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ORIGINAL REPORT

Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients

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ABSTRACT

Purpose
Chronic inflammation is believed to contribute to the development and progression of breast cancer. Systemic C-reactive protein (CRP) and serum amyloid A (SAA) are measures of low-grade chronic inflammation and potential predictors of cancer survival.

Patients and Methods
We evaluated the relationship between circulating markers of inflammation and breast cancer survival using data from the Health, Eating, Activity, and Lifestyle (HEAL) Study (a multiethnic prospective cohort study of women diagnosed with stage 0 to IIIA breast cancer). Circulating concentrations of CRP and SAA were measured approximately 31 months after diagnosis and tested for associations with disease-free survival (approximately 4.1 years of follow-up) and overall survival (approximately 6.9 years of follow-up) in 734 disease-free breast cancer survivors. Cox proportional hazards models were used with adjustment for potential confounding factors to generate hazard ratios (HRs) and 95% CIs.

Results
Elevated SAA and CRP were associated with reduced overall survival, regardless of adjustment for age, tumor stage, race, and body mass index (SAA *P* trend < .0001; CRP *P* trend = .002). The HRs for SAA and CRP tertiles suggested a threshold effect on survival, rather than a dose-response relationship (highest *v* lowest tertile: SAA HR = 3.15; 95% CI, 1.73 to 5.65; CRP HR = 2.27; 95% CI, 1.27 to 4.08). Associations were similar and still significant after adjusting for self-reported history of cardiovascular events and censoring cardiovascular disease deaths. Elevated CRP and SAA were also associated with reduced disease-free survival, although these associations were of borderline significance (SAA *P* trend = .04; CRP *P* trend = .07).

Conclusion
Circulating SAA and CRP may be important prognostic markers for long-term survival in breast cancer patients, independent of race, tumor stage, and body mass index.

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INTRODUCTION

Chronic inflammation is a key contributor to cancer development and progression.[1] Cancer survivors with chronic inflammation may have an elevated risk of recurrence as a result of the effects of inflammatory processes on cell growth[1] or the presence of cancer cells that induce inflammation.

C-reactive protein (CRP) and serum amyloid A (SAA) are nonspecific, acute-phase, hepatic proteins secreted in response to cytokines including interleukin-1, interleukin-6, and tumor necrosis factor α.[2] CRP has several immune-related functions, such as opsonization (for phagocytosis) and activation of classical complement binding, whereas SAA participates in cholesterol transport, extracellular matrix degradation, and the recruitment of inflammatory cells.[2-4] These biomarkers are also measures of low-grade chronic inflammation and potential predictors of cancer risk and/or survival. Elevated CRP has been associated with poor survival in metastatic prostate (CRP measured during treatment),[5] gastroesophageal (measured before resection),[6] colorectal (measured before and after resection),[7,8] inoperable non–small-cell lung (measured before treatment),[9] and pancreatic (measured at diagnosis)[10] cancers. Similarly, preoperative SAA has been associated with survival in gastric cancer patients (receiving gastrectomy)[11] and renal cell carcinoma patients (receiving nephrectomy).[12]

Inflammatory status may also be a prognostic factor for beast cancer. Clinical and experimental

data suggest that chronic inflammation promotes mammary tumor development through mechanisms involving chronic activation of humoral immunity and infiltration of Th2 cells and polarized innate inflammatory cells.[13] Breast cancer patients have elevated concentrations of CRP before surgery, more so in women with advanced disease,[14,15] suggesting that CRP may be related to tumor burden or progression. Elevated pretreatment CRP and low albumin were associated with decreased cancer-specific survival in a study of 96 breast cancer patients presenting with metastatic relapse.[16] Similarly, in a study of 85 metastatic breast cancer patients, elevated pretreatment CRP was associated with decreased survival.[17] However, in a study of 300 patients with invasive primary operable breast cancer, CRP was not associated with survival.[18] CRP is also a risk factor for cardiovascular disease, which is more common among breast cancer patients after radiation treatment.[19]

No studies have assessed the relationship between biomarkers of inflammation and long-term survival in breast cancer patients. Furthermore, SAA has not yet been evaluated in the context of breast cancer prognosis. The aim of this study was to determine whether circulating markers of inflammation (CRP and SAA), measured approximately 31 months after diagnosis, predict disease-free and overall survival in a population-based cohort of 734 breast cancer survivors.

PATIENTS AND METHODS

Study Setting, Participants, and Recruitment

The Health, Eating, Activity, and Lifestyle (HEAL) Study is a population-based, multicenter, multiethnic prospective cohort study that has enrolled 1,183 breast cancer patients who are being observed to determine whether weight, physical activity, diet, sex hormones, mammographic density, and other factors affect breast cancer prognosis. Women were recruited into the HEAL study through Surveillance, Epidemiology, and End Results (SEER) registries in New Mexico, Los Angeles County in California, and western Washington. Details of the aims, study design, and recruitment procedures have been published previously.[20-22]

Briefly, in New Mexico, we recruited 615 women, age 18 years or older, diagnosed with in situ to stage IIIA breast cancer between July 1996 and March 1999 and living in Bernalillo, Sante Fe, Sandoval, Valencia, or Taos Counties. In western Washington, we recruited 202 women, between the age of 40 and 64 years, diagnosed with in situ to stage IIIA breast cancer between September 1997 and September 1998 and living in King, Pierce, or Snohomish Counties. In Los Angeles County, we recruited 366 black women with stage 0 to IIIA primary breast cancer who had participated in the Los Angeles portion of the Women's Contraceptive and Reproductive Experiences Study, a case-control study of invasive breast cancer, or who had participated in a parallel case-control study of in situ breast cancer. Los Angeles participants were US-born, English-speaking women age 35 to 64 years and diagnosed with breast cancer between May 1995 and May 1998.

Participants completed in-person interviews at baseline (within their first year after diagnosis; on average, 7.5 months after diagnosis) and 24 months after the baseline visit (within their third year of diagnosis; on average, 31 months after diagnosis). Written informed consent was obtained from each patient. The study was performed with the approval of the institutional review boards of participating centers, in accord with an assurance filed with and approved by the US Department of Health and Human Services.

Biomarkers

A 30-mL fasting blood sample was collected at the follow-up interview, targeted for 24 months after enrollment. The sample was processed within 3 hours of collection, and serum was stored at −70 to −80°C until analysis. CRP and SAA were measured by latex-enhanced nephelometry using high-sensitivity assays on the Behring Nephelometer II analyzer (Dade Behring Diagnostics, Deerfield, IL) at the University of Washington. The lower detection limits for CRP and SAA assays were 0.2 and 0.7 mg/L, respectively. Interassay coefficients of variation were 5% to 9% for CRP and 4% to 8% for SAA. Control materials from Bio-Rad Laboratories (Hercules, CA) were run with each assay for quality control purposes.

Outcome Assessment

Information on vital status was obtained from SEER. If alive, individuals were observed through their last follow-up assessment or SEER vital status update, whichever was most recent. Information on breast cancer recurrences and new primary breast cancers was obtained by self-report at 24-month and 5-year interviews and confirmed using a combination of medical records and SEER data. Overall survival time was the time from the 24-month follow-up interview to death from any cause or end of follow-up; events were limited to data collected through April 30, 2008. Disease-free survival time was defined as the time from the 24-month follow-up interview to diagnosis of nonfatal breast cancer (recurrence or diagnosis of a second primary), death from any cause, or end of follow-up, updated through September 30, 2004. Individuals lost to follow-up were censored at last date of contact (3% for reasons other than death).

Disease Stage and Treatment

We obtained data on disease stage from the local SEER registries before recruitment. Participants were classified as having in situ, stage I (localized), or stage II to IIIA (regional) breast cancer based on American Joint Committee on Cancer stage of disease classification contained within SEER. Estrogen receptor (ER) and progesterone receptor (PR) status of tumors was categorized as positive, negative, or unknown/borderline. Treatment and additional clinical data were obtained from medical records. Adjuvant treatment was categorized into the following four mutually exclusive groups: surgery only; surgery and radiation; surgery and chemotherapy; or surgery, radiation, and chemotherapy.

Anthropometrics

With the women wearing light clothing and no shoes, weight was measured at the 24-month follow-up assessment to the nearest 0.1 kg using a balance-beam laboratory scale at New Mexico and Washington and a portable

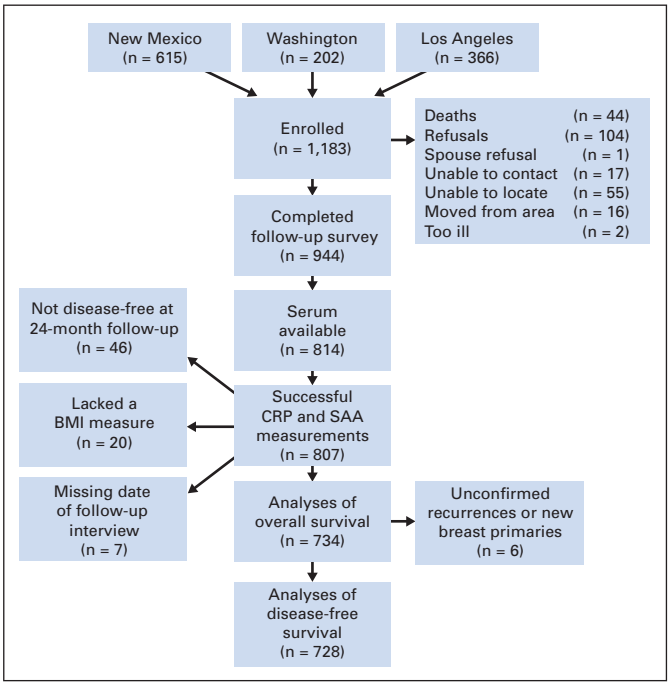

Fig 1. Cohort definition and exclusions. CRP, C-reactive protein; SAA, serum amyloid A; BMI, body mass index.

Table 1. Demographics and Clinical Characteristics of HEAL Study Participants Stratified by Race

Patient Demographics and Clinical Characteristics	All Patients* (N = 734)	Non-Hispanic Whites (n = 446)	African Americans (n = 191)	Hispanics (n = 77)
Median follow-up time,† years				
Overall survival	6.9	6.9	7.3	6.8
Disease-free survival	4.1	4.0	4.5	4.3
Age, years, % of patients				
30-39	1.5	0.7	2.1	5.1
40-49	22.9	17.3	36.7	21.8
50-59	36.8	36.1	36.7	35.9
60-69	25.9	27.7	24.6	20.5
70-79	9.5	13.1	0.0	14.1
80-89	3.4	5.1	0.0	2.5
Mean	57.5	59.6	53.0	57.1
Standard deviation	10.4	10.7	7.7	11.7
Education, % of patients				
High school only	26.6	19.1	36.7	43.6
College	54.8	56.3	54.5	47.4
Graduate school	18.5	24.4	8.9	9.0
Missing	0.1	0.2	0.0	0.0
Study site, % of patients				
Seattle	22.0	31.0	0.5	3.9
New Mexico	52.4	69.0	0.0	96.2
Los Angeles	25.6	0.0	99.5	0.0
Body mass index, kg/m^2				
Mean	27.6	26.3	30.9	27.0
SD	6.5	5.6	7.6	5.0
Menopause status, % of patients				
Premenopausal	18.1	18.0	17.3	21.8
Postmenopausal	75.8	78.3	73.8	69.2
Unknown	6.1	3.8	8.9	9.0
Self-reported cardiovascular events, % of patients				
History of myocardial infarction	3.2	3.1	2.0	2.1
History of heart failure	2.3	2.2	3.1	0.0
CRP, mg/L				
Mean	4.5	3.7	6.4	4.2
SD	8.3	5.5	13.3	5.2
Median	2.2	1.9	2.9	2.8
IQR	0.8-5.0	0.8-4.0	1.1-7.2	1.0-5.3
SAA, mg/L				
Mean	10.3	10.5	10.5	8.8
SD	29.4	27.4	39.3	7.0
Median	5.7	5.7	5.4	6.3
IQR	3.5-10.1	3.5-9.7	3.0-10.7	4.4-10.5
SEER summary stage, % of patients				
In situ	23.3	25.1	19.4	21.8
Localized	54.5	57.2	45.0	62.8
Regional	22.3	17.7	35.6	15.4
ER/PR status, % patients				
ER positive/PR positive	42.0	48.7	25.7	40.3
ER positive/PR negative	8.6	9.6	7.3	6.5
ER negative/PR positive	2.3	0.9	6.3	1.3
ER negative/PR negative	12.4	9.4	18.9	15.6
Unknown	34.7	31.4	41.9	36.3
Treatment (after surgery), % of patients				
No chemotherapy or radiation	31.7	29.9	35.1	35.9
Radiation only	38.2	44.1	24.1	37.2
Chemotherapy only	9.9	6.4	18.9	7.7
Radiation and chemotherapy	20.2	19.5	22.0	19.2

Abbreviations: HEAL, Health, Eating, Activity, and Lifestyle; CRP, C-reactive protein; IQR, interquartile range; SAA, serum amyloid A; SEER, Surveillance, Epidemiology, and End Results; SD, standard deviation; ER, estrogen receptor; PR, progesterone receptor.

*Twenty women did not fall into any of the three race categories (five American Indians, 11 Asians, and four others).

†Follow-up was initiated at the time of blood draw, approximately 31 months after diagnosis.

Thinner Digital Electronic Scale (Conair, East Windsor, NJ) at Los Angeles. Height was measured, without shoes, to the nearest 0.1 cm using a stadiometer at New Mexico and Washington and a tape measure at Los Angeles. All measurements were performed twice and averaged. Body mass index (BMI) was computed as kilograms per meter squared.

Other Variables

Standardized questionnaire information was collected at baseline and 24-month follow-up on medical history and demographic characteristics. Postmenopausal status, which was assessed at the follow-up interview, was defined as age ≥ 55 years, not menstruating in the last 12 months, an oophorectomy, or a hysterectomy. Use of oral hormone replacement therapy was defined as any use of estrogen or progesterone since diagnosis. Women were defined as users of tamoxifen, nonsteroidal anti-inflammatory drugs, beta-blockers, angiotensin-converting enzyme inhibitors, lipid-lowering medications, or vitamin E supplements if they reported current use at the follow-up interview. History of medical conditions related to cardiovascular disease and inflammation (eg, heart failure, myocardial infarction) was self-reported at the follow-up interview. Information on physical activity collected at follow-up was used to compute total average metabolic equivalent task (MET) hours per week of moderate and/or vigorous sport and recreational activities for the year before follow-up.[21]

Exclusions

Among the 1,183 eligible women enrolled at baseline, 944 women completed the follow-up survey (Fig 1). Reasons for nonparticipation included death (n = 44), refusal (n = 104), spouse refusal (n = 1), unable to contact (n = 17), unable to locate (n = 55), moved from study area (n = 16), and illness (n = 2). Serum samples were available for 814 participants, and CRP and SAA were measured successfully for 807 participants. Of these 807 participants, 46 were not disease free at the 24-month follow-up, 20 lacked a BMI measure, and seven were missing a 24-month follow-up interview date, resulting in a sample size of 734 participants for analyses of overall survival. For analyses of disease-free survival, we excluded six additional women whose reported recurrence or new breast primary was not confirmed by medical records.

Statistical Analysis

We assigned women with unknown or unclassifiable race or ER/PR status to "unknown" categories for analytic purposes. A race/study site variable was created to adjust for confounding because race and study site were highly correlated. This variable had the following four categories: non-Hispanic white at University of Southern California, non-Hispanic white at Fred Hutchinson Cancer Research Center, Hispanic, and African American.

Hazard ratios (HRs) and 95% CIs for CRP and SAA tertiles were estimated using Cox proportional hazards regression. The four models were adjusted for age, tumor stage at diagnosis, and race/study site (model 1); model 1 covariates and BMI (model 2); model 2 covariates and ER/PR status (model 3); and model 3 covariates and history of self-reported cardiovascular events known to be associated with CRP and SAA in the HEAL cohort (heart failure and myocardial infarction; model 4).[23] ER/PR status was classified as ER/PR positive, ER positive/PR negative, ER negative/PR positive, ER/PR negative, or missing. Wald *P* values for trend were computed by treating the tertiles as an ordinal variable. CRP and SAA values were log transformed to account for skewness, and HRs and 95% CIs were generated for these continuous measures.

Table 2. HRs for Disease-Free and Overall Survival by CRP and SAA Tertiles

Tertile	No. of Events	No. of Participants	Model 1* HR	Model 1* 95% CI	Model 1* *P* (trend)	Model 2† HR	Model 2† 95% CI	Model 2† *P* (trend)	Model 3‡ HR	Model 3‡ 95% CI	Model 3‡ *P* (trend)	Model 4§ HR	Model 4§ 95% CI	Model 4§ *P* (trend)
Overall survival‖	88	734												
CRP, mg/L														
≤ 1.2	24	260	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
1.3-3.8	22	237	0.81	0.44 to 1.48		0.92	0.50 to 1.73		0.91	0.48 to 1.69		0.94	0.50 to 1.76	
≥ 3.9	42	237	1.90	1.11 to 3.23	.01	2.31	1.30 to 4.12	.002	2.27	1.27 to 4.08	.002	2.05	1.14 to 3.69	.01
SAA, mg/L														
≤ 4.2	18	252	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
4.3-8.0	20	238	0.89	0.46 to 1.73		0.98	0.50 to 1.90		0.99	0.51 to 1.93		0.97	0.49 to 1.89	
≥ 8.1	50	244	2.67	1.53 to 4.63	.0001	3.08	1.73 to 5.47	.0001	3.15	1.76 to 5.65	.0001	2.91	1.61 to 5.26	.0001
lnCRP	88	734	1.28	1.06 to 1.53		1.39	1.14 to 1.70		1.39	1.14 to 1.69		1.25	1.02 to 1.54	
lnSAA	88	734	1.42	1.24 to 1.88		1.55	1.29 to 1.99		1.61	1.30 to 2.01		1.42	1.10 to 1.82	
Disease-free survival¶	91	728												
CRP, mg/L														
≤ 1.2	23	260	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
1.3-3.8	31	236	1.40	0.80 to 2.44		1.59	0.89 to 2.84		1.56	0.87 to 2.80		1.58	0.88 to 2.83	
≥ 3.9	37	232	1.71	0.99 to 2.95	.05	2.08	1.14 to 3.77	.02	1.99	1.09 to 3.65	.03	1.91	1.04 to 3.51	.04
SAA, mg/L														
≤ 4.2	26	251	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
4.3-8.0	24	237	0.93	0.52 to 1.65		0.97	0.55 to 1.72		1.02	0.57 to 1.81		1.00	0.56 to 1.79	
≥ 8.1	41	240	1.54	0.92 to 2.57	.08	1.65	0.97 to 2.81	.05	1.67	0.97 to 2.86	.05	1.62	0.94 to 2.80	.07
lnCRP	91	728	1.15	0.97 to 1.37		1.23	1.01 to 1.49		1.21	1.00 to 1.48		1.17	0.97 to 1.44	
lnSAA	91	728	1.29	1.02 to 1.63		1.34	1.05 to 1.71		1.35	1.06 to 1.73		1.29	0.99 to 1.68	

Abbreviations: HR, hazard ratio; CRP, C-reactive protein; SAA, serum amyloid A; BMI, body mass index.
*Adjusted for age (continuous), disease stage, and race/study site.
†Adjusted for age (continuous), disease stage, race/study site, and BMI (continuous).
‡Adjusted for age (continuous), disease stage, race/study site, BMI (continuous), and estrogen receptor/progesterone receptor status.
§Adjusted for age (continuous), disease stage, race/study site, BMI (continuous), estrogen receptor/progesterone receptor status, and cardiovascular events (histories of heart failure and myocardial infarction).
‖Data on overall survival collected through April 30, 2008.
¶Data on disease-free survival collected through September 30, 2004.

To further evaluate associations in the context of cardiovascular disease, analyses were also performed excluding individuals reporting cardiovascular events and censoring deaths caused by cardiovascular and cerebrovascular disease. Additional analyses were conducted excluding women with in situ cancer (n = 169, eight deaths, 14 disease-free survival events).

Furthermore, we explored associations after stratifying on ER/PR status. Only participants with localized and regional stage disease were included in these analyses because of the limited data on ER/PR status of in situ cancers (92% and 94% reported as having borderline/unknown ER and PR status, respectively).

RESULTS

Characteristics of HEAL participants are listed in Table 1. Median follow-up times for overall survival and disease-free survival were 6.9 and 4.1 years, respectively. The mean age at 24-month follow-up was 57.5 years; mean BMI was 27.6 kg/m^2; and the percentages of participants with in situ, localized, and regional stage disease were 23.3%, 54.5%, and 22.3%, respectively. A high percentage of participants had unknown ER (28%) or PR status (35%), but the majority of these participants were diagnosed with in situ disease (75% of unknown ER and 66% of unknown PR). There were 88 deaths (before April 30, 2008) and 91 disease-free survival events (before September 30, 2004). Common causes of death were breast neoplasms (n = 34), other neoplasms (n = 10), diseases of the circulatory system (n = 10), and respiratory diseases (n = 6).

HRs and 95% CIs comparing tertiles of CRP and SAA for disease-free and overall survival are listed in Table 2. Higher concentrations of SAA were associated with decreased overall survival (P trend $< .0001$ in all models; lnSAA, $P < .01$ in all models). The HRs for SAA tertiles suggest a threshold effect rather than a dose-response relationship. In model 2 for example, when compared with the lowest SAA tertile ($\leq$ 4.2 mg/L), women in the middle tertile had comparable overall survival (HR = 0.98; 95% CI, 0.50 to 1.90), whereas the highest SAA tertile ($\geq$ 8.1 mg/L) was associated with decreased survival (HR = 3.08; 95% CI, 1.73 to 5.47). Adjustment for ER and PR status did not alter the association substantially (model 3), whereas further adjustment for cardiovascular comorbidities resulted in slightly attenuated, yet still highly significant associations (model 4). Similarly, elevated CRP ($>$ 3.9 mg/L) showed a statistically significant association with reduced survival; however, HRs were somewhat lower than for SAA. CRP was associated with disease-free survival across all models in a dose-response fashion (P trend = .04 in model 4). SAA showed borderline associations with reduced disease-free survival (P trend = .07 in model 4), but the highest SAA tertile ($\geq$ 8.1 mg/L) was driving this association. All of the associations observed in this analysis were slightly attenuated after adjustment for cardiovascular events (model 4 compared with model 3), suggesting that these factors

Table 3. HRs for Disease-Free Survival and Overall Survival by CRP and SAA Tertiles, Excluding Individuals With Self-Reported Cardiovascular Events or In Situ Disease Stage

	Excluding Participants Reporting Cardiovascular Events*					Excluding Participants With In Situ Disease†				
Tertile	No. of Events	No. of Participants	HR	95% CI	P (trend)	No. of Events	No. of Participants	HR	95% CI	P (trend)
Overall survival‡	75	697				80	565			
CRP, mg/L										
≤ 1.2	22	253	1.00	Reference		23	203	1.00	Reference	
1.3-3.8	20	229	0.97	0.50 to 1.89		19	181	0.85	0.44 to 1.64	
≥ 3.9	33	215	2.32	1.22 to 4.39	.006	38	181	1.96	1.07 to 3.61	.02
SAA, mg/L										
≤ 4.2	16	248	1.00	Reference		16	189	1.00	Reference	
4.3-8.0	17	225	1.00	0.49 to 2.05		18	190	0.98	0.48 to 1.98	
≥ 8.1	42	224	3.32	1.78 to 6.17	.0001	46	186	2.88	1.54 to 5.37	.0002
lnCRP	75	697	1.43	1.14 to 1.79		47	565	1.24	1.01 to 1.54	
lnSAA	75	697	1.86	1.41 to 2.45		47	565	1.37	1.05 to 1.79	
Disease-free survival§	83	692				77	559			
CRP, mg/L										
≤ 1.2	22	253	1.00	Reference		23	203	1.00	Reference	
1.3-3.8	29	228	1.54	0.84 to 2.81		27	180	1.40	0.76 to 2.57	
≥ 3.9	32	211	1.91	1.01 to 3.61	.05	27	176	1.34	0.70 to 2.57	.40
SAA, mg/L										
≤ 4.2	24	247	1.00	Reference		22	188	1.00	Reference	
4.3-8.0	21	224	0.98	0.53 to 1.80		21	189	1.02	0.55 to 1.90	
≥ 8.1	38	221	1.75	1.00 to 3.07	.04	34	182	1.58	0.87 to 2.86	.12
lnCRP	83	692	1.23	0.99 to 1.53		77	559	1.05	0.86 to 1.31	
lnSAA	83	692	1.46	1.09 to 1.95		77	559	1.16	0.87 to 1.53	

Abbreviations: HR, hazard ratio; CRP, C-reactive protein; SAA, serum amyloid A; BMI, body mass index.
*Adjusted for age (continuous), disease stage, race/study site, BMI (continuous), and estrogen receptor/progesterone receptor status.
†Adjusted for age (continuous), disease stage, race/study site, BMI (continuous), estrogen receptor/progesterone receptor status, and self-reported history of cardiovascular events (heart failure and myocardial infarction).
‡Data on overall survival collected through April 30, 2008.
§Data on disease-free survival collected through September 30, 2004.

may partially confound the relationship between inflammatory markers and survival.

However, similar if not stronger associations were observed after restricting analyses to individuals not reporting a history of cardiovascular events (Table 3) and in analyses censoring deaths caused by cardiovascular disease (n = 4) and cerebrovascular disease (n = 4) (Table 4). Analyses excluding participants with in situ disease resulted in HRs similar in magnitude to those in the analysis of the full cohort, although perhaps slightly attenuated (Table 3). Restricting analyses to non-Hispanic whites or individuals with more than 6 months of survival resulted in similar HRs as those observed in analyses of all participants (data not shown). Models adjusted for smoking, physical activity, and estradiol generated HRs similar to those obtained from models that were not adjusted for these covariates (data not shown). In analyses stratified by ER/PR status, we did not observe substantial differences between strata, although slightly stronger associations were observed in the ER-negative and ER-negative/PR-negative strata (Tables 5 and 6).

DISCUSSION

To our knowledge, this is the largest population-based cohort study to date examining associations between systemic inflammation and breast cancer survival and the first to evaluate SAA as a prognostic marker for breast cancer. We observed significant associations between reduced overall survival and elevated concentrations of the inflammatory biomarkers SAA and CRP (measured approximately 31 months after diagnosis and 24 months after enrollment). Similarly, we observed a significant association between elevated CRP and reduced disease-free survival and a borderline association between elevated SAA and reduced disease-free survival. All associations were independent of disease stage, BMI, self-reported cardiovascular events, estradiol concentrations, smoking, and physical activity and did not seem to be modified by ER status, PR status, or disease stage.

Results from smaller studies[16-18] suggest that an association between CRP and survival may be present only in groups of patients with metastatic disease. However, the results from our population-based study of nonmetastatic breast cancer (stage 0 to IIIA) indicate that this association is also present in patients with less advanced disease. The discrepancy between our work and that of Al Murri et al[18] may be attributable to the timing of biomarker measurement, which was approximately 31 months after treatment in our study but before treatment in the study by Al Murri et al. Our study reflects longer term survival among breast cancer patients and is unlikely to be impacted by tumor burden.

The associations of SAA and CRP with disease-free survival were weaker in magnitude than the associations for SAA and CRP with overall survival, suggesting that these markers may be more closely related to overall survival than breast cancer. However, the CIs for these associations indicate that their magnitudes may be similar.

Table 4. HRs for Overall and Disease-Free Survival by CRP and SAA Tertiles with Censoring of Deaths Caused by Cardiovascular and Cerebrovascular Disease

	Censoring Deaths Caused by Cardiovascular Disease*					Censoring Deaths Caused by Cardiovascular or Cerebrovascular Disease*				
Tertile	No. of Events	No. of Participants	HR	95% CI	*P* (trend)	No. of Events	No. of Participants	HR	95% CI	*P* (trend)
Overall survival†	84	734				80	734			
CRP, mg/L										
≤ 1.2	23	260	1.00	Reference		22	260	1.00	Reference	
1.3-3.8	21	237	1.03	0.54 to 1.96		19	237	1.00	0.52 to 1.93	
≥ 3.9	40	237	2.23	1.22 to 4.08	.007	39	237	2.23	1.20 to 4.16	.008
SAA, mg/L										
≤ 4.2	18	252	1.00	Reference		17	252	1.00	Reference	
4.3-8.0	19	238	0.94	0.48 to 1.85		19	238	1.02	0.51 to 2.04	
≥ 8.1	47	244	2.82	1.55 to 5.13	.0002	44	244	2.80	1.51 to 5.20	.003
lnCRP	84	734	1.27	1.06 to 4.57		80	734	1.27	1.03 to 1.57	
lnSAA	84	734	1.36	1.05 to 1.77		80	734	1.35	1.04 to 1.76	
Disease-free survival‡	88	728				84	728			
CRP, mg/L										
≤ 1.2	22	260	1.00	Reference		21	260	1.00	Reference	
1.3-3.8	30	236	1.71	0.94 to 3.10		28	236	1.68	0.91 to 3.09	
≥ 3.9	36	232	2.14	1.15 to 3.99	.02	35	232	2.09	1.11 to 3.94	.02
SAA, mg/L										
≤ 4.2	26	251	1.00	Reference		25	251	1.00	Reference	
4.3-8.0	23	237	0.96	0.53 to 1.72		23	237	1.04	0.57 to 1.88	
≥ 8.1	39	240	1.59	0.92 to 2.76	.08	36	240	1.53	0.87 to 2.69	.13
lnCRP	88	728	1.21	0.99 to 1.48		84	728	1.20	0.97 to 1.47	
lnSAA	88	728	1.27	0.97 to 1.66		84	728	1.26	0.96 to 1.66	

Abbreviations: HR, hazard ratio; CRP, C-reactive protein; SAA, serum amyloid A.
*Adjusted for age (continuous), disease stage, race/study site, body mass index (continuous), estrogen receptor/progesterone receptor status, and cardiovascular events (histories of heart failure and myocardial infarction).
†Data on overall survival collected through April 30, 2008.
‡Data on disease-free survival collected through September 30, 2004.

Table 5. HRs for Disease-Free and Overall Survival for One-Unit Increases in lnCRP and lnSAA, Stratified by ER and PR Status (localized and regional stage disease only)

	ER Positive				ER Negative				PR Positive				PR Negative			
Survival	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI
Overall survival																
lnCRP	55	403	1.31	1.01 to 1.69	18	110	1.80	0.98 to 3.30	43	319	1.45	1.09 to 1.93	22	151	1.17	0.72 to 1.89
lnSAA	55	403	1.58	1.15 to 2.18	18	110	2.36	1.02 to 5.44	43	319	1.89	1.31 to 2.73	22	151	1.70	0.85 to 3.40
Disease-free survival																
lnCRP	52	399	1.07	0.81 to 1.42	19	109	1.09	0.60 to 1.97	38	316	0.97	0.69 to 1.35	24	149	1.34	0.81 to 2.23
lnSAA	52	399	1.30	0.88 to 1.91	19	109	1.46	0.64 to 3.34	38	316	1.28	0.80 to 2.03	24	149	2.22	1.00 to 4.93

NOTE. HRs were adjusted for age (continuous), disease stage, race/study site, body mass index (continuous), and comorbidities (histories of heart failure and myocardial infarction.

Abbreviations: HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor.

Further investigation is needed to characterize these associations and get more precise risk estimates.

SAA and CRP are regulated by related cytokines, and the amplitude of the inflammation-related increase in SAA is similar to, or slightly greater than, that of CRP.[24] However, the physiologic level of circulating SAA is approximately 10-fold greater than that of CRP, suggesting that SAA may be more useful for detecting slight elevations in systemic or chronic inflammation.[24] SAA is superior to CRP for detecting inflammation associated with severe burns, viral infections, kidney transplantation, Crohn's disease, and ulcerative colitis.[24]

The mechanism by which chronic inflammation is related to breast cancer prognosis is unclear. Chronic inflammation may promote carcinogenesis through complex processes such as polarization of M2 tumor-associated macrophages via cytokines and the subsequent production of tumor growth factors or promotion of angiogenesis.[13,25] In addition, inflammatory status is correlated with several prognostic factors such as body fatness, physical activity, and cardiovascular comorbidity, which may affect prognosis through alternate mechanisms. It is also possible that inflammation is a response to the presence of undetected cancer cells, rather than being solely a contributor to tumor promotion.

CRP and SAA were associated with a self-reported history of myocardial infarction and history of heart failure in the HEAL study,[23] and these histories seem to partially confound the relationship between these inflammatory markers and survival. However, the associations of SAA and CRP with overall survival remained strong after adjustment for these factors. In addition, our analyses restricted to patients not reporting a history cardiovascular events and focused on noncardiovascular deaths suggest that the associations of SAA and CRP with overall survival are not attributable to cardiovascular disease.

To our knowledge, this is the first large, population-based study of post-treatment inflammatory markers and breast cancer survival. Our study measured biomarkers of inflammation at a later time point (approximately 31 months after diagnosis) and thus concerns longer term survival. Accordingly, we did not assess the relationship between inflammation and survival in participants who were not disease free at the time of biomarker measurement (n = 46). However, the timing minimizes the effect of treatment on inflammatory markers, resulting in measurements that reflect long-term inflammatory status. It is possible that acute inflammatory conditions at the time of blood collection caused elevated CRP and SAA concentrations that do not reflect long-term inflammatory status. However, this bias will be nondifferential (ie, similar among those with and without events) and bias risk estimates toward a null effect, unless such acute conditions are also associated with the event. Aromatase inhibitors were not yet available when HEAL patients were diagnosed, and data on HER-2/*neu* status were not routinely collected in SEER; therefore, we could not assess

Table 6. HRs for Disease-Free and Overall Survival for One-Unit Increases in lnCRP and lnSAA, Stratified by Joint ER/PR Status (localized and regional stage disease only)

	ER Positive/PR Positive				ER Negative/PR Negative				ER Negative/PR Positive				ER Positive/PR Negative			
Survival	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI	No. of Events	No. of Participants	HR	95% CI
Overall survival																
lnCRP	41	302	1.39	1.05 to 1.86	14	88	2.01	0.96 to 4.02	2	16	—*	—*	7	62	—*	—*
lnSAA	41	302	1.89	1.30 to 2.74	14	88	2.84	1.03 to 7.86	2	16	—*	—*	7	62	—*	—*
Disease-free survival																
lnCRP	36	299	0.98	0.69 to 1.39	14	87	1.49	0.71 to 3.13	2	16	—*	—*	9	61	—*	—*
lnSAA	36	299	1.36	0.83 to 2.15	14	87	2.09	0.81 to 5.39	2	16	—*	—*	9	61	—*	—*

NOTE. HRs were adjusted for age (continuous), disease stage, race/study site, body mass index (continuous), and comorbidities (histories of heart failure and myocardial infarction.

Abbreviations: HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor.

*There were too few events for regression to converge.

their effects on the associations observed in this study. Furthermore, power to assess associations within and compare associations between ER and PR subgroups was limited as a result of the small number of events in each group. We acknowledge that multiple statistical tests were conducted in this analysis, emphasizing the importance of follow-up studies to confirm these results.

In summary, this study suggests that systemic inflammation, as measured by circulating CRP and SAA, may be an important long-term prognostic factor for breast cancer, even after adjustment for age, stage, BMI, and cardiovascular events. The strong associations between overall survival and these inflammatory biomarkers require further investigation, as do the effects of reductions in inflammatory markers on breast cancer recurrence and survival (either using medications, such as nonsteroidal anti-inflammatory drugs/statins, or lifestyle changes, such as weight loss in overweight/obese patients).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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