### Title

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# Chronic oxidative stress as a marker of long-term radiation-induced cardiovascular outcomes in breast cancer

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## Abstract

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**Author contributions:** Alexi Vasbinder, Richard K. Cheng, Kerryn W. Reding, Lisa Johnson, Rachel Yung, Electra Paskett, Oleg Zaslavsky, Hilaire Thompson, Susan R. Heckbert contributed to the conception and design of the study. Rowan T. Chlebowski, Jean Wactawski-Wende, Electra D. Paskett contributed to the access of data. Alexi Vasbinder analyzed the data. The first draft of the manuscript was written by Alexi Vasbinder. All authors read, provided substantial revisions, and approved the final manuscript.

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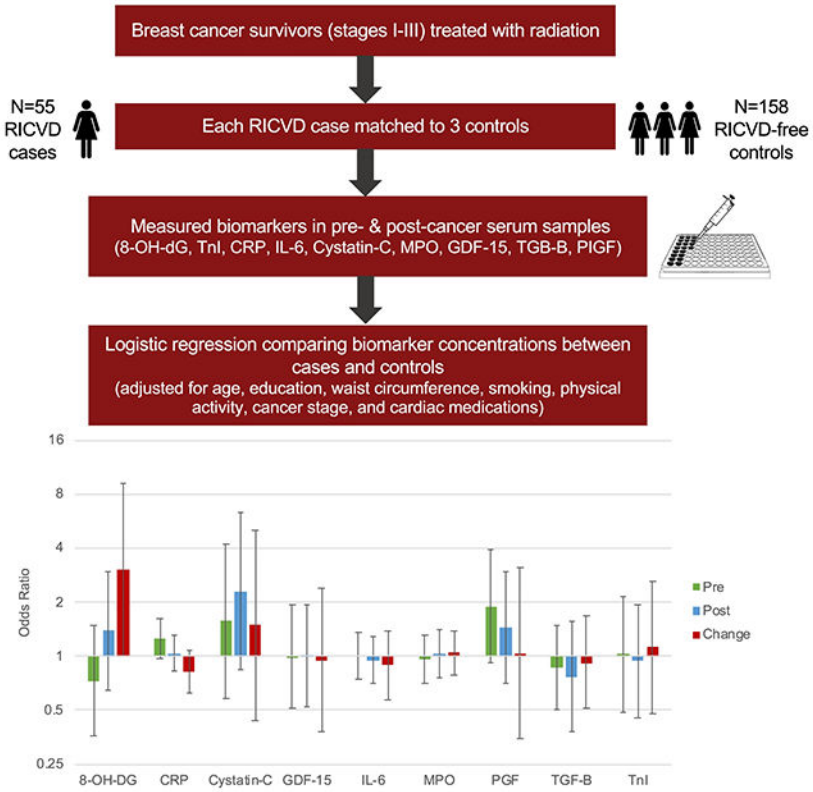
**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was 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.

**Animal studies**: No animal studies were carried out by the authors for this article.

While biomarkers have been proposed to identify individuals at risk for radiation-induced cardiovascular disease (RICVD), little is known about long-term associations with cardiac events. We examined associations of biomarkers of oxidative stress (myeloperoxidase, growth differentiation factor-15, 8-hydroxy-2'-deoxyguanosine [8-OH-dG], placental growth factor), cardiac injury (troponin-I, cystatin-C), inflammation (interleukin-6, C-reactive protein), and myocardial fibrosis (transforming growth factor-ß) with long-term RICVD in breast cancer (BC) survivors. We conducted a nested case-control study within the Women's Health Initiative of postmenopausal women with incident BC stages I-III, who received radiation and had pre- and post-BC diagnosis serum samples. Cases (n=55) were defined as developing incident, physician-adjudicated myocardial infarction, coronary heart disease death, other CVD death, heart failure, or stroke after BC. Cases were matched to three controls (n=158). After adjustment, a higher 8-OH-dG ratio was significantly associated with an elevated long-term risk of RICVD, suggesting oxidative DNA damage may be a putative pathway for RICVD.

## Graphical Abstract




### Keywords

breast cancer; radiation; cardiovascular disease; biomarkers; inflammation; oxidative stress

## Introduction

Over 50% of breast cancer (BC) patients receive radiation as primary or adjuvant therapy [1]. Although radiation has contributed to improved survival from breast cancer, many survivors experience long-term treatment-related adverse effects including radiation-induced cardiovascular disease (RICVD) [2–4]. RICVD is associated with increased morbidity and mortality among breast cancer survivors [5]. RICVD can manifest as heart failure (HF), myocardial ischemia, acute coronary syndrome, valvular heart disease, pericardial disease, and is also associated with higher risk of cardiac mortality [6–9]. The reported incidence of RICVD in breast cancer survivors varies; however, a large meta-analysis documented an absolute risk increase of 76.4 cases per 100,000 person-years and 125.5 cases per 10,000 person-years for coronary heart disease and CVD mortality, respectively, for radiation recipients compared to those who did not receive radiation [10].

Currently, it is not known how to best identify individuals who will develop long-term RICVD, cardiovascular outcomes occurring years after treatment, as it is frequently neglected in research due to the challenges of following individuals long-term [11; 12]. As a result, the primary body of literature examines short-term cardiac outcomes, mainly related to chemotherapy [11; 13–16]. Based on these studies, biomarkers of cardiac damage, inflammation, and oxidative stress have been identified as acute contributors of cardiovascular events. While these pathways likely influence RICVD, this has not been definitively tested in research studies.

Thus, the aim of this analysis was to examine the association of biomarkers of oxidative stress, cardiac damage, and inflammation with long-term RICVD in breast cancer survivors. Specifically, we examined the following biomarkers: *oxidative stress*: 8-hydroxy-2'-deoxyguanosin (8-OH-dG), myeloperoxidase (MPO); *cardiac damage*: cardiac troponin-I (TnI), cystatin-C*; inflammation*: interleukin-6 (IL-6), C-reactive protein (CRP), growth differentiation factor-15 (GDF-15), placental growth factor (PGF); and *myocardial fibrosis:* transforming growth factor-ß (TGF-B).

## Methods

### Study design and population

We conducted a nested case-control study within the Women's Health Initiative (WHI). Details of the WHI study design and conduct have been published [17]. In summary, 161,808 postmenopausal women aged 50-79 years were enrolled in an Observational Study or at least one of four randomized Clinical Trials at 40 US clinical centers between 1993 and 1998. All participants provided written consent and the protocols were approved at each site's institutional review board. Participants were initially followed through 2005; women subsequently enrolled in the first Extension Study (2005-2010) and the second Extension Study (2010-ongoing) for further follow-up [17]. A sub-cohort of cancer survivors, the Life and Longevity After Cancer (LILAC) study, was established in 2013 [18]. A primary goal of LILAC was to collect information on cancer treatment and outcomes in women diagnosed with incident cancer. Eligible women were without cancer prior to WHI enrollment with

incident breast, endometrial, ovary, lung, or colorectal cancer, melanoma, lymphoma, or leukemia.

This analysis included participants with breast cancer who were enrolled in LILAC or in Medicare fee-for-service at the time of their cancer diagnosis and had breast cancer treatment data available. In addition, women were eligible for this analysis if they met the following criteria: 1) had both pre- and post-breast cancer diagnosis serum samples available and 2) had documented receipt of radiation therapy treatment (right- or left-sided) either through medical record abstraction, Medicare claims data, or self-report. The percent agreement between medical record abstraction/Medicare claims data and self-report data in 204 LILAC participants with both types of data available was 98%, which corresponds to a Kappa of 0.95 (95% CI: 0.91, 0.99). Women were ineligible if they 1) had an adjudicated major adverse cardiac event (MACE) or HF outcome prior to breast cancer, 2) were diagnosed with metastatic disease or were missing stage, or 3) self-reported a history of breast, lung, lymphoma, Hodgkin's, or thyroid cancers at WHI baseline given prior radiation exposure is a risk factor for later cardiac outcomes. A total of 409 participants met the criteria for this study.

## Case selection

Cases were defined as having an incident, physician-adjudicated MACE (coronary heart disease, which includes myocardial infarction and coronary heart disease death, CVD death not classified as coronary heart disease, and stroke) or HF event after breast cancer (i.e., the second serum collection time-point). Of the eligible sample, there were 55 cases, and all were included in this study.

WHI cardiac adjudication methods have been in described in detail elsewhere [19]. In summary, potential outcomes were identified through semi-annual or annual medical history self-report forms. If an event was self-reported, medical records were requested and events were physician-adjudicated using standardized criteria for each outcome. Deaths and cause of death were verified by medical record or death certificate review. Mortality findings were enhanced through serial linkage with the National Death Index. Specific definitions for each cardiac outcome are provided in the Supplemental Material. Coronary heart disease, stroke, and CVD death events were adjudicated on all participants through 2010, whereas incident HF was adjudicated through 2005.

## Control selection & matching

Controls were defined as participants with breast cancer who received radiation therapy but did not have a self-reported or an adjudicated MACE or HF outcome during WHI study follow-up. There were 354 eligible controls. We randomly selected controls without replacement to achieve a ratio of 1:3 cases to controls. Controls were frequency matched to cases on age at WHI enrollment (5-year categories), visit year of the pre-breast cancer specimen draw, treatment ascertainment (self-report or medical record abstraction/ Medicare), and LILAC enrollment (yes/no). The matching algorithm was allowed to select the closest matches, based on criteria to minimize an overall distance measure. Matching was done in a time-forward manner, selecting up to three controls for each case from the

risk set at the time of the case's event (i.e., days to adjudicated MACE or HF outcome), ensuring that each control had at least as much follow-up time as its corresponding case. This resulted in 158 controls being selected. Five cases did not have three corresponding controls; however, each had at least one matched control.

## Exposures

The exposures for this study were pre-breast cancer, post-breast cancer, and the ratio of pre- to post-breast cancer biomarker levels of 8-OH-dG, CRP, cystatin-C, GDF-15, IL-6, MPO, PGF, TGF-B, and TnI.

The WHI has detailed protocols regarding specimen collection, handling, preparation and storage [17]. All WHI staff were trained in standardized methods of specimen acquisition and processing to minimize variation and ensure accuracy of biomarker results. Samples were stored at −80 degrees Celsius prior to analysis.

All biomarkers were measured using commercially available enzyme-linked immunosorbent assay kits except IL-6 and TnI (Supplemental Table 1). IL-6 and TnI were measured using ProQuantum real time-PCR kits. All assays were conducted in the University of Washington School of Nursing Office for Nursing Research Laboratory. Samples measured using enzyme-linked immunosorbent assays were tested in duplicate, whereas those measured by real time-PCR kits were tested in triplicate, and the averages were used in the analysis. For samples that were below the detectable limit, we used a value that was halfway between zero and the lower limit of detection. All participants were randomly intermixed on each plate and laboratory personnel were blinded to case status. However, samples were provided such that cases and the matched controls, as well as the pre- and post-cancer biomarkers, were assayed on the same plate. Lastly, to ensure quality control, the WHI included 22 blind duplicate sample pairs. All biomarker assays had an intra-assay CV < 10% and inter-assay CV < 15% (Supplemental Table 1).

## Additional variables

Demographic information, such as age at WHI enrollment, race, ethnicity, and income, were collected at baseline on self-report questionnaires. Lifestyle factors, including smoking, physical activity, and alcohol consumption, were recorded at multiple time points during WHI follow-up on self-report questionnaires. BMI ($kg/m^2$) and waist circumference (cm) were measured in-person at WHI clinic visits. Comorbidities, such as diabetes and hypertension, were reported annually on self-report medical history questionnaires. Cardiac medication use, including calcium channel blockers, beta-blockers, diuretics, ACE inhibitors, angiotensin receptor blockers, or statins, was recorded at multiple time points during WHI follow-up. Lastly, cancer characteristics such as stage, chemotherapy and targeted therapy use, and laterality of breast cancer were recorded from medical records. For variables measured at multiple time points, the value closest, but prior to the pre-cancer serum collection was used in the analysis.

## Statistical Analysis

Baseline characteristics were compared between cases and controls. Normality was assessed visually for continuous variables. Characteristics were summarized with mean and standard deviations or median with interquartile range (IQR) as appropriate for continuous variables and proportions for categorical variables. Differences in mean values or proportions were determined by unpaired t-test and chi-square test, respectively.

Distributions of pre- and post-cancer biomarkers were described using both means with standard deviations and medians with IQR stratified by case status and displayed using boxplots. Differences in medians between pre- and post-cancer biomarkers by case status were tested using Wilcoxon rank tests given the non-normal distribution of the biomarkers.

Logistic regression was used to evaluate the associations between pre-cancer, post-cancer, and change from pre- to post-cancer biomarkers and risk of MACE or HF [20; 21]. The odds ratio (OR) and 95% confidence interval (CI) are reported. A separate model was created for each biomarker. The change of each biomarker was modeled as the ratio of the post-cancer value relative to the pre-cancer biomarker as has been done in prior research [14]. Given the non-normal distribution of the biomarkers, the biomarkers and the change ratio were log transformed to base 2. Each unit difference in the log base 2-transformed biomarker or biomarker ratio represents a doubling in value. However, an exception to this occurred for TnI and PGF. Both biomarkers had over 50% of values below detection and, thus, violated the linearity assumption. These biomarkers were modeled as categorical variables and defined as either above or below detection based on the defined limit of detection (Supplemental Table 1). Confounders were selected *a priori* based on the relationship of each variable with both the exposures and outcome. Matching variables were included in the model if they are known to be associated with the biomarker level (i.e., age [5-year categories]) [21]. Multivariable models were additionally adjusted for income (< $34,000, $35,000 - $74,999, > $75,000), waist circumference (cm), smoking (pack-years), physical activity (total MET-minutes/week), cancer stage (local vs. regional), and cardiac medications (yes/no). There was no violation of the collinearity assumption as measured by variance inflation factors and no influential values were identified by Cook's distance values.

We ran these exploratory and pre-planned sensitivity analyses: 1) repeated the proposed analyses adjusted for chemotherapy in the subset of LILAC participants with abstracted treatment data, 2) performed a stratified analysis based on the time from breast cancer to the post-cancer serum collection, and 3) stratified the analyses by left vs. right-sided breast cancer. For sensitivity analysis 1, we investigated whether chemotherapy was a substantial confounder in our data given the established cardiotoxic effects of chemotherapy. For this analysis, we compared unadjusted models with those adjusted for chemotherapy (yes vs. no). For sensitivity analysis 2, we created a variable to represent the timing of post-cancer biomarker collection as either < 1 year, 1- 2 years, or > 2 years after breast cancer diagnosis. To test whether there were any differences in the OR among the three groups, an interaction term was included in the models between this timing variable and each biomarker. The overall interaction was tested using the likelihood ratio test. For sensitivity analysis 3, we included an interaction term between each biomarker and laterality of breast cancer (right vs. left).

All analyses were conducted using R Version 4.0.1 (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 characteristics of cases and controls

Of the 213 participants, the mean (SD) age at enrollment was 69.2 (5.5) years, 196 (92%) were non-Hispanic White women, and 163 (76.5%) were enrolled in LILAC. Of the 55 cases, the initial events were classified as 15 CHD, 10 stroke, 22 CVD death, and 8 HF. More than half of participants were diagnosed with right-sided (60.6%) and local (78.5%) breast cancer. The median (IQR) time from the pre-cancer serum collection to breast cancer diagnosis was 1.8 (0.8, 2.6) years and the median (IQR) time from breast cancer diagnosis to the post-cancer serum collection was 1.4 (0.7, 2.4) years. Lastly, the median (IQR) interval between breast cancer diagnosis and either MACE or HF or end of follow-up was 11.0 (8.9, 12.6) years.

Cardiac risk factors were similar when comparing cases and controls for smoking, BMI, waist circumference, physical activity, and hypertension. However, cases were more likely to be on cardiac medications (25.5% vs 10.1%, p = 0.005) including beta-blockers (7.3% vs 0.6%, p=0.010) and calcium channel blockers (5.5% vs 0.6%, p=0.039) (Table 1).

### Distribution of biomarkers pre- and post-cancer stratified by case status

Serum concentrations were above the limit of detection for all biomarkers except for PGF and TnI. For PGF, 119 (55.9%) and 126 (59.2%) participants had undetectable concentrations for PGF for pre- and post-cancer time points, respectively. For TnI, 110 (51.6%) and 109 (51.2%) participants had undetectable concentrations for pre- and post-cancer time points, respectively. When comparing whether pre- or post-cancer serum biomarkers differed between cases and controls, we found no significant differences (Figure 2). We also examined whether there was a difference between pre- and post-cancer serum biomarkers for controls and cases separately. For controls, the median concentrations for TGF-B (p = 0.007) and CRP (p = 0.002) were significantly lower post-cancer compared to pre-cancer, whereas the concentrations for GDF-15 (p <0.001) were significantly higher post-cancer. For cases, the median concentrations of both cystatin-C (p = 0.03) and GDF-15 (<0.001) were higher post-cancer and CRP (p =0.002) was lower post-cancer compared to pre-cancer (Figure 2).

### Adjusted associations between each biomarker and RICVD

After adjustment, higher 8-OH-dG ratios were significantly associated with higher odds of MACE or HF (p=0.047). For a doubling in the biomarker ratio comparing post- to pre-cancer biomarkers, the odds of MACE or HF was 3.04 times higher (95% CI: 1.01, 9.21). In adjusted analyses, there were no significant associations for any other biomarker (Figure 3, Supplemental Table 2).

### Sensitivity analyses

Besides IL-6, there were no significant interactions when stratified by timing of the biomarker in relation to breast cancer diagnosis (Supplemental Table 3). We additionally repeated the main analysis in a subset of LILAC participants with abstracted treatment data available (n=131). In this subset, cases were more likely to receive docetaxel (5.7% vs. 0.0%, P=0.004) (Table 1). No other significant differences were noted between type of chemotherapy received. When chemotherapy was added to the unadjusted models for participants in LILAC, there was minimal change in the odds ratio suggesting that chemotherapy is not a significant confounder in these data (Supplemental Table 4). Last, there were no significant interactions between any biomarker and breast cancer laterality (data not shown).

## Discussion

This is the first study, to our knowledge, to examine the associations of biomarkers with long-term cardiac events in breast cancer survivors who received radiation therapy. The association of radiation treatment with cardiac events has received less attention than chemotherapy. Additionally, this study examined long-term cardiac outcomes, rather than surrogate endpoints such as early changes in left ventricular ejection fraction (LVEF). The findings from this study demonstrate that 8-OH-dG, a marker of oxidative stress and DNA damage, was associated with the odds of long-term cardiac outcomes in breast cancer survivors treated with radiation.

8-OH-dG is a ubiquitous marker of oxidative stress and has been examined extensively as a biomarker of endogenous oxidative DNA damage in the context of chronic HF [22], CVD [23], and cancer [24]. Reactive oxygen species oxidize nuclear and mitochondrial DNA damaging DNA and creating 8-OHdG. There is evidence that anthracycline administration is associated with an increase in mitochondrial and nuclear DNA adducts of 8-OH-dG in liver and heart cells and plasma 8-OH-dG in rats [25; 26] and one study showed continued elevation of 8-OH-dG DNA adducts 5 weeks after the last treatment [25]. Rat studies also have shown that there is an increase in 8-OH-dG adducts to mitochondrial DNA in liver and cardiac tissue and greater selectively of adducts in cardiac tissue, furthering support for using 8-OH-dG as a biomarker of cardiotoxicity [25; 27]. However, this is the first study to examine 8-OH-dG as a marker of RICVD in breast cancer survivors. Our findings suggest that post-cancer 8-OH-dG concentrations may be independently associated with long-term cardiac outcomes after radiation therapy.

We did not find any association between TnI concentrations and long-term cardiac outcomes, which is consistent with prior studies that found radiation to have little to no effect on cardiac troponin concentrations [28–31]. This is in contrast with studies examining the association of chemotherapy with cardiac troponin concentrations, which have reported higher troponin concentrations to be associated with an elevated risk of short-term cardiac dysfunction defined as decrement in LV systolic function [13; 32; 33]. A prior study in breast cancer patients receiving adjuvant radiation therapy reported radiation was associated with an increase in high-sensitivity troponin-T after treatment and a positive correlation between troponin concentrations and cardiac dose of radiation [34]. This study suggests

radiation may be associated with acute subclinical cardiac changes although they did not investigate long-term cardiac endpoints. However, it is important to note that despite using a high-sensitivity assay to measure TnI, we still had a substantial proportion of participants with values below detection. This suggests that TnI may not be a valuable biomarker for assessing cardiac events in breast cancer survivors treated with radiation, especially long-term cardiac outcomes; this is not surprising as TnI would more likely reflect acute injury as suggested in prior studies [34].

We also did not find any association between MPO, GDF-15, or PGF and long-term cardiac outcomes in this study. Post-radiation GDF-15 and PGF have recently been identified as two promising biomarkers for predicting acute cardiac dysfunction, defined as a drop in LVEF [14; 28]. In a prior study, GDF-15 and PGF were associated with cardiac dysfunction in lung and lymphoma patients; however, no associations were found in breast cancer patients [28]. This was attributed to the lower mean heart doses of radiation in breast cancer patients compared to lung and lymphoma patients. While we are unable to obtain the radiation dose, most women in our study received radiation between 1993 and 1998. Thus, they likely received higher doses of radiation to the heart compared to modern radiation therapies based on common practices at that time [35]. Despite the likelihood that women in our study received higher doses of radiation, we did not report any significant associations between GDF-15 or PGF concentrations and long-term cardiac outcomes. We also did not find an association between MPO and long-term cardiac outcomes in contrast to prior studies focused on chemotherapy [13; 14; 32]. However, this is the first study to examine MPO as a marker for radiation-induced cardiovascular disease in breast cancer survivors.

Of importance, there are major differences between the current study and prior studies examining biomarkers and treatment-induced cardiotoxicity. First, prior studies typically defined changes in LVEF or global longitudinal strain as primary outcomes. While important surrogate endpoints, it remains unclear whether early changes in LVEF or strain increases long term risk for cardiovascular events. Though HF and cardiomyopathy are included in RICVD outcomes, RICVD also can manifest as pericardial disease, myocardial fibrosis, coronary artery disease, valvular disease, and arrhythmias [36]. Additionally, the length of follow-up time in the current study was substantially longer than previous studies [32]. Thus, these biomarkers may be more sensitive to cardiac changes that occur shortly after treatment rather than long-term cardiac outcomes.

## Study Limitations

Our study has some limitations. Given this study was observational, there is a possibility of residual confounding. However, we were able to adjust for known cardiac risk factors, such as age, smoking, BMI, and cardiac medications. With the testing of multiple biomarkers, there is a higher probability of a type I error. Given the p-value for 8-OH-dG was close to 0.05, these results should be interpreted with caution and these findings should be validated in a larger sample size. Our sample was limited based on the availability of serum in eligible participants and the limited documentation of radiation treatment, thus selection bias is possible. However, when this analytic sample is compared to the subset of participants in LILAC and the overall breast cancer cohorts in the WHI and in LILAC,

the participant characteristics were similar (Supplemental Table 5). Additionally, only a subset of participants had detailed chemotherapy data available. Thus, we were unable to fully account for the effects of concurrent chemotherapy and residual confounding is possible. Due to resource restraints, we were unable to assess associations between ß-natriuretic peptide and RICVD. Evidence of an association between of ß-natriuretic peptide and subclinical cardiac damage has been demonstrated in prior studies and should be investigated further with long-term RICVD [37; 38].

Most women (89.0%) in this study received radiation prior to 2000, when radiation doses were higher than currently, which may reduce the generalizability of these results to breast cancer survivors who received contemporary radiation. Although modern radiation therapy reduces the total cardiac dose, evidence suggests cardiac exposure is not eliminated [10; 39]. We were unable to estimate total radiation doses as this data was not available. However, based on data from large cohorts, the mean heart dose of breast radiotherapy has declined over time with an average of 4.7 Gy in the 1990s compared to 2.3 Gy in 2006 [40; 41]. Additionally, we found no effect modification by laterality of breast cancer, which could be due to lack of power, inability to assess radiation doses, or timing of biomarker collection. Further research is needed in the context of contemporary radiation to determine the impact of radiation dose on these associations and whether these associations differ by laterality of radiation.

Despite these limitations, our study has many strengths. This study focused on RICVD, which has received less attention in comparison to chemotherapy. While still small, our study is the largest study to examine biomarkers associated with RICVD with other studies ranging from n = 23 – 87 [1; 28; 31; 34; 37; 42; 43]. This study also focused on long-term cardiac events, rather than surrogates such as changes in LVEF. Lastly, cardiac outcomes were physician-adjudicated, minimizing the possibility of misclassification.

## Conclusions

In breast cancer survivors treated with radiation, a higher pre- to post-cancer ratio of 8-OH-dG was associated with higher odds of long-term RICVD. This suggests that oxidative DNA damage may be a putative pathway for RICVD. However, given the small sample size and multiple testing, this study should be repeated with a larger sample to confirm findings. The study of biomarkers in the context of cancer treatment-related cardiotoxicities is an evolving field. Identification of biomarkers associated with RICVD may improve the understanding of the mechanisms underlying RICVD and the improve the identification of RICVD in cancer survivors. While formal clinical guidelines regarding the use of biomarkers in identifying RICVD are lacking, there is evidence that biomarkers may be useful in improving the risk prediction of RICVD, especially when used in combination or with other modalities such as cardiac imaging. This line of work could lead to reductions in RICVD risk by targeting interventions to those at highest risk, such as increasing surveillance or introduction of cardioprotective medications. Further research is needed to determine the optimal frequency and relevant biomarker cut off values to predict and identify RICVD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data availability:

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).

## Abbreviations

| | |
|---|---|
| **8-OH-dG** | 8-hydroxy-2'-deoxyguanosine |
| **CRP** | C-reactive protein |
| **GDF-15** | growth differentiation factor-15 |
| **HF** | heart failure |
| **IL-6** | interleukin-6 |
| **LVEF** | left ventricular ejection fraction |
| **LILAC** | Life and Longevity After Cancer study |
| **MACE** | major adverse cardiac event |
| **MPO** | myeloperoxidase |
| **PGF** | placental growth factor |

| | |
|---|---|
| **RICVD** | radiation-induced cardiovascular disease |
| **TnI** | troponin I |
| **WHI** | Women's Health Initiative |

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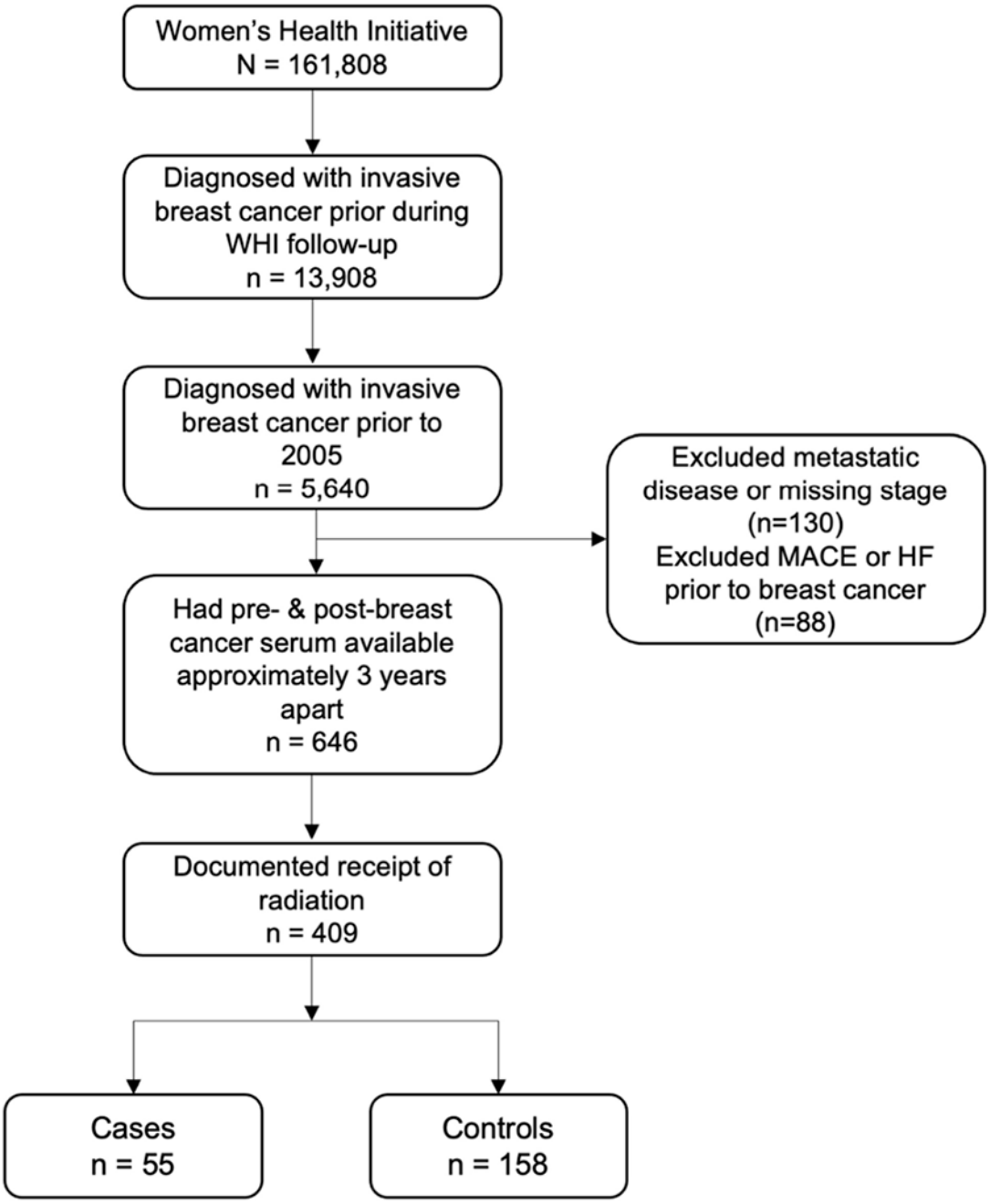


**Fig 1. Sample flow chart.**

Participants were eligible 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. Cases are defined as participants who developed a major adverse cardiac event (MACE) or heart failure (HF) after breast cancer.

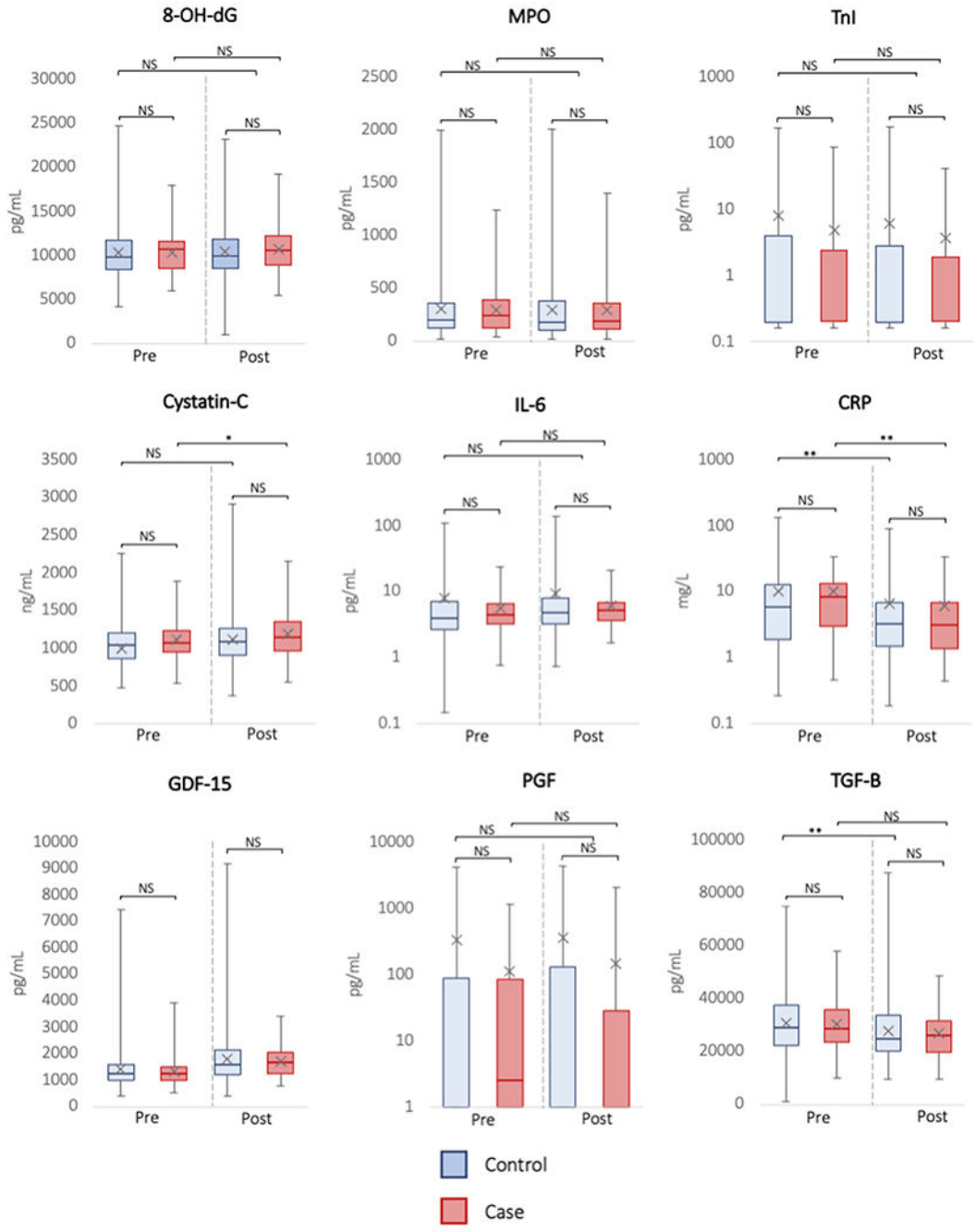


**Fig 2. Boxplots for the distribution of pre- and post-breast cancer biomarker concentrations by case status.**

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. *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.

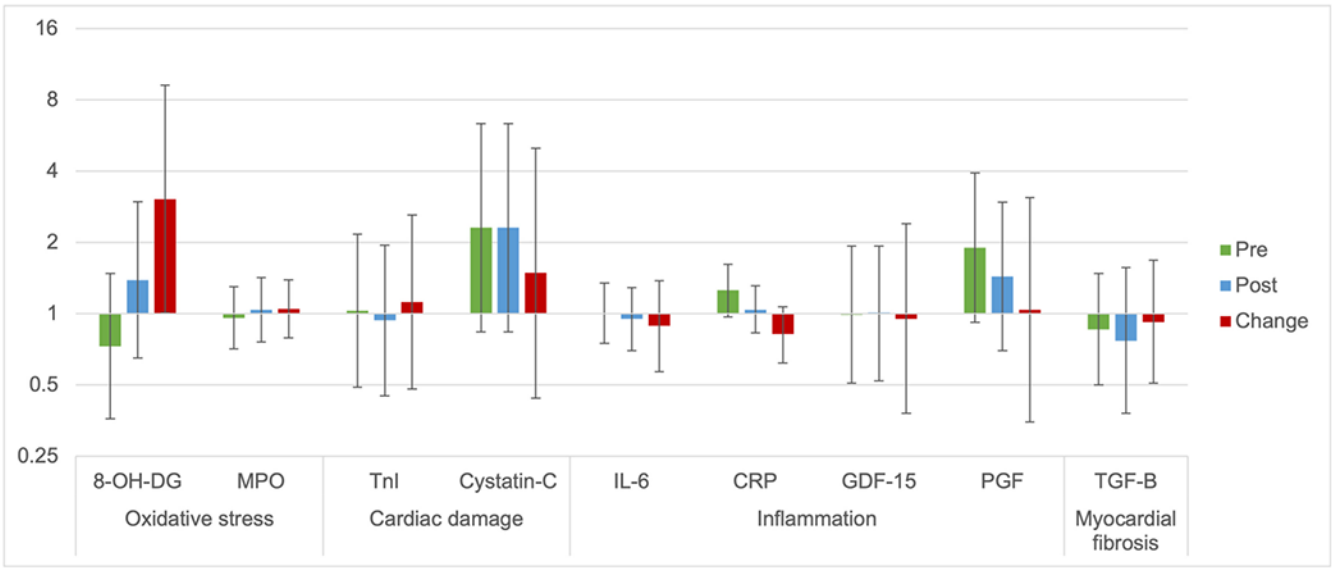


**Fig 3. Adjusted associations of pre-cancer biomarkers, post-cancer biomarkers, and biomarker change ratios with MACE or HF.**

Pre- and post-cancer biomarkers are log transformed to base 2. Change is modeled as the $\log_2$ ratio of post-cancer relative to pre-cancer concentration; each unit difference in the biomarker ratio corresponds to a doubling in value compared to pre-cancer. PGF and TNI are categorized as above vs. below (reference) detection; the change value is adjusted for pre-cancer biomarker. Models are adjusted for age (5-year categories), income (< $34,999, $35,000 - $74,999, >$75,000), waist circumference (cm), smoking (pack-years), physical activity (total MET-minutes/week), cancer stage (local vs. regional), cardiac medications (yes/no).

**Table 1.**

Baseline (i.e., pre-cancer) characteristics stratified by case status

| | Controls (N = 158) | Cases (N = 55) |
|---|---|---|
| **Demographics** | | |
| Age at Diagnosis, mean (SD) | 69.2 (5.5) | 69.5 (5.5) |
| Age at WHI Enrollment, mean (SD) | 66.9 (5.6) | 67.0 (5.5) |
| Race/Ethnicity, n (%) | | |
| Non-Hispanic Black | 4 (2.5) | 2 (3.6) |
| Non-Hispanic White | 147 (93.0) | 49 (89.1) |
| Other[a] | 7 (4.4) | 4 (7.3) |
| Income, n (%) | | |
| < $34,999 | 47 (29.7) | 19 (34.5) |
| $35,000 - $74,999 | 68 (43.0) | 19 (34.5) |
| > $ 75,000 | 35 (22.2) | 14 (25.5) |
| **Cardiac risk factors** | | |
| Smoking (pack-years), mean (SD)[b] | 11.3 (18.3) | 12.4 (18.6) |
| BMI (kg/m$^2$), mean (SD) | 27.2 (6.8) | 27.7 (5.8) |
| Waist circumference (cm), mean (SD) | 83.1 (12.0) | 86.7 (14.11) |
| Physical Activity (MET-hours/week), mean (SD) | 15.5 (14.9) | 18.3 (16.2) |
| Alcohol (servings/week), mean (SD) | 3.25 (5.0) | 3.6 (9.6) |
| Hypertension, n (%) | 3 (1.9) | 4 (7.3) |
| Diabetes, n (%) | 2 (1.3) | 0 (0.0) |
| **Cardiac Medications, n (%)** | 16 (10.1) | 14 (25.5) |
| Beta blockers | 1 (0.6) | 4 (7.3) |
| Calcium channel blockers | 1 (0.6) | 3 (5.5) |
| Statins | 5 (3.2) | 3 (5.5) |
| ACEi/ARB | 5 (3.2) | 4 (7.3) |
| Diuretics | 6 (3.8) | 1 (1.8) |
| **Cancer characteristics** | | |
| Cancer stage, n (%) | | |
| Local | 124 (78.5) | 43 (78.2) |
| Regional | 34 (21.5) | 12 (21.8) |
| Laterality, n (%) | | |
| Right | 96 (60.8) | 33 (60.0) |
| Left | 62 (39.2) | 22 (40.0) |
| **Enrolled in LILAC, n (%)** | 121 (76.6) | 42 (76.4) |
| **Chemotherapy, n (%)**[c,d] | | |
| 5-FU | 10 (10.4) | 3 (8.6) |
| Cyclophosphamide | 22 (22.9) | 8 (22.9) |
| Doxorubicin | 16 (16.7) | 5 (14.3) |
| Docetaxel | 0 (0.0) | 2 (5.7) |

| | Controls (N = 158) | Cases (N = 55) |
|---|---|---|
| Methotrexate | 9 (9.4) | 3 (8.6) |
| Paclitaxel | 4 (4.2) | 3 (8.6) |
| Chemotherapy, not specified | 6 (6.3) | 2 (5.7) |
| **Treatment Source, n (%)** | | |
| Abstraction/Medicare | 133 (84.2) | 48 (87.3) |
| Self-Report | 25 (15.8) | 7 (12.7) |

[a]This category includes Hispanic/Latino, Asian or Pacific Islander, American Indian or Alaskan Native, and those who self-reported as “other”

[b]Pack-years calculated among all participants; never smokers were coded as zero pack-years

[c]Among participants in LILAC with chemotherapy data abstracted (n=131; control [n=96], case [n=35])

[d]Participants may have received more than one type of chemotherapy

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; LILAC, Life and Longevity After Cancer; MET, metabolic equivalent; SD, standard deviation