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Original article

Tired of feeling tired – The role of circulating inflammatory biomarkers and long-term cancer related fatigue in breast cancer survivors

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ABSTRACT

Background: Low-grade inflammation has been associated with cancer related fatigue (CRF). However, most studies focused on CRF during or shortly after treatment. Longitudinal studies are rare with inconsistent results. We assessed the association of inflammatory biomarkers with total CRF and all subdomains (physical, cognitive, affective) in long-term breast cancer survivors.

Method: Patients recruited between 2002 and 2005 provided information on CRF at first follow-up (FU1) (N = 1292) and second follow-up (FU2) (N = 1205), after a median of 6.2 years and 11.7 years, respectively. Associations of 11 inflammatory biomarkers with CRF at FU1 and at FU2 were assessed using linear regression models. Logistic regression models were used to compare patients fatigued at both timepoints and those never fatigued (N = 932).

Results: C-reactive protein (CRP) was significantly associated with total CRF at FU1 ($\beta = 1.47$, 95%CI = 0.62 −2.31, $p = 0.0007$), at FU2 ($\beta = 1.98$, 95 %CI = 0.96−2.99, $p = 0.0001$) and with persistent CRF (OR = 1.29, 95%CI = 1.13−1.47, $p < 0.0001$). IL-6 levels were associated with total CRF at FU1 ($\beta = 1.01$, 95%CI = 0.43 −1.59, $p = 0.0006$), but not with CRF at FU2 or persistent CRF. No association remained significant after adjustment for relevant covariates.

Discussion: CRP and Il-6 were associated with risk of CRF in long-term breast cancer survivors, but were not independent of other known risk factors, suggesting that currently studied inflammatory markers are not suitable to identify patients at risk of long-term CRF.

1. Introduction

Over the last decade breakthroughs in breast cancer screening and treatment have led to improved survival times and a steadily increasing number of breast cancer survivors [1]. Hence, quality of life[1] (QoL) of long-term breast cancer survivors has become a topic of great research interest. Noticeably, recent studies have pointed out that otherwise healthy breast cancer survivors report lower QoL than cancer-free controls even years after treatment [2,3]. One of the most burdensome and long lasting side-effects is cancer related fatigue (CRF) [4]. It affects up to 99% of patients during treatment [5,6], but, maybe more importantly, continues to be a burden after completion of treatment in one quarter to one third of survivors, limiting activities of day to day life and the resumption of pre-cancer lifestyles [7–11]. To diagnose patients the European Society for Medical Oncology (ESMO) clinical practice guidelines for

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[1] Abbreviations: CRF: Cancer related fatigue; QoL: Quality of life; FU: Follow-Up; ESMO: European Society for Medical Oncology; IL: Interleukin; Ra: Receptor agonist; CRP: C-reactive protein; SAA: Serum amyloid A; ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion protein; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor; CI: Confidence interval; FAQ: Fatigue Assessment Questionnaire; MARIE: Mamma Carcinoma Risk Factor Investigation; CV: Coefficient of variability; MET: Metabolic equivalent of task.

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CRF diagnosis and treatment [12] recommends a 10-point numerical rating scale as a screening tool [12]. Routine screenings are recommended from the point of diagnosis onward, at regular intervals during therapy and aftercare. However, support for long-term breast cancer survivors still suffering from CRF after routine care is lacking. Long-term CRF cannot be explained by type of treatment or tumor characteristics and the underlying biological mechanisms remain unclear [13,14] therefore, patients at risk of long-term CRF cannot yet be identified. Most frequently, but inconclusively studied, are associations between inflammatory biomarkers and CRF. A review by Saligan, Olson [13] concluded that interleukin (IL)-6 or its receptors have most often been investigated in association with CRF, but yielded mixed results. Inconsistent results were also reported for Interleukin 1 receptor antagonist (IL1-ra), soluble tumor necrosis factor receptor type 2 (sTNF-R2) as well as C-reactive protein (CRP). The vast majority of studies assessed fatigue either during or shortly after treatment, neglecting the growing number of long-term survivors still burdened by CRF years later. Longitudinal studies are rare [15–19] and were either exploratory (without controlling for multiple testing) or of relatively small sample size. Assuming moderate associations with individual biomarkers, large prospective studies are required to identify inflammatory biomarkers associated with persistent CRF in long-term breast cancer survivors, which may not only help to identify patients at risk but also yield appropriate targets for intervention.

The study therefore assessed whether 11 inflammatory biomarkers (IL-1β, IL-5, IL-6, IL-8, IL-10, TNF-α, CRP, serum amyloid A (SAA), vascular endothelial growth factor (VEGF), intercellular adhesion molecule (ICAM), and vascular cell adhesion protein (VCAM)) have predictive value for CRF and its subdomains in long-term breast cancer survivors.

2. Material and methods

2.1. Participants

Incident breast cancer patients recruited from 2002 to 2005 into the case–control MARIE (Mamma Carcinoma Risk Factor Investigation) study [20] were re-contacted in 2009 (follow-up 1 (FU1), median 6.2 years) and 2014 (follow-up 2 (FU2), median 11.7 years). Eligible patients were aged 50–74 years at diagnosis with a histologically confirmed primary invasive or in situ breast cancer, had undergone breast surgery and were a resident of one of the study regions (Hamburg or Rhine-Neckar-Karlsruhe).

At baseline/recruitment 3813 patients gave comprehensive information on personal and lifestyle factors through a face-to-face interview. Current clinical data, tumor characteristics and treatment data were abstracted from medical and pathology records. Blood samples were collected at baseline as well as at FU1. Updated information on lifestyle factors at FU1 and FU2 was obtained through computer-assisted telephone interviews. Information on levels of CRF was collected at FU1 and FU2 using the self-administered Fatigue Assessment Questionnaire (FAQ). In addition, pre-diagnosis fatigue was assessed retrospectively at FU1. The studies were approved by the ethics committees of the University of Heidelberg, the State of Rhineland-Palatinate, and the Hamburg Medical Council and were conducted in accordance with the Declaration of Helsinki. All study participants provided informed written consent.

At FU1 3300 patients were eligible and 2326 (70%) returned the fatigue questionnaire. Comparable to our previous work [21] exclusion criteria included recurrences before FU1 (n = 136), missing total fatigue scores (n = 4), missing pre-diagnosis fatigue information (n = 33), missing baseline blood draw (n = 444), and blood draw 7 days or less after surgery (n = 254). We additionally excluded survivors (n = 136) who reported high pre-diagnosis fatigue levels (values ≥ 7 on a 0–10 scale), because these women either suffered from fatigue already before cancer treatment or might have misinterpreted the 0–10 scale. After exclusion 1292 patients, remained for analysis of CRF at FU1 (Fig. 1A), 1205 patients for CRF at FU2 (Figs. 1B) and 932 patients for the analysis of CRF with respect to both time-points (Fig. 1C).

2.3. Fatigue measurement

The FAQ is a 20-item, multidimensional self-assessment questionnaire that has been validated for a German-speaking population [22,23]. It covers the physical, affective, and cognitive dimension of CRF. Possible values for these items were 0 = not at all, 1 = a little, 2 = quite a bit, 3 = very much. The total CRF score was calculated by adding these 20 item scores and standardizing the sum to values from 0 to 100, with higher scores indicating more severe fatigue. Subscores for all CRF dimensions were derived by using the same procedure for the appropriate items. Mean imputation of missing data was performed if less than half of the questions used to calculate the respective score were missing. Scores were used as continuous variables to describe CRF at FU1 and at FU2. To identify patients with persistent CRF, defined as having a high CRF score at both FU time-points, fatigue scores were dichotomized. As in our previous work [21] CRF was considered present when the fatigue score was ≥44, which was the upper tertile score at FU1.

2.4. Circulating biomarker information

Post-diagnosis non-fasting blood samples were processed, divided into aliquots and stored at −80 °C. Biomarkers were measured for patients with available serum or plasma samples for baseline as well as FU1. The median time between surgery and blood draw at baseline was 6.8 months. The MesoScale Discovery (MSD) Electrochemiluminescence platform with multiplex capacity was used to analyse 11 pro- or anti-inflammatory cytokines (IL-1ß, IL-6, IL-8, IL-10, TNF-α (Proinflammatory Panel 1 [human], K15049G); IL-5, VEGF (Cytokine Panel 1 [human], K15050G); SAA, CRP, VCAM-1, ICAM-1 (Vascular Injury Panel 2 [human], K15198G) for 2775 blood samples. Intra- and inter-batch coefficient of variability (CV) ranged from 2.1 to 5.8% and from 2.4 to 10.9%, respectively. For all markers, except IL-6, a normal distributed standard curve was observed. Therefore, non-detectable values for IL-6 were set to missing, while non-detectable values for the remaining inflammatory markers were set to half the detection limit due to highly skewed distributions [24]. Missing values above fit curve range as well as extreme outliers were set to missing, resulting in available biomarker concentrations for between 2729 (e.g., IL-6) and 2761 patients (e.g., TNF-α) [25] (Supplementary Tables 1A and 1B).

3. Statistical analysis

Linear regression models were conducted to assess the effects of inflammatory biomarkers measured at baseline and FU1 on CRF assessed at FU1 and FU2, respectively. Additionally, logistic regression was used to investigate associations of circulating biomarkers at FU1 with patients reporting persistent CRF compared to never fatigued patients. Since some markers were strongly correlated (Supplementary Tables 2A and 2B), we applied further analyses whereby highly correlated biomarkers (Spearman's rank r ≥ 0.5) were added into the model simultaneously to investigate

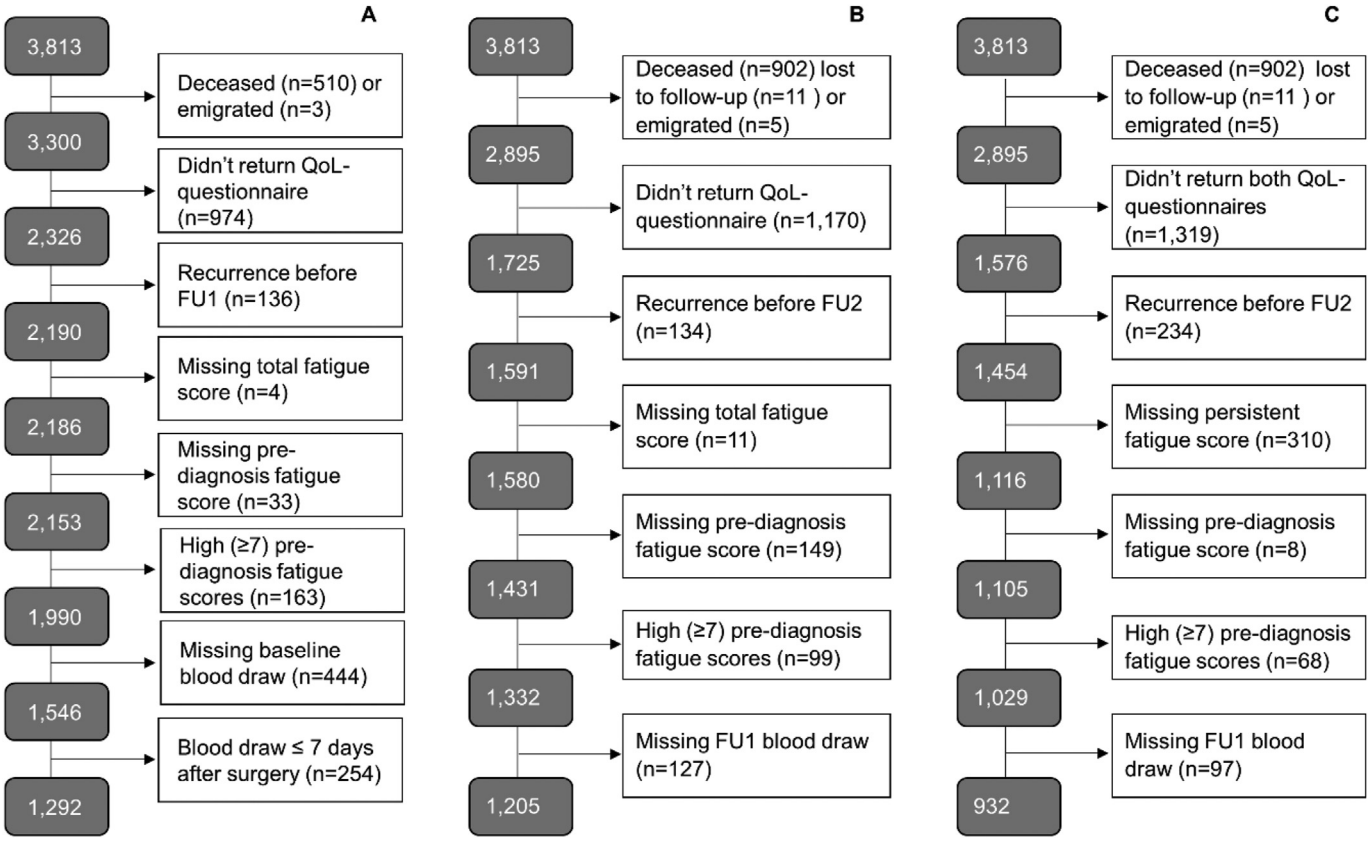

Fig. 1. Patient flow at (A) follow-up 1 (B) follow-up 2 (C) follow-up 1 and 2 combined.

their independent effects.

Fatigue scores for both primary (total CRF) and secondary (physical, cognitive, and affective CRF) outcomes were used as untransformed scores. Since they were slightly right-skewed we explored different transformations (i.e. logarithmic, square root), however none led to a better model fit. Circulating biomarker levels were entered as continuous variables using log transformations to adjust for skewed distributions. To account for the influence of pre-existing fatigue levels unrelated to the disease on subsequent CRF levels, all regression models (basic and fully adjusted) were adjusted for patient's pre-diagnosis fatigue scores. Additional a priori determined covariates included age at diagnosis (continuous), education status (low, medium, high), inflammation related chronic comorbidities (chronic pulmonary diseases, chronic liver disease, chronic bowl disease, chronic bladder and kidney disease, and thyroid disease (yes/no)), BMI (<22.5, 22.5–25, 25.5–30, >30 kg/m^2), and physical activity levels before diagnosis (MET (metabolic equivalent of task) hours/week), depression (yes/no), as well as time between surgery and blood draw [26]. Depression scores were available at FU1 only and used for analyses of CRF at FU1 and FU2. Updated information at FU1 for chronic inflammatory diseases, BMI, and physical activity was available, and was employed for analysis of biomarker with respect to CRF at FU2 and persistent CRF. Multiple testing was adjusted for using the Bonferroni method, thus a significance level of $p = 0.0015$ ($0.05/11 \times 3$) for 11 cytokines analyzed at three endpoints was applied. SAS version 9.4 was used for all statistical analyses.

4. Results

The distribution of CRF scores at FU1 and FU2 is shown in Fig. 2A and B and 3. The median total CRF score at FU1 was 33.3 (Q1 = 16.7, Q3 = 53.3) while the median score at FU2 was slightly lower at 26.7 (Q1 = 13.3, Q3 = 48.3). CRF scores at FU1 and FU2 were highly correlated (Spearman's rank r = 0.64). Table 1 presents the patient characteristics for patients categorized as being fatigued or not for FU1 and FU2 separately as well as for both time-points (persistent CRF). Higher CRF scores at FU1 were associated with higher co-morbidity burden, less physical activity, lower education levels, a higher BMI, and higher pre-diagnosis fatigue scores (Supplemental Table 4). Similar associations were found between these covariates assessed at FU1 and CRF levels at FU2 as well as persistent CRF.

4.1. Association of circulating inflammatory biomarkers with total fatigue

In basic linear regression models, baseline concentrations of IL-6 ($\beta = 1.01$, 95%CI = 0.43–1.59, $p = 0.0006$), and CRP ($\beta = 1.47$, 95% CI = 0.62–2.31, $p = 0.0007$) were significantly associated with CRF scores at FU1 after Bonferroni correction (Table 2A). After adjustment for relevant covariates none of the associations remained significant.

Concentrations of CRP ($\beta = 1.98$, 95%CI = 0.96–2.99, $p = 0.0001$) and SAA ($\beta = 1.67$, 95%CI = 0.68–2.66, $p = 0.0009$) at FU1 were significantly associated with CRF scores at FU2 after correction for multiple testing. When the highly correlated biomarkers CRP and SAA (Spearman's rank r = 0.67) were assessed simultaneously only CRP concentrations at FU1 remained significantly associated with CRF at FU2 ($\beta = 1.97$, 95%CI = 0.96–2.99, $p = 0.0001$). After adjustment for relevant covariates, CRP no longer showed a significant association with CRF at FU2 (Table 2B).

Circulating CRP was the only biomarker associated with an

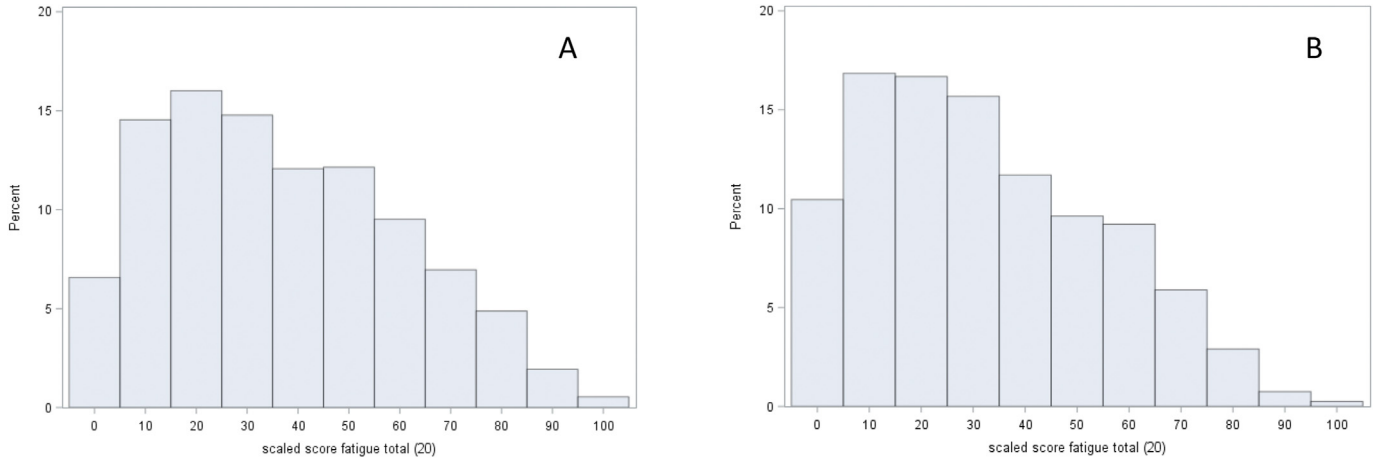

Fig. 2. Distribution of CRF scores at (A) FU1 and (B) FU2.

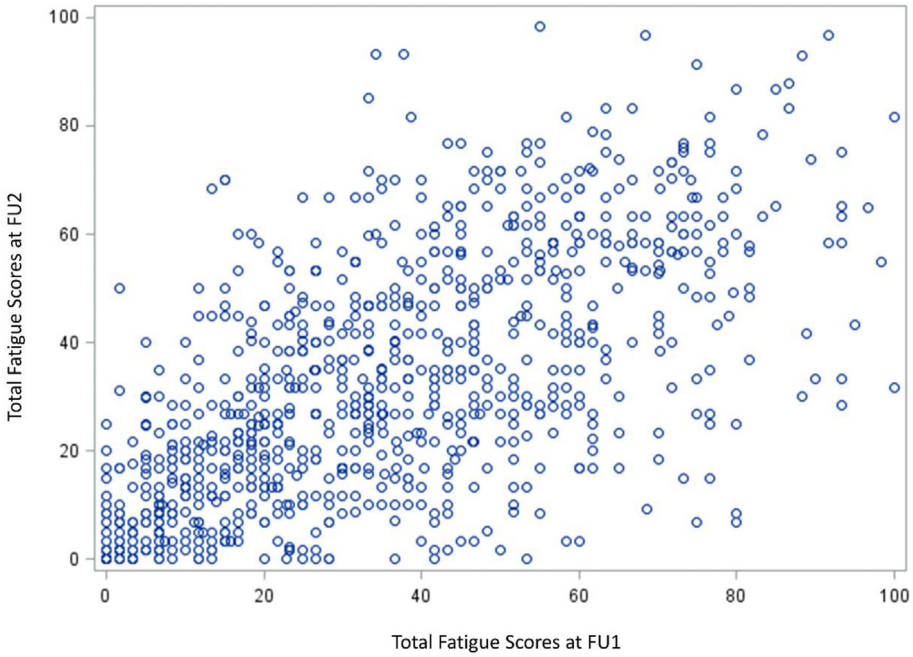

Fig. 3. Scatterplot for CRF distribution assessed at FU1 and FU2.

increased risk of persistent CRF (OR = 1.29, 95%CI = 1.13–1.47, $p \leq 0.0001$) after correction for multiple testing. After adjustment for relevant covariates, the association was no longer significant (Table 2C).

4.2. Circulating inflammatory biomarkers associations for fatigue subdomains

Associations between biomarkers and physical CRF were comparable to those found for total CRF but generally stronger (Supplementary Table 5). For affective and cognitive CRF, there was no association with any biomarker (data not shown).

5. Discussion

We found most consistent associations between the nonspecific inflammatory biomarker CRP and long-term CRF. Elevated levels of CRP were associated with CRF at all time-points. Less consistent results were found for IL-6. Circulating IL-6 had an effect on CRF at FU1, but was not significantly associated with CRF at FU2 or with persistent CRF. CRF subdomain analyses revealed associations of inflammatory biomarkers only with physical CRF, which were comparable to those found for total CRF. Moreover, the associations between inflammatory biomarkers and CRF disappeared after adjusting for relevant covariates, indicating that the associations found are not independent of other determinants for CRF.

5.1. Chronic low-grade inflammation and CRF

Our findings for CRP are in line with those of previous studies that reported associations of long-term CRF and CRP levels both assessed at a mean of four years [15] as well as three months to two

Table 1
Patient characteristics according to fatigue status at FU1, FU2 and persistent CRF.

		Fatigue at FU1[a]				*Fatigue at FU2*[a]				*Persistent Fatigue*[a]			
		No (n = 826)		Yes (n = 466)		No (=860)		Yes (=345)		No (N = 702)		Yes (N = 230)	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Depression[b]	No	732	(89.1)	326	(70.0)	746	(86.7)	249	(72.2)	634	(90.3)	154	(67.0)
	Yes	47	(5.7)	115	(24.7)	79	(9.2)	82	(23.8)	40	(5.7)	67	(29.1)
Chronic Inflam. disease[c]	No	493	(59.7)	212	(45.5)	435	(50.6)	116	(33.6)	361	(51.4)	72	(31.3)
	Yes	331	(40.3)	254	(54.5)	395	(45.9)	219	(63.5)	318	(45.3)	152	(66.1)
Physical Activity	Mean MET hours/week	9.4		6.5		15.2		12.6		16.3		13.0	
Education level	Low	434	(52.5)	314	(67.4)	440	(51.2)	212	(61.4)	342	(48.7)	156	(67.8)
	Medium	246	(29.8)	104	(22.3)	270	(31.4)	78	(22.6)	226	(32.2)	45	(19.6)
	High	146	(17.7)	48	(10.3)	150	(17.4)	55	(15.9)	134	(19.1)	29	(12.6)
BMI	22.5 - <25	189	(22.9)	86	(18.5)	166	(19.3)	59	(17.1)	231	(32.9)	36	(15.7)
	<22.5	235	(28.5)	102	(21.9)	265	(30.8)	65	(18.8)	146	(20.8)	38	(16.5)
	25 - <30	309	(37.4)	172	(36.9)	290	(33.7)	138	(40.0)	222	(31.6)	86	(37.4)
	≥30	93	(11.3)	106	(22.7)	108	(12.6)	73	(21.2)	79	(11.3)	64	(27.8)
Pre-Diagnosis Fatigue score[d]	0	409	(49.5)	112	(24.0)	397	(46.2)	96	(27.8)	350	(49.9)	42	(18.3)
	1	172	(20.8)	85	(18.2)	192	(22.3)	62	(18.0)	151	(21.5)	41	(17.8)
	2	117	(14.2)	104	(22.3)	131	(15.2)	73	(21.2)	103	(14.7)	59	(25.7)
	3	53	(6.4)	72	(15.5)	62	(7.2)	45	(13.0)	42	(6.0)	34	(14.8)
	4	27	(3.3)	36	(7.7)	29	(3.4)	27	(7.8)	21	(3.0)	23	(10.0)
	5	29	(3.5)	39	(8.4)	28	(3.3)	26	(7.5)	20	(2.8)	18	(7.8)
	6	19	(2.3)	18	(3.9)	21	(2.4)	16	(4.6)	15	(2.1)	13	(5.7)
Stage (S)	1	387	(46.9)	237	(50.9)	437	(50.8)	165	(47.8)	352	(50.1)	107	(46.5)
	2a/2b	301	(36.4)	146	(31.3)	293	(34.1)	119	(34.5)	239	(34.0)	80	(34.8)
	3a/3b/3c	56	(6.8)	34	(7.3)	49	(5.7)	25	(7.2)	42	(6.0)	16	(7.0)
	4	4	(0.5)	0	(0.0)	4	(0.5)	0	(0.0)	4	(0.6)	0	0
	In situ	14	(1.7)	16	(3.4)	18	(2.1)	10	(2.9)	51	(7.3)	19	(8.3)
	Neo adj. CTX	64	(7.7)	33	(7.1)	59	(6.9)	26	(7.5)	14	(2.0)	8	(3.5)
Grade (G)	Low	175	(21.2)	98	(21.0)	178	(20.7)	75	(21.7)	148	(21.1)	49	(21.3)
	Moderate	400	(48.4)	240	(51.5)	431	(50.1)	163	(47.2)	343	(48.9)	106	(46.1)
	High	171	(20.7)	78	(16.7)	171	(19.9)	70	(20.3)	144	(20.5)	47	(20.4)
Nodal Status (N)	0	558	(67.6)	308	(66.1)	592	(68.8)	226	(65.5)	481	(68.5)	147	(63.9)
	1–3	147	(17.8)	82	(17.6)	154	(17.9)	63	(18.3)	126	(17.9)	44	(19.1)
	4–9	32	(3.9)	21	(4.5)	32	(3.7)	12	(3.5)	25	(3.6)	8	(3.5)
	>10	11	(1.3)	6	(1.3)	5	(0.6)	8	(2.3)	5	(0.7)	4	(1.7)
Tumor Size (T)	<2 cm	471	(57.0)	283	(60.7)	525	(61.0)	198	(57.4)	423	(60.3)	131	(57.0)
	2–4 cm	246	(29.8)	120	(25.8)	233	(27.1)	101	(29.3)	191	(27.2)	64	(27.8)
	≥5 cm	19	(2.3)	11	(2.4)	16	(1.9)	8	(2.3)	14	(2.0)	6	(2.6)
	Into Chest	12	(1.5)	3	(0.6)	9	(1.0)	2	(0.6)	9	(1.3)	2	(0.9)
Age at Diagnosis	median	62		62		61		63		61		62	

[a] Baseline and FU1 values were used to describe patients at FU1 and at FU2/persistent fatigue, respectively.
[b] Depression scores were only available at FU1.
[c] Chronic inflammatory diseases include chronic pulmonary diseases, chronic liver disease, chronic bowl disease, chronic bladder and kidney disease, and thyroid disease.
[d] For pre-diagnosis fatigue scores patients with high scores (≥7) are excluded as described in the exclusion criteria.

years after primary treatment [19]. Another longitudinal-study found that CRP measured 30 months post diagnosis had predictive value for CRF assessed 39 months post diagnosis. Yet, in a study of 84 HER2-negative breast cancer survivors, no association was found for CRP and CRF measured concurrently before, during and two year after treatment [18]. Our results for IL-6 are corroborated by a small study of 50 breast cancer patients at a mean of 5 years post diagnosis, which reported associations with IL-1ra, IL-6 and TNF-α [10,17], but not by another study that reported no association for IL-6, IL-1ra, sTNF-R1 or neopterin [15]. Differences in results may be explained by several methodological aspects. First, the definition of CRF is heterogeneous. Questionnaires time of administration differed between studies. Secondly, a clear cut-off point for clinical relevant CRF scores has not been defined. Therefore, dichotomization of fatigued versus non-fatigued patients, as applied in most studies, is still based on subjective evaluation. Several studies support the use of fatigue as a continuous outcome [27–29]. Thus, we analyzed associations of inflammatory biomarkers with CRF as a continuous variable in addition to dichotomization for persistent CRF. In this way potential bias or loss of power due to exclusion of a subset of the study subjects was avoided. Thirdly, time between blood draw and CRF assessment differed between our and other studies. We aimed to investigate the hypothesis that chronic low grade inflammation, which can last for years [30], is associated with persistent CRF. Since biological mechanisms of chronic and acute inflammation differ, discrepancies between our results and studies using a simultaneous assessment of CRF and biomarkers are to be expected.

Noteworthy, inconsistent results for both CRP and IL-6 underline a recognized opinion that the independent diagnostic value of the two markers may be limited [31]. The production of CRP is part of the nonspecific acute-phase response to most forms of inflammation, tissue damage or infection and is stimulated by the cytokine IL-6. However, CRP and IL-6 may also be elevated in the absence of inflammation and even have anti-inflammatory effects [32]. Therefore, it has been argued that unambiguous inflammatory biomarkers such as IL-1β and TNF-α may be needed to identify chronic low-grade inflammation. Our data showed that neither IL-1β nor TNF- α had a significant effect on CRF at FU1, FU2 or on persistent CRF (Tables 2A,2B and 2C). These results suggest that if low grade inflammation is the underlying biological mechanism causing long-term CRF in breast cancer patients, the relevant biomarkers have not yet been identified or the existing studies including our own are not adequately powered to detect very moderate effects.

Table 2
Associations of circulating biomarkers with total CRF at FU1 (A), FU2 (B) and persistent CRF (C).

	Basic[a]		Multivariate[b]	
	β (95% CI)	p	β/OR (95% CI)	p
A. CRF at FU1[c]				
IL-1β	0.38 (−0.17, 0.94)	0.17	0.23 (−0.30, 0.76)	0.39
IL-5	0.40 (−0.66, 1.46)	0.46	−0.11 (−1.13, 0.90)	0.83
IL-6	**1.01 (0.43, 1.59)**	**0.0006**	0.31 (−0.27, 0.89)	0.30
Il-8	0.61 (0.19, 1.03)	0.004	0.32 (−0.10, 0.74)	0.13
IL-10	1.42 (0.49, 2.34)	0.003	0.30 (−0.61, 1.21)	0.51
TNF-α	0.96 (0.01, 1.92)	0.05	0.29 (−0.63, 1.21)	0.54
CRP	**1.47 (0.62, 2.31)**	**0.0007**	0.47 (−0.39, 1.34)	0.28
SAA	0.63 (−0.21, 1.47)	0.14	0.01 (−0.82, 0.84)	0.99
ICAM	1.33 (−0.75, 3.41)	0.21	0.78 (−1.19, 2.74)	0.44
VCAM	0.31 (−2.02, 2.64)	0.80	−0.16 (−2.37, 2.05)	0.89
VEGF	1.19 (−0.18, 2.56)	0.09	0.65 (−0.67, 1.96)	0.33
B. CRF at FU2[c]				
IL-1β	0.13 (−0.29, 0.54)	0.55	0.17 (−0.23, 0.58)	0.27
IL-5	1.12 (−0.11, 2.36)	0.08	0.75 (−0.48, 1.98)	0.52
IL-6	0.19 (−0.25, 0.63)	0.40	0.16 (−−0.27, 0.59)	0.30
Il-8	−0.05 (−0.46, 0.36)	0.81	0.07 (−0.33, 0.47)	0.21
IL-10	0.61 (−0.36, 1.59)	0.22	0.52 (−0.43, 1.48)	0.46
TNF-α	0.39 (−0.22, 1.00)	0.21	0.40 (−0.19, 1.00)	0.47
CRP	**1.98 (0.96, 2.99)**	**0.0001**	0.59 (−0.48, 1.66)	0.44
SAA	**1.67 (0.68, 2.66)**	**0.001**	0.88 (−0.11, 1.87)	0.42
ICAM	2.84 (0.70, 4.99)	0.01	2.20 (0.10, 4.30)	1.00
VCAM	2.55 (0.02, 5.08)	0.05	1.11 (−1.39, 3.62)	1.13
VEGF	0.12 (−1.09, 1.32)	0.85	0.14 (−1.02, 1.30)	0.67
C. Persistent CRF[c]				
IL-1β	1.01 (0.96, 1.07)	0.66	1.01 (0.95, 1.07)	0.73
IL-5	1.04 (0.89, 1.22)	0.60	1.01 (0.84, 1.21)	0.93
IL-6	1.03 (0.97, 1.09)	0.34	1.02 (0.95, 1.09)	0.58
Il-8	0.99 (0.94, 1.04)	0.62	0.99 (0.94, 1.05)	0.80
IL-10	1.05 (0.93, 1.19)	0.42	1.04 (0.90, 1.20)	0.58
TNF-α	1.02 (0.94, 1.10)	0.64	1.02 (0.93, 1.11)	0.73
CRP	**1.29 (1.13, 1.47)**	**< .0001**	1.12 (0.96, 1.31)	0.15
SAA	1.19 (1.06, 1.35)	0.005	1.15 (0.99, 1.32)	0.06
ICAM	1.28 (0.96, 1.71)	0.09	1.26 (0.91, 1.75)	0.17
VCAM	1.23 (0.88, 1.74)	0.23	1.16 (0.78, 1.70)	0.47
VEGF	1.11 (0.95, 1.31)	0.19	1.11 (0.93, 1.33)	0.24

bold = significant after Bonferroni correction
[a] Basic models are adjusted for pre-diagnosis fatigue.
[b] Multivariate models are additionally adjusted for BMI, physical activity, education, comorbidities (depression and chronic inflammatory diseases), and time of blood draw (for analyses on CRF at FU1 only).
[c] Biomarker measurements at baseline and FU1 were used to describe patients at FU1 and FU2/persistent fatigue, respectively.

5.2. Other determinants of long-term CRF

Our analyses showed that education levels, BMI, physical activity levels, chronic inflammatory diseases as well as depression and pre-diagnosis fatigue scores were strong predictors for long-term CRF. After adjustment for these factors all previously strongly associated biomarkers lost their independent effects on CRF. These results as well as inconsistent findings on inflammatory biomarkers and CRF suggest that lifestyle, comorbidities and socioeconomic factors may be more suitable to identify patients at risk of long-term CRF than circulating inflammatory biomarkers. While biomarkers are highly sensitive to external factors, lifestyle and socioeconomic factors as well as inflammation related comorbidities have been shown to be reliable determinants throughout the trajectory of the disease and its treatment [33,34].

Modifiable lifestyle factors such as physical activity and BMI are not only consistently associated with CRF, they have also been shown to be effective targets in the treatment of CRF. A review on the role of exercise in reducing CRF states that research supports the ability of exercise to reduce CRF, especially when conducted in supervised interventions [35]. Nevertheless, authors concluded that the prescription of exercise is far from becoming a part of standard care and further intervention studies are necessary to endorse clinical implementation. It is of interest to note that a recent randomized controlled intervention trial in breast cancer patients demonstrated that an exercise intervention lead to reduced CRF leves, but inflammatory biomarkers (Il-6 and TNF-α) did not differ between experimental and control group [36].

5.3. Strengths and limitations

We accounted for pre-diagnosis fatigue levels, which have been repeatedly shown to be more strongly associated with CRF than other relevant factors [37,38]. It is well established that chronic fatigue syndrome, an illness unrelated to cancer and its treatment, and CRF are distinct diseases, with different biological underpinnings [39]. Therefore, accounting for pre-diagnosis fatigue levels is crucial to ensuring a homogeneous phenotype.

In addition, our study took into account that CRF is multimodal. We were able to show that associations for physical CRF were comparable to those found for total CRF, whereas cognitive and affective CRF were not associated with any biomarker under investigation. The comparability in results for total and physical CRF could in part be explained by questionnaire design. While physical CRF is derived from eleven out of 20 items, only five items are used to build the affective subdomain and three items for cognitive CRF. Null findings for affective and cognitive CRF, however, suggest that CRF needs to be treated as a multimodal disease with independent subdomains that might have different underlying biological mechanisms.

It has to be noted that information on pre-diagnosis fatigue was assessed retrospectively at FU1 rather than before cancer onset. Even though, the high correlation between pre-diagnosis fatigue scores and CRF found in our data is in accordance to that reported by previous studies, we cannot exclude the possibility that the retrospective assessment of pre-diagnosis fatigue may have led to some over-adjustment for pre-diagnosis fatigue and thus conservative findings.

5.4. Sensitivity analyses

We conducted sensitivity analyses to account for varying time intervals between surgery and blood draw at baseline as well as follow-up times between baseline blood draw and assessment of CRF at FU1. The associations of CRF with both IL-6 (β = 0.8, 95% CI = 0.2–1.4, p = 0.0095) and CRP (β = 1.2, 95%CI = 0.3–2.1, p = 0.0076) remained significant on a nominal level but were not significant after Bonferroni correction. Since pre-diagnosis fatigue was retrospectively assessed and prone to recollection bias we conducted sensitivity analyses excluding pre-diagnosis fatigue scores from the adjusted models. Associations between biomarkers and CRF remained non-significant at all time-points. Further sensitivity analyses were conducted excluding all patients with biomarker concentrations 20% or more above highest standard. Again results did not change (data not shown) at either time-point.

6. Conclusion

We were able to confirm previous findings for circulating CRP and Il-6 in association with risk of CRF in long-term breast cancer survivors. However, findings do not provide support for suspected pathways of low-grade inflammation as underlying biological mechanisms of long-term CRF. The currently associated inflammatory markers are not independent of other known risk factors and therefore not suitable to identify patients at risk. However, research on inflammatory biomarkers to better understand biological pathways associated with CRF is still warranted to develop

targeted pharmaceutical therapy options.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2021.02.008.

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