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Abstract

Purpose: Studies examining biomarkers associated with fatigue in breast cancer survivors treated with radiation are limited. Therefore, we examined the longitudinal association between serum biomarkers and post-breast cancer fatigue in survivors treated with radiation: [oxidative stress] 8-hydroxyguanosine, myeloperoxidase; [inflammation] interleukin-6 (IL-6), c-reactive protein, growth differentiation factor-15 (GDF-15), placental growth factor, transforming growth factor-beta, [cardiac damage] cystatin-C, troponin-I.

Methods: In a secondary analysis, we included participants from the Women's Health Initiative if they had: a previous breast cancer diagnosis (stages I-III), no prior cardiovascular diseases, pre-and post-breast cancer serum samples drawn approximately 3 years apart, and fatigue measured using the Short-Form 36 vitality subscale at both serum collections. Biomarkers were measured using ELISA or RT-qPCR and modeled as the $\log_2$ post-to pre-breast cancer ratio.

Results: Overall, 180 women with a mean (SD) age of 67.0 (5.5) years were included. The mean (SD) vitality scores were 66.2 (17.2) and 59.7 (19.7) pre- and post-breast cancer, respectively. Using multivariable weighted linear regression, higher biomarker ratios of cystatin-C, IL-6, and GDF-15 were associated with a lower vitality score (i.e., higher fatigue). For example, for each 2-fold difference in cystatin-C biomarker ratio, the vitality score was lower by 7.31 points (95% CI: −14.2, −0.45).

Conclusion: Inflammatory and cardiac damage biomarkers are associated with fatigue in breast cancer survivors treated with radiation; however, these findings should be replicated in a larger sample. Biomarkers could be measured in clinical practice or assessed in risk prediction models to help identify patients at high risk for fatigue.

Keywords

breast cancer, fatigue, radiation, biomarkers, inflammation, oxidative stress

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Survival rates for breast cancer have improved and there are an estimated 3.8 million breast cancer survivors in the United States as of 2021 (Miller et al., 2016). Radiation therapy is an effective therapy for breast cancer and is administered to approximately 50% of breast cancer patients (Bower, 2014; Clark et al., 2016; Gilliam & St Clair, 2011; Neilsen et al., 2017). However, despite treatment benefits, radiation is a substantial contributor to fatigue. Fatigue occurs in approximately 33% of all breast cancer survivors and is associated with decrements in activities of daily living, increased depression, reduced quality of life, and increased mortality (Aversa et al., 2017; Bower, 2014; Hofman et al., 2007).

While there is an abundance of literature examining biomarkers of fatigue associated with systemic therapies, studies focused specifically on fatigue associated with radiation treatment are limited. Based on few prior studies, the leading hypotheses linking radiation to fatigue involve inflammation, as studies have shown associations between increased inflammatory markers and fatigue in breast cancer patients receiving radiation (Bower, 2014; LaVoy et al., 2016; Saligan & Kim, 2012; Saligan et al., 2015). However, based on the biological consequences of radiation, there may be additional mechanisms contributing to fatigue such as oxidative stress and cardiac damage. Oxidative stress has been proposed as an underlying mechanism explaining fatigue in the context of chemotherapy; yet it has been investigated minimally in the context of radiation (Hockenberry et al., 2014; Repka & Hayward, 2018). Cardiac damage is also a well-recognized complication of radiation therapy through a complex process of direct tissue injury, acute and chronic inflammation, oxidative stress, and impaired remodeling (Lenneman & Sawyer, 2016; Taunk et al., 2015). Cardiac dysfunction has been associated with reductions in cardiorespiratory fitness, exercise intolerance, and fatigue (Haykowsky et al., 2016). However, cardiac damage biomarkers have not been investigated as potential markers of fatigue in cancer survivors.

The purpose of this study was to examine biomarkers of oxidative stress [8-OH-dG, myeloperoxidase (MPO)], inflammation [c-reactive protein (CRP), growth differentiation factor-15 (GDF-15), interleukin-6 (IL-6), placental growth factor (PGF), transforming growth factor-beta (TGF-B)], and cardiac damage [cystatin-C, troponin-I] in the development of fatigue in breast cancer survivors treated with radiation.

Methods

Study Population and Design

We conducted a secondary analysis of data collected as part of an ancillary case-control study conducted within the Women's Health Initiative (WHI). Detailed descriptions of the overall WHI study design and recruitment have been published elsewhere (Anderson et al., 2003). In brief, the WHI is a longitudinal, prospective cohort study of 161,808 women aged 50–79 who were enrolled at 40 clinical centers nationwide beginning in 1993. The WHI has two main components, a randomized Clinical Trial and an Observational Study. Data were collected in the main study until 2005 and women were asked to participate in follow-up extension studies for an additional 5 years of follow-up through 2010 and again through 2015 (Anderson et al., 2003). In 2013, women with no prior cancer diagnosis at WHI enrollment were invited to participate in the Life and Longevity After Cancer (LILAC) study, a cancer survivorship cohort study, if they developed 1 of 8 cancers during WHI follow-up (breast, colorectal, endometrial, ovarian, lung, melanoma, leukemia and lymphoma) (Paskett et al., 2018). All participants provided written informed consent prior to data collection.

The current analysis uses data collected as part of an original case-control study within the WHI aimed at investigating biomarkers associated with cardiovascular events in breast cancer survivors treated with radiation. In the original case-control study, eligible women were those diagnosed with incident invasive breast cancer (stages I-III) and had documentation of radiation treatment. Radiation treatment was documented if a participant was either enrolled in LILAC or were enrolled in Medicare fee-for-service at the time of their breast cancer diagnosis. For participants in LILAC, radiation was documented either through medical record abstraction, Medicare fee-for-service data, or by self-report on the LILAC questionnaires. Incident breast cancer was defined as the first invasive cancer adjudicated during WHI follow-up through medical chart review. The original case-control required pre- and post-breast cancer diagnosis serum samples drawn approximately three follow-up years apart with the breast cancer diagnosis occurring in between the two blood collections. Women were excluded from the original case-control study if they (1) had metastatic disease or missing stage, (2) had an adjudicated or self-reported cardiovascular outcome (i.e., heart failure, coronary heart disease, or stroke) at WHI baseline or before the second blood draw, or (3) self-reported a history of breast, lung, lymphoma, Hodgkin's, or thyroid cancers at WHI baseline. In the original case-control study, participants who developed a cardiovascular outcome after the second blood draw were matched to participants who did not develop a cardiovascular outcome after the second blood draw in a 1:3 ratio without replacement on age at WHI enrollment (5-year categories), visit year of the pre-breast cancer specimen draw, treatment ascertainment (self-report or Medicare/abstraction), and LILAC enrollment (yes/no). The original case-control study included a total of 213 women (55 cases and 158 controls).

For the current analysis, we further excluded women in the original case-control study who did not have fatigue measured at both timepoints of serum collection ($n = 33$). A total of 180 participants were included in the current analysis (Figure 1). We used inverse probability weighting to account for the selection into the original case-control study. Methods have been developed to allow for analyses of secondary outcomes using case-control data while considering the sampling design

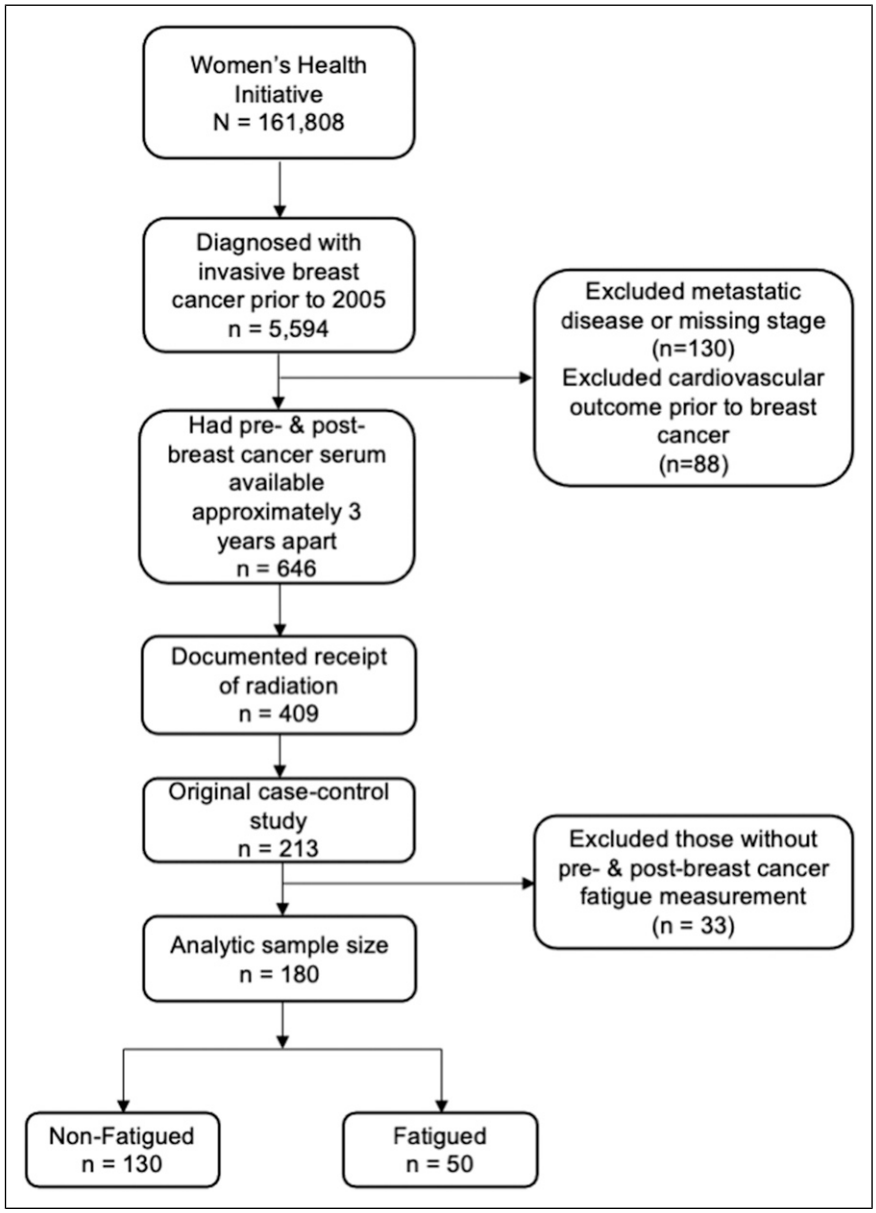

Figure 1. Sample flow chart. Participants were eligible for the original case-control study if they were diagnosed with invasive breast cancer prior to 2005 and met the following criteria: (1) had a pre- and post-breast cancer diagnosis serum sample available approximately 3 years apart and (2) had documented receipt of radiation treatment either through medical record abstraction, Medicare claims data, or self-report. For this analysis, participants were excluded if they were missing a pre- or post-breast cancer fatigue measurement (n = 33). A total of 180 participants were included.

of the original case-control study (Reilly et al., 2005; Richardson et al., 2007; Schifano, 2019). This method uses sampling weights to create data, which resembles a random sampling design with respect to the secondary outcome (i.e., fatigue).

Outcome

The primary outcome in this analysis was post-breast cancer fatigue, which was measured at the same time as the post-cancer diagnosis biomarkers. Fatigue was measured in the WHI using the Short-Form (SF)-36 vitality subscale. In summary, participants were asked how often they felt full of pep, worn out, tired, or had a lot of energy during the last 4 weeks. Individual questions were scored from 1 to 6, which were transformed to create a total index score, which ranges from 0–100. Higher scores indicate less fatigue. Fatigue in cancer populations has been phenotypically characterized with the SF-36 using a cutoff of 50, with scores <50 being "fatigued" and scores ≥50 being "non-

fatigued" (Bower et al., 2002). Additionally, prior studies have reported a change of five points on the SF-36 to represent a clinically minimal important difference (Bjorner et al., 2007). The SF-36 has high internal consistency in cancer populations with alpha coefficients ranging from 0.89 to 0.91 (Brown et al., 2011; Stein et al., 2004).

Exposure

The exposures for this study were post-cancer diagnosis biomarker concentrations. The WHI has detailed protocols regarding specimen collection, handling, preparation, and storage. In summary, WHI staff were trained in standardized methods of specimen collection and processing. Serum was centrifuged and separated from blood samples within 1 hour after collection. Samples were maintained at 4°C during handling. After separation, samples were separated into 0.25 mL aliquots and placed into a −80°C freezer within 2 hours of collection for future use.

All biomarkers were measured using commercially available assay kits (Supplemental Table 1). Except for IL-6 and Troponin-I, biomarkers were measured using enzyme-linked immunosorbent assays (ELISAs). TnI and IL-6 were measured using ProQuantum RT-PCR kits. The biomarkers assays were conducted in the University of Washington School of Nursing Office for Nursing Research laboratory. All biomarkers were tested and analyzed following manufacturer's protocols. For ELISAs, samples were tested in duplicates and in triplicates for RT-PCR. The average of the replicates was used in the analysis. All participants were randomly intermixed on each plate and laboratory personnel were blinded to sample IDs. Lastly, to ensure quality control, the WHI included 22 pairs (i.e., 44 samples) of blinded duplicate samples, with approximately half measured on the same plates and half measured on different plates to account for within and between plate variation. All biomarker kits had an intra-assay coefficient of variation <10% and inter-assay coefficient of variation <15%.

Additional Variables

Demographic information on age at WHI enrollment, education, and self-identified race/ethnicity were collected from WHI baseline questionnaires. Data on pre-cancer fatigue, sleep disturbance, emotional well-being, pain, and physical function were collected from self-report questionnaires. Pre-cancer fatigue, emotional well-being, pain, and physical function were measured using the SF-36 vitality, emotional well-being, pain, and physical functioning subscales, respectively. All subscales range from 0–100 points, with higher scores indicating a better health status. Sleep disturbance was measured using the WHI Insomnia Rating Scale. The Insomnia Rating Scale scores range from 0–20 with higher scores indicating greater sleep disturbance.

Lifestyle factors of smoking status, alcohol consumption, and physical activity were recorded from self-reported questionnaires. Smoking status was reported as current, former, or never smoker. Alcohol consumption was calculated by the number of alcoholic servings per week which includes beer, wine, and/or liquor, based on standard serving sizes (i.e., 12 oz of beer, 6 oz of wine, and 1.5 oz of liquor). Physical activity was recorded as the number of total-metabolic equivalents hours per week (metabolic equivalent-hours/week) (Ainsworth et al., 2000; McTiernan et al., 2003).

Physical measurements including height, weight, and waist circumference (cm) were measured in-person at baseline clinic visits by trained WHI personnel. Body mass index was calculated as weight in kg/height in m^2.

Cancer characteristics such as stage, laterality of breast cancer, concurrent chemotherapy treatment, and initiation of radiation treatment were obtained from medical records or self-report questionnaires. Stage was classified according to the Surveillance, Epidemiology, and End Results coding rules. For participants in LILAC, data on concurrent chemotherapy treatment and timing of radiation therapy initiation were available.

Statistical Analysis

Participants' baseline characteristics were compared between those with and without fatigue using a cut point of 50. Normality of continuous variables was visually assessed. Characteristics were summarized with mean and standard deviations for continuous variables and proportions for categorical variables. Differences in mean values or proportions were determined by unpaired t-tests and chi-square tests, respectively.

Distributions of pre- and post-cancer biomarkers were calculated using both means with standard deviations and medians with interquartile range (IQR) stratified by fatigue using a cut point of 50. Differences in medians between pre- and post-cancer biomarkers by fatigue status were tested using Wilcoxon rank tests given the non-normal distribution of the biomarkers.

Weighted multiple variable linear regression was used to evaluate the association of independent variables (post-cancer diagnosis biomarkers) with fatigue post-cancer diagnosis as the dependent variable. All biomarkers, except TnI and PGF, were modeled as the ratio of the post-cancer value relative to the pre-cancer biomarker. Given the non-normal distribution of the biomarkers, this ratio was log transformed to base 2. Each unit difference in the biomarker ratio represents a doubling in the biomarker value compared to pre-cancer. Given TnI and PGF were under the detection limit in approximately 50% of participants, the linearity assumption was violated. Thus, we modeled TnI and PGF as categorical variables defined as either above or below detection (reference) (Supplemental Table 1). Models for TnI and PGF were also adjusted for the $\log_2$ of the pre-cancer biomarker

concentrations. Fatigue was analyzed as a continuous variable. A separate model was created for each biomarker. Covariates were selected a priori based on scientific and clinical rationale. Multivariable models were adjusted for age (5 year-categories), education (high school/GED or less, > high school – bachelor's degree, > bachelor's degree), smoking (pack-years), BMI (kg/m^2), physical activity (total metabolic equivalent-hours/week), alcohol consumption (servings per week), cancer stage (local/regional, distant), and pre-cancer emotional well-being, physical function, pain, sleep disturbance, and fatigue. Stratified sampling fractions based on the original case-control study matching criteria were calculated and were used in the regression model to account for participant selection into the original case-control study. Confidence intervals were calculated using robust standard errors to account for the correlation among weighted observations as described previously (Monsees et al., 2009).

Two pre-specified exploratory sensitivity analyses were conducted. First, concurrent chemotherapy was only obtained in participants enrolled in LILAC. To determine if inclusion of concurrent chemotherapy in the models influenced the results, we repeated the main analysis in participants in LILAC with chemotherapy treatment data available (n = 105). We ran the original models with the addition of chemotherapy (yes/no) as a covariate. Additionally, to determine if the timing of serum collection in relation to breast cancer diagnosis influenced the results, we created a variable to represent the timing of biomarker collection as either <1 year, 1–2 years, or >2 years after breast cancer diagnosis. We included an interaction term in the main models between this timing variable and each biomarker. The overall interaction was tested using the likelihood ratio test.

All analyses were conducted using R Version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Two-sided p-values are reported with an alpha of 0.05 used to determine statistical significance.

Results

Baseline Sample Characteristics

Of the 180 participants, 50 (27.8%) were classified as fatigued and 130 (72.2%) were classified as non-fatigued based on a SF-36 vitality subscale cut point of 50. The mean (SD) age at WHI enrollment was 67.0 (5.5) years and the majority of participants self-identified as non-Hispanic White (93.9%), had at least a high school diploma (48.3%) with many having earned at least a bachelor's degree (40.6%), and were diagnosed with regional cancer (77.8%) (Table 1). Among those in LILAC with chemotherapy data available, 29 (27.6%) received chemotherapy. The median (IQR) time from breast cancer diagnosis to the post-cancer serum collection was 1.4 (0.7, 2.3) years. Among those in LILAC with available data on the timing of radiation, the median (IQR) time from breast cancer diagnosis to radiation was 63 (40, 97) days and the median (IQR) time from radiation treatment to post-cancer serum collection was 1.0 (0.4, 2.0) years.

When comparing participants with and without fatigue, individuals with fatigue were more likely to have a higher BMI and waist circumference, engage in less physical activity, have higher rates of pain, emotional well-being, pre-cancer fatigue, and sleep disturbance, and report lower physical function (Table 1).

Distribution of Pre- and Post-Cancer Biomarkers

Serum concentrations were above the limit of detection for all biomarkers except for PGF and TNI. For PGF, 102 and 106 participants had undetectable concentrations for pre- and post-cancer time points, respectively. For TNI, 94 and 88 participants had undetectable concentrations for pre- and post-cancer time points, respectively. When comparing pre-cancer serum biomarkers between participants with and without fatigue, those with fatigue had higher concentrations of CRP (median 9.1 vs. 5.1 mg/L), IL-6 (median 5.7 vs. 4.1 pg/mL), and cystatin-C (median 1103 vs. 992 ng/mL) (Figure 2, Supplemental Table 2). For post-cancer serum biomarkers, participants with fatigue had significantly higher concentrations of CRP compared to those without fatigue (4.4 vs. 2.6 mg/L). When comparing differences in concentrations between pre- and post-cancer biomarkers, participants without fatigue had higher concentrations of cystatin-C and GDF-15 and lower concentrations of TGF-B and CRP post-cancer. Participants with fatigue had higher concentrations of GDF-15 and lower concentrations of CRP and MPO post-cancer.

Weighted Linear Regression for Association of Post-Cancer Biomarkers With Fatigue Scores

After multivariable adjustment, higher ratios of cystatin-C, IL-6, and GDF-15 levels were all associated with a lower SF-36 vitality score indicating higher fatigue (Figure 3). For a 2-fold difference in each biomarker ratio, the SF-36 vitality score was lower by 7.31 (95% CI: −14.2, −0.45), 4.45 (95% CI: −7.62, −1.29), and 6.67 (95% CI: −12.3, −0.99) points for cystatin-C, IL-6, and GDF-15, respectively (Supplemental Table 3).

Exploratory Sensitivity Analyses

Of the 105 participants with chemotherapy data available, 29 received chemotherapy. The most common therapies administered were cyclophosphamide, doxorubicin, and fluorouracil (Supplemental Table 4). In the restricted analysis among LILAC participants, higher IL-6 ratios were associated with greater fatigue after adjustment for chemotherapy (Supplemental Table 5). Additionally, significant associations emerged such that higher MPO and TNI ratios were associated with lower fatigue (Supplemental Table 5).

Table 1. Baseline (i.e., Pre-Cancer) Participant Characteristics Stratified by Fatigue Scores.

	Overall (*N* = 180)	Non-fatigued[a] (*N* = 130)	Fatigued[a] (*N* = 50)	*p*-Value
Demographics				
Age at breast cancer diagnosis, mean (SD)	69.1 (5.5)	68.6 (5.4)	70.3 (5.5)	0.067
Age at WHI enrollment (5 years), *n* (%)				0.100
55–59	23 (12.8)	16 (12.3)	7 (14.0)	
60–64	28 (15.6)	24 (18.5)	4 (8.0)	
65–69	62 (34.4)	46 (35.4)	16 (32.0)	
70–74	59 (32.8)	41 (31.5)	18 (36.0)	
75–79	8 (4.4)	3 (2.3)	5 (10.0)	
Race/Ethnicity, *n* (%)				0.711
Non-hispanic white	169 (93.9)	121 (93.1)	48 (96.0)	
Non-hispanic black	4 (2.2)	3 (2.3)	1 (2.0)	
Other[b]	7 (3.9)	6 (4.6)	1 (2.0)	
Education, *n* (%)				0.231
High School/GED or less	20 (11.1)	12 (9.2)	8 (16.0)	
> High School – Bachelor's	87 (48.3)	61 (46.9)	26 (52.0)	
> Bachelor's	73 (40.6)	57 (43.8)	16 (32.0)	
Clinical characteristics, mean (SD)				
Smoking (pack-years)[c]	11.6 (18.7)	11.6 (18.6)	11.6 (19.2)	0.988
Body mass index (kg/m^2)	27.3 (6.8)	26.6 (6.3)	29.0 (7.6)	0.038*
Waist circumference (cm)	83.2 (12.2)	82.1 (11.5)	86.0 (13.7)	0.062
Physical activity (MET-hours/week)	16.3 (15.3)	17.7 (16.5)	12.8 (11.0)	0.058
Alcohol consumption (servings/week)	3.5 (6.9)	3.8 (7.8)	2.8 (3.7)	0.386
Pre-cancer symptoms, mean (*SD*)				
Pain	75.2 (23.0)	79.8 (20.9)	63.5 (24.3)	<0.001***
Emotional well-being	80.1 (14.7)	81.9 (14.2)	75.3 (14.8)	0.006**
Fatigue	66.3 (17.2)	71.9 (12.9)	51.6 (18.4)	<0.001***
Physical function	82.6 (17.4)	86.1 (14.5)	73.4 (21.0)	<0.001***
Sleep disturbance	7.1 (4.3)	6.6 (4.3)	8.1 (4.20)	0.038*
Cancer characteristics, *n* (%)				
Cancer stage, *n* (%)				0.965
Local	140 (77.8)	101 (77.7)	39 (78.0)	
Regional	40 (22.2)	29 (22.3)	11 (22.0)	
Enrolled in LILAC	135 (75.0)	97 (74.6)	38 (76.0)	0.848
Treatment source				0.413
Abstraction/Medicare	150 (83.3)	106 (70.1)	44 (88.0)	
Self-report	30 (16.7)	24 (18.5)	6 (12.0)	
Chemotherapy[d]	29 (27.6)	21 (24.7)	11 (34.3)	0.305

Note. Significant at alpha level: *0.05, **0.01, ***0.001.
LILAC: Life and Longevity After Cancer Study; MET: metabolic equivalents; SD: standard deviation; WHI: Women's Health Initiative.
[a]Non-fatigued: SF-36 vitality score ≥50, fatigued SF-36 vitality score <50.
[b]Other race/ethnicity categories include American Indian/Alaskan Native, Asian, Native Hawaiian/Other Pacific Islander, or unknown.
[c]Among all participants; never smokers were coded as zero pack-years.
[d]Total number of participants in LILAC with chemotherapy data available is 105.

To explore if the timing of serum collection in relation to breast cancer diagnosis influenced the results, we conducted a stratified analysis based on the timing of post-cancer serum collection in relation to breast cancer diagnosis (either <1 year, 1–2 years, or >2 years). When comparing all three groups, there was a significant interaction between the timing of post-cancer serum collection and biomarker concentrations for CRP (Supplemental Table 6). Higher CRP ratios were significantly associated with greater fatigue if measured 1–2 years after cancer diagnosis [β: −4.74 (95% CI: −7.82, −1.66)].

Discussion

In this secondary analysis of post-menopausal women in the WHI, we found that higher levels of cystatin-C, IL-6, and

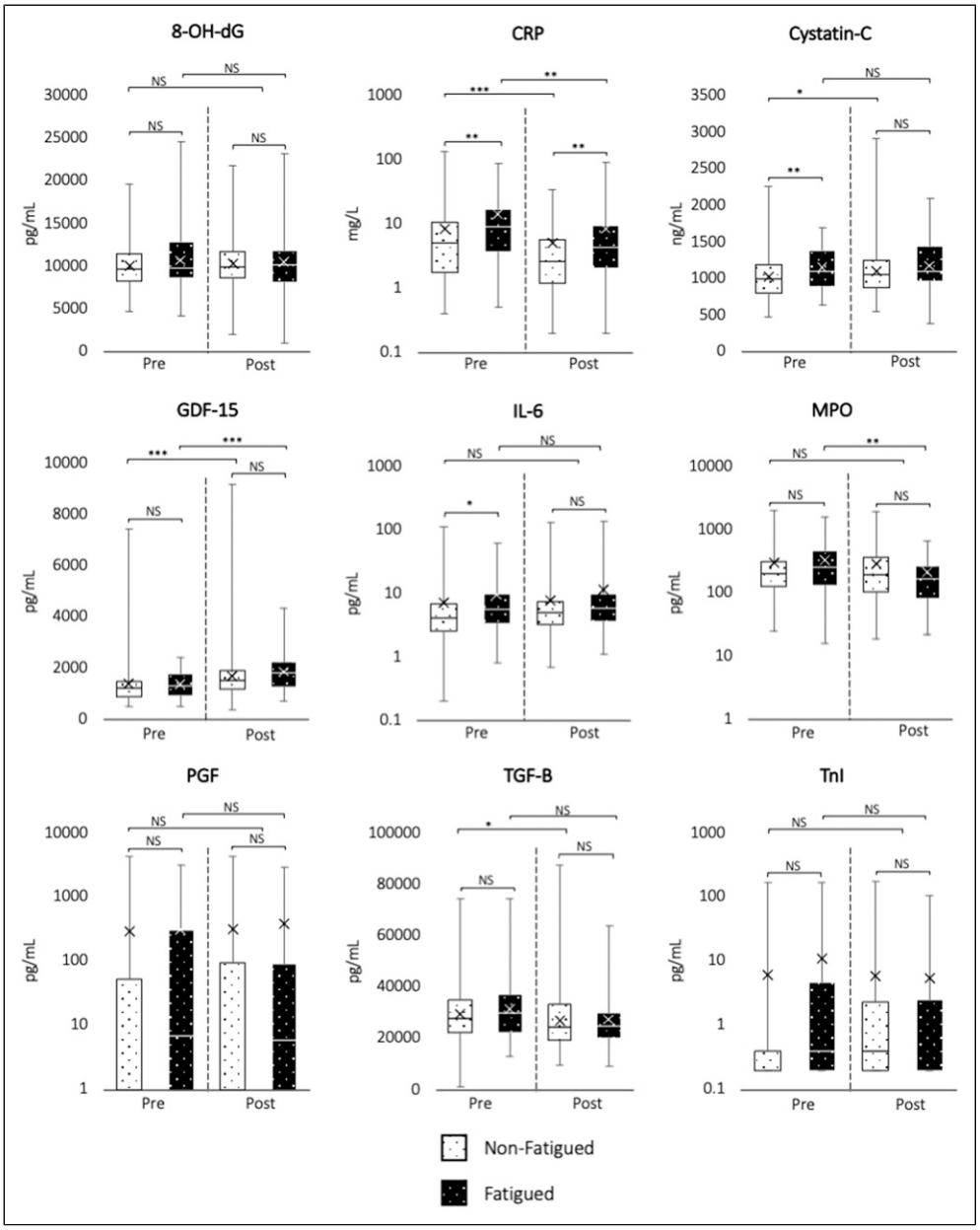

Figure 2. Boxplots for the distribution of pre- and post-breast cancer biomarker concentrations by fatigue. Lower and upper box boundaries depict 25th and 75th percentiles; line inside box represents the median; whiskers extend to maximum and minimum values; "x" represents the mean. Comparisons were made using Wilcoxon-rank sum tests. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: 8-OH-dG, 8-hydroxy-2/-deoxyguanosin; c-reactive protein, CRP; GDF-15, growth differentiation factor-15; IL-6, interleukin-6; MPO, myeloperoxidase; NS, not significant at alpha 0.05; PGF, placental growth factor; TGF-B, transforming growth factor-beta; TnI, troponin-I.

GDF-15 were associated with higher fatigue in breast cancer survivors treated with radiation, after adjustment for relevant lifestyle, demographic, and psychosocial characteristics. The difference in SF-36 effect estimates for both cystatin-C and GDF-15 was greater than 5, the minimal clinically important difference. No associations were found for CRP, 8-OH-dG, PGF, MPO, or TnI in the main models.

The pathophysiology of fatigue in cancer survivors treated with radiation is complex; however, immune dysregulation and chronic inflammation have been widely accepted as putative causes of fatigue in cancer survivors. There is strong evidence that levels of proinflammatory cytokines, including IL-6, increase during and after radiation treatment, and these cytokines have been associated with fatigue symptoms in cancer survivors, particularly those treated with chemotherapy (Hsiao et al., 2016; Saligan et al., 2015; Wang & Woodruff, 2015). However, limited research has examined the role of inflammatory biomarkers in breast cancer survivors treated

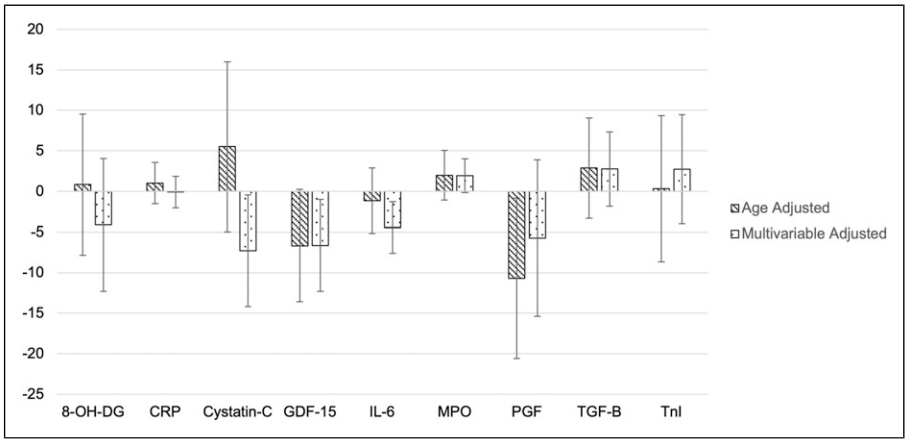

Figure 3. Linear regression results for association of post-cancer biomarker on continuous fatigue scores. Height of the bar represents the β coefficient with whiskers corresponding to the lower and upper 95% confidence intervals. Biomarker ratios (post/pre) were log transformed to base two; β corresponds to 2-fold difference in biomarker ratio. PGF and TNI are categorized as above versus below (reference) detection. All models are adjusted for pre-cancer biomarker. Age-adjusted model (blue) is further adjusted for age (5-year categories). Multivariable model (red) is further adjusted for age (5-year categories), education (HS/GED or <, > HS – Bachelor's, > Bachelor's), BMI (kg/m2), smoking (pack-years), physical activity (total MET-minutes/week), alcohol consumption (servings per week), cancer stage (local/regional, distant), and pre-cancer emotional well-being, physical function, pain, sleep disturbance, and fatigue. Abbreviations: 8-OH-dG, 8-hydroxy-2/-deoxyguanosin; c-reactive protein, CRP; GDF-15, growth differentiation factor-15; IL-6, interleukin-6; MPO, myeloperoxidase; NS, not significant at alpha 0.05; PGF, placental growth factor; TGF-B, transforming growth factor-beta; TnI, troponin-I.

specifically with radiation. Two prior studies examined IL-6 and fatigue in breast cancer patients who were undergoing radiation therapy; however, these studies did not detect any association between changes in IL-6 and fatigue (Bower et al., 2009; Shi et al., 2020). The findings from this current study provide support for the role of IL-6 in radiation-induced fatigue. Possible explanations for the lack of association in the previous two studies could be related to small sample size which ranged from 28 to 147 participants. Additionally, previous studies did not adjust for a comprehensive set of potential confounding variables.

This study also examined novel biomarkers, which have yet to be examined in fatigue in breast cancer survivors in the context of chemotherapy or radiation. We found that higher concentrations of cystatin-C and GDF-15 were associated with greater fatigue. Cystatin-C is a marker of renal function correlated with glomerular filtration. Evidence from prior studies have shown that concentrations of cystatin-C are higher in oncology patients prior to chemotherapy compared to a reference population and concentrations of cystatin-C increase during chemotherapy without accompanying changes in renal function (Jones et al., 2017; Li et al., 2020). This suggests that chemotherapy may influence cystatin-C levels through other pathways. One possible pathway is through cardiac dysfunction. Cystatin-C has recently been used in epidemiological studies to predict cardiovascular disease (Ix et al., 2007; Sarnak et al., 2005). Fatigue is a commonly reported symptom in patients with cardiovascular disease (Casillas et al., 2006). Likewise, GDF-15 has been implicated in cardiac dysfunction in cancer survivors (Putt et al., 2015). The findings from cystatin-C and GDF-15 may be indicative of an underlying pathophysiologic process, which is contributing to fatigue symptoms such as cardiovascular dysfunction.

GDF-15 may also play an important role in treatment-related cachexia, a condition characterized by loss of lean body mass which is highly associated with fatigue symptoms (Alesi & del Fabbro, 2014; Kilgour et al., 2010). Thus, the results of GDF-15 in this study could be reflective of fatigue associated with body composition changes, although detailed body composition data are not available to examine in this study. Future studies should look at the associations between cystatin-C, GDF-15, and fatigue with longitudinal changes in body composition, specifically muscle mass, and cardiac function to further explore the possible mechanisms by which these biomarkers are associated with fatigue.

A strength of this study is its efficiency in leveraging available biomarker data and is the largest study, to our knowledge, to examine biomarkers associated with fatigue in breast cancer survivors treated with radiation. We also examined a variety of biomarkers, including novel biomarkers, which may suggest possible mechanisms associated with fatigue in breast cancer survivors treated with radiation. While prior research, and the results from this study, support the role of inflammation in fatigue in breast cancer survivors treated with radiation, there may be other possible contributing factors of fatigue such as underlying cardiac damage or body composition changes. Future research is needed to validate these novel biomarkers and further elucidate possible mechanistic pathways in other breast cancer populations.

Our study also has limitations. As this analysis used data from an observational study with post-cancer biomarkers and

fatigue measured at the same time point, reverse causation is possible. To counteract this, we adjusted for pre-cancer biomarkers and fatigue. Residual confounding is also a possibility, although we were able to adjust for a range of psychosocial and clinical characteristics, including the receipt of chemotherapy, which are highly associated with fatigue (Bower, 2014). However, given the limited sample size and availability of data, we were not able to further assess the effects of specific types of chemotherapies, particularly cardiotoxic chemotherapies, or radiation dose, which have been recognized as potential contributors of fatigue (Abrahams et al., 2016; Kowalczyk et al., 2021). The original case-control study excluded women who developed cardiovascular outcomes prior to breast cancer. Thus, these results may not be generalizable to the general breast cancer population. However, when this sample is compared to the overall breast cancer cohort in the WHI and in LILAC, the participant characteristics were similar. Additionally, most women in this study received radiation in the late 1990s, when radiation doses were higher than currently, which may reduce the generalizability of these results to breast cancer survivors who received contemporary radiation. Another limitation is that fatigue in the WHI was measured using the SF-36, which was not created specifically for cancer-related fatigue and lacks a multi-dimensional component. However, it is commonly used as a measure of fatigue in cancer survivors and has been used to detect biomarker differences (Alfano et al., 2012; Bower et al., 2002, 2005; Brown et al., 2011; Collado-Hidalgo et al., 2008; Ware, 2000). We also tested multiple biomarkers, which could increase the possibility of a type I error. Further studies are needed to validate findings from this study. Lastly, there was a wide variation in the timing from breast cancer to post-breast cancer serum collection. We were able to perform a stratified analysis, which showed there is likely variation in the estimates depending on when the biomarkers were collected in relation to breast cancer, especially for CRP. Future prospective studies could measure biomarkers at consistent times before, during, and after radiation.

Conclusions

Findings from this study suggest that inflammation and cardiac damage biomarkers, such as IL-6, cystatin-C, and GDF-15, are associated with fatigue in breast cancer survivors treated with radiation. While inflammatory pathways have been studied frequently in the context of chemotherapy, results from this study suggest mechanisms of fatigue in breast cancer survivors treated with radiation is likely multifactorial and may also be, in part, suggestive of underlying pathological processes related to cardiac dysfunction or body composition changes in addition to inflammation. However, further studies are needed to replicate these findings in larger samples accounting for more refined treatment characteristics and contemporary radiation. The focus on radiation treatment in the development of cancer fatigue is an understudied area lending to underreporting and undertreatment of this debilitating symptom. The study of biomarkers in the context of cancer fatigue of is an evolving field. Identifying biomarkers associated with fatigue could help provide a better understanding of the mechanisms of fatigue and could be used in clinical practice or included in risk prediction models to identify cancer survivors treated with radiation who may be at higher risk for fatigue. Early identification of individuals at risk for fatigue could lead to reductions in fatigue by targeting interventions to those at highest risk identified by specific biological pathways.

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Declaration of Conflicting Interests

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Author Contributions

AV, KWR, EDP, OZ, HT, and SRH contributed to the conception and design of the study. RTC and EDP contributed to the access of data. AV analyzed the data. The first draft of the manuscript was written by AV. All authors read, provided substantial revisions, and approved the final manuscript.

Availability of Data

The datasets generated and analyzed during the current study are not publicly available in accordance with policies developed by the

National Heart, Lung, and Blood Institute and the Women's Health Initiative. Data requests must be approved by the Fred Hutchinson Cancer Research Center, which currently serves as the institutional review board of record for the Women's Health Initiative. Data requests may be made by emailing helpdesk@WHI.org. The following supporting documents are available: the WHI protocol and informed consent form (https://www.whi.org/page/protocols-and-study-consents).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the University of Washington Institutional Review Board.

Consent to Participate

Written informed consent was obtained on all participants in the Women's Health Initiative prior to enrollment in the study. In addition, women provided consent to have their stored biological samples be used for future research. Both occurred prior to collecting serum samples or questionnaire data.

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Supplemental Material

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