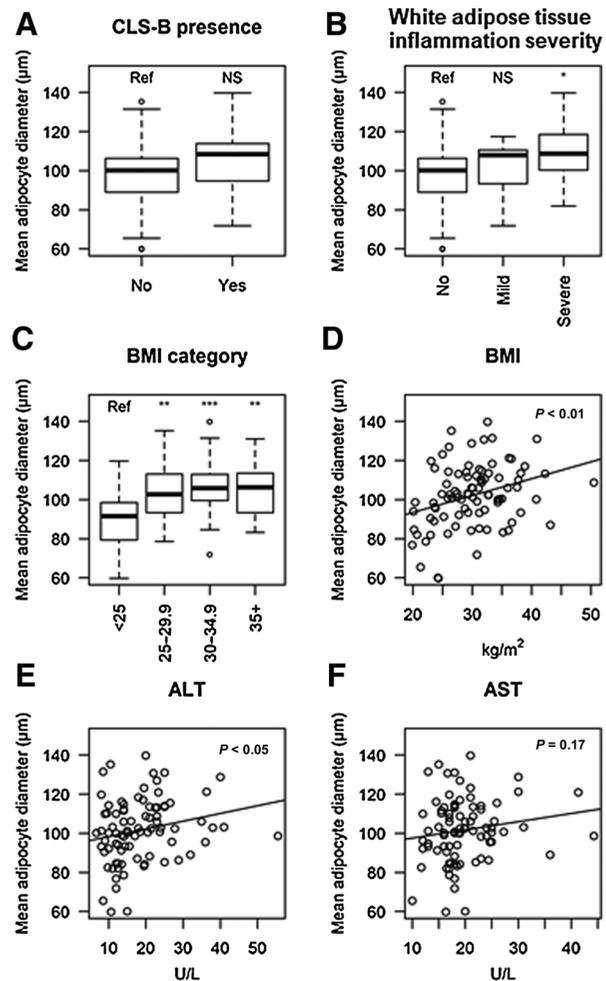


Figure 2. Association between average adipocyte diameter and biomarkers of liver function. **A–C**, The box plots compare the mean adipocyte diameters between groups, and **D–F**, the scatter plots and fitted linear regression lines show the correlation between mean adipocyte diameter and each biomarker. Patients with higher BMI and more severe BWATi had larger adipocyte diameter (all $P < 0.05$). For box plots, statistically significant differences between groups were denoted by *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant; BMI, body mass index; CLS-B, crown-like structure of the breast; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2. Association of breast white adipose tissue inflammation severity with demographic and clinical characteristics

Characteristics		Total N	Severe ^a (N = 19) N (%)	Mild ^a (N = 22) N (%)	None (N = 50) N (%)	P ^b	
Age	Median (range)	91	74 (53–91)	69 (36–89)	66 (39–96)	0.15	
Country of origin	Dominican Republic	57	8 (42%)	15 (68%)	34 (68%)	0.03	
	Puerto Rican	15	8 (42%)	2 (9%)	5 (10%)	—	
	South American	2	0 (0%)	0 (0%)	2 (4%)	—	
	Mexican	2	0 (0%)	2 (9%)	0 (0%)	—	
	Cuban	0	0 (0%)	0 (0%)	0 (0%)	—	
Education	Other Hispanic	15	3 (16%)	3 (14%)	9 (18%)	—	
	High school	31	7 (37%)	7 (32%)	17 (34%)	0.40	
	College	22	5 (26%)	2 (9%)	15 (30%)	—	
Employment	Graduate school	24	4 (21%)	8 (36%)	12 (24%)	—	
	Employed	18	4 (21%)	3 (14%)	11 (22%)	0.26	
	Unemployed	25	2 (11%)	8 (36%)	15 (30%)	—	
Income	Retired	27	10 (53%)	6 (27%)	11 (22%)	—	
	Homemaker	8	1 (5%)	2 (9%)	5 (10%)	—	
	0–\$15,000	49	11 (58%)	16 (73%)	22 (44%)	0.41	
Postmenopause	\$15,000+	21	4 (21%)	4 (18%)	13 (26%)	—	
	Yes	72	17 (89%)	16 (73%)	39 (78%)	0.45	
Current smoking	No	19	2 (11%)	6 (27%)	11 (22%)	—	
	Yes	8	1 (5%)	5 (23%)	2 (4%)	0.04	
History of smoking	No	71	16 (84%)	14 (64%)	41 (82%)	—	
	Current	7	0 (0%)	5 (23%)	2 (4%)	0.10	
	Ever	11	3 (16%)	2 (9%)	6 (12%)	—	
Current alcohol use	Never	52	11 (58%)	11 (50%)	30 (60%)	—	
	Yes	25	6 (32%)	6 (27%)	13 (26%)	0.95	
Stage	No	55	11 (58%)	14 (64%)	30 (60%)	—	
	0	9	0 (0%)	5 (23%)	4 (8%)	0.18	
	I	36	9 (47%)	6 (27%)	21 (42%)	—	
	II	35	6 (32%)	8 (36%)	21 (42%)	—	
ER	III	11	4 (21%)	3 (14%)	4 (8%)	—	
	Positive	78	16 (84%)	18 (82%)	44 (88%)	0.62	
PR	Negative	11	2 (11%)	4 (18%)	5 (10%)	—	
	Positive	64	13 (68%)	16 (73%)	35 (70%)	1.00	
HER2 ^c	Negative	25	5 (26%)	6 (27%)	14 (28%)	—	
	Positive	7	1 (5%)	4 (18%)	2 (4%)	0.24	
	Negative	42	10 (53%)	7 (32%)	25 (50%)	—	
	Indeterminate	29	7 (37%)	6 (27%)	16 (32%)	—	
Neoadjuvant chemotherapy	Not applicable	7	0 (0%)	3 (14%)	4 (8%)	—	
	Yes	15	5 (26%)	6 (27%)	4 (8%)	0.04	
No	75	13 (68%)	16 (73%)	46 (92%)	—		
Biological markers		N	N (Mean, SD)	N (Mean, SD)	P	N (Mean, SD)	P
Liver function tests							
	ALT (U/L)	86	19 (24.4, 8.8)	20 (16.9, 9.6)	0.01	47 (17.5, 9.1)	0.01
	AST (U/L)	86	19 (22.8, 6.6)	20 (18.5, 5.4)	0.03	47 (19.5, 6.0)	0.05
Adipocyte diameter (μm)							
	Mean (SD)	89	19 (108.3, 16.6)	21 (103.2, 11.4)	0.31	49 (99.3, 16.9)	0.04
	Range		(81.9–139.8)	(71.8–117.5)	—	(59.7–135.2)	—

NOTE: For all variables, the number of participants who did not respond to the question were not displayed in this table, therefore the percentages may not add up to 100%.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ER, estrogen receptor; PR, progesterone receptor.

^aInflammation severity is defined categorically as follows and using the value of 0.27 CLS-B/cm² as the cutoff: no inflammation (absent CLS-B), mild inflammation (≤ 0.27 CLS-B/cm²), and severe inflammation (> 0.27 CLS-B/cm²).

^bFisher exact test excluded the "Unknown" category.

^cPatients with stage 0 breast cancer were considered not applicable for IHC testing.

However, adipocyte size was not significantly associated with BWATi when evaluated as a categorical variable (CLS-B absent/present). The relatively higher BMI and larger adipocyte diameters in our sample of Hispanic/Latina breast cancer survivors versus non-Hispanic white patients in our previous studies may explain the marginal differences in adipocyte diameters between patients with and without CLS-B (16, 19). For example, in one of our previous studies of 237 predominantly non-Hispanic white women with a median BMI of 25.3 kg/m² (19), the

average adipocyte diameter was 92 μm (SD = 16 μm) in women without CLS-B, compared with 107 μm (SD = 12 μm) in women with CLS-B. In comparison, in this study, median BMI was 29.9 kg/m², which is higher than the median BMI in our previous population of non-Hispanic white women and may explain the narrower difference in adipocyte diameter between women without CLS-B (mean = 99 μm, SD = 17 μm) and with CLS-B (mean = 106 μm, SD = 14 μm). It is also possible that the normal range of adipocyte diameter varies by racial/ethnic groups,

Table 3. Association of breast white adipose tissue inflammation with chemotherapy, smoking, ALT, and AST, adjusting for BMI

	Unadjusted				Adjusted for BMI ^c			
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Presence of CLS-B^a	CLS-B present vs. absent	—	—	—	CLS-B present vs. absent	—	—	—
Neoadjuvant chemotherapy received Yes vs. no	4.4 (1.4–16.9)	0.02	—	—	4.3 (1.2–18.3)	0.03	—	—
Severity of breast white adipose tissue inflammation^b	Severe vs. no inflammation		Mild vs. no inflammation		Severe vs. no inflammation		Mild vs. no inflammation	
Country of origin								
Dominican Republic vs. other Hispanic	0.9 (0.2–3.8)	0.85	1.0 (0.3–3.3)	0.96	0.6 (0.1–3.1)	0.57	0.8 (0.2–3.0)	0.74
Puerto Rican vs. other Hispanic	5.9 (1.1–32.0)	0.04	0.9 (0.1–6.2)	0.90	5.0 (0.9–28.8)	0.07	0.8 (0.1–6.1)	0.83
Current smoking								
Smoker vs. nonsmoker	1.3 (0.1–15.1)	0.84	7.3 (1.3–42.1)	0.03	1.3 (0.1–16.4)	0.84	6.4 (1.0–39.2)	0.04
Neoadjuvant chemotherapy received Yes vs. no	4.4 (1.0–18.9)	0.04	4.3 (1.1–17.3)	0.04	5.2 (1.1–25.7)	0.04	3.8 (0.9–16.2)	0.07
ALT								
Per 1 interquartile range increase	2.1 (1.1–4.0)	0.02	0.9 (0.4–1.9)	0.78	2.0 (1.1–3.9)	0.03	0.8 (0.3–1.7)	0.52
AST								
Per 1 interquartile range increase	1.6 (1.0–2.8)	0.07	0.8 (0.4–1.6)	0.47	1.5 (0.9–2.7)	0.12	0.7 (0.3–1.5)	0.39

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

^aPresence of CLS-B was analyzed using logistic regression with "CLS-B absent" as the referent.

^bSeverity of breast white adipose tissue inflammation was a 3-level categorical variable, and was analyzed using multinomial logistic regression with "no inflammation" as the referent group.

^cBMI was analyzed as a categorical variable (<25 kg/m², 25–29.9 kg/m², 30–34.9 kg/m², and 35+ kg/m²).

and that specific racial/ethnic groups may have a greater propensity to develop CLS-B due to having larger adipocytes. Future studies can test this hypothesis by examining the interaction between race/ethnicity and adipocyte diameter in relation to CLS-B.

Several novel associations were observed in this study. To our knowledge, this is the first report indicating that smoking history and preoperative neoadjuvant chemotherapy were associated with CLS-B. Smoking is known to induce inflammation, however an association between smoking and adipose inflammation has not been previously described. Although there were only 8 current smokers in our sample, contributing to the large variance of the OR for smoking, the association between smoking and BWATi is biologically plausible. Smoking causes atherosclerosis (35), and cigarettes contain vasoactive compounds for example, nicotine, which can lead to tissue hypoxia (36). Adipocyte death in hypoxic fat pads could lead to inflammation and the formation of CLS-B. Thus, smoking may contribute to BWATi by inducing a hypoxic state. However, mechanistic studies are needed to better understand the association between smoking and BWATi.

Of particular interest and novelty, we found an association between abnormal LFTs and severity of BWATi in Hispanic/Latina patients with breast cancer. Elevated transaminases are indicative of underlying hepatic dysfunction which is most commonly due to nonalcoholic fatty liver disease (NAFLD) in obese individuals. The association between elevated ALT and BWATi is likely to be indicative of underlying NAFLD. While the finding of NAFLD in individuals with elevated BMI is not surprising, the association between elevated ALT and BWATi was independent of BMI. This independent association is consistent with our

prior studies reporting BWATi in a subset of normal BMI individuals who are likely to have metabolic obesity (37). Furthermore, our findings suggest that measurement of

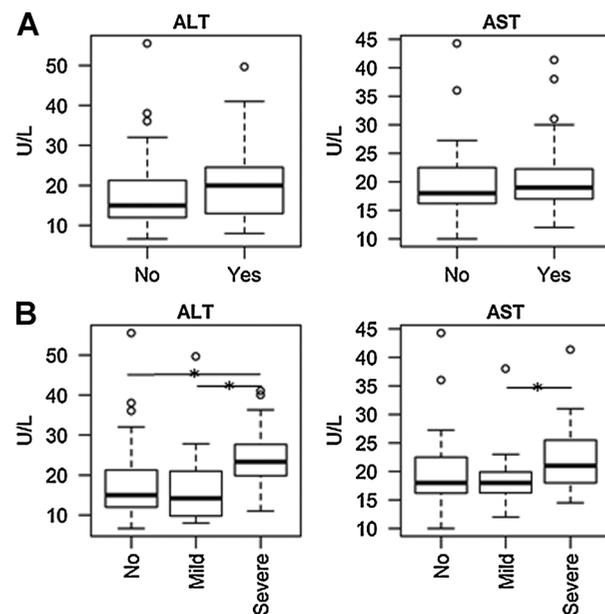


Figure 3. Biomarkers of liver function by presence of CLS-B (A) and severity of breast white adipose tissue inflammation (B). The boxplot shows the median (dark horizontal line inside box), the 25% percentile (lower bound of box), and the 75% percentile (upper bound of box) of each biomarker by inflammation status. The horizontal bar with a star denotes statistically significant differences between groups. Patients with severe BWATi had higher ALT compared with patients with mild ($P = 0.02$) or no BWATi ($P = 0.01$). Patients with severe BWATi had higher AST compared with patients with mild BWATi ($P = 0.03$). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

LFTs, which are routinely followed in clinical practice, could be developed as a noninvasive method to diagnose BWATi. These novel findings warrant future studies with larger sample sizes to better understand the prevalence and correlates of BWATi among diverse populations of patients with breast cancer.

Our study contributes to a growing body of literature examining the association between preoperative neoadjuvant chemotherapy and the presence of BWATi. In our study, Hispanic/Latina patients with breast cancer who received neoadjuvant chemotherapy had five-time higher odds of having CLS-B compared with patients who received adjuvant or no chemotherapy. Another study of 237 patients with breast cancer reported that BWATi was observed in 19 of 34 (56%) patients who received preoperative chemotherapy, which was numerically but not statistically significantly higher compared with patients who did not receive preoperative chemotherapy (51%, $P = 0.71$; ref. 33). It is possible that the types of neoadjuvant chemotherapy varied in these two studies accounting for the different findings. Although the mechanisms linking chemotherapy use to BWATi are unclear, it is possible that cytotoxic chemotherapy could induce breast adipocyte death, leading to the formation of CLS-B. Dead and dying adipocytes are known to recruit macrophages, which leads to the formation of CLS (12, 14). Future studies need to examine associations between receipt of neoadjuvant chemotherapy, subsequent development of BWATi, and measurable clinical outcomes.

Although our study identified new and potentially clinically relevant correlates of BWATi, we did not observe an association between BWATi and postmenopausal status as in previous studies (18, 19). Notably, the median age of our study population was higher than in prior studies, thereby reducing the power of this study to detect differences in menopausal status by white adipose tissue inflammation.

Our study is limited by its retrospective design. Specifically, detailed information on diet, physical activity, and alcohol drinking were not collected prospectively using standard questionnaires, making it difficult to examine the causal relations between lifestyle factors and BWATi. Furthermore, this study relied on clinically measured LFTs collected retrospectively from medical records rather than single-batch measurement of markers in prospectively collected blood from fasting women. Prospectively designed studies in ethnically diverse populations are needed. The relatively small sample size also limited our power to analyze multiple factors simultaneously. Finally, 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. Nonetheless, this is the first study to evaluate BWATi in a cohort of exclusively Hispanic/Latina women with breast

cancer. Our findings establish the rationale for further studies of BWATi in this population.

In conclusion, BWATi is associated with higher BMI in Hispanic/Latina patients with breast cancer. Smoking history, preoperative chemotherapy, and abnormal LFTs were associated with BWATi in this cohort, independent of BMI. However, a causal association between these factors would require replication in a prospective cohort study. Understanding the mechanisms contributing to worse breast cancer outcomes in obese individuals, such as BWATi, will provide key insights into selecting intervention strategies that are likely to be effective. Moreover, elucidating the intersections among race/ethnicity and BWATi is a novel and biologically informed approach to combating the obesity epidemic which has disproportionately affected minority populations. As BWATi has been associated with increased risk of breast cancer and worse breast cancer prognosis, the findings here support the need to develop noninvasive strategies to identify women with BWATi and to identify effective interventions to reduce BWATi and reduce breast cancer risk and/or improve breast cancer prognosis. Efforts are underway to develop noninvasive biomarkers to identify women with CLS-B. Furthermore, it is possible that lifestyle interventions (e.g., diet, physical activity, and/or weight loss) could reduce BWATi and reduce risk. Similarly, for women who are found to have BWATi at the time of mastectomy, antiinflammatory interventions may improve prognosis. These findings are provocative and require future prospectively designed studies with larger sample sizes to better understand the prevalence and correlates of BWATi among diverse populations of patients with breast cancer.

Disclosure of Potential Conflicts of Interest

A.J. Dannenberg is a consultant/advisory board member at SynDevRx. N.M. Iyengar is a consultant/advisory board member at Novartis and Puma Biotechnology. No potential conflicts of interest were disclosed by other authors.

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Other (reviewed the patient's pathology reports and microscopic evaluation of paraffin-embedded breast tissue slides with documentation of the related pathology findings and diagnostic interpretation.): A. Ahmed

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