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Research Article

Postdiagnosis C-Reactive Protein and Breast Cancer Survivorship: Findings from the WHEL Study

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Abstract

Background: Serum C-reactive protein (CRP) is a marker of acute inflammatory response and has been associated with health outcomes in some studies. Inflammation and immune response may have potential prognostic implications for breast cancer survivors.

Methods: The Women's Healthy Eating and Living Study includes 2,919 early-stage breast cancer survivors with serum collected 2 years postdiagnosis and follow-up for clinical outcomes over approximately 7 years. CRP concentrations were measured using high-sensitivity electrochemiluminescence assay. Outcomes, including all-cause mortality, breast cancer–specific mortality, and additional breast cancer events were oncologist verified from medical records and death certificates. Cox proportional hazards models were conducted with adjustment for potential confounding factors to generate HRs and 95% confidence intervals (CI).

Results: CRP concentrations in women diagnosed with breast cancer were associated with death due to any cause, death due to breast cancer, and additional breast cancer events, after adjustment for sociodemographic and cancer characteristics (lnCRP: $P < 0.05$ for all three outcomes). The HR for women with (vs. without) acute inflammation suggests a threshold effect on overall survival, rather than a dose–response relationship ($\geq$10.0 mg/L vs. <1 mg/L: HR, 1.96; 95% CI, 1.22–3.13). Associations were similar for breast cancer–specific mortality (HR, 1.91; 95% CI, 1.13–3.23) and any additional breast cancer–related event (HR, 1.69; 95% CI, 1.17–2.43).

Conclusions: Acute inflammation status (CRP $\geq$ 10 mg/L) may be an important independent biomarker for long-term survival in breast cancer survivors.

Impact: Interventions to decrease circulating CRP concentrations in breast cancer survivors with acute inflammation may improve prognosis. *Cancer Epidemiol Biomarkers Prev; 23(1); 189–99. ©2013 AACR.*

Introduction

An estimated 2.9 million women in the United States live with a history of breast cancer and these women are susceptible to various comorbidities, including disease recurrence or new primary cancer (1, 2). Evidence from both laboratory and epidemiologic data suggest chronic inflammation facilitates tumor growth and metastasis through the modification of tumor cell biology, activation of stromal cells in the tumor microenvironment, cancer cell invasion by the conditioning of vasculature to enhance the extravasation, engraftment and growth of micrometastases, or reactivate dormant tumors at distant sites (3–6). Cancer survivors with persistent inflammation may have an elevated risk of recurrence or new primary as a result of the effects of inflammatory processes or the presence of cancer cells that induce inflammation (7). Thus, understanding the association between postdiagnosis inflammation and prognosis is a high research priority.

A number of studies have examined inflammatory markers as predictors of disease recurrence and death in cancer populations (7–12). Although several biomarkers of inflammation have been examined, including white blood cells, fibrinogen, interleukin-6, TNF-α, serum amyloid A, and C-reactive protein (CRP); the most consistent association with prognosis has been with CRP (2, 12–15). CRP is a nonspecific, acute-phase protein produced by the liver in response to inflammation, infection, and tissue damage (6). As one of the most sensitive acute-phase reactants, circulating concentrations of CRP increases rapidly in response to numerous pathologic and disease conditions (16). Although the acute-phase response is not diagnostic for any particular disease, elevated concentrations of CRP, conventionally defined as circulating concentrations of CRP $\geq$3 mg/L, have proved useful in the clinical setting to monitor infections and postoperative complications, to assess the effectiveness of treatments on the course of a disease, and to estimate risk of future cardiovascular events (17). With improved measurement techniques, specifically high-sensitivity assays, researchers can detect minute concentrations of circulating CRP (minimum detectable limit

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0.02 mg/mL). Advancements in measurement of CRP, combined with knowledge of the role that inflammation plays in cancer development and progression have led to a growing interest in determining the clinical utility of CRP for predicting recurrence risk, for monitoring risk reduction, and for guiding preventive approaches in women previously diagnosed with breast cancer.

Longitudinal studies in women diagnosed with breast cancer have reported conflicting results in relation to inflammation and prognosis, with some studies showing an association between elevated CRP and poor prognosis (18–22) and others showing no relationship (23–25). In 2011, a meta-analysis was conducted using 10 studies ($n = 4{,}502$) in women diagnosed with breast cancer. Han and colleagues reported a pooled HR for overall survival at 1.62 [95% confidence interval (CI), 1.20–2.18] and a higher pooled HR for cancer-specific survival (HR, 2.08; 95% CI, 1.48–2.94; ref. 26) for elevated CRP measured across the continuum of the breast cancer experience from pre- to postdiagnosis (26). It is plausible that the variation in the CRP–survival relationship seen in previous reports could be partially attributed to different cut points. Thus, it is unclear whether clinically relatable cut points for CRP values are associated with breast cancer prognosis among women diagnosed with breast cancer, specifically categories used for cardiovascular risk prediction: low risk (<1.0 mg/L), moderate risk (1.0–3.0 mg/L), high risk (>3–10 mg/L), and values indicating acute infection (≥10 mg/L; ref. 17).

Here, we report on the prevalence of inflammatory status, using posttreatment serum concentrations of CRP measured on average 24 months postdiagnosis, and the association with all-cause and breast cancer–specific mortality and additional breast cancer events in a cohort of 2,919 women following a diagnosis of invasive breast cancer (stage I–IIIA, American Joint Committee on Cancer IV classification). We also examine the influence of individual and breast cancer clinical characteristics on inflammatory status.

Materials and Methods

Design overview, participants, and methods

The Women's Healthy Eating and Living (WHEL) study was a randomized controlled trial aimed at examining the effects of a high-vegetable, low-fat diet in reducing additional breast cancer events and early death in women diagnosed with breast cancer. Study details and the intervention have been described (27). In brief, between 1995 and 2000, 3,088 women were enrolled and followed through 2006. Participants were enrolled an average of 2 years postdiagnosis, were diagnosed with stage I to III invasive breast cancer, and had completed active treatment. Participants were 27 to 74 years of age and had no evidence of disease within 12 months of study enrollment. The study was performed with the approval of the Institutional Review Boards of the University of California, San Diego and 6 other participating centers. All participants provided informed written consent.

At baseline, the mean age of participants was 53 years, 85.5% were non-Hispanic white, 84.9% had had stage I or II breast cancer, 56.5% had well or moderately differentiated tumors, 77.8% had estrogen receptor-positive (ER^+) and/or progesterone receptor-positive (PR^+) tumors, 61.6% had received radiation therapy, 69.3% had received adjuvant chemotherapy, and 61.5% reported taking antiestrogen medication at study entry. Women included in this study were followed semiannually and had a median follow-up of 7.4 years from the time of study enrollment.

There was no intervention effect on additional breast cancer events or mortality during the 7.4-year follow-up period (28). Accordingly, we treated the WHEL study as a cohort study.

Serum CRP assay

Using stored fasting serum specimens drawn a mean 23.6 months postdiagnosis (median: 21.7 months; range: 2–48 months), we measured serum concentrations of CRP using a high-sensitivity electrochemiluminescence assay (MesoScale Discovery) at the Laboratory for Clinical Biochemistry Research, University of Vermont. The lower detection limit for this assay platform was 0.02 mg/L. For each assay, we included blinded duplicates for quality control assessment. The interassay coefficients of variation were between 7% and 12%.

Outcomes and follow-up

Women were followed for vital status from study entry until end of study, June 1, 2006. Information on outcomes, including all-cause and breast cancer–specific mortality and additional breast cancer events [defined as recurrence (85%) or new primary breast cancer (15%)], were obtained by self-report every 6 months throughout the study and confirmed by an oncologist using medical records and death certificates. Causes of death were coded using International Classification of Diseases, 9th Revision (ICD-9) codes. Approximately 4% of study participants were lost to follow-up and these were censored at date of last contact.

Anthropometrics

At the baseline clinical visit, each participant had height, weight, waist, and hip measurements conducted by trained staff using standardized measurement protocols (27). Body mass index (BMI, kg/m^2) was calculated from weight and height, measured to the nearest 0.1 kg and 0.1 cm, respectively, with a balance beam scale and stadiometer.

Other variables

Standardized questionnaire information was collected at baseline on medical history, demographic characteristics, and lifestyle factors. At enrollment, women were considered postmenopausal if they were amenorrheic for at least 12 months and premenopausal if they reported at

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least one menstrual cycle during the last 3 months. Women were classified as users of anti-estrogen medication (99.7% of which was selective ER modulators), if they reported current use at the baseline clinic visit. History of inflammatory-related conditions [e.g., cardiovascular disease (CVD), arthritis, and diabetes] was self-reported at baseline. Recreational physical activity at time of study entry was computed using a self-report validated scale designed for the Women's Health Initiative (29). Self-report of current and former smoking status was recorded and alcohol and dietary intake data were obtained via repeated 24-hour dietary recalls (described elsewhere in more detail; ref. 30). We converted physical activity into metabolic equivalent task hours/week (31), tobacco use was converted to pack-years and the multiple dietary recalls were used to characterize participant alcohol consumption (g/day), fruit and vegetable intake (servings/day), dietary fiber intake (g/day), and percentage energy from fat.

Exclusions

Among the 3,088 eligible women enrolled at baseline, serum samples were available for 3,023 participants. We excluded those with outcome events within 9 months of the baseline blood draw ($n = 85$) as well as those for whom CRP was not measured successfully ($n = 19$), resulting in a final sample size of 2,919 participants.

Statistical analysis

Our analytic goals were to describe the distribution of serum CRP in this cohort of breast cancer survivors and characterize the association of inflammatory status with all-cause mortality, breast cancer mortality, or additional breast cancer events. We modeled inflammatory status using two methods: the first classified serum CRP concentrations into four categories of inflammatory status based on CVD prediction cut points: no inflammation (<1 mg/L), low inflammation (1–3 mg/L), moderate inflammation (>3 to <10 mg/L), and acute inflammation (≥10 mg/L; ref. 17); the second treated CRP as a log-transformed continuous variable to reduce skewness of the distribution. For categorical analyses, the reference group was women with no inflammation. Trend tests, which used a single ordinal indicator variable based on inflammatory status (coded 1 for no inflammation to 4 for acute inflammation), were used to assess dosage.

We employed delayed-entry Cox proportional hazards models (with entry time at baseline blood draw) to estimate the age-adjusted and multivariable-adjusted HRs and 95% CIs for death due to any cause, breast cancer–specific death, and disease recurrence by inflammatory status (32). Time since diagnosis (years) was used as the time metric for all regression analysis. Variables were retained in the final model (model 1) if they were associated with elevated CRP status (>3 mg/L), were associated with overall mortality in women with no inflammation (CRP < 1 mg/L), and had altered the risk estimate of the model containing inflammatory status plus age by at least 10%. We further adjusted for factors that improved model fit (model 2), and/or allowed comparison to the published literature (model 3; refs. 16, 18, 19, 33). The three models were adjusted for age at diagnosis, time since diagnosis, race–ethnicity, and tumor stage and grade (model 1); model 1 covariates and BMI (model 2); model 2 covariates plus anti-estrogen medication use and ER/PR status (model 3). Additional variables considered for adjustment included intervention arm (intervention/control), menopausal status (pre- and perimenopausal/postmenopausal), treatment used (chemotherapy, radiation, or both), physical activity (MET-h/wk), and tobacco use (pack-year); however, adjusting for these covariates did not materially change the risk estimates for CRP (data not shown). We tested and confirmed nonviolation of the proportionality assumption based on a graphical approach (log(–log) plots; ref. 32) and the goodness-of-fit test using Schoenfeld residuals (34). Given the high proportion of deaths due to breast cancer in this cohort, we also considered whether the association between inflammation and breast cancer–specific death (79.7% of the deaths) varied according to subgroups defined by age (>55 years), postmenopausal status (yes/no), BMI (≥25 kg/m^2), tumor stage (≥stage II), tumor hormone receptor status (ER$^+$), and inflammatory-related illness, including CVD (yes/no), arthritis (yes/no), and diabetes (yes/no). Effect modification by these factors was tested by adding a product term for inflammatory status and each of the aforementioned covariates. Statistical tests were performed using Stata (version 11.1; StataCorp LP) and SAS (version 9.3) software. All tests were two-sided and statistical significance was set at $P < 0.05$.

Results

In this cohort of 2,919 women with a history of breast cancer, the distribution of serum concentrations of CRP was markedly skewed with 91.3% of survivors having concentrations less than 10 mg/L. The geometric mean for serum CRP was 1.71 mg/L (interquartile range, 0.67–4.24) and median was 3.83 ± 6.7 mg/L. Here, we summarize statistically significant participant and tumor characteristics associated with this biomarker of inflammation.

Serum CRP increased with age, BMI, and postmenopausal status and the distributions of CRP concentrations varied across racial–ethnic groups (Table 1). Asian women had the lowest average CRP concentration (0.91 mg/L), non-Hispanic black women had higher concentrations (3.38 mg/L), and non-Hispanic white and Hispanic women were within those bounds (1.67 and 2.13, respectively). Less healthful behaviors were associated with increased CRP, such as tobacco use, lack of physical activity, alcohol consumption, and poor dietary habits (e.g., <5 servings/day of fruits and vegetables, <20 g/day of dietary fiber intake, and ≥30% energy intake from fat). Women with more aggressive tumor characteristics, specifically tumor stage, and estrogen and PR negative tumors (ER$^-$/PR$^-$ vs. ER$^+$/PR$^+$) had higher CRP values (Table 2). Women using

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Table 1. Serum concentrations of CRP by participant characteristics in breast cancer survivors of the WHEL study

			Distribution of inflammatory status								
			No inflammation <1 mg/L		Low inflammation 1–3 mg/L		Moderate inflammation ≥3 to <10 mg/L		Acute inflammation ≥10 mg/L		
	n (%)	Serum CRP mg/L Geometric mean	No.	%	No.	%	No.	%	No.	%	*P*
Total	2,919	1.7	981	33.6	933	31.96	750	25.69	255	8.74	
Age at study entry, y											<0.001
20–29	6 (0.2)	0.40	5	0.5	1	0.1	0	0.0	0	0.0	
30–39	188 (6.4)	1.19	83	8.5	57	6.1	34	4.5	14	5.5	
40–49	872 (29.9)	1.57	331	33.7	244	26.2	219	29.2	78	30.6	
50–59	1,159 (39.7)	1.74	382	38.9	382	40.9	297	39.6	98	38.4	
60–69	610 (20.9)	2.04	162	16.5	218	23.4	172	22.9	58	22.7	
70+	84 (2.9)	2.32	18	1.8	31	3.3	28	3.7	7	2.7	
Race/ethnicity											<0.001
White, non-Hispanic	2,495 (85.5)	1.67	853	87.0	797	85.4	641	85.5	204	80.0	
Black, non-Hispanic	110 (3.8)	3.38	12	1.2	40	4.3	34	4.5	24	9.4	
Hispanic	154 (5.3)	2.13	44	4.5	50	5.4	47	6.3	13	5.1	
Asian	91 (3.1)	0.91	49	5.0	26	2.8	15	2.0	1	0.4	
Other	69 (2.4)	1.95	23	2.3	20	2.1	13	1.7	13	5.1	
BMI (kg/m^2)											<0.001
<18.5, underweight	27 (0.9)	0.48	21	2.1	3	0.3	2	0.3	1	0.4	
18.5–24.9, normal weight	1,222 (41.9)	0.83	699	71.3	363	38.9	134	17.9	26	10.2	
25–29.9, overweight	905 (31.0)	2.02	211	21.5	382	40.9	250	33.3	62	24.3	
30 to <35, obese class I	453 (15.5)	3.93	35	3.6	134	14.4	213	28.4	71	27.8	
>35 to <40, obese class II	200 (6.9)	4.77	13	1.3	43	4.6	103	13.7	41	16.1	
>40, obese class III	112 (3.8)	9.03	2	0.2	8	0.9	48	6.4	54	21.2	
Waist/hip ratio											<0.001
<0.80	1,525 (52.2)	1.11	720	73.4	472	50.6	263	35.1	69	27.1	
≥0.80	1,384 (47.4)	2.75	258	26.3	454	48.7	485	64.7	185	72.5	
Menopausal status											<0.001
Premenopausal	323 (11.1)	1.16	148	15.1	89	9.5	65	8.7	21	8.2	
Perimenopausal	272 (9.3)	1.37	114	11.6	77	8.3	62	8.3	19	7.5	
Postmenopausal	2,324 (79.6)	1.86	719	73.3	767	82.2	623	83.1	215	84.3	

(Continued on the following page)

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Table 1. Serum concentrations of CRP by participant characteristics in breast cancer survivors of the WHEL study (Cont'd)

			Distribution of inflammatory status								
			No inflammation <1 mg/L		Low inflammation 1–3 mg/L		Moderate inflammation >3 to <10 mg/L		Acute inflammation ≥10 mg/L		
	n (%)	Serum CRP mg/L Geometric mean	No.	%	No.	%	No.	%	No.	%	*P*
Lifestyle factors											
Tobacco use (pack-years)											0.001
None	1,558 (53.4)	1.64	540	55.0	496	53.2	390	52.0	132	51.8	
<20	967 (33.1)	1.65	337	34.4	312	33.4	247	32.9	71	27.8	
≥20	349 (12.0)	2.35	86	8.8	112	12.0	105	14.0	46	18.0	
Alcohol consumption, 10 g/d											<0.001
None	1,588 (54.4)	1.95	482	49.1	489	52.4	456	60.8	161	63.1	
<1	784 (26.9)	1.48	289	29.5	255	27.3	181	24.1	59	23.1	
≥1	540 (18.5)	1.44	209	21.3	187	20.0	109	14.5	35	13.7	
Fruit/vegetable, servings per day											<0.001
<5	1,133 (38.8)	1.99	329	33.5	357	38.3	326	43.5	121	47.5	
≥5	1,779 (61.0)	1.55	651	66.4	574	61.5	420	56.0	134	52.5	
Dietary fiber, g/d											<0.001
<20	1,485 (50.9)	2.02	419	42.7	483	51.8	430	57.3	153	60.0	
≥20	1,427 (48.9)	1.43	561	57.2	448	48.0	316	42.1	102	40.0	
% Energy from fat											<0.001
<30	1,674 (57.4)	1.45	652	66.5	525	56.3	383	51.1	114	44.7	
≥30	1,238 (42.4)	2.14	328	33.4	406	43.5	363	48.4	141	55.3	
Physical activity (MET-h/wk)											<0.001
Q1 (0–2)	572 (19.6)	2.75	109	11.1	167	17.9	217	28.9	79	31.0	
Q2 (2.25–7.2)	553 (18.9)	2.24	137	14.0	186	19.9	160	21.3	70	27.5	
Q3 (7.25–14)	572 (19.6)	1.65	205	20.9	185	19.8	139	18.5	46	18.0	
Q4 (14.1–24)	560 (19.2)	1.35	232	23.6	179	19.2	121	16.1	28	11.0	
Q5 (24.2–107)	565 (19.4)	1.07	263	26.8	187	20.0	90	12.0	22	8.6	

NOTE: *P* values were calculated using χ^2 tests for categorical variables.

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Table 2. Serum concentrations of CRP by tumor characteristics in breast cancer survivors of the WHEL study

			Distribution of inflammatory status								
		Serum CRP mg/L	No inflammation <1 mg/L		Low inflammation 1–3 mg/L		Moderate inflammation >3 to <10 mg/L		Acute inflammation ≥10 mg/L		
	n (%)	Geometric mean	No.	%	No.	%	No.	%	No.	%	*P*
Tumor stage											0.007
I	1,144 (39.2)	1.54 (0.58, 3.91)	418	42.6	360	38.6	277	36.9	89	34.9	
II	1,333 (45.7)	1.73 (0.71, 4.17)	527	53.7	538	57.7	424	56.5	150	58.8	
IIIA	442 (15.1)	2.2 (0.94, 5.55)	36	3.7	35	3.8	49	6.5	16	6.3	
Tumor grade											0.097
1, Well differentiated	463 (15.9)	1.51 (0.54, 4.23)	181	18.5	131	14.0	109	14.5	42	16.5	
2, Moderately differentiated	1,184 (40.6)	1.64 (0.66, 3.88)	397	40.5	395	42.3	302	40.3	90	35.3	
3, Poorly differentiated	1,027 (35.2)	1.92 (0.77, 4.87)	316	32.2	331	35.5	278	37.1	102	40.0	
Unspecified	245 (8.4)	1.66 (0.63, 4.04)	87	8.9	76	8.1	61	8.1	21	8.2	
Estrogen/PR status											0.003
ER^+/PR^+	1,818 (62.3)	1.60 (0.64, 4.02)	631	64.3	597	64.0	459	61.2	135	52.9	
ER^+/PR^-	331 (11.3)	1.53 (0.63, 3.83)	121	12.3	106	11.4	84	11.2	22	8.6	
ER^-/PR^+	121 (4.2)	1.73 (0.57, 4.35)	42	4.3	33	3.5	34	4.5	12	4.7	
ER^-/PR^-	582 (19.9)	2.20 (0.85, 5.50)	170	17.3	179	19.2	154	20.5	78	30.6	
Unknown	67 (2.3)	2.20 (0.91, 6.06)	17	1.7	18	1.9	19	2.5	8	3.1	
HER2 receptor status											0.605
Positive	360 (12.3)	1.72 (0.66, 4.22)	123	12.5	108	11.6	100	13.3	29	11.4	
Negative	1,672 (57.3)	1.74 (0.70,4.26)	542	55.2	543	58.2	438	58.4	149	58.4	
Unknown	887 (30.4)	1.66 (0.65, 4.17)	316	32.2	282	30.2	212	28.3	77	30.2	
Treatment (after surgery)											0.597
No chemotherapy or radiation	321 (11.0)	1.56 (0.58, 3.86)	112	11.4	106	11.4	74	9.9	29	11.4	
Radiation only	569 (19.5)	1.63 (0.66, 4.00)	196	20.0	193	20.7	140	18.7	40	15.7	
Chemotherapy only	794 (27.2)	1.76 (0.66, 4.17)	255	26.0	258	27.7	205	27.3	76	29.8	
Radiation and chemotherapy	1,230 (42.1)	1.76 (0.71, 4.56)	415	42.3	375	40.2	331	44.1	109	42.7	
Unknown	5 (0.17)	0.85 (0.25, 2.68)	3	0.3	1	0.1	0	0.0	1	0.4	
Current anti-estrogen use											<0.001
Yes	1,796 (61.5)	1.54 (0.63, 3.92)	640	65.2	586	62.8	449	59.9	121	47.5	
No	1,119 (38.3)	2.03 (0.80, 5.10)	341	34.8	343	36.8	301	40.1	134	52.5	
Unknown	4 (0.13)	1.75 (1.29, 2.37)	0	0.0	4	0.4	0	0.0	0	0.0	

NOTE: *P* values were calculated using χ^2 tests for categorical variables.

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Table 3. Risk of all-cause mortality, breast cancer–specific mortality, and additional breast cancer event by inflammatory status using serum CRP concentrations in breast cancer survivors in the WHEL study

Models of association by serum CRP	No. of events	*N*	Model 1[a]			Model 2[b]			Model 3[c]		
			HR	95% CI	P_{trend}	HR	95% CI	P_{trend}	HR	95% CI	P_{trend}
All-cause mortality											
Inflammatory status					<0.001			0.005			0.006
No inflammation, CRP <1 mg/L	61	981	1.00	Referent		1.00	Referent		1.00	Referent	
Low, CRP 1–3 mg/L	66	933	1.01	0.71–1.44		0.99	0.69–1.43		0.98	0.68–1.42	
Moderate, CRP >3 to <10 mg/L	73	750	1.41	0.99–2.00		1.32	0.89–1.96		1.30	0.88–1.93	
Acute, CRP ≥10 mg/L	36	255	2.12	1.38–3.23		1.96	1.22–3.13		1.92	1.20–3.08	
lnCRP	236	2,919	1.21	1.09–1.34		1.19	1.06–1.34		1.19	1.05–1.34	
Breast cancer mortality											
Inflammatory status					0.004			0.03			0.03
No inflammation	51	981	1.00	Referent		1.00	Referent		1.00	Referent	
Low Inflammation	56	933	1.10	0.75–1.62		1.07	0.71–1.59		1.07	0.72–1.59	
Moderate inflammation	53	750	1.33	0.90–1.97		1.22	0.78–1.90		1.22	0.78–1.91	
Acute inflammation	28	255	2.10	1.30–3.38		1.91	1.13–3.23		1.88	1.11–3.18	
lnCRP	188	2,919	1.18	1.06–1.33		1.16	1.02–1.32		1.16	1.01–1.31	
Additional breast cancer events											
Inflammatory status					0.01			0.02			0.03
No inflammation	126	981	1.00	Referent		1.00	Referent		1.00	Referent	
Low inflammation	126	933	1.06	0.82–1.36		1.08	0.82–1.40		1.06	0.82–1.38	
Moderate inflammation	111	750	1.12	0.86–1.45		1.14	0.85–1.53		1.12	0.83–1.50	
Acute inflammation	54	255	1.67	1.20–2.31		1.69	1.17–2.43		1.65	1.15–2.38	
lnCRP	417	2,919	1.12	1.04–1.21		1.14	1.04–1.24		1.13	1.03–1.24	

[a]Adjusted for age (continuous), time since diagnosis, disease stage, disease grade, and race–ethnicity.
[b]Adjusted for age (continuous), time since diagnosis, disease stage, disease grade, race–ethnicity, and BMI (categorical).
[c]Adjusted for age (continuous), time since diagnosis, disease stage, disease grade, race–ethnicity, BMI (categorical), anti-estrogen use, and ER/PR status.

anti-estrogen medication had lower CRP concentrations than women who did not (1.54 and 2.03 mg/L, respectively; Table 2).

A total of 236 women died, including 188 deaths due to breast cancer, and 417 women experienced an additional breast cancer event (recurrence [85%] or new breast cancer primary [15%]) during a median follow-up period of 7.4 years. Higher versus lower concentrations of CRP were associated with increased risk for all-cause mortality, breast cancer–specific mortality, and additional breast cancer events (Ptrend < 0.05 in all models; lnCRP, $P < 0.05$ in all models; Table 3). The 5-year unadjusted overall survival rates in women with acute, moderate, low, and no inflammation were 96.1%, 98.4%, 98.4%, and 97.8%, respectively (log rank $P <$ 0.001, data not shown). The comparable 10-year figures were 85.8%, 89.8%, 92.6%, and 93.0%, respectively (log rank $P = 0.002$, data not shown).

A one-unit increase in lnCRP, which corresponds to an approximately 2.7-fold increase in CRP level, was associated with a 21% increased risk of all-cause mortality (model 1) and an 18% increased risk of breast cancer–specific mortality (model 1). For example, a lnCRP increase from 0 to 1 translates to a serum CRP concentration increase from 1 to 2.7 mg/L and an associated HR for all-cause mortality of 1.21 and HR for breast cancer–specific mortality of 1.18 (Table 3); a lnCRP value of 2, equates to a serum CRP concentration of 7.4 mg/L with respective HRs of 1.46 and 1.39; and a lnCRP value of 3, is a serum CRP concentration >20 mg/L with HRs of 1.77 and 1.64, respectively, compared with CRP level of 1 mg/L. When CRP was modeled using four categories of inflammatory status, we saw a threshold effect. Compared with women with no inflammation (<1 mg/L), those with values in the acute range (≥10 mg/L) had an approximate 2-fold increased risk of mortality due to breast cancer and/or any cause. Women with moderate inflammation (>3 to <10 mg/L) had an increased risk of mortality due to any cause or breast cancer that ranged between 33% and 41%, although these associations were not statistically significant. Women with no inflammation and low inflammation (1–3 mg/L) had comparable risks for all-cause and breast cancer–specific mortality. Adjusting for BMI (model 2) plus anti-estrogen use and tumor hormone receptor status (model 3) did not meaningfully alter the CRP–survival relationship (Table 3).

Women with acute inflammation (vs. no inflammation) had a 67% increase risk of an additional breast cancer–

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Table 4. Subgroup analysis of elevated CRP (>10 mg/L) and risk of breast cancer–specific mortality in breast cancer survivors in the WHEL study

Stratified models of association by upper quartile of CRP	No. of events	*N*	Stratified multivariable-adjusted model		*P*[a]
			HR	95% CI	
Age, y					0.92
<55	115	1,936	1.42	0.81–2.50	
≥55	73	983	2.08	1.07–4.05	
Postmenopausal					0.74
No	38	595	0.86	0.26–2.66	
Yes	150	2,324	1.89	1.19–3.02	
BMI, kg/m^2					0.56
18.5–24.9	70	1,249	1.08	0.26–4.56	
≥25	119	1,697	1.84	1.18–2.88	
Tumor stage					0.32
I	25	1,144	2.51	0.82–7.64	
II–III	163	1,775	1.55	0.97–2.47	
Tumor ER					0.18
ER positive	120	2,171	1.98	1.17–3.38	
ER negative	66	710	1.33	0.65–2.72	
CVD					0.99
No history of CVD	111	1,801	1.44	0.93–2.22	
History of CVD	39	605	1.17	0.56–2.43	
Arthritis					0.64
No history of arthritis	121	1,944	1.47	0.94–2.59	
History of arthritis	29	462	2.82	0.99–8.00	
Type 2 diabetes					0.65
No history of T2DM	139	2,295	1.46	0.86–2.50	
History of T2DM	11	111	2.27	0.55–9.44	

NOTE: Adjusted for the following variables, including age, time since diagnosis tumor, postmenopausal status, tumor stage, tumor grade, race–ethnicity, BMI, anti-estrogen use, and ER/PR status, unless stratified by specific variable.

[a]Two-sided *P* for interaction.

related event, whereas women with low to moderate inflammation (vs. no inflammation) had comparable HRs for an additional breast cancer–related event. Acute inflammatory status remained an independent predictor following adjustment for BMI (model 2) plus anti-estrogen use and tumor hormone status (model 3). In a sensitivity analysis, we limited the outcomes to breast cancer recurrences, and the findings were not meaningfully changed (data not shown).

We also examined the association between very high CRP (≥10 mg/L) and breast cancer–specific mortality by select subgroups. The adverse association between very high CRP and death due to breast cancer was stronger in certain subgroups (Table 4). These subgroups included older women (≥55 years of age), postmenopausal women, those with excessive adiposity (BMI ≥ 25 kg/m^2), and women whose tumors were ER$^+$. However, interaction terms for CRP with these factors were not statistically significant. Further, separate models examined the CRP–survival relationship stratified by (i) intervention arm and (ii) time interval from diagnosis to blood draw; the hazard estimates for CRP did not vary by intervention arm or time interval value (data not shown).

Discussion

This analysis assessed the distribution and the prognostic value of serum CRP with all-cause and breast cancer–specific mortality and additional breast cancer–related events among a cohort of women following a diagnosis of breast cancer. In this cohort, we observed a prevalence (34.4%) of elevated CRP (>3 mg/L) that is similar to that of the general population (ranging 25–43%; ref. 35). This concurrence suggests that circulating concentrations of CRP return to relatively normal ranges following cancer diagnosis and treatment. Compared with women with the lowest concentrations of CRP (<1 mg/L), those with values of CRP ≥10 mg/L had an approximate 2-fold increased risk of all-cause and breast cancer–specific mortality, and a 67% increased risk of an additional breast cancer–related events in models

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adjusted for prognostic confounders. We also found that acute inflammation–survival relationship was not modified by age, postmenopausal status, BMI, tumor stage, ER^+ receptor tumor status, or inflammatory-related conditions.

These results, including the size of the HRs and statistical significance, are comparable with reports from two other observational prospective cohort studies of women following a diagnosis of breast cancer. The larger report was conducted in a cohort of 2,910 Danish women diagnosed with invasive breast cancer (36). Women whose blood was drawn before treatment, Allin and colleagues reported women with CRP levels >3.24 mg/L (highest tertile vs. the lowest tertile, <1.04 mg/L) had increased risk of all-cause mortality (HR, 1.84; 95% CI, 1.39–2.45), an increased risk of death due to breast cancer (HR, 1.66; 95% CI, 1.15–2.41), and a suggested increased risk of disease recurrence (HR, 1.45; 95% CI, 0.93–2.26), independent of age, tumor characteristics (size, grade, ER/PR receptor status, HER2 status), lymph node status, presence of distant metastases, lifestyle factors (smoking, alcohol consumption), BMI, and CVD (18). In another cohort of 734 U.S. postmenopausal breast cancer survivors (stage I–IIIA) whose blood was drawn following disease treatment, Pierce and colleagues reported that women in the highest tertile ($\geq$3.9 mg/L) of CRP concentrations (vs. lowest tertile, $\leq$1.2 mg/L) had a 2-fold increased risk of all-cause mortality (HR, 2.31; 95% CI, 1.30–4.12) and disease-free survival (HR, 1.99; 95% CI, 1.09–3.65) after adjusting for age, disease stage, race/study site, BMI, and ER/PR receptor status (19). Conversely, results from smaller studies (23–25) suggest no association between CRP and prognosis. The differences in HRs and statistical significance may be due to confounding, timing of blood draw with respect to disease treatment, or may suggest that these studies were inadequately powered to evaluate the association in the presence of potential confounding.

The role of CRP as a biological indicator of prognosis versus as a contributor to carcinogenesis is unclear. CRP has some immune-related functions, including activation of classical complement binding and opsonization (for phagocytosis; ref. 37). In a recent study of genetic variants in the *CRP* gene, certain variants were associated with altered plasma CRP concentrations, but not cancer risk (2). In this context, our finding that very high concentrations of circulating CRP (>10 mg/L) was associated with increased risk of mortality due to breast cancer and additional breast cancer events suggest that CRP may be a biological indicator of carcinogenesis, rather than a direct contributor.

Strengths of this analysis include the cohort study design, almost complete outcomes follow-up conducted with medical and death records, a reasonably large sample size, and relatively long follow-up time. Furthermore, the sample size of this cohort was sufficient to assess the effect of acute inflammation (>10 mg/L) and outcomes in breast cancer survivors, in contrast with the other papers cited in which "moderate" and "acute" inflammation (as defined here) were combined. The inclusion of the acute inflammation category leads to the suggestion of a possible threshold effect. Thus, acute inflammation status (CRP $\geq$ 10 mg/L) may be an important independent biomarker for long-term survival in breast cancer survivors. A limitation of our study was that our samples were obtained at one time point following completion of active treatment (on average 2 years following diagnosis); consequently, no conclusions can be drawn as to whether elevated CRP persisted over the long term. Future studies using blood samples from multiple time points before and after cancer diagnosis may provide important information clarifying the temporal relationship of biomarkers with cancer survival. Finally, our results are only generalizable to women who have completed treatment and survived at least 2 years after diagnosis of breast cancer.

In summary, our results indicate that the risk of mortality and disease recurrence among women diagnosed with breast cancer is associated with acute inflammation (>10 mg/L) and possibly moderate inflammation (CRP > 3 mg/L). Combined with previous research, this study appears to confirm the association of circulating CRP levels with increased risk of poor breast cancer outcomes. It is particularly notable that there was an average of 7 years from the baseline CRP assessment and recurrence or mortality, which indicates that CRP is not a transient measure of acute inflammation in this cohort but offers long-term prognostic information. Arguably, the most important next step is to determine whether this association is causal. If CRP plays a causal role in breast cancer outcomes, then future research should be focused on understanding how lifestyle interventions, such as diet and physical activity, can reduce the circulating concentrations of this risk factor. Alternatively, if CRP is simply a prognostic marker, then further research is needed to understand whether it is responsive (i.e., has utility) to drug and lifestyle interventions designed to decrease risk of recurrence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Villaseñor, L. Natarajan, R.E. Patterson
Development of methodology: A. Villaseñor, L. Natarajan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.P. Pierce, R.E. Patterson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Villaseñor, S.W. Flatt, L. Natarajan, R.E. Patterson
Writing, review, and/or revision of the manuscript: A. Villaseñor, S.W. Flatt, C. Marinac, L. Natarajan, J.P. Pierce, R.E. Patterson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Villaseñor, S.W. Flatt, C. Marinac, R.E. Patterson
Study supervision: J.P. Pierce

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References

1. American Cancer Society. Breast cancer facts & figures 2011–2012. Atlanta: American Cancer Society, Inc.; 2012.
2. Allin KH, Nordestgaard BG, Zacho J, Tybjærg-Hansen A, Bojesen SE. C-reactive protein and the risk of cancer: a Mendelian randomization study. J Natl Cancer Inst 2009;102:202–6.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–44.
4. Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett 2008;267:204–15.
5. Chiang AC, Massague J. Molecular basis of metastasis. N Engl J Med 2008;359:2814–23.
6. Favaro E, Amadori A, Indraccolo S. Cellular interactions in the vascular niche: implications in the regulation of tumor dormancy. APMIS 2008;116:648–59.
7. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 2008;8:887–99.
8. Ikeda M, Natsugoe S, Ueno S, Baba M, Aikou T. Significant host- and tumor-related factors for predicting prognosis in patients with esophageal carcinoma. Ann Surg 2003;238:197–202.
9. McMillan DC, Canna K, McArdle CS. Systemic inflammatory response predicts survival following curative resection of colorectal cancer. Br J Surg 2003;90:215–9.
10. Hilmy M, Bartlett JM, Underwood MA, McMillan DC. The relationship between the systemic inflammatory response and survival in patients with transitional cell carcinoma of the urinary bladder. Br J Cancer 2005;92:625–7.
11. Jamieson NB, Glen P, McMillan DC, McKay CJ, Foulis AK, Carter R, et al. Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas. Br J Cancer 2005;92:21–3.
12. Roxburgh CS, McMillan DC. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. Future Oncol 2010;6:149–63.
13. Schmid M, Schneitter A, Hinterberger S, Seeber J, Reinthaller A, Hefler L. Association of elevated C-reactive protein levels with an impaired prognosis in patients with surgically treated endometrial cancer. Obstet Gynecol 2007;110:1231–6.
14. Polterauer S, Grimm C, Tempfer C, Sliutz G, Speiser P, Reinthaller A, et al. C-reactive protein is a prognostic parameter in patients with cervical cancer. Gynecol Oncol 2007;107:114–7.
15. O'Dowd C, McRae LA, McMillan DC, Kirk A, Milroy R. Elevated preoperative C-reactive protein predicts poor cancer specific survival in patients undergoing resection for non-small cell lung cancer. J Thorac Oncol 2010;5:988–92.
16. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. CRC Crit Rev Clin Lab Sci 2011;48:155–70.
17. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.
18. Allin KH, Nordestgaard BG, Flyger H, Bojesen SE. Elevated pretreatment levels of plasma C-reactive protein are associated with poor prognosis after breast cancer: a cohort study. Breast Cancer Res 2011;13:R55.
19. Pierce BL, Ballard-Barbash R, Bernstein L, Baumgartner RN, Neuhouser ML, Wener MH, et al. Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. J Clin Oncol 2009;27:3437–44.
20. Al Murri AM, Bartlett JM, Canney PA, Doughty JC, Wilson C, McMillan DC. Evaluation of an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer. Br J Cancer 2006;94:227–30.
21. Albuquerque KV, Price MR, Badley RA, Jonrup I, Pearson D, Blamey RW, et al. Pre-treatment serum levels of tumour markers in metastatic breast cancer: a prospective assessment of their role in predicting response to therapy and survival. Eur J Surg Oncol 1995;21:504–9.
22. Williams MR, Turkes A, Pearson D, Griffiths K, Blamey RW. An objective biochemical assessment of therapeutic response in metastatic breast cancer: a study with external review of clinical data. Br J Cancer 1990;61:126–32.
23. Al Murri AM, Wilson C, Lannigan A, Doughty JC, Angerson WJ, McArdle CS, et al. Evaluation of the relationship between the systemic inflammatory response and cancer-specific survival in patients with primary operable breast cancer. Br J Cancer 2007;96:891–5.
24. Pasanisi P, Venturelli E, Morelli D, Fontana L, Secreto G, Berrino F. Serum insulin-like growth factor-I and platelet-derived growth factor as biomarkers of breast cancer prognosis. Cancer Epidemiol Biomarkers Prev 2008;17:1719–22.
25. Ravishankaran P, Karunanithi R. Clinical significance of preoperative serum interleukin-6 and C-reactive protein level in breast cancer patients. World J Surg Oncol 2011;9:18.
26. Han Y, Mao F, Wu Y, Fu X, Zhu X, Zhou S, et al. Prognostic role of C-reactive protein in breast cancer: a systematic review and meta-analysis. Int J Biol Markers 2011;26:209–15.
27. Pierce JP, Faerber S, Wright FA, Rock CL, Newman V, Flatt SW, et al. A randomized trial of the effect of a plant-based dietary pattern on additional breast cancer events and survival: the Women's Healthy Eating and Living (WHEL) Study. Control Clin Trials 2002;23:728–56.
28. Pierce JP, Natarajan L, Caan BJ, Parker BA, Greenberg ER, Flatt SW, et al. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. JAMA 2007;298:289–98.
29. Johnson-Kozlow M, Rock CL, Gilpin EA, Hollenbach KA, Pierce JP. Validation of the WHI brief physical activity questionnaire among women diagnosed with breast cancer. Am J Health Behav 2007;31:193–202.
30. Thomson CA, Giuliano A, Rock CL, Ritenbaugh CK, Flatt SW, Faerber S, et al. Measuring dietary change in a diet intervention trial: comparing food frequency questionnaire and dietary recalls. Am J Epidemiol 2003;157:754–62.
31. Hong S, Bardwell WA, Natarajan L, Flatt SW, Rock CL, Newman VA, et al. Correlates of physical activity level in breast cancer survivors participating in the Women's Healthy Eating and Living (WHEL) Study. Breast Cancer Res Treat 2007;101:225–32.
32. Kleinbaum DG, Klein M. Evaluating the proportional hazards assumption. New York: Springer; 2005. p. 131–71
33. Nguyen XM, Lane J, Smith BR, Nguyen NT. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. J Gastrointest Surg 2009;13:1205–12.

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34. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361–87.
35. Ford ES, Giles WH, Mokdad AH, Myers GL. Distribution and correlates of C-reactive protein concentrations among adult US women. Clin Chem 2004;50:574–81.
36. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. J Clin Oncol 2009;27:2217–24.
37. Schultz DR, Arnold PI. Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, alpha 1-acid glycoprotein, and fibrinogen. Semin Arthritis Rheum 1990;20:129–47.

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