Research Article

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Inflammatory Biomarker C-Reactive Protein and Radiotherapy-Induced Early Adverse Skin Reactions in Patients with Breast Cancer

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Abstract

Background: Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American women. Postsurgery adjuvant radiotherapy (RT) significantly reduced the local recurrence rate. However, many patients develop early adverse skin reactions (EASR) that impact quality of life and treatment outcomes.

Methods: We evaluated an inflammatory biomarker, C-reactive protein (CRP), in predicting RT-induced EASRs in 159 patients with breast cancer undergoing RT. In each patient, we measured pre- and post-RT plasma CRP levels using a highly sensitive ELISA CRP assay. RT-induced EASRs were assessed at weeks 3 and 6 using the National Cancer Institute Common Toxicity Criteria (v3.0). Associations between EASRs and CRP levels were assessed using logistic regression models after adjusting for potential confounders.

Results: RT-induced grade 2+ EASRs were observed in 8 (5%) and 80 (50%) patients at weeks 3 and 6 (end of RT), respectively. At the end of RT, a significantly higher proportion of African Americans developed grade 3 EASRs (13.8% vs. 2.3% in others); grade 2+ EASRs were significantly associated with: change of CRP > 1 mg/L [odds ratio (OR), 2.51; 95% confidence interval (CI), 1.06–5.95; $P = 0.04$], obesity (OR, 2.08; 95% CI, 1.03–4.21; $P = 0.04$), or combined both factors (OR, 5.21; 95% CI, 1.77–15.38; $P = 0.003$).

Conclusion: This is the first study to demonstrate that an inflammatory biomarker CRP is associated with RT-induced EASRs, particularly combined with obesity.

Impact: Future larger studies are warranted to validate our findings and facilitate the discovery and development of anti-inflammatory agents to protect normal tissue from RT-induced adverse effects and improve quality of life in patients with breast cancer undergoing RT. *Cancer Epidemiol Biomarkers Prev; 23(9); 1873–83. ©2014 AACR.*

Introduction

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American women (1). There are more than 2 million American breast cancer survivors and it is important to address cancer survivorship issues related to treatment adverse responses that significantly impact quality of life. Compared with breast conserving surgery alone, the addition of radiotherapy (RT) to breast cancer therapy reduced the rate of local cancer recurrence (2). However, it is not yet clear which patients can be successfully treated with lumpectomy alone. Although well tolerated by most patients, even with improved RT technology, patients with breast cancer experience moist desquamation as early adverse skin reactions (EASR) during or up to 6 weeks after RT; 31.2% with intensity-modulated RT and 47.8% with standard treatment, respectively (3). The breast remains tender to palpation and the skin remains hyperpigmented for 6 to 9 months after treatment. The most common permanent effects on normal tissue are minor changes in the aesthetic appearance of the breast resulting from volume loss, fibrosis, or retraction at the tumor-bed site (4, 5). Breast or chest wall pain, increased risk of rib fracture, increased risk of cardiac morbidity, and lymphedema are also known late side effects of radiation (6, 7). Increasing evidence has suggested that individual genetic variations may play a significant role in the development of adverse radiation responses (8–10).

Inflammation may play critical roles in RT-induced EASRs and previous studies showed that RT induces changes in pro-inflammatory (IL1α, IL4, IL2, IL6, IL8,

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IL10, TNFα, IFNγ), pro-fibrotic (TGFβ1), pro-angiogenic (VEGF), and stem cell mobilizing (GM-CSF) cytokines and growth factors that may contribute to normal tissue toxicities or tumor control (11, 12). In addition, an inflammatory biomarker, C-reactive protein (CRP) has been associated with elevated risk for vascular atherosclerosis, insulin resistance, type 2 diabetes mellitus, and cancer (13–15). CRP levels were associated with fatigue and sleep quality in patients with breast cancer and RT-induced mucositis in patients with head and neck cancer (16–18). Furthermore, CRP levels also have prognostic value in patients with (i) breast cancer, (ii) loco-regionally advanced laryngeal carcinoma, or (iii) advanced esophageal cancer (14, 19, 20).

Using the plasma samples from the first 159 patients with breast cancer undergoing adjuvant RT to the intact breast in an ongoing prospective study, we pilot tested the hypothesis that higher pre- and post-RT CRP levels are associated with RT-induced EASRs. To the best of our knowledge, this is the first study investigating CRP in RT-induced EASRs of patients with breast cancer.

Materials and Methods

Study population

We used the plasma samples/data from the first 159 patients recruited during the period of December of 2008 and June of 2011 from an ongoing study to conduct this pilot study. Women diagnosed with breast carcinoma, stage 0 to III (American Joint Committee on Cancer) after breast conserving surgery were recruited from the Radiation Oncology Departments at the Sylvester Comprehensive Cancer Center and Jackson Memorial Hospital in Miami, Florida. Each patient was asked to complete a self-administered questionnaire to collect information on (i) demographic factors, (ii) race/ethnicity, and (iii) smoking history/status. Blood samples (20 mL) were collected from each individual before the initiation of RT and immediately after completion of RT. They were processed within 2 hours of phlebotomy and plasma was stored at −80°C until assay. This study was approved by the institution's review board at the University of Miami Miller School of Medicine and the Jackson Memorial Hospital. After receiving a detailed description of the study protocol, signed informed consent was obtained from each participant.

RT and EASRs assessment

Patients with breast cancer usually begin RT about 4 to 6 weeks after surgery or completion of chemotherapy. RT to the whole breast was given using standard opposed tangential fields alone, or to the whole breast plus regional lymph nodes at the treating physician's discretion. A regular dose of 45 to 50.4 Gy, in 1.8 or 2.0 Gy per fraction was delivered to the whole breast with or without regional nodes using 6 and/or 10 MV photons. In selected cases, a medial electron field was used for inclusion of the internal mammary nodes and/or cardiac sparing. A boost dose of 10 to 16 Gy was delivered to the lumpectomy cavity in the majority of case. Two radiation oncologists used the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Effects (CTCAE, version 3.0; http://ctep.cancer.gov) to evaluate EASRs grade and the presence or absence of moist desquamation at week 3 and at the completion of RT (week 6). The NCI CTCAE for radiation dermatitis contains 4 severity grades: grade 1, faint erythema or dry desquamation; grade 2, moderate erythema, noncontiguous patchy moist desquamation and moderate edema; grade 3, contiguous moist desquamation and bleeding induced by minor trauma or abrasion; grade 4, necrosis or ulceration and spontaneous bleeding from involved site. Within grade 2, patients may or may not have moist desquamation, a clinically relevant endpoint.

High-sensitivity CRP assay

CRP levels in the pre- and post-RT plasma samples were measured using the high-sensitivity C-Reactive Protein ELISA Kit (Calbiotech) according to the manufacturer's protocol. The standard curve was generated with each batch of samples based on the CRP ranged from 0.2 to 10 mg/L. We always re-run subset of samples that are outside the detection range by adjusting the dilution ratio from the standard 1:100 to either more concentrate (e.g., 1:50) or less concentrate (e.g., 1:200, 1:500, or 1:1,000) for repeated assay to ensure that the diluted samples are within the linear range of the standard curve of 0.2 and 10 mg/L. Briefly, frozen plasma sample was thawed and centrifuged at 10,000 rpm for 3 minutes. The clarified supernatant was diluted and 10 μL was added to duplicate CRP-coated wells. One hundred microliters of the enzyme conjugate was added and the plate was agitated briefly to mix. Following 1 hour incubation at room temperature, unbound mixture was removed and the wells washed 3 times with wash buffer. The plate was blotted on paper towels to remove residual wash solution. One hundred microliters of substrate was added and the plate was incubated for 15 minutes at room temperature. Fifty microliters of stop solution was then added and the plate was agitated to mix. The absorbance at 450 mm was determined using the Synergy HT microplate reader (BioTek Instruments). A standard curve using known concentrations of CRP was generated and levels of CRP were extrapolated based on the standard curve. The average coefficient of variation of duplicate samples was 8.3% and the interassay variation was <10%.

Statistical analysis

We first determined the distributions of demographics (age, race, and ethnicity) and other patient characteristics (body mass index [BMI], smoking status, and breast cancer stage), as well as EASR grade at weeks 3 and 6 respectively overall and by race. Test of symmetry was used to compare the marginal distributions of matching pairs data on EASR grade at weeks 3 and 6. χ^2 or Fisher exact test was used to test the association between patient demographics and other characteristics and EASRs grade at weeks 3 and 6. A paired t test was used to compare CRP

pre- and post-RT, using CRP log-base$_2$–transformed data for better fit of normal distribution. Analysis of variance (ANOVA) was conducted for comparisons of CRP log-base$_2$ transformed data, either at pre-RT or post-RT, by categories of demographic and other patient characteristic variables (not shown). Alternatively, we used nonparametric methods (Wilcoxon–Mann–Whitney test, or Kruskal–Wallis test for ANOVA, Wilcoxon signed-rank test for paired sample test) for corresponding analysis of CRP raw data when distributions were significantly right skewed.

Logistic regressions were used to test whether pre-RT CRP ($\geq$2 mg/L vs. <2 mg/L), post-RT CRP ($\geq$2 mg/L vs. <2 mg/L), or the CRP change (>1 mg/L vs. $\leq$1 mg/L, post-RT minus pre-RT) were significantly associated with grade 2/3 versus 0/1 RT-induced EASRs at weeks 3 and 6, respectively. Optimal cut-off values, 2 for CRP and 1 for CRP change, were selected based on estimates of sensitivity, specificity, and accuracy from receiver operating characteristic (ROC) univariate logistic regression analyses including CRP variables as continuous. High sensitivity was prioritized to select common cut-off value for pre-/post-CRP, and cut-off for CRP change. Multiple logistic regression models were used to evaluate the association between EASR grade and CRP or CRP change, after adjustment for BMI ($\geq$30 vs. <30), ethnicity (Hispanic vs. non-Hispanic), race (African Americans vs. non–African Americans), and age (in years). Odds ratios (OR) and 95% confidence intervals (95% CI) are reported. All statistical analysis was carried out in SAS v 9.3 (SAS Institute, Cary, NC), and test results were considered significant at the 2-sided 5% level.

Results

Study population characteristics

As summarize in Table 1, the study population consists of 29 African Americans (18.2%), 96 Hispanic Whites (60.4%), 29 non–Hispanic Whites (18.2%), and 5 other (3.1%). There were significant racial/ethnic differences in BMI distributions; about 62.1% African Americans were obese compared with 36.5% and 27.6% in Hispanic Whites and non–Hispanic Whites, respectively. No significant racial/ethnic differences were observed for clinical stage, smoking history, and smoking status. There was no significant racial/ethnic difference in skin toxicity grade at week 3 or 6. At week 3, lower proportion of Hispanic Whites had a grade 2 skin toxicity (2.1%) compared with African Americans (10.7%) or non–Hispanic Whites (10.3%). At week 6, higher proportion of African Americans had a grade 3 skin toxicity (13.8%) compared with Hispanic Whites (2.1%) or non–Hispanic Whites (3.4%). There was no significant racial/ethnic difference in the distribution of total number of 11 comorbidity conditions or total RT doses. However, there was a significant racial/ethnic difference ($P < 0.0001$) in breast volume; African American patients have the highest breast volume (mean $\pm$ SD = 1,358 $\pm$ 647) compared with that in Hispanic Whites (905 $\pm$ 437), non–Hispanic Whites (808 $\pm$ 408), or other (587 $\pm$ 251).

Progression of EASRs from week 3 to week 6 (end of RT)

In Table 2, we demonstrate a significant RT dosage-dependent progression of EASRs from week 3 to week 6 ($P < 0.001$). There was only one patient who had grade 0 at both weeks 3 and 6. At week 3, 15.8%, 79.1%, and 5.1% patients had grades 0, 1, and 2 EASRs, respectively. At week 6, there was a significant increase in the severity of RT-induced EASRs: 1.3%, 48.1%, 46.2%, and 4.4% patients had grade 0, 1, 2, and 3 EASRs, respectively.

EASR grade by patient characteristics

In Table 3, we summarize the results of EASR grade by patient characteristics. At week 3, there was a marginally significant ethnic different in EASR grade ($P = 0.07$); Hispanic patients have less grade 2 toxicity (2.0% vs. 10.3% in non-Hispanic). At week 6, a higher proportion of African Americans developed grade 3 EASRs (13.8% vs. 2.3% in others). A significantly higher proportion of obese (BMI $\geq$ 30) women experienced grade 3 EASRs at week 6 compared with those with BMI < 30 (8.2% vs. 2.0%; $P = 0.02$). A significantly higher proportion of current smoker experienced grade 3 EASRs at week 6 compared with nonsmoker (33.3% vs. 4.7%; $P = 0.01$). In addition, patients with above-median breast volume have a higher risk of developing grade 3 EASRs at week 6 compared with patients with below-median breast volume (9.0% vs. 0%; $P = 0.007$).

CRP levels by BMI and other patient characteristics

As shown in Fig. 1, there were significant correlations among individual's pre- and post-RT CRP levels ($R = 0.437$, $P < 0.0001$) and BMI (pre-RT: $R = 0.294$, $P = 0.0002$; post-RT: $R = 0.171$, $P = 0.032$). The pre- and post-RT CRP levels by patient characteristics were summarized in Table 4. The mean $\pm$ SD of pre- and post-RT CRP levels were 4.93 $\pm$ 6.65 and 5.26 $\pm$ 8.59 mg/L, respectively. For the pre-RT CRP levels, obese patients with BMI $\geq$ 30 have a significantly higher pre-RT CRP level vs. patients with BMI < 30 ($P < 0.0001$). For post-RT CRP, in addition to the differences by obesity, significantly higher levels were observed in African Americans compared with other racial/ethnic groups (6.44 $\pm$ 6.11 vs. 5.00 $\pm$ 9.05, $P = 0.02$). Patients with multiple comorbidity conditions have significantly higher pre-RT and post-RT CRP values. When we evaluated the change of CRP between pre- and post-RT, patients with at least 3 comorbidity conditions have a significant increase (6.50 $\pm$ 20.51; $P = 0.02$) compared with other groups. Patients with above-median breast volume have significantly higher pre-RT and post-RT CRP values.

Association between EASRs and CRP and/or obesity

In multivariable logistic regression models, we evaluated the associations between specific CRP variable and

Table 1. Study variables by race/ethnicity

Variable	Patients *N* (%)	AA[a] *N* (%)	HW[a] *N* (%)	NHW[a] *N* (%)	Other *N* (%)	*P*[b]
Study population	159 (100)	29 (18.2)	96 (60.4)	29 (18.2)	5 (3.1)	
Age, mean (SD)	56.3 (9.3)	55.1 (7.4)	57.2 (9.4)	55.5 (10.5)	50.2 (9.2)	0.30
BMI (kg/m^2)						
<25	47 (29.6)	4 (13.8)	24 (25.0)	15 (51.7)	4 (80.0)	0.003
25–29.99	51 (32.1)	7 (24.1)	37 (38.5)	6 (20.7)	1 (20.0)	
≥30	61 (38.4)	18 (62.1)	35 (36.5)	8 (27.6)	0 (0)	
Clinical stage						
0	33 (20.8)	5 (17.2)	22 (22.9)	4 (13.8)	2 (40.0)	0.23
IA and IB	79 (49.7)	11 (37.9)	51 (53.1)	16 (55.2)	1 (20.0)	
IIA–IIIC	47 (29.6)	13 (44.8)	23 (24.0)	9 (31.0)	2 (40.0)	
Smoking history						
Never	108 (67.9)	18 (62.1)	8 (70.8)	18 (62.1)	4 (80.0)	0.53
Ever	51 (32.1)	11 (37.9)	28 (29.2)	11 (37.9)	1 (20.0)	
Smoking status						
Never	108 (67.9)	18 (62.1)	68 (70.8)	18 (62.1)	4 (80.0)	0.30
Former	45 (28.3)	8 (27.6)	26 (27.1)	10 (34.5)	1 (20.0)	
Current	6 (3.8)	3 (10.3)	2 (2.1)	1 (3.4)	0 (0)	
Week 3 EASR grade						
0	25 (15.8)	5 (17.9)	16 (16.7)	2 (6.9)	2 (40.0)	0.10
1	125 (79.1)	20 (71.4)	78 (81.3)	24 (82.8)	3 (60.0)	
2	8 (5.1)	3 (10.7)	2 (2.1)	3 (10.3)	0 (0)	
Week 6 EASR grade						
0	2 (1.3)	1 (3.4)	1 (1.0)	0 (0)	0 (0)	0.17[c]
1	77 (48.4)	12 (41.4)	46 (47.9)	15 (51.7)	4 (80.0)	
2	73 (45.9)	12 (41.4)	47 (49.0)	13 (44.8)	1 (20.0)	
3	7 (4.4)	4 (13.8)	2 (2.1)	1 (3.4)	0 (0)	
Pre-RT CRP (mg/L)						
<10	145 (91.2)	24 (82.8)	90 (93.8)	27 (93.1)	4 (80.0)	0.25
≥10	14 (8.8)	5 (17.2)	6 (6.3)	2 (6.9)	1 (20.0)	
<2	58 (36.5)	8 (27.6)	36 (37.5)	13 (44.8)	1 (20.0)	0.39
≥2	101 (63.5)	21 (72.4)	60 (62.5)	16 (55.2)	4 (80.0)	
Post-RT CRP (mg/L)						
<10	141 (88.7)	23 (79.3)	87 (90.6)	27 (93.1)	4 (80.0)	0.20
≥10	18 (11.3)	6 (20.7)	9 (9.4)	2 (6.9)	1 (20.0)	
<2	56 (35.2)	7 (24.1)	34 (35.4)	13 (44.8)	2 (40.0)	0.25
≥2	103 (64.8)	22 (75.9)	62 (64.6)	16 (55.2)	3 (60.0)	
Post-RT − pre-RT CRP (mg/L)						
<0	71 (44.7)	12 (41.4)	41 (42.7)	14 (48.3)	4 (80.0)	0.36
0	10 (6.3)	0 (0)	8 (8.3)	2 (6.9)	0 (0)	
>0 and <1	44 (27.7)	7 (24.1)	30 (31.3)	6 (20.7)	1 (20.0)	
≥1	34 (21.4)	10 (34.5)	17 (17.7)	7 (24.1)	0 (0)	
No. of comorbidities[d]						
0	65 (40.9)	6 (20.7)	42 (43.8)	14 (48.3)	3 (60.0)	0.13
1	56 (35.2)	12 (41.4)	35 (36.5)	9 (31.0)	0 (0)	
2	28 (17.6)	8 (27.6)	15 (15.6)	3 (10.3)	2 (40.0)	
≥3	10 (6.3)	3 (10.3)	4 (4.2)	3 (10.3)	0 (0)	
Total RT dose (Gy)[e]						
<60	33 (20.8)	5 (17.2)	18 (18.8)	9 (31.0)	1 (20.0)	0.31
60	108 (67.9)	20 (69.0)	69 (71.9)	15 (51.7)	4 (80.0)	
>60	18 (11.3)	4 (13.8)	9 (9.4)	5 (17.2)	0 (0)	

(Continued on the following page)

Table 1. Study variables by race/ethnicity (Cont'd)

Variable	Patients *N* (%)	AA[a] *N* (%)	HW[a] *N* (%)	NHW[a] *N* (%)	Other *N* (%)	*P*[b]
Breast volume (cc)						
Mean (SD)	958 (509)	1,358 (647)	905 (437)	808 (408)	587 (251)	<0.0001
Median	864	1357	831	774	593	
Below median (<864)	77 (49.7)	8 (28.6)	49 (52.7)	16 (55.2)	4 (80.0)	0.06
Above median (≥864)	78 (50.3)	20 (71.4)	44 (47.3)	13 (44.8)	1 (20.0)	

[a]AA, Black or African American; HW, Hispanic Whites; NHW, non–Hispanic Whites.
[b]χ^2 or Fisher's exact tests excluding the other race category and missing.
[c]Combined EASR grades 0 and 1.
[d]Sum of 11 comorbidity conditions: diabetes, hypertension, heart disease, lung disease, thyroid condition, cirrhosis liver, stroke, chronic bronchitis, hepatitis, tuberculosis, and other.
[e]Sum of total breast and boost radiation doses; the range was between 50 and 66.4 Gy except for one patient at 38.5 Gy.

obesity (BMI $\geq$ 30 vs. <30) and EASRs after adjustment for age (years), race (African Americans vs. others), ethnicity (Hispanic or non-Hispanic), and tumor stage (II–IV or I vs. 0). Because interactions between specific CRP variable with BMI were not significant, OR estimates for combination high CRP and obesity vs. low CRP and nonobesity were calculated by multiplying the individual ORs. As shown in Table 5, neither pre-RT CRP nor obesity alone was significantly associated with EASRs at week 3 or 6. However, there was a significant association between combined higher pre-RT CRP with obesity and grade 2+ EASRs at week 6 (OR, 3.03; 95% CI, 1.28–7.17; $P = 0.01$). Grades 2/3 EASRs at week 6 were significantly associated with a higher post-RT CRP combined with obesity (OR, 2.70; 95% CI, 1.18–6.19; $P = 0.02$). Grades 2/3 EASRs at week 6 were significantly associated with change (post-RT minus pre-RT) of CRP >1 mg/L (OR, 2.51; 95% CI, 1.06–5.95; $P = 0.04$), and obesity (OR, 2.08; 95% CI, 1.03–4.21; $P = 0.04$). Furthermore, grades 2/3 EASRs at week 6 was significantly associated with combined elevated CRP (>1 mg/L) and obesity (BMI $\geq$ 30) versus CRP $\leq$ 1 and nonobesity (OR, 5.21; 95% CI, 1.77–15.38; $P = 0.003$).

Discussion

After breast-conserving surgery, adjuvant RT to the breast contributes to improved local regional recurrence and survival in patients with breast cancer. However, whole breast RT is associated with EASRs, increased cardiovascular mortality, and lung cancer development. In general, African Americans have worse treatment-related side effects and survival. Therefore, we designed this tri-racial/ethnic study to evaluate whether inflammatory biomarker CRP is related to RT-induced EASRs. To the best of our knowledge, this is the first study to date investigating the association between CRP and RT-induced EASRs in breast cancer. We reported significant association between elevated risk of RT-induced EASRs and higher levels of CRP, particularly the change between pre-RT and post-RT, combined with obesity. We also observed that African Americans have higher CRP and are more susceptible to RT-induced EASRs compared with Whites.

Our data showed that African Americans are more susceptible to RT-induced EASRs, which is consistent with the data from a previous study using a self-administered questionnaire to assess skin reactions (21). In our study, 2 radiation oncologists used the NCI CTC v3.0, a well-established tool for assessing RT-induced EASRs, which may present a more consistent and objective evaluation of EASRs. Patients with pre-RT or post-RT CRP levels greater than 2 mg/L or patients experienced an increase in CRP levels greater than 1 mg/L have a higher risk for grade 2+ EASRs at week 6. This is supported by a

Table 2. Progression of EASR from week 3 to week 6 (end of RT)

	Week 6 grade					
Week 3 grade	0	1	2	3	Total	*P*[a]
0	1	14	9	1	25 (15.8%)	<0.001
1	1	62	57	5	125 (79.1%)	
2	0	0	7	1	8 (5.1%)	
Total	2 (1.3%)	76 (48.1%)	73 (46.2%)	7 (4.4%)	158	

[a]Wilcoxon signed-rank test.

Table 3. EASR grade by patient characteristics

	Week 3 grade				Week 6 grade				
	0	1	2		0	1	2	3	
Variable	***N* (%)**	***N* (%)**	***N* (%)**	***P*[a]**	***N* (%)**	***N* (%)**	***N* (%)**	***N* (%)**	***P*[a]**
Total patient	25 (15.8)	125 (79.1)	8 (5.0)		2 (1.3)	77 (48.4)	73 (45.9)	7 (4.4)	
Age (y)									
<50	6 (13.3)	34 (75.6)	5 (11.4)	0.35	—	19 (42.2)	25 (55.6)	1 (2.2)	0.13
50–59	10 (18.5)	43 (79.6)	1 (1.9)		1 (1.9)	29 (53.7)	19 (35.2)	5 (9.3)	
≥60	9 (15.3)	48 (81.4)	2 (3.2)		1 (1.7)	29 (48.3)	29 (48.3)	1 (1.7)	
Ethnicity									
Non-Hispanic	9 (15.5)	43 (74.1)	6 (10.3)	0.07	1 (1.7)	29 (50.0)	24 (41.4)	4 (6.9)	0.24
Hispanic	16 (16.0)	82 (82.0)	2 (2.0)		1 (1.0)	48 (47.5)	49 (48.5)	3 (3.0)	
Race/ethnicity									
AA	5 (17.9)	20 (71.4)	3 (10.3)	0.10	1 (3.4)	12 (41.4)	12 (41.4)	4 (13.8)	0.22
HW	16 (16.7)	78 (81.3)	2 (2.1)		1 (1.0)	46 (47.9)	47 (49.0)	2 (2.1)	
NHW	2 (6.9)	24 (82.8)	3 (10.3)		0 (0)	15 (51.7)	13 (44.8)	1 (3.4)	
Other	2 (40.0)	3 (60.0)	0 (0)		0 (0)	4 (80.0)	1 (20.0)	0 (0)	
AA	5 (17.9)	20 (71.4)	3 (10.7)	0.22	1 (3.4)	12 (41.4)	12 (41.4)	4 (13.8)	0.02
Non–AA	21 (16.2)	104 (80.0)	5 (3.8)		1 (0.8)	65 (50.0)	61 (46.9)	3 (2.3)	
BMI (kg/m^2)									
<25	9 (19.1)	37 (78.7)	1 (2.1)	0.83	—	30 (63.8)	16 (34.0)	1 (2.1)	0.06
25–29.99	7 (14.0)	40 (80.0)	3 (5.9)		1 (2.0)	25 (49.0)	24 (47.1)	1 (2.0)	
≥30	9 (14.8)	48 (78.7)	4 (6.6)		1 (1.6)	22 (36.1)	33 (54.1)	5 (8.2)	
<30	16 (16.5)	77 (79.4)	4 (4.1)	0.78	1 (1.0)	55 (56.1)	40 (40.8)	2 (2.0)	0.02
≥30	9 (14.8)	48 (78.7)	4 (6.6)		1 (1.6)	22 (36.1)	33 (54.1)	5 (8.2)	
Clinical stage									
0	7 (21.2)	25 (75.8)	1 (3.0)	0.60	2 (6.1)	20 (60.6)	10 (30.3)	1 (3.0)	0.29
IA–IB	9 (11.5)	65 (83.3)	4 (5.1)		—	36 (45.6)	39 (49.4)	4 (5.1)	
IIA–IIIC	9 (19.1)	35 (74.5)	3 (6.4)		—	21 (44.7)	24 (51.1)	2 (4.3)	
Smoking history									
Never	19 (17.8)	83 (77.6)	5 (4.7)	0.61	1 (0.9)	53 (49.1)	49 (45.4)	5 (4.7)	1.00
Ever	6 (11.8)	42 (82.4)	3 (5.9)		1 (2.0)	24 (47.1)	24 (47.1)	2 (3.9)	
Smoking status									
Never	19 (17.8)	83 (77.6)	5 (4.7)	0.78	1 (0.9)	53 (49.1)	49 (45.4)	5 (4.7)	0.01
Former	5 (11.1)	37 (82.2)	3 (6.7)		1 (2.2)	20 (44.4)	24 (53.3)	0 (0)	
Current	1 (16.7)	5 (83.3)	0 (0)		0 (0)	4 (66.7)	0 (0-)	2 (33.3)	
No. of comorbidities									
0	7 (10.9)	55 (85.9)	2 (3.2)	0.25	0 (0)	37 (56.9)	26 (40.0)	2 (3.1)	0.15
1	10 (17.9)	40 (71.4)	6 (10.7)		0 (0)	25 (44.6)	30 (53.6)	1 (1.8)	
2	6 (21.4)	22 (78.6)	0 (0)		2 (7.1)	12 (42.9)	12 (42.9)	2 (7.1)	
≥3	2 (20.0)	8 (80.0)	0 (0)		0 (0)	3 (30.0)	5 (50.0)	2 (20.0)	
RT dose (Gy)									
<60	2 (6.3)	28 (87.5)	2 (6.3)	0.30	0 (0)	17 (51.5)	14 (42.4)	2 (6.1)	0.90
60	18 (16.7)	84 (77.8)	6 (5.6)		1 (0.9)	52 (48.1)	51 (47.2)	4 (3.7)	
>60	5 (27.8)	13 (72.2)	0 (0)		1 (5.6)	8 (44.4)	8 (44.4)	1 (5.6)	
Breast volume (cc)									
<864	13 (16.9)	62 (80.5)	2 (2.6)	0.65	2 (2.6)	42 (54.5)	33 (42.9)	0 (0)	0.007
≥864	12 (15.6)	60 (77.9)	5 (6.5)		0 (0)	33 (42.3)	38 (48.7)	7 (9.0)	

Abbreviations: AA, Black or African American; HW, Hispanic Whites; NHW, non–Hispanic Whites.

[a]*P* value of the χ^2 test or the Fisher's exact test excluding missing; for week 6, grade 0/1 vs. 2 vs. 3.

previous study that expression of human CRP in mice was correlated with upregulation of the TGFβ/Smad3 signaling pathway, which has been associated with RT-induced fibrosis or moist desquamation of the skin (22). Other risk factors, such as obesity and breast volume, have been related to late effects (23). This could be because of

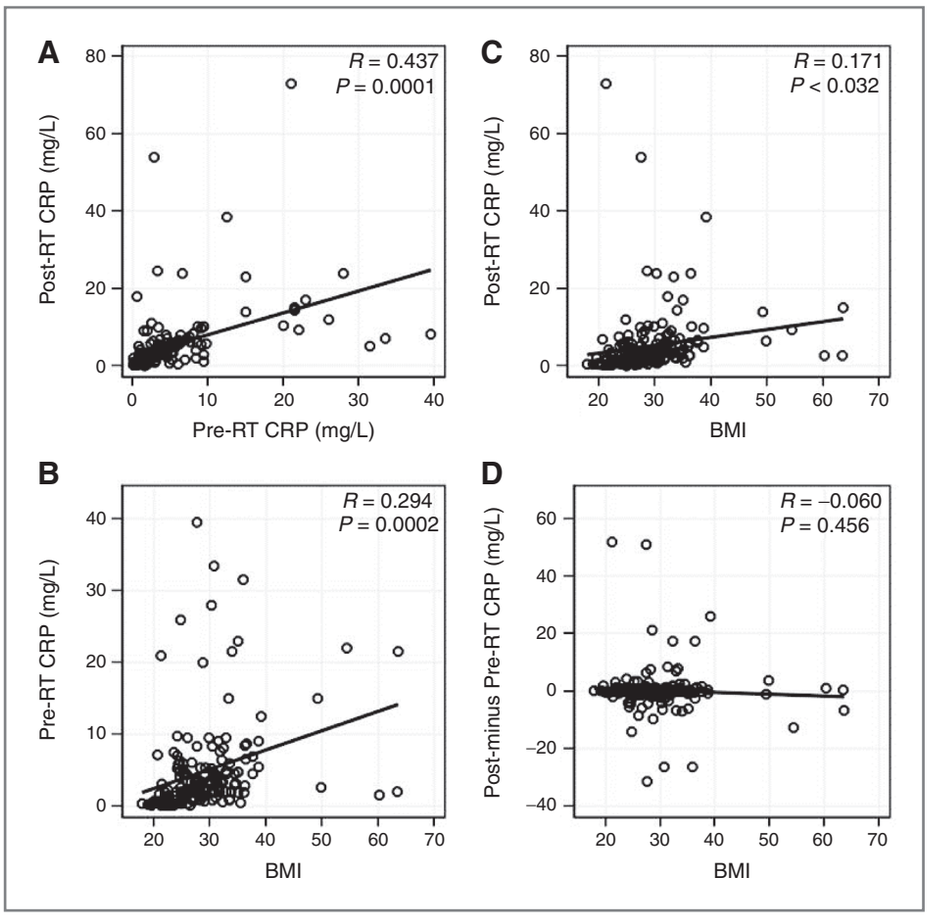

Figure 1. Correlations among pre-RT CRP, post-RT CRP, and BMI. The mean of pre-RT CRP was 4.93 mg/L (SD = 6.65, median = 2.8, and range = 0.1–39.5); the mean of post-RT CRP was 5.26 mg/L (SD = 8.59, median = 3.1, and range = 0.1–73). CRP data were substantially skew. Comparison of $\log_2$ transformed data indicated no statistically significant difference between pre-RT versus post-RT CRP (mean of $\log_2$ CRP: 1.33 vs. 1.42, $P = 0.34$). Correlations were 0.437 ($P < 0.0001$) between pre- and post-RT CRP, 0.171 ($P = 0.03$) for post-RT CRP and BMI, and 0.294 ($P < 0.001$) for pre-RT CRP and BMI. After excluding the 2 largest outliers for post-RT CRP (values 54 and 73), the correlations were 0.529 for pre- and post-RT, and 0.373 for post-RT CRP versus BMI.

dosimetric variation across the breast related to skin folding in patients with higher BMI and/or breast volume. Similarly, we reported an association between obesity and RT-induced EASRs, although larger breast volume was not significantly associated with EASRs.

The CRP level in normal human serum ranges from 0.2 to 10 mg/L, where 90% of apparently healthy individuals have CRP levels <3 mg/L and only 1% have levels >10 mg/L. As shown in our study, 14 (8.8%) and 18 (11.3%) patients have pre-RT and post-RT CRP ≥10 mg/L, respectively. We also observed that higher proportion of African Americans have CRP ≥10 mg/L at both pre-RT (17.2%) and post-RT (20.7%). This is consistent with the previous finding that higher CPR levels were reported in African Americans compared with Whites, Chinese, or Japanese in a multiethnic study in noncardiovascular disease women (24, 25). Furthermore, racial/ethnic differences have been documented in multiple inflammatory cytokine polymorphisms indicating that groups of the African or black Americans have higher frequency of cytokine variants responsible for the regulation of immune/inflammatory responses (26, 27). Similarly, we report that African-American patients had higher pre-RT CRP levels compared with Whites. Although our data suggest that higher pre-RT CRP may be associated with extremely early onset of grade 2+ EASRs at week 3 (OR, 4.90; 95% CI, 0.50–48.32); the association was not significant probably because of small sample size of patients with grade 2+ EASRs at week 3 ($n = 8$). Another consideration is that racial/ethnic differences in EASRs may be attributed to multiple genetic risk factors. For example, previous studies have shown that racial/ethnic differences in RT-induced skin reactions in *ATM* sequence variant carriers: 17% Hispanics, 23% African Americans, and 8% Whites (9, 28). Therefore, future large genetic studies are warranted to elucidate contribution of genetic variants in racial/ethnic differences of RT-induced EASRs and late effects.

Previous studies suggest that RT-induced changes in pro-inflammatory cytokines and growth factors may contribute to normal tissue toxicities (11, 12). Our current data provide new evidence that the inflammatory biomarker, plasma CRP levels are associated with RT-induced EASRs in patients with breast cancer undergoing RT. Our current findings will have several clinical implications. First, although CRP produced by tumor cells may act as an opsonin, mediating tumor cell lysis, or it may also stimulate production of prostaglandins, which would facilitate tumor progression; although tumor CRP synthesis may be too low to influence circulating CRP levels.

Table 4. CRP levels (mg/L) by demographic factors and other patient characteristics

		Pre-RT CRP				Post-RT CRP				Post- and pre-RT CRP			
Variable	***N***	**Median**	**Mean**	**SD**	***P*[a]**	**Median**	**Mean**	**SD**	***P*[a]**	**Median**	**Mean**	**SD**	***P*[a]**
Total	159	2.80	4.93	6.65		3.10	5.26	8.59		0.00	0.33	8.25	
Age (y)													
<50	45	2.10	4.39	6.32	0.12	2.70	5.45	11.32	0.04	0.10	1.06	9.42	0.29
50—59	54	2.40	4.46	5.95		2.25	3.83	4.35		−0.20	−0.63	3.66	
≥60	60	3.35	5.75	7.47		4.20	6.41	9.04		0.05	0.66	10.13	
Ethnicity													
Non-Hispanic	58	2.65	5.54	7.70	0.98	2.75	6.06	11.92	0.66	−0.05	0.52	11.64	0.52
Hispanic	101	3.00	4.58	5.98		3.20	4.80	5.92		0.10	0.23	5.50	
Race/ethnicity													
AA	29	4.50	6.67	7.79	0.16	4.70	6.44	6.11	0.04	0.40	−0.23	7.43	0.27
HW	96	2.90	4.54	6.02		3.15	4.62	5.74		0.00	0.08	5.54	
NHW	29	2.20	4.02	6.66		2.20	6.50	16.09		0.00	2.48	14.53	
Other	5	4.20	7.50	10.51		2.00	3.64	4.84		−0.80	−3.86	5.93	
AA	29	4.50	6.67	7.79	0.08	4.70	6.44	6.11	0.02	0.40	−0.23	7.43	0.24
Non—AA	130	2.65	4.54	6.34		2.80	5.00	9.05		0.00	0.46	8.44	
BMI (kg/m^2)													
<25	47	0.90	2.93	5.02	<0.0001	1.20	3.55	10.63	<0.0001	0.00	0.61	8.09	0.91
25–29.99	51	2.50	4.14	5.96		3.20	4.84	8.07		0.10	0.69	9.37	
≥30	61	4.60	7.12	7.68		4.70	6.94	6.93		0.10	−0.18	7.45	
<30	98	2.00	3.56	5.54	<0.0001	1.75	4.22	9.36	<0.0001	0.00	0.66	8.73	0.46
≥30	61	4.60	7.12	7.68		4.70	6.94	6.93		0.10	−0.18	7.45	
Clinical stage													
0	33	3.70	5.03	5.98	0.70	3.30	4.99	5.71	0.75	0.10	−0.04	2.44	0.93
IA—IB	79	2.80	4.56	5.82		2.60	6.04	11.30		0.00	1.48	10.20	
IIA—IIIC	47	2.30	5.46	8.31		3.20	4.14	3.43		0.00	−1.33	6.92	
Smoking history													
Never	108	2.75	4.87	6.59	0.87	3.20	5.37	9.31	0.74	0.00	0.50	8.90	0.67
Ever	51	2.90	5.05	6.84		2.80	5.04	6.89		0.10	−0.01	6.73	
Smoking status													
Never	108	2.75	4.87	6.59	0.84	3.20	5.37	9.31	0.96	0.00	0.50	8.90	0.87
Former	45	2.50	5.16	7.21		2.80	5.17	7.24		0.10	0.01	6.98	
Current	6	3.55	4.22	2.92		2.05	4.07	3.57		0.40	−0.15	4.95	
Week 3 EASR grade													
0	25	3.60	4.80	5.19	0.27	5.20	5.61	4.93	0.08	0.20	0.82	6.06	0.29
1	125	2.30	4.68	6.48		2.60	5.24	9.37		0.00	0.56	8.54	
2	8	3.65	9.15	11.89		3.25	4.71	4.85		−0.60	−4.44	9.49	
Week 6 EASR grade													
0/1	79	3.00	4.45	5.60	0.22	2.20	5.21	10.03	0.13	0.00	0.76	7.65	0.07
2	73	2.90	5.73	7.82		3.90	5.39	7.17		0.10	−0.34	9.15	
3	7	2.00	1.91	0.94		2.70	4.49	4.21		0.60	2.57	3.56	
No. of comorbidities													
0	65	2.10	3.66	4.16	0.02	1.70	4.60	10.25	<0.001	−0.20	0.93	7.91	0.02
1	56	2.75	4.77	6.81		3.20	4.36	4.86		0.20	−0.42	4.20	
2	28	5.35	7.80	7.99		4.65	6.05	4.96		−0.25	−1.76	7.29	
≥3	10	2.70	5.94	11.84		8.60	12.44	16.21		3.95	6.50	20.51	
Total RT dose (Gy)													
<60	33	3.20	6.48	7.51	0.20	3.30	7.22	13.11	0.45	−0.20	0.74	9.83	0.35
60	108	2.55	4.81	6.81		2.70	4.99	7.38		0.00	0.17	8.40	
≥60	18	2.50	2.77	1.79		3.30	3.33	2.75		0.50	0.56	2.52	
Breast volume (cc)													
<864	77	2.00	3.74	5.95	<0.001	2.00	4.46	10.41	<0.001	0.00	0.72	9.31	0.98
≥864	78	3.40	5.99	7.02		3.85	6.05	6.43		0.05	0.06	7.30	

Abbreviations: AA, Black or African American; HW, Hispanic Whites; NHW, non–Hispanic Whites.

[a]*P* value, Wilcoxon–Mann–Whitney or Kruskal–Wallis test (except the first line).

Table 5. Association between EASRs and CRP and/or obesity

		Week 3 grade ($n = 158$)					Week 6 grade ($n = 159$)				
		Total	0/1	2	Multivariable		Total	0/1	2/3	Multivariable	
Variable	**Level**	***N* (%)**	***N* (%)**	***N* (%)**	**OR (95% CI)[a]**	***P***	**N (%)**	**N (%)**	**N (%)**	**OR (95% CI)[a]**	***P***
Pre-RT CRP (mg/L)	<2	58 (36.7)	57 (38.0)	1 (12.5)	Reference		58 (36.5)	34 (43.0)	24 (30.0)	Reference	
	≥2	100 (63.3)	93 (62.0)	7 (87.5)	4.90 (0.50–48.32)	0.17	101 (63.5)	45 (57.0)	56 (70.0)	1.56 (0.76–3.21)	0.22
BMI	<30	97 (61.4)	93 (62.0)	4 (50.0)	Reference		98 (61.6)	56 (70.9)	42 (52.5)	Reference	
	≥30	61 (38.6)	57 (38.0)	4 (50.0)	1.18 (0.23–6.21)	0.84	61 (38.4)	23 (29.1)	38 (47.5)	1.94 (0.94–4.02)	0.08
Pre-RT CRP ≥ 2 and BMI ≥ 30 vs. CRP <2 and BMI <30					5.81 (0.49–69.23)[b]	0.16				3.03 (1.28–7.17)[b]	0.01
Post-RT CRP (mg/L)	<2	56 (35.4)	53 (35.3)	3 (37.5)	Reference		56 (35.2)	33 (41.8)	23 (28.8)	Reference	
	≥2	102 (64.6)	97 (64.7)	5 (62.5)	0.82 (0.13–5.15)	0.84	103 (64.8)	46 (58.2)	57 (71.3)	1.38 (0.65, 2.94)	0.40
BMI	<30	97 (61.4)	93 (62.0)	4 (50.0)	Reference		98 (61.6)	56 (70.9)	42 (52.5)	Reference	
	≥30	61 (38.6)	57 (38.0)	4 (50.0)	2.02 (0.30–13.63)	0.47	61 (38.4)	23 (29.1)	38 (47.5)	1.96 (0.92, 4.17)	0.08
Post-RT CRP ≥ 2 and BMI ≥ 30 vs. CRP <2 and BMI <30					1.66 (0.27–10.32)[b]	0.59				2.70 (1.18, 6.19)[2]	0.02
Post-RT minus pre-RT CRP	≤1	125 (79.1)	118 (78.7)	7 (87.5)	Reference		126 (79.2)	69 (87.3)	57 (71.3)	Reference	
	>1	33 (20.9)	32 (21.3)	1 (12.5)	0.47 (0.05–4.38)	0.51	33 (20.8)	10 (12.7)	23 (28.8)	2.51 (1.06–5.95)	0.04
BMI	<30	97 (61.4)	94 (62.0)	4 (50.0)	Reference		98 (61.6)	56 (70.9)	42 (52.5)	Reference	
	≥30	61 (38.6)	57 (38.0)	4 (50.0)	2.09 (0.39–11.14)	0.39	61 (38.4)	23 (29.1)	38 (47.5)	2.08 (1.03–4.21)	0.04
CRP change > 1 and BMI ≥ 30 vs. change ≤ 1 and BMI <30					0.97 (0.08–11.97)[b]	0.98				5.21 (1.77–15.38)[b]	0.003

[a]OR estimates of CRP variables (high vs. low category, mg/L), BMI (≥30 vs. <30 kg/m^2), or combination, obtained from logistic regression models including the specific CRP variable, BMI, age (years), ethnicity (Hispanic vs. non-Hispanic), race (AA vs. others), and tumor stage (II–IV or I, vs. 0).

[b]Interactions between specific CRP variable with BMI were not significant, OR estimates for combination high CRP and obesity vs. low CRP and nonobesity were calculated by multiplying the individual ORs.

Second, elevated circulating CRP has been associated with cancer prognosis, vascular atherosclerosis, insulin resistance, and type 2 diabetes mellitus that may also impact overall survival. Therefore, patients with CRP levels $\geq$10 mg/L (8.8% pre-RT and 10.3% post-RT) in our study population will need to be monitored for cancer recurrence and other clinical conditions. Third, considering the involvement of CRP in fatigue and prognosis of patients with breast cancer, our future follow-up study will focus on monitoring CRP levels, quality of life, and clinical outcomes, including fatigue, late effects, and recurrence/metastasis.

Intriguingly, we observed a subset of patients with breast cancer who experienced extreme hypersensitivity to RT and developed grade 2+ EASRs within the first 3 weeks and majority of patients developed RT-induced EASRs (98%) at the end of RT in a dose-dependent manner. In addition, we also observe that patients with at least 3 comorbidity conditions have significantly elevated CRP post-RT. Thus, the development of accurate prediction models in identifying high-risk patients and targets for personalized intervention to minimize EASRs in patients with breast cancer may be essential. In summary, this is the first study to demonstrate racial/ethnic differences in CRP levels and disparities in RT-induced EASRs in patients with breast cancer undergoing RT. Inflammatory biomarker CRP is associated with RT-induced EASRs, particularly when combined with obesity. With limited sample size, our results should be interpreted with caution. Future larger studies are warranted to confirm our findings. We are currently conducting 2 larger studies with a total sample size of 1,400 to validate our promising findings. If confirmed, our data will support and facilitate the application of anti-inflammatory agents in protecting normal tissue from RT-induced EASRs and improving quality of life in patients with breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.L. Rodriguez-Gil, C. Takita, J. Wright, J.J. Hu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.L. Rodriguez-Gil, J. Wright, I.M. Reis, W. Zhao, B.E. Lally, J.J. Hu
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.L. Rodriguez-Gil, J.J. Hu
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