Impact of radiotherapy-induced inflammatory responses on progression-free survival in a tri-racial/ethnic breast cancer population

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Abstract

Background

Inflammatory biomarker C-reactive protein (CRP) is associated with breast cancer risk and survival. We examined whether CRP levels before radiotherapy (pre-RT), after RT (post-RT), and RT-induced change impact breast cancer progression-free survival (PFS).

Methods

Plasma high-sensitivity CRP was measured, and patients were followed for up to 13 years after RT. PFS was calculated from the date of diagnosis to the date of disease progression or the last date of follow-up. Univariable and multivariable Cox proportional hazards regression models were used to evaluate the associations between CRP and PFS adjusted for other patient/clinical variables.

Results

In 469 patients (64 non-Hispanic Whites, 303 Hispanic Whites, and 102 African Americans), post-RT CRP levels were significantly higher in patients with progression compared to progression-free patients (mean±SD: 12.2±15.4 mg/L vs. 7.3±11.5, p=0.011). In univariable analyses, worse PFS was significantly associated with post-RT CRP ≥5.1 mg/L (hazard ratio [HR]: 2.67; 95% confidence interval [95% CI]: 1.65-4.30) and CRP change ≥2.3 mg/L (HR: 3.55; 95% CI: 2.25-5.64). In multivariable models, post-RT CRP ≥5.1 mg/L was associated with worse PFS in all (HR: 2.10; 95% CI: 1.29-3.42) or patients with tumor stage III (HR: 2.93, 95% CI: 1.20-7.18). CRP change ≥2.3 mg/L was associated with worse PFS in all (HR: 2.38; 95% CI: 1.45-3.92) or patients with tumor stage III (HR: 2.41, 95% CI: 1.09-5.33).

Conclusions

Our data suggest that an RT-induced hyper-inflammatory response may contribute to worse breast cancer PFS. Future larger studies are warranted to validate our findings and guide follow-up surveillance and targeted interventions.

Background

Breast cancer remains the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among women in the United States [1]. Early detection and advances in treatment modalities have improved breast cancer survival. Adjuvant radiotherapy (RT) has been the standard of care following breast-conserving surgery and has contributed to improved locoregional recurrence and survival of breast cancer patients [2, 3]. However, there are adverse responses to RT, including pain, acute skin toxicity, lymphedema, fibrosis, and potential cardiotoxicity [4–8]. Radiation exposure to normal tissues adjacent to the breast and regional lymph nodes may affect the heart and pose a risk to cardiovascular health [7, 9]. Some pathophysiological and lifestyle-associated risk factors shared between cancer and chronic diseases may also contribute to worse overall survival among cancer patients, such as systemic inflammation [10–12].

Inflammation plays roles in breast cancer risk and prognosis [13]. Several epidemiological studies have reported that elevated levels of an inflammatory biomarker, C-reactive protein (CRP), increased risk for breast cancer and RT-related skin toxicities and reduced progression-free and overall survival [14–19]. The high-sensitivity CRP (hsCRP) assay has been used to detect low-grade systemic inflammatory and a prognostic biomarker for different cancers, including breast cancer [15, 20]. Further exploring the link between RT-related inflammatory responses using CRP and progression-free survival (PFS) may identify high-risk patients for worse PFS, implement rigorous follow-up surveillance, and explore targeted interventions [21].

Due to its ability to be easily measured and standardized, CRP is a useful indicator to assess and monitor the presence, severity, and course of the inflammatory response. Although CRP is considered a valuable biomarker in predicting breast cancer prognosis and the clinical outcomes of many diseases, previous studies have not evaluated whether RT-induced changes in CRP may impact breast cancer PFS. Therefore, we conducted this prospective study to evaluate pre-RT, post-RT, and RT-induced changes in CRP in predicting the PFS of breast cancer patients.

Methods

Study design and patient population

CRP data for the current study were obtained from a prospective cohort study evaluating the effects of RT on skin toxicities in a racially and ethnically diverse population of breast cancer patients in Miami, Florida, as detailed in previous manuscripts [19, 22–24]. In brief, we recruited 516 breast cancer patients at the radiation oncology clinics at the University of Miami Sylvester Comprehensive Cancer Center and Jackson Memorial Hospital in Miami, Florida from December 2008 to August 2014. The study was approved by the Institutional Review Boards of the University of Miami and Jackson Memorial Hospital, and all patients provided written informed consent. The inclusion criteria were: (1) female subjects newly diagnosed with breast carcinoma, tumor stage 0–III; (2) post-lumpectomy, quadrantectomy, or mastectomy; (3) planned to receive adjuvant RT to the whole breast or chest wall +/- regional lymph nodes, total dose ≥40 Gy, dose per fraction ≥1.8 Gy, use of 2D, 3D conformal, or intensity-modulated RT allowed; (4) able and willing to sign a protocol consent form; (5) age ≥18 years old; and (6) self-identified race/ethnicity as non-Hispanic white (NHW), Hispanic white (HW), or Black/African American (AA). The exclusion criteria were:

(1) tumor stage IV; (2) prior radiation to the involved breast or chest wall; (3) concurrent chemotherapy; (4) unable or unwilling to sign informed consent; and (5) unable to speak English or Spanish.

In the current study, we had a final sample size of 469 after the further exclusion of participants with missing information on both pre- and post-RT CRP levels or incomplete information on clinicopathologic details. At the time of enrollment, each participant completed a self-administered questionnaire about basic demographic/patient information.

Assessment of CRP

Blood samples (20 mL) were collected on the first and the last day of RT, processed within 2 hours of phlebotomy, and the aliquoted plasma samples were stored at -80°C until assay. Plasma CRP levels were measured using a hsCRP enzyme-linked immunosorbent assay (ELISA) kit (Calbiotech, Spring Valley, CA), according to the manufacturer's protocol. A standard curve was generated for each batch of samples based on CRP concentrations, which ranged from 0.2 to 10.0 mg/L. To ensure that the diluted samples were within the linear range of the standard curve, we re-ran the assays by adjusting the dilution ratio if samples were outside the detection range. The average coefficient of variation was 8.3%, and the inter-assay variation was less than 10%. Although CRP level has been commonly dichotomized using 10 mg/L as a clinically meaningful cut-off value for elevated inflammatory responses in cardiovascular disease and breast cancer, we found 5.1 mg/L to be a better cut-off in our study population using Contal and O'Quigley's maximized log-rank statistic method [25–27]. Change in CRP was calculated as the difference between the post-RT and pre-RT levels and dichotomized into low or high groups using 2.3 mg/L as the cut-off. These cut-offs were obtained using the Evaluate Cutpoints application in R (R Core Team, Vienna, Austria,) which determines cutpoints for continuous predictors based on the highest log-rank statistic (Contal and O'Quigley method) [27, 28].

Assessment of progression-free survival (PFS)

Patients in the current study were followed for up to 13 years after the completion of RT through the regular review of electronic medical records, last completed in July 2021. Progression-free was defined as a patient who was alive and did not have recurrence, metastasis, or a second primary cancer by the last date of follow-up. PFS was defined as the elapsed time between the date of breast cancer diagnosis and the earliest date of documented disease progression, including recurrence, distant metastasis, second primary cancer, or death. Progression-free patients were censored at the last date of follow-up.

Assessment of covariates

We assessed race (white and Black/AA), ethnicity (non-Hispanic and Hispanic), age at diagnosis (under 60 years and 60 years or older), and smoking history (never and current/former) as patient covariates. Current/former smokers were defined as those who had smoked at least 100 cigarettes in their lifetimes. Clinical variables, such as triple-negative status (yes and no) and clinical tumor stage (0-II and III) were ascertained using medical records. The clinical tumor stage was based on the American Joint Committee on Cancer staging scheme, 8th edition [29].

Statistical analysis

We first compared the means of pre-RT and post-RT CRP by disease progression status using paired samples t-tests. We also assessed pre-RT, post-RT, and change in CRP levels, separately, of disease progression and progression-free patients overall and within patient/clinical characteristics using independent samples t-tests. We compared the distributions of patient/clinical characteristics by CRP (cut-off value of 5.1 mg/L for pre- and post-RT, 2.3 mg/L for RT-induced CRP change) using Pearson's chi-square test or Fisher's exact test. PFS curves by covariate levels were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariable and multivariable Cox proportional hazards regression was conducted to test whether CRP, race/ethnicity, age at diagnosis, smoking history, triple-negative status, and tumor stage were associated with PFS. Multivariable analysis was performed, with each model including a CRP variable and adjusted for the aforementioned covariates, all of which have been widely shown in the literature to be associated with breast cancer. Hazard ratios (HRs) and corresponding 95% confidence intervals (95% CIs) were reported. Considering that tumor stage is the most important factor for PFS, analyses were conducted in all stages and stratified into stage 0-II and stage III patients. When testing for the interaction effects between CRP and tumor stage in separate multivariable models that only included a CRP variable, stage, and the interaction between the two variables, a p-value of 0.143 was reported as the lowest p-value for the interaction (CRP change and stage). The joint effects of CRP and smoking history on PFS were also analyzed based on the observed elevated pre- and post-RT CRP levels in patients with smoking history and progression. More importantly, smoking may impact CRP levels and/or breast cancer survival [30–32]. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA) and R 4.1.1 (R Core Team, Vienna, Austria).

Results

CRP levels and distributions by disease progression status and patient/clinical characteristics

The study population consisted of 469 breast cancer patients, 386 of whom were progression-free by the last follow-up and 83 of whom experienced disease progression (21 deaths, 14 recurrences, 35 metastases, 12 second primaries, 1 with both second primary and death) (Table 1). 78% of the patients self-identified as white and 22% as Black/AA; 32% identified as non-Hispanic, while 68% identified as Hispanic. Details on other patient/clinical characteristics are shown in Table 1. Post-RT CRP was significantly higher than pre-RT CRP in patients with progression (mean±SD: 12.2±15.4 vs. 7.2±9.2, p=0.001). Post-RT CRP and RT-induced CRP changes were significantly higher in patients with disease progression compared to progression-free patients (post-RT CRP mean±SD: 12.2±15.4 vs 7.3±11.5, p=0.011; CRP change mean±SD: 5.3±13.1 vs 0.8±11.3, p=0.003).

Table 1. CRP levels and change by disease progression status and patient/clinical variables

Variable	Total		Progression-Free							Disease Progression					
	N	%	N	Pre-RT CRP		Post-RT CRP		CRP Change		p*	N	Pre-RT CRP		Post-RT CRP		CRP Change	
				Mean	SD	Mean	SD	Mean	SD			Mean	SD	Mean	SD	Mean	SD
Total	469	100	386	6.6	9.4	7.3	11.5	0.8	11.3	0.186	83	7.2	9.2	12.2	15.4	5.3	13.1
Race																	
White	367	78	306	6.3	9.2	6.7	11.4	0.7	11.2	0.302	61	7.5	9.2	12.5	15.4	5.5	12.4
Black/African American	102	22	80	7.9	9.7	9.6	11.8	1.3	11.8	0.383	22	6.3	9.3	11.0	15.7	4.6	15.3
Ethnicity																	
Non-Hispanic	152	32	128	7.1	10.6	7.2	11.9	0.1	12.8	0.921	24	6.2	8.9	12.6	16.5	6.5	15.9
Hispanic	317	68	258	6.4	8.7	7.3	11.4	1.1	10.6	0.103	59	7.6	9.4	12.0	15.1	4.8	12.0
Age at Diagnosis																	
<60	328	70	278	6.4	9.4	6.5	9.9	0.3	10.0	0.668	50	7.1	9.3	11.9	14.8	4.6	9.7
≥60	141	30	108	7.3	9.2	9.2	14.8	2.1	14.0	0.132	33	7.3	9.2	12.6	16.5	6.2	16.9
Smoking History																	
Never	310	66	260	6.2	8.8	7.1	11.1	0.9	11.0	0.222	50	5.5	6.3	9.2	12.3	3.6	12.4
Current/Former	159	34	126	7.6	10.3	7.7	12.5	0.7	12.1	0.569	33	9.6	12.1	16.2	18.4	7.5	13.8
Triple-Negative																	
No	392	84	327	6.5	9.1	7.1	11.0	0.7	10.5	0.283	65	7.2	9.2	12.5	16.0	5.5	11.8
Yes	77	16	59	7.4	10.5	8.3	14.3	1.7	15.4	0.441	18	7.3	9.6	11.1	13.5	4.3	17.6
Tumor Stage																	
0-II	378	81	330	6.3	9.1	6.3	9.3	0.1	9.2	0.923	48	6.7	9.2	9.9	14.1	3.9	11.6
III	91	19	56	8.3	10.8	13.2	19.2	5.3	19.3	0.060	35	7.8	9.4	15.1	16.7	7.0	14.7

p*: p-value from paired samples t test (comparing post- and pre-RT CRP); p**: p-value from independent samples t test (comparing CRP in disease progressio
SD: standard deviation

Among patients with disease progression, post-RT CRP levels were significantly higher than pre-RT CRP in those who were white (p=0.002), Hispanic (p=0.005), <60 years (p=0.004), had a history of smoking (p=0.005), non-triple-negative breast cancer (p=0.001), stage 0-II (p=0.039), and stage III (p=0.011). Significantly higher post-RT CRP levels in patients with progression than their progression-free counterparts were observed in those who were white (p=0.009), Hispanic (p=0.035), <60 years (p=0.026), had a history of smoking (p=0.019), and non-triple-negative breast cancer (p=0.017). In addition, patients with progression who were white (p=0.005), Hispanic (p=0.025), <60 years (p=0.010), had a history of smoking (p=0.008), non-triple-negative breast cancer (p=0.002), and stage 0-II (p=0.049) had significantly higher change in CRP than their progression-free counterparts.

Using 5.1 mg/L as the cut-off, a significantly higher percentage of stage III patients had higher pre-RT CRP levels compared to patients who were stage 0-II (49% vs. 36%, p=0.020) (Table 2). A significantly higher percentage of Black/AA patients had elevated post-RT CRP levels compared to white patients (58% vs. 41%, p=0.004), and a higher percentage of tumor stage III patients had elevated post-RT CRP levels compared to tumor stage 0-II patients (63% vs. 40%, p=0.002). A higher percentage of stage III patients also had CRP change ≥2.3 mg/L compared to stage 0-II patients (50% vs. 15%, p<0.001).

Table 2. Distributions of binary CRP variables (low vs high) by patient/clinical characteristics

Variable	Total		Pre-RT CRP (n=461)					Post-RT CRP (n=427)					Change in CRP (n=419)				
			<5.1 mg/L		≥5.1 mg/L		p*	<5.1 mg/L		≥5.1 mg/L		p*	<2.3 mg/L		≥2.3 mg/L		p*
	N	%	N	%	N	%		N	%	N	%		N	%	N	%	
Total	469	100	283	61	178	39		238	56	189	44		326	78	93	22	
Race							0.115					**0.004**					0.133
White	367	78	229	63	133	37		201	59	138	41		265	79	69	21	
Black/African American	102	22	54	55	45	46		37	42	51	58		61	72	24	28	
Ethnicity							0.961					0.874					0.638
Non-Hispanic	152	32	90	61	57	39		76	56	59	44		103	79	27	21	
Hispanic	317	68	193	62	121	39		162	56	130	45		223	77	66	23	
Age at Diagnosis							0.149					0.248					0.547
<60	328	70	204	64	117	36		171	58	126	42		228	79	62	21	
≥60	141	30	79	56	61	44		67	52	63	49		98	76	31	24	
Smoking History							0.099					0.866					0.747
Never	310	66	196	64	110	36		158	56	124	44		215	77	63	23	
Current/Former	159	34	87	56	68	44		80	55	65	45		111	79	30	21	
Triple-Negative							0.929					0.630					0.404
No	392	84	236	61	149	39		203	56	158	44		278	79	76	22	
Yes	77	16	47	62	29	38		35	53	31	47		48	74	17	26	
Tumor Stage							**0.020**					**<0.001**					**<0.001**
0-II	378	81	238	64	134	36		207	60	136	40		285	85	52	15	
III	91	19	45	51	44	49		31	37	53	63		41	50	41	50	

p*: p-value from Chi-square or Fisher's exact test

Association between CRP, patient/clinical characteristics, and progression-free survival

The median follow-up times for disease progression and progression-free patients were 3.3 years (range: 0.3 to 11.1 years) and 7.4 years (range: 0.8 to 13.0 years), respectively. Considering all patients, median PFS was not reached, and the 5-year PFS rate was 90% (95% CI: 86-92%). Figure 1 shows PFS by pre-RT CRP (A-C), post-RT CRP (D-F), and CRP change (G-I) in all patients (A, D, and G), tumor stage 0-II patients (B, E, and H), and tumor stage III patients (C, F, and I). As shown in panels A-C, PFS did not differ by pre-RT CRP in any of the three tumor stage groups. As shown in panels D-F, PFS significantly differs by post-RT CRP in all stages (p<0.001), tumor stage 0-II (p=0.049), tumor stage III patients (0.012). Finally, as shown in panels G-I, PFS differs by CRP change in all stages (p<0.001), tumor stage 0-II (p=0.040), and tumor stage III patients (p=0.006).

As shown in Table 3, in univariable analysis, there was significantly worse PFS in patients with post-RT CRP ≥5.1 mg/L (HR: 2.67, 95% CI: 1.65-4.30), RT-induced CRP change ≥2.3 mg/L (HR: 3.55, 95% CI: 2.55-5.64), age ≥60 years at diagnosis (HR: 1.66, 95% CI: 1.07-2.57), and tumor stage III (HR: 3.92, 95% CI: 2.53-6.08) (Table 3). In stratified analysis, worse PFS in tumor stage 0-II patients was associated with elevated CRP change (HR: 2.04, 95% CI: 1.02-4.07) and identifying as Black/AA (HR: 1.91, 95% CI: 1.05-3.48). Worse PFS in tumor stage III patients was associated with elevated post-RT CRP (HR: 2.97, 95% CI: 1.22-7.23) and CRP change (HR: 2.80, 95% CI: 1.29-6.06).

Table 3. Univariable Cox regression analysis of potential factors associated with PFS by tumor stage

Variable	All Stages				Stage 0-II				Stage III			
	PF*	PD**	HR (95% CI)	p***	PF*	PD**	HR (95% CI)	p***	PF*	PD**	HR (95% CI)	p***
Pre-RT CRP												
<5.1 mg/L	239	44	Ref		212	26	Ref		27	18	Ref	
≥5.1 mg/L	140	38	1.53 (0.99-2.36)	0.057	113	21	1.64 (0.92-2.92)	0.092	27	17	0.92 (0.47-1.79)	0.804
Post-RT CRP												
<5.1 mg/L	212	26	Ref		187	20	Ref		25	6	Ref	
≥5.1 mg/L	141	48	**2.67 (1.65-4.30)**	**<0.001**	114	22	1.82 (1.00-3.34)	0.052	27	26	**2.97 (1.22-7.23)**	**0.017**
Change in CRP												
<2.3 mg/L	287	39	Ref		255	30	Ref		32	9	Ref	
≥2.3 mg/L	59	34	**3.55 (2.25-5.64)**	**<0.001**	41	11	**2.04 (1.02-4.07)**	**0.044**	18	23	**2.80 (1.29-6.06)**	**0.009**
Race												
White	306	61	Ref		262	32	Ref		44	29	Ref	
Black/African American	80	22	1.37 (0.84-2.22)	0.210	68	16	**1.91 (1.05-3.48)**	**0.035**	12	6	0.80 (0.33-1.93)	0.621
Ethnicity												
Non-Hispanic	128	24	Ref		112	16	Ref		16	8	Ref	
Hispanic	258	59	1.20 (0.75-1.93)	0.453	218	32	1.06 (0.58-1.93)	0.858	40	27	1.28 (0.58-2.82)	0.541
Age at Diagnosis												
<60	278	50	Ref		233	28	Ref		45	22	Ref	
≥60	108	33	**1.66 (1.07-2.57)**	**0.025**	97	20	1.72 (0.97-3.05)	0.065	11	13	1.85 (0.93-3.67)	0.079
Smoking History												
Never	260	50	Ref		223	26	Ref		37	24	Ref	
Current/Former	126	33	1.32 (0.85-2.04)	0.220	107	22	1.66 (0.04-2.93)	0.081	19	11	0.97 (0.48-1.99)	0.942
Triple-Negative												
No	327	65	Ref		283	43	Ref		44	22	Ref	
Yes	59	18	1.68 (1.00-2.83)	0.052	47	5	0.82 (0.33-2.08)	0.680	12	13	1.97 (0.99-3.91)	0.054
Tumor Stage												
0-II	330	48	Ref									
III	56	35	**3.92 (2.53-6.08)**	**<0.001**								

PF*: progression-free; P**: progressive disease; p***: p-value

Multivariable Cox regression models for PFS including each of the three binary CRP variables (pre-RT, post-RT, and RT-induced CRP change) were obtained considering all patients and separately in early and advanced tumor stage patients (Table 4). For all patients (A), worse PFS remained significantly associated with elevated post-RT CRP (HR: 2.10, 95% CI: 1.29-3.42) and elevated CRP change (HR: 2.38, 95% CI: 1.45-3.92). None of the CRP variables was significantly associated with PFS in the tumor stage 0-II models (B) at the 5% significance level. In tumor stage III patients (C), elevated post-RT CRP (HR: 2.93, 95% CI: 1.20-7.18) and CRP change (HR: 2.41: 95% CI: 1.09-5.33) are significantly associated with worse PFS.

Table 4. Multivariable Cox regression analysis of potential factors associated with PFS by tumor stage

(A) All Tumor Stages			(B) Tumor Stage 0-II			(C) Tumor Stage III		
Variable	**HR (95% CI)**	**p**	**Variable**	**HR (95% CI)**	**p**	**Variable**	**HR (95% CI)**	**p**
Pre-RT CRP*			**Pre-RT CRP****			**Pre-RT CRP*****		
<5.1 mg/L	Ref		<5.1 mg/L	Ref		<5.1 mg/L	Ref	
≥5.1 mg/L	1.22 (0.78-1.90)	0.386	≥5.1 mg/L	1.36 (0.76-2.46)	0.302	≥5.1 mg/L	1.07 (0.54-2.10)	0.856
Post-RT CRP*			**Post-RT CRP****			**Post-RT CRP*****		
<5.1 mg/L	Ref		<5.1 mg/L	Ref		<5.1 mg/L	Ref	
≥5.1 mg/L	**2.10 (1.29-3.42)**	**0.003**	≥5.1 mg/L	1.51 (0.81-2.81)	0.194	≥5.1 mg/L	**2.93 (1.20-7.18)**	**0.019**
CRP Change*			**CRP Change****			**CRP Change*****		
<2.3 mg/L	Ref		<2.3 mg/L	Ref		<2.3 mg/L	Ref	
≥2.3 mg/L	**2.38 (1.45-3.92)**	**0.001**	≥2.3 mg/L	1.76 (0.87-3.58)	0.118	≥2.3 mg/L	**2.41 (1.09-5.33)**	**0.030**

*Covariates for all tumor stage models (A): race, ethnicity, age at diagnosis, smoking history, triple-negative status, tumor stage

**Covariates for tumor stage 0-II models (B): race, ethnicity, age at diagnosis, smoking history, triple-negative status

***Covariates for tumor stage III models (C): age at diagnosis, triple-negative status

Table 5 presents the results of Cox regression models of the joint effects of smoking history and (A) pre-RT CRP, (B) post-RT CRP, and (C) CRP change on PFS. The joint effects of elevated CRP or CRP change and smoking history were significantly associated with worse PFS in all models except for the pre-RT CRP and post-RT CRP models in stage III patients. Elevated CRP change combined with smoking history had the strongest joint effect on PFS in all patients (HR: 6.13, 95% CI: 3.19-11.76).

Table 5. The joint effects of CRP and smoking history on PFS by tumor stage

		All Stages					Stage 0-II					Stage III				
CRP	Smoking History	Total	PF*	PD**	HR (95% CI)	p***	Total	PF*	PD**	HR (95% CI)	p***	Total	PF*	PD**	HR (95% CI)	p***
Pre-RT																
<5.1 mg/L	Never	196	168	28	Ref		163	148	15	Ref		33	20	13	Ref	
<5.1 mg/L	Current/Former	87	71	16	1.36 (0.74-2.52)	0.323	75	64	11	1.64 (0.76-3.58)	0.210	12	7	5	1.40 (0.50-3.93)	0.522
≥5.1 mg/L	Never	110	89	21	1.55 (0.88-2.73)	0.131	83	73	10	1.54 (0.69-3.42)	0.293	27	16	11	1.11 (0.50-2.48)	0.799
≥5.1 mg/L	Current/Former	68	51	17	**1.90 (1.04-3.48)**	**0.037**	51	40	11	**2.64 (1.21-5.75)**	**0.015**	17	11	6	0.84 (0.32-2.22)	0.731
Post-RT																
<5.1 mg/L	Never	158	141	17	Ref		138	126	12	Ref		20	15	5	Ref	
<5.1 mg/L	Current/Former	80	71	9	1.04 (0.47-2.34)	0.920	69	61	8	1.32 (0.54-3.23)	0.544	11	10	1	0.33 (0.04-2.83)	0.312
≥5.1 mg/L	Never	124	98	26	**2.22 (1.20-4.09)**	**0.011**	89	79	10	1.41 (0.61-3.26)	0.425	35	19	16	2.01 (0.74-5.51)	0.174
≥5.1 mg/L	Current/Former	65	43	22	**3.66 (1.94-6.89)**	**<0.001**	47	35	12	**3.16 (1.42-7.04)**	**0.005**	18	8	10	2.65 (0.90-7.76)	0.076
Change																
<2.3 mg/L	Never	215	192	23	Ref		190	173	17	Ref		25	19	6	Ref	
<2.3 mg/L	Current/Former	111	95	16	1.30 (0.69-2.45)	0.426	95	82	13	1.44 (0.70-2.97)	0.322	16	13	3	0.68 (0.17-2.71)	0.580
≥2.3 mg/L	Never	63	44	19	**3.06 (1.66-5.62)**	**<0.001**	34	30	4	1.20 (0.40-3.59)	0.740	29	14	15	2.11 (0.82-5.44)	0.124
≥2.3 mg/L	Current/Former	30	15	15	**6.13 (3.19-11.76)**	**<0.001**	18	11	7	**5.06 (2.10-12.23)**	**<0.001**	12	4	8	**3.32 (1.15-9.57)**	**0.027**

*PF: progression-free; **PD: progressive disease; ***p: p value

Discussion

In the current study, we evaluated whether an inflammatory biomarker, CRP at pre-RT, post-RT, or RT-induced change was associated with breast cancer PFS. To the best of our knowledge, this is the first study with mainly minority (86.4%) breast cancer patients to date reporting a significant association between PFS and post-RT CRP and RT-induced change in CRP. Intriguingly, our results also showed strong joint effects between CRP and smoking history on breast cancer RFS.

The CRP level in normal human serum ranges from 0.2 to 10 mg/L; healthy individuals have CRP levels < 3 mg/L and less than 5% of the general population have levels ≥ 10 mg/L [33, 34]. As shown in Table 2, 39% and 44% of patients had pre-RT and post-RT CRP ≥ the optimal cutoff value of 5.1 mg/L, respectively. The high CRP levels may reflect cancer status, transient inflammation, infection, tissue damage, or other acute phase response. We also observed that a higher proportion of Black/AA patients had CRP ≥5.1 mg/L at post-RT (58%). This is consistent with the previous findings that higher CRP levels were reported in Black/AA patients compared to whites, Chinese, or Japanese [35, 36]. Multiple genetic and environmental factors may contribute to racial/ethnic differences in CRP levels. Serum CRP concentrations may be positively associated with sugar intake and negatively associated with dietary intakes of minerals, vitamins, and fruit and vegetables [37, 38]. Therefore, CRP concentrations may be modulated by dietary modification as a promising intervention strategy.

Determining which subset of patients may have worse post-RT PFS is an important clinical question in breast cancer care. Previous studies showed that breast cancer patients with higher levels of baseline CRP had lower mean survival times [39, 40]. Other studies reported that increased CRP was associated with a higher risk for worse disease-free and overall survival among patients with breast or other cancers [16, 41–46]. However, previous studies investigated

levels of CRP before RT or other treatments. Our present study introduced new data that post-RT CRP levels and change in CRP by RT may contribute to worse breast cancer PFS. Immune and inflammatory cells interact with malignant cells in tumor microenvironments to promote tumor growth, ultimately leading to invasion and metastasis [47–50]. Inflammation has also been linked to pain, fatigue, and other negative conditions in breast cancer patients after treatment, which suggests a shared etiology among various post-treatment cancer outcomes [22, 51, 52]. Moreover, continued exposure to RT may exacerbate inflammatory responses to the initial radiation-induced injury [53, 54]. Our current results suggest that elevated post-RT CRP levels may contribute to worse breast cancer PFS. Furthermore, our data suggest that an RT-induced hyper-inflammatory response, as measured by an elevated change in CRP, may contribute to worse breast cancer PFS, particularly in patients with a smoking history.

Considering tumor stage is the most important factor for survival, we also stratified Cox models by tumor stage to demonstrate differential effects of pre-RT, post-RT, and RT-induced CRP change on PFS in tumor stage 0-II and III patients. Previous studies have reported high levels of pre-operative and post-operative serum CRP as prognostic markers of cancer-specific and recurrence-free survival in early-stage patients with breast, gastric, and colorectal cancer [26, 55–57]. In our stratified analyses, post-RT CRP and CRP change were significantly associated with worse PFS in all stages and tumor stage 0-II patients. Preoperative CRP was associated with worse overall survival in patients with tumor stage IV colorectal cancer and metastatic breast cancer [40, 58]. However, our data only showed a significant association between post-RT CRP and CRP change and worse PFS in tumor stage III breast cancer patients.

In a previous study, pre-operative CRP was used to predict recurrence-free survival in patients with invasive breast cancer. Using the cut-off value of 12 mg/L, elevated CRP was significantly associated with poorer recurrence-free survival [59]. Researchers have also explored different cut-offs for CRP values in building more effective prediction models of cancer outcomes [43, 60, 61]. Therefore, we used Contal and O'Quigley's re-scaled rank statistic method to determine the optimal cut-off values of pre-RT, post-RT, and RT-induced change in CRP [27, 28]. Using the cut-off of 5.1 mg/L for pre- and post-RT CRP and 2.3 mg/L for change in CRP, we showed a strong association between post-RT CRP and RT-induced CRP change and worse PFS in all stages and tumor stage III patients but not tumor stage 0-II patients.

Previous studies reported that cigarette smoking exacerbated inflammation, increased circulating CRP levels, and increased risk of death compared to patients who had never smoked [30–32]. We demonstrate that among patients who had a history of smoking and experienced disease progression, CRP was significantly higher at post-RT than pre-RT. Intriguingly, breast cancer patients with a smoking history who experienced progression had over 2-fold higher post-RT CRP levels compared to patients who did not have progression (mean±SD: 16.2±18.4 vs. 7.7±12.5). Our data suggest that breast cancer patients with a smoking history may develop hyper-inflammatory responses to RT that may contribute to worse PFS. Future studies are warranted to evaluate the potential mechanisms that genomic predisposition and epigenomic changes by tobacco smoking may play critical roles in such hyper-inflammatory responses. Our results suggest that RT may contribute to worse PFS in patients with elevated CRP and smoking history (Table 5). The clinical implication is that breast cancer patients with elevated CRP and smoking history should be placed under more rigorous surveillance during follow-up.

Radiation-induced changes in the inflammatory microenvironment leading to increased cellular plasticity were also observed after surgery [62–64]. Inflammation and reactive oxygen species (ROS) are two inter-related molecular mechanisms that contribute to aging- and radiation-related biological effects on carcinogenesis and PFS of cancer survivors. In a long-term follow-up study of atomic bomb survivors, serum CRP levels increased significantly with increasing age and radiation dose [65]. They also showed that intracellular ROS levels in blood cells increased due to aging 60 years after radiation exposure, particularly in individuals with high serum CRP levels [65]. Their results suggest that radiation exposure may lead to mitochondrial oxidative metabolism in the tissue-damaging response and contribute to inflammation. Although ROS plays an important role in immune responses, excessive radiation-related ROS production and accumulation might enhance the risk of inflammation-related diseases. Our study findings highlight the need for a more comprehensive assessment of the late effects in breast cancer survivors and support the application of inflammatory biomarkers as a tool to identify high-risk patients for more rigorous follow-up surveillance and targeted interventions, such as antioxidants and anti-inflammatory agents. For example, clinical RT-protective applications, as well as mitigation of radiation injury in a possible radiation disaster, have focused on the potential radioprotective effects of melatonin on several signaling pathways, such as inflammatory responses, antioxidant defense, DNA repair response enzymes, and pro-oxidant enzymes [66].

This study has several strengths. First, we used a prospective study design to collect plasma CRP data at both pre- and post-RT to assess RT-induced changes of CRP in PFS, while previous studies used one measurement of CRP before the initiation of any treatment. This allowed us to further evaluate the effect of adjuvant RT on CRP at the individual level. Second, because our patients returned to the same clinic for follow-up appointments, we were able to collect information for up to 13 years after diagnosis using electronic medical records. Third, our study has focused on minority health with a very high proportion of racial/ethnic minorities (22% Black/AA and 68% Hispanic patients). However, this study also has a few limitations. First, our patient variables were self-reported and collected at one time-point, before RT. Therefore, we were unable to assess the effect of changes in health status on progression-free survival. Second, we decided to not analyze the self-reported medication usage at study entry that may influence the level of CRP, such as anti-inflammatory agents, due to the amount of missing data. Finally, our multivariable models include a limited number of clinically relevant patient/clinical variables to avoid overfitting due to the relatively small number of patients with disease progression [67]. Therefore, our multivariable results must be interpreted with these limitations in mind. Given the ease and low cost of hsCRP assays, future larger studies are warranted to validate these results and to assess the role of CRP in conjunction with other biomarkers, additional patient/clinical variables, and treatment characteristics in predicting PFS outcomes among breast cancer patients.

Conclusions

In summary, our study results showed that elevated post-RT and change in CRP are significantly associated with worse breast cancer PRS. Our findings have several clinical implications. First, elevated plasma 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 elevated post-RT CRP levels should be actively monitored for breast cancer recurrence, metastasis, and other medical conditions that may impact overall survival. Second, considering the involvement of CRP in pain, fatigue, and

prognosis of breast cancer patients, our future follow-up study should also focus on monitoring CRP levels in quality of life, continued PFS, and targeted interventions.

Abbreviations

AA: African American; CI: confidence interval; CRP: C-reactive protein; HR: hazard ratio; hsCRP: high-sensitivity CRP; HW: Hispanic white; NHW: non-Hispanic white; ROS: reactive oxygen species; RT: radiotherapy; SD: standard deviation

Declarations

Ethics approval and consent to participate

All women who participated in the study provided written informed consent. The study was approved by the Institutional Review Boards of the University of Miami and the Jackson Memorial Hospital.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GRY, IMR, and JJH designed the study. CT and JLW were in charge of radiotherapy, patient enrollment, and clinical outcome assessment. EAS, EL, OLN, JLW, and JJH collected the laboratory and questionnaire data. GRY, LGA, and IMR helped with data curation and conducted the statistical data analysis. GRY, IMR, CT, JM, and JJH interpreted results. GRY and LGA drafted the manuscript. All authors read and approved the final manuscript.

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Figures

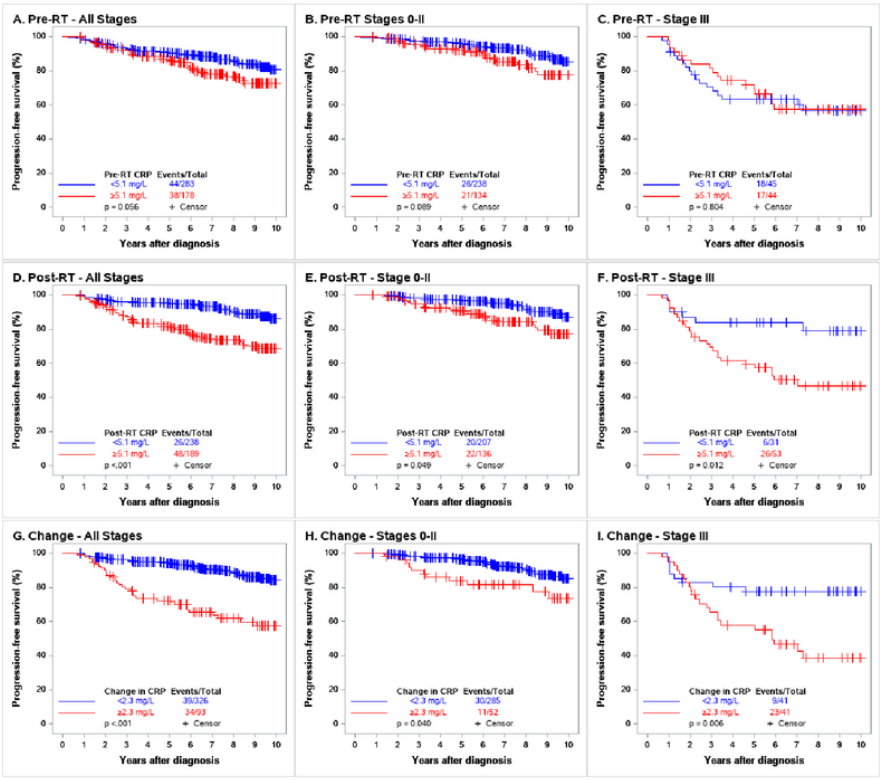

Figure 1

Progression-free survival curves by pre-RT CRP in (A) all tumor stages, (B) stage 0-II, and (C) stage III; post-CRP in (D) all stages, (E) stage 0-II, and (F) stage III; RT-induced CRP change in (G) all stages, (H) stage 0-II, and (I) stage III.