Radiation-Induced Cardiovascular Disease and Fatigue in Breast Cancer Survivors: Understanding Biological Mechanisms and Risk Factors

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Abstract

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Although survival rates have improved, many breast cancer survivors experience adverse effects related to the physiologic consequences of cancer treatment including an increased risk cardiovascular disease (CVD) and symptoms, such as fatigue. Currently, it is not known how to best identify breast cancer survivors at risk for CVD and fatigue. Cancer treatments are known to be associated with both CVD and fatigue independently. At the same time, cardiotoxicity and fatigue may share common physiologic mechanisms such as chronic inflammation and oxidative stress, which directly target cardiac tissue to produce CVD and produce circulating inflammatory markers that may lead to fatigue. It is hypothesized that cancer treatments, through the complex relationship with oxidative stress and inflammation, create a pro-fibrotic environment, which contributes to heart disease and heart failure. While cancer treatments can independently cause fatigue, fatigue is also a commonly reported symptom in patients with heart failure, coronary artery disease, pericardial disease, and valvular disease, which are all potential cardiovascular outcomes associated with radiation treatment. Therefore, it is not only important to examine the

association between inflammatory and oxidative stress biomarkers and fatigue but also look at cardiac damage and fibrosis markers in persons who have received radiation treatment. Additionally, there is evidence in non-cancer populations that fatigue itself, and other symptoms associated with cancer treatments, are associated with the risk of CVD. Therefore, the overall purpose of this dissertation is to better understand risk factors for CVD and symptoms after cancer treatment in breast cancer survivors and to better understand how these factors relate to one another. Specifically, this dissertation has the following aims: 1) to assess the association of post-cancer biomarkers and CVD events in breast cancer survivors treated with radiation; 2) to examine the associations of post-cancer biomarkers and post-cancer fatigue in breast cancer survivors treated with radiation; and 3) examine whether physical and mental health-related quality of life (which incorporates a measure of fatigue) or sleep disturbances scores are associated with CVD risk in breast cancer survivors.

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DISCLAIMER

The opinions, ideas, and interpretations included in this dissertation are those of Ms. Vasbinder and her committee and not those of the WHI investigators.

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CHATPER 1

Introduction

Due to improvements in cancer treatments, survival rates have improved and, as such, as of 2016 there are an estimated 3.5 million breast cancer survivors in the United States.[1] Radiation therapy is common in the treatment of cancer, as over 50% of breast cancer patients receive radiation.[2] Although radiation has contributed to improved survival from breast cancer, many survivors experience late treatment-related adverse effects including cardiovascular disease (**CVD**) and symptoms, such as fatigue.[3-5] An understanding of the pathophysiology of these effects and translation of this knowledge into clinical practice can lead to improvements in risk prediction and early identification, which can, in turn, guide management and intervention strategies.

Radiation-induced cardiovascular disease (RICVD)

The National Cancer Institute (**NCI)** broadly defines cardiotoxicity as "toxicity that affects the heart".[6] While traditional definitions of cardiotoxicity have focused on changes in left ventricular ejection fraction (**LVEF)**, this definition is evolving as the evidence is mounting for an expanded impact of cardiotoxicity.[7] Radiation contributes to cardiotoxicity by promoting the development of CVD and can manifest as pericardial disease, myocardial fibrosis, cardiomyopathy, coronary artery disease (**CAD**), valvular disease, and arrhythmias.[8] The incidence of radiation-induced cardiovascular disease (**RICVD**) varies among breast cancer survivors depending on the definition used; however epidemiologic studies estimate a 25% 40-year cumulative incidence of RICVD. RICVD occurs, on average,10 years after radiation.[9] RICVD is of great concern as it is associated with substantial morbidity and mortality and is likely irreversible.[4, 10, 11] In particular, radiation in the treatment of cancers, such as breast, is more likely to cause cardiotoxicity given the proximity to the heart. Further research is needed to identify biological targets associated with RICVD, which can further be used in risk prediction.

Pathophysiology of RICVD.

Radiation is responsible for anti-tumor effects by directly damaging DNA in cells beyond repair, thus halting the cell cycle and initiating cell death. While the goal of radiation is to target tumor cells, radiation can also cause direct injury to surrounding tissues such as cardiac tissue. The pathophysiology of RICVD is a complex process of acute cellular injury resulting in DNA strand breaks and the formation of reactive oxygen species (**ROS**), acute inflammation as a result of tissue injury, oxidative stress-mediated chronic inflammation, and impaired remodeling, atherosclerosis, and fibrosis (Figure 1). While the pathophysiology is heterogeneous, inflammatory and oxidative processes appear to be common in both ischemic vascular effects and cardiomyocyte damage. The end result leads to microvascular and macrovascular changes, and dysfunctional cardiac tissue that leads to reduction in ejection fraction, atherosclerosis with thrombus formation, pericardial disease, and arrhythmias.

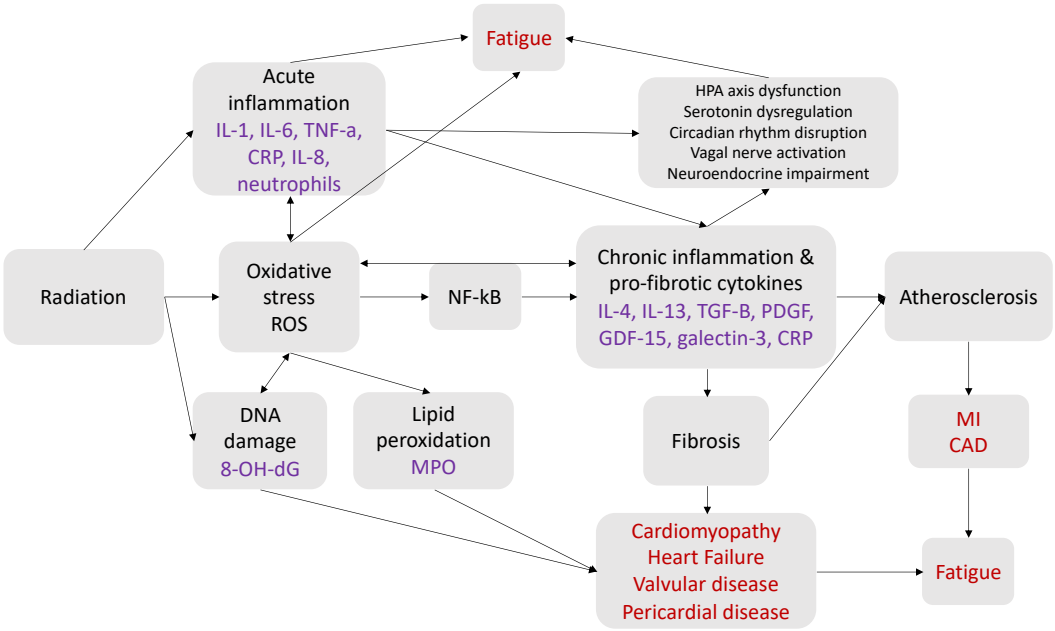

Figure 1 provides a summary of the pathophysiologic pathways that are hypothesized to be involved in RICVD and radiation-induced fatigue (RIF). This figure also highlights the possible relationship between RICVD and RIF. From this figure, it highlights the roles of acute inflammation, oxidative stress, and chronic inflammation, as well as the role of RICVD as a mediator of RIF in breast cancer survivors treated with radiation. RICVD and RIF outcomes are highlighted in red. Plausible biomarkers to reflect these pathways are highlighted in purple.

Radiation acutely results in direct tissue injury and DNA damage, acute inflammatory processes, and the production of ROS. Similar to normal cellular damage responses, there is an initial neutrophilic response with the release of pro-inflammatory cytokines resulting in vasodilation and increased vascular permeability.[12, 13] Additionally, damaged endothelial cells release cellular adhesion molecules and growth factors, further promoting an acute inflammatory response.[13, 14] Acute inflammatory mediators thought to play a role in RICVD are tumor necrosis factor alpha (**TNF-a**), interleukin (**IL**)-1, IL-6, and IL-8.[13, 15] Lastly, microvascular damage results in initial fibrin deposition as a result of activation of coagulation pathways, which is thought to contribute to chronic fibrosis.[13]

In addition to an acute inflammatory process, radiation causes direct DNA damage, which results in cell cycle arrest and apoptosis depending on the degree of damage.[15] A downstream effect of cell death signaling pathways is the production of ROS, which can result in oxidative stress. Oxidative stress is a physiologic state that occurs when there is a disturbance in the balance between the production of reactive oxygen species and antioxidant defenses.[16] These highly reactive molecules are able to bind to cellular structures, such as phospholipid membranes, which are abundant in cardiomyocytes particularly sensitive to ROS. This can lead to structural and functional defects, causing impaired energy metabolism and contractile ability.[14] ROS can also oxidize nucleoside bases resulting in lesions in DNA and strand breaks.[17] This can further trigger cell death signaling if not repaired. ROS can persist long after radiation therapy has ended and has been shown to persist for at least 6 months in animal models.[15]

Oxidative stress is also hypothesized to play a crucial role in the transition between acute and chronic inflammation by activating nuclear factor-kappa B (**NF-kB**), a protein heavily involved in immune regulation and cell survival.[12, 13, 15] Upregulation of NF-kB is thought to lead

to a chronic, pro-inflammatory state, which results in ineffective remodeling and recruitment of further inflammatory mediators and adhesion molecules.[12] This chronic attempt to repair damaged tissue leads to a vicious cycle of further increased ROS and inflammation.

Chronic inflammation and oxidative stress are thought to be responsible for the development of fibrosis and atherosclerosis. Fibrosis is the key contributor to the long-term effects of RICVD and can affect numerous cardiac structures. Fibrosis is the result of a complex process that converts fibroblasts into myofibroblasts, which secrete more collagen. Chronic, pro-fibrotic markers secreted by immune cells, such as IL-13, IL-4, transforming growth factor-beta (TGF-β), and platelet-derived growth factor are thought to promote this transformation.[12] Fibrosis can occur in various cardiac structures such as the myocardium, valves, pericardium, and coronary arteries.[18] Fibrosis in the myocardium ultimately results in decreased tissue elasticity and impaired muscle contraction leading to reductions in ejection fraction and heart failure. Additionally, fibrosis is the main contributor for pericardial disease, valvular disease, and conduction abnormalities.[18]

A pro-fibrotic state and compensatory mechanisms to repair endothelial damage as a result of chronic inflammation lead to vascular thickening, accumulation of endothelial injuries, and thrombi formation in arteries. These sequelae of chronic inflammation can progress to atherosclerosis and CAD.[12] Radiation can induce atherosclerosis by itself, or by accelerating already existing processes, making RICVD more likely to occur in those with traditional cardiac risk factors such as hyperlipidemia, smoking, hypertension, and diabetes.[19] CAD can be fatal as it can develop into a myocardial infarction (**MI**) and other major cardiac events, which is often associated with the increases in cardiac mortality and morbidity associated with RICVD.

Identifying individuals at risk for long-term RICVD has been a challenge and is often

neglected in research; however, biomarkers of acute-onset RICVD are promising for a role in long-term RICVD. Identifying individuals at risk for RICVD is difficult as it can occur several years after treatment. LVEF is typically used to monitor RICVD, but early changes in LVEF may not be predictive, and late changes may not be detectable until significant, irreversible damage has occurred.[20-24] Identifying biomarkers to classify individuals at risk has been a focus of recent research as biomarkers may be able to detect underlying mechanisms of disease progression prior to clinical manifestations. Cardiac damage and inflammatory biomarkers have been proposed to characterize and identify cancer survivors at risk for cardiotoxicity; however, studies have neglected to examine long-term RICVD outcomes, as only a few studies have focused on RICVD with most studies having only a 12-month follow-up period. Cystatin-C is an additional cardiac damage biomarker that has been shown to significantly predict CVD death, MI, and heart failure in individuals with CVD. There is evidence that it may be a valuable predictive biomarker of chemotherapy cardiotoxicity in murine models.[25, 26] However, this biomarker has not been evaluated as a marker of RICVD risk in cancer survivors.

While cardiac damage biomarkers may be able to identify damage associated with treatment, oxidative stress and inflammatory markers may be better able to identify individuals at risk for RICVD prior to any significant cardiac damage as these biological changes are likely occurring prior to cardiac damage. Therefore, further research is needed to investigate the association between oxidative stress and inflammation as potential biomarkers for RICVD, in addition to cardiac damage biomarkers.

Radiation-Induced Fatigue (RIF)

Fatigue one of the most commonly reported symptoms in cancer survivors and which affects up to 90% of survivors.[3, 27] While rates of fatigue are highest during treatment, it is

estimated approximately 33% of survivors experience fatigue up to 10 years after treatment.[27] Fatigue has been associated with many adverse outcomes including impaired activities of daily living, depression, reduced quality of life, and increased mortality.[3, 28] Given the substantial detrimental effects of fatigue, there is a need to understand its underlying pathophysiology in order to identify individuals at risk.

Fatigue is a common result of a variety of cancer treatments, including radiation, and the mechanisms driving fatigue are dependent on treatment type.[29] As such, it is important to examine the biological mechanisms within the context of a specific treatment. While the literature on the potential mechanisms of chemotherapy-induced fatigue is robust, radiation has received less attention. Therefore, it is important to summarize the pathophysiology focused on radiation-induced fatigue (**RIF**) to better understand the mechanisms.

Pathophysiology of RIF

While radiation therapy produces local effects to damage cancer cells, radiation has been shown to result in an increase in inflammatory circulatory markers, which are thought to drive fatigue. Immune dysregulation and chronic inflammation have been widely accepted as leading theories of RIF; however, it is not fully understood if inflammatory markers independently produce fatigue or exacerbate fatigue symptoms through downstream effects. There is strong evidence that levels of proinflammatory cytokines, such as IL-6, IL-1, and TNF-a, increase during and after radiation treatment; these cytokines have been associated with fatigue symptoms.[30-32] Increases in pro-inflammatory markers are also thought to contribute to fatigue by activating a cascade of events leading to hypothalamic-pituitary-adrenal (**HPA**) axis disruption, serotonin dysregulation, circadian rhythm disruption, vagal afferent nerve activation, and neuroendocrine impairment.[31]

Chronic inflammation is thought to disrupt the HPA axis through various pathways. The HPA axis plays an important role in cytokine and cortisol production and has strong anti-inflammatory effects.[3] With acute stress, there is typically an increase in corticotropin-releasing hormone (**CRH**), which results in increased adrenocorticotropic hormone (**ACTH**) release and cortisol. IL-6, IL-1, and TNF-a are stimulators of the HPA axis, which stimulate the production of cortisol; however, with increased exposure, blunting of this response occurs. Chronic inflammation has been shown to result in reduced CRH release with reductions in cortisol, and studies have shown decreases in cortisol are associated with fatigue.[33-35] Additionally, cortisol has a suppressive effect on the production of pro-inflammatory cytokines. Thus, decreased cortisol production with chronic exposure to pro-inflammatory cytokines leads to an even greater production of pro-inflammatory cytokines.[33, 34]

There is also evidence that inflammatory cytokines influence serotonin (**5-HT**) metabolism, which in turn has effects on the HPA axis and cortisol production. 5-HT regulates release of CRH in the HPA-axis by activating serotonin receptors in the hypothalamus stimulating cortisol and cytokine production. It is hypothesized that pro-inflammatory cytokines can lead to reductions in 5-HT by activating indoleamine 2,3-dioxygenase, a degrading enzyme thereby altering metabolism.[33, 34] This results in decreased production of cortisol and potential fatigue.[33]

Cortisol disruption as a result of HPA axis dysfunction is thought to play a role in fatigue by altering circadian rhythms. Research has shown that flatter diurnal cortisol slopes and a slower decline of evening cortisol are associated with greater fatigue in cancer patients.[33-36] This results in a loss of a diurnal rhythm and elevated levels of cortisol at night. Disrupted circadian rhythm is supported by evidence of abnormal sleep patterns in those with fatigue with higher

wakefulness during normal sleep hours and greater fatigue during the day.[33] Inflammation is also thought to be related to circadian rhythms as they regulate the immune system and may result in increased pro-inflammatory markers.[33]

Lastly, inflammatory cytokines are thought to also play a role in vagal afferent nerve activation. Stimulation of the vagal afferent nerve is thought to lead to suppression of somatic muscle activity and "sickness behavior". Pro-inflammatory cytokines, 5-HT, and prostaglandins can stimulate the vagal afferent nerve, resulting in decreased muscle tone and a general sense of weakness, which may cause symptoms of fatigue. Additionally, activation of the vagal nerve can cause "sickness behavior", which is includes symptoms of fatigue, malaise, increased sleep, and poor concentration.[33] While this hypothesis has support in animal models, the role in fatigue in humans remains elusive.

While inflammation is highly involved in the etiology of RIF, radiation is also thought to contribute to fatigue through the production of ROS and oxidative stress. The production of ROS damages cellular structures, including the mitochondria, which leads to defects in oxidative phosphorylation and depletion of adenosine triphosphate (**ATP**). ATP is a major source of energy for muscle function, and reduction in ATP can cause contractile dysfunction and precipitate fatigue.[30] Evidence also supports the role of oxidative stress in RICVD. Prior research has found changes in fatigue were significantly correlated with changes in measures of plasma oxidative stress (**8-OH-dG**) in cancer survivors receiving an exercise intervention compared to healthy controls.[37] A separate study found increases in oxidative stress during treatment were significantly associated with symptoms of fatigue in children with leukemia.[38]

While there are multiple hypotheses of RIF, inflammation and oxidative stress appear to be leading contributors. Refer to Figure 1 for a representation of these relationships. The

literature has examined mechanisms of fatigue in cancer populations receiving chemotherapy or a combination of chemotherapy and radiation, but not radiation alone. Therefore, the specific mechanisms related to radiation needs to be explored. While radiation leads to increases in circulating markers of inflammation and oxidative stress, differences may exist between systemic therapies (chemotherapy) and local therapies (radiation).

Assessing and predicting fatigue is difficult due to multiple mechanisms likely causing fatigue and a lack of consistent definition of RIF. Numerous investigators have examined mechanisms of fatigue and associations between IL-6 and CRP and post-treatment fatigue in cancer survivors[3, 31, 39, 40], however there are still gaps that need to be addressed. First, there is a need to disentangle the multiple potential mechanisms leading to RIF in order to improve its assessment. Specifically, the cardiotoxic nature of radiation may increase RIF by adding additional cardiac defects. Cardiac damage biomarkers may improve assessment in patients receiving radiation by accounting for potential impairments in cardiac function, which could cause symptoms of RIF. Second, issues in defining and characterizing clear RIF case definitions have yielded inconsistent results in search of fatigue biomarkers[40] , which are needed to provide a detailed characterization of the phenotype needed, translate findings, and identify appropriate biomarkers. Additionally, the influence of covariates, such as depression and comorbidities, on biomarkers should be explored to explain inter-individual variation in fatigue. Multiple factors may influence the association between radiation and fatigue including treatment effects, concurrent treatment (such as skeletal muscle damage due to chemotherapy), comorbidities, and lifestyle factors.[3] Thus, studies investigating RIF must have detailed data on these factors.

Relationship between RICVD and Fatigue

While radiation therapy can independently contribute to RICVD and fatigue, breast

cancer survivors present a unique situation that can lead to RICVD-mediated fatigue given the proximity of radiation to the heart. As previously mentioned, RICVD and RIF may share common physiologic mechanisms such as chronic inflammation and oxidative stress, which directly target cardiac tissue to produce RICVD or produce circulating inflammatory markers that lead to fatigue (Figure 1). It is hypothesized that radiation, through the complex relationship with oxidative stress and inflammation, create a pro-fibrotic environment, which contributes to cardiomyopathies and heart failure. Fatigue is a commonly reported symptom in patients with heart failure and CVD due to cardiac dysfunction.[41] Fibrosis due to RICVD can cause systolic heart failure (reductions in LVEF, a measure of how much blood the left ventricle pumps out with each contraction) and diastolic heart failure (heart failure with preserved LVEF). Reductions in LVEF can directly cause fatigue symptoms, and diastolic heart failure has been associated with reductions in cardiorespiratory fitness, exercise intolerance, and fatigue.[42] Fatigue is also a commonly reported symptom in patients with heart failure, coronary artery disease, pericardial disease, and valvular disease, which are all potential cardiovascular outcomes associated with radiation treatment.

Inflammation and oxidative stress are also likely independent contributors to fatigue. Treatment that directly damages heart tissue, such as radiation, may be more likely to cause fatigue symptoms. Therefore, it is not only important to examine inflammatory and oxidative stress biomarkers and fatigue but also look at cardiac damage and fibrosis markers. Given the potential for radiation to cause fatigue through inflammation, oxidative stress, and cardiac damage, studies investigating this relationship are warranted. This work may provide insight on contribution of RICVD in fatigue of breast cancer survivors treated with radiation. Additionally, there is evidence in non-cancer populations that fatigue itself, and other symptoms associated

with cancer treatments, are associated with the risk of CVD.

Conceptual Model

The National Institute for Nursing Research (**NINR**) developed the National Institute of Health Symptom Science Model (**NIH-SSM**) to guide researchers using 'omics'-based studies.[43] The model suggests that research should start by identifying a symptom or symptom cluster, followed by a characterization of the symptom phenotype. Once a symptom has been phenotypically characterized, 'omics' approaches can be used to identify biomarkers. These can be used to identify individuals at risk for symptoms and, ultimately, be used as targets for bio-behavioral interventions (Figure 2).[43] Recently, NINR built upon this model to include precision health concepts, which are important in symptom science and symptom management. Precision health is an emerging field aimed at focusing on the individual taking into account genetic, environmental, and lifestyle factors. This revised model, named the Nursing Science Precision Health Model (**NSPHM**), includes four precision concepts related to each component of the NIH-SSM: 1) measurement, 2) characterization of phenotype, 3) characterization of genotype and biomarkers, and 4) intervention target discovery, design, and delivery (Figure 2).[44] This model also incorporates translational research concepts as biological discoveries need translated to 1) humans and 2) clinical practice in order to improve symptoms. Precision in characterizing symptom phenotypes and biomarker measurements are necessary to translate findings into practice.[44]

Precision health concepts greatly align with symptom science as the goal is to understand individual and biological characteristics associated with symptoms in order to develop personalized strategies to manage and treat symptoms. Research aimed at understanding the biological mechanisms associated with symptoms can lead to targeted interventions reflecting

biological, lifestyle, environmental, and behavioral factors. This will, in turn, provide information on how these interventions affect symptom phenotypes and their underlying biological etiologies.[44] The NSPHM was used to guide this dissertation by providing a framework to characterize RICVD and RIF to identify potential biomarkers related to inflammation, cardiac damage, and oxidative stress in RICVD and RIF. The pathophysiology of RICVD is complex, however phenotypically presents as heart failure, CAD, or MI, which can result in the need for coronary revascularization or cardiac death. Therefore, in this dissertation, RICVD is defined has having either a heart failure, MI, coronary heart disease, stroke, or CVD-death outcome. Additionally, fatigue has been phenotypically characterized with the SF-36 using a cutoff of 50, with scores < 50 being "fatigued" and scores ≥ 50 being "non-fatigued".[35, 36, 45, 46] In previous studies, this phenotypic characterization of fatigue has been able to detect differences in IL-6 and CRP in fatigued vs. non-fatigued cancer survivors, suggesting this phenotypic characterization is appropriate for identifying biological markers associated with fatigue. With appropriate characterization of RICVD and RIF, biomarkers can be identified that correspond with the pathophysiology of RICVD and RIF, which this study aims to address.

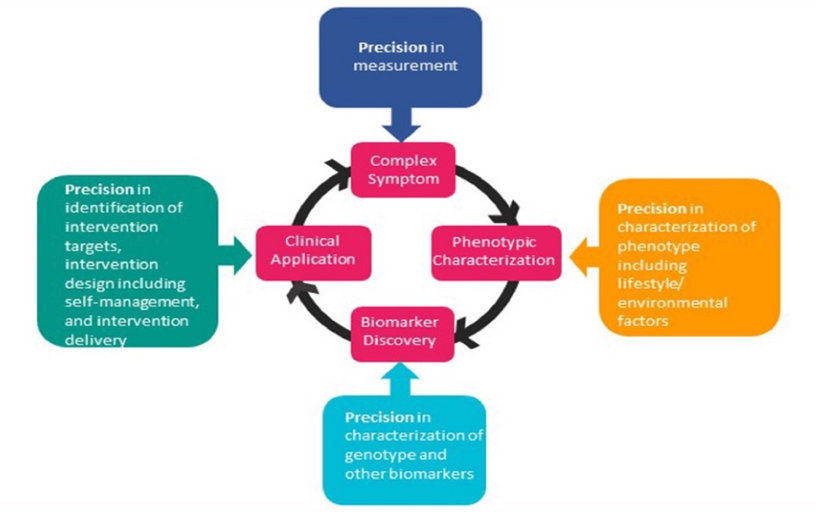

Figure 2. Nursing Science Precision Health Model conceptual framework. The inner circle represents the original Symptom Science Model. The arrows are the four additional precision concepts.

Dissertation Purpose & Elements

The overall purpose of this dissertation is to better understand biomarker associated with cardiovascular disease and symptoms after radiation treatment in breast cancer survivors and to better understand the inter-relatedness of these outcomes (Figure 3). In particular, this study examined the role of oxidative stress, cardiac damage, fibrosis, and inflammation in the development of RICVD and RIF in breast cancer survivors treated with radiation as well as examined the role of symptoms, such as fatigue, in the risk of RICVD in breast cancer survivors. A brief overview of each chapter is provided.

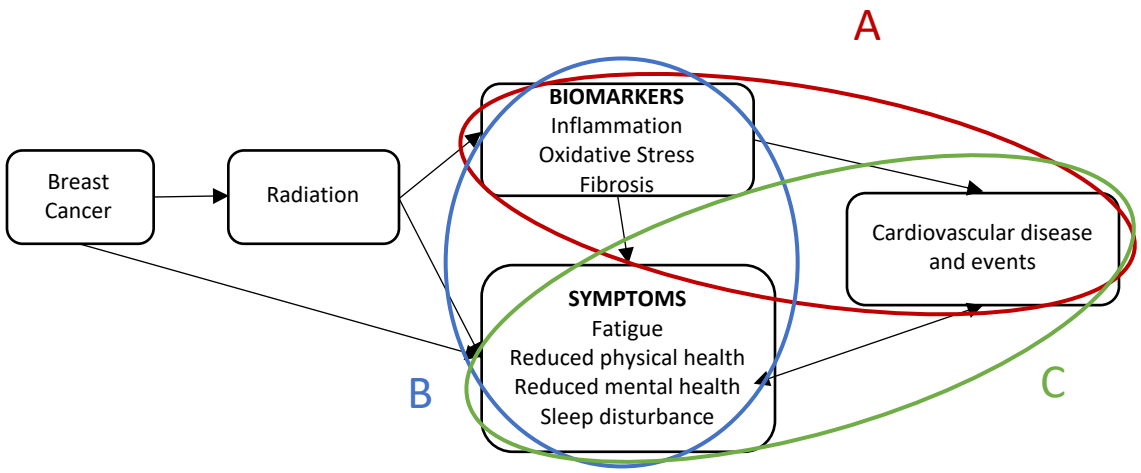

Figure 3. Conceptual framework for dissertation chapters

Chapter 2: This chapter aims to assess the association of post-cancer biomarkers and CVD events in breast cancer survivors treated with radiation (Figure 3, A). This study is a nested case-control study within the Women's Health Initiative (**WHI**), which included 55 RICVD cases and 158 non-RICVD controls. Biomarkers were analyzed using enzyme-linked immunosorbent assays (**ELISAs**) or RT-qPCR in serum samples collected both pre- and post-breast cancer diagnosis. Multi-variable adjusted logistic regression models were used to test the study aims.

Chapter 3: This chapter examines the association of post-cancer biomarkers and fatigue in breast cancer survivors treated with radiation (Figure 3, B). Using the sample selected in chapter 3, a secondary analysis was conducted. Associations between post-breast cancer biomarker concentrations and post-breast cancer fatigue, measured using the SF-36 Vitality subscale, were analyzed using multi-variable adjusted weighted linear regression equations. Inverse probability weights were applied to account for sample selection into the original case-control study described in chapter 3.

Chapter 4: This chapter examines the relationship between post-cancer health-related quality of life and sleep disturbance measures and risk of CVD in breast cancer survivors within the WHI (Figure 3, C). This study included women diagnosed with breast cancer during WHI follow-up without a history of CVD. Post-breast cancer physical and mental health-related quality of life was determined by the SF-36 Physical and Mental Component Scores while sleep disturbance was measured by the WHI Insomnia Rating Scale. Individual SF-36 subscales, including fatigue, pain, and depression, were also assessed. Time-dependent Cox proportional hazard models were used to test the study aims.

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CHAPTER 2

Biomarkers of oxidative stress, inflammation, and cardiac damage as markers of long-term radiation-induced cardiovascular outcomes in breast cancer

Abstract

Background: Although radiation has contributed to improved survival, many breast cancer (BC) survivors experience radiation-induced cardiovascular disease (RICVD). Currently, it is not known how to best identify individuals who will develop RICVD. Thus, the aim of this study was to examine associations between biomarkers of oxidative stress, cardiac damage, and inflammation and long-term RICVD in BC survivors treated with radiation.

Methods: In an ancillary, nested case-control study within the Women's Health Iniative, postmenopausal women with incident BC stages I-III, who received RT, and had pre- and post-BC diagnosis serum samples available not more than 3 years apart were eligible. Cases were defined as developing incident, physician-adjudicated coronary heart disease, myocardial infarction, CVD death, heart failure, or stroke after BC. Each case was matched to 3 controls on enrollment age and visit year of pre-BC blood draw. Biomarkers were analyzed using enzyme-linked immunosorbent assays (CRP, cystatin-C, 8-OH-dG, MPO, GDF-15, TGF-B, PGF) or RT-qPCR (IL-6, troponin-I). Each biomarker was recorded as the ratio of the post-BC value relative to the pre-BC biomarker and log transformed to base 2. Logistic regression models were adjusted for relevant demographic, lifestyle, and cancer characteristics.

Results: A total of 55 cases and 158 controls were included with a mean (SD) age of 66.9 (5.5) years and a median (IQR) time from BC diagnosis to post-BC serum collection of 1.4 (0.7, 2.4) years. After adjustment, a higher 8-OH-dG ratio was significantly associated with an elevated risk of RICVD (Table). For a doubling in the biomarker ratio, RICVD risk was 3.04-fold higher (95% CI: 1.01, 9.21). Other biomarkers were not associated with RICVD risk.

Conclusions: In this study, higher concentrations of 8-OH-dG were associated with a higher risk of RICVD. This suggests that oxidative DNA damage may be a putative pathway for

delayed RICVD. However, given the sample size and multiple testing, further studies are needed to confirm or refute these findings.

Introduction

Radiation therapy is a common cancer treatment across all breast cancer stages, with over 50% of patients receiving radiation as primary or adjuvant therapy.[2] Although radiation has contributed to improved survival from breast cancer, many survivors experience treatment-related chronic adverse effects including cardiotoxicity.[3-5] In particular, radiation is known to increase the risk of cardiovascular disease (CVD) known as radiation-induced cardiovascular disease (RICVD), which is a substantial cause of increased morbidity and mortality among breast cancer survivors.[5] RICVD can manifest as heart failure (both diastolic and systolic), myocardial ischemia, acute coronary syndrome, valvular heart disease, pericardial disease, and increased risk of cardiac mortality.[3,6-9] The incidence of RICVD in breast cancer survivors varies depending on the definition used by researchers. However a large meta-analysis documented an incident rate of 76.4 and 125.5 cases per 100,000 person-years coronary heart disease and CVD-specific mortality, respectively, in breast cancer patients treated with radiation compared to those without.[47]

The pathophysiology of RICVD is a complex process of acute cellular injury resulting in DNA strand breaks and the formation of reactive oxygen species (ROS), acute inflammation as a result of tissue injury, oxidative stress-mediated chronic inflammation, and impaired remodeling, atherosclerosis, and fibrosis. Chronic oxidative stress and a pro-inflammatory state result in the formation of fibrotic tissue and atherosclerosis.[13, 48] A pro-fibrotic state and compensatory mechanisms to repair endothelial damage as a result of chronic inflammation lead to vascular thickening, accumulation of endothelial injuries, and thrombi formation in arteries. These sequelae can progress to atherosclerosis and coronary artery disease (CAD).[12] Radiation can induce atherosclerosis by itself, but can also accelerate already existing processes, making

RICVD more likely to occur in those with traditional cardiac risk factors such as hyperlipidemia, smoking, hypertension, and diabetes.[19] CAD can be fatal as it can result in myocardial infarction (MI) and other major cardiac events, which are responsible , in part, for the increased cardiac mortality associated with RICVD. Fibrosis can also lead to decreased tissue elasticity of the ventricles and reduced ejection fraction or diastolic dysfunction, presenting as heart failure.[13, 49]

Currently, it is not known how to best identify individuals who will develop RICVD, as it often occurs years after treatment and is often neglected in research due to the challenges of following patients for long-term outcomes.[9, 50] As a result, the primary body of literature in cardio-oncology examines short-term cardiac outcomes, mainly related to chemotherapy.[11] Based on these studies, biomarkers of cardiac damage and inflammation have been identified as acute contributors of cardiotoxicity. While these pathways likely influence RICVD, this has not been definitively tested in research studies. In addition, mechanistically, RICVD is hypothesized to be associated with pathways of chronic oxidative stress, fibrosis, and inflammation; however few studies have examined this.[10-13]

Thus, the aim of this study is to examine the role of biomarkers of oxidative stress, cardiac damage, and inflammation in the prediction of RICVD in breast cancer survivors. We conducted a nested case-control study of RICVD within the Women's Health Initiative (WHI) to examine if post-breast cancer diagnosis biomarkers were associated with the risk of major adverse cardiovascular events or heart failure (MACE/HF) in breast cancer survivors treated with radiation. Specifically, we examined the following biomarkers: oxidative stress: 8-hydroxy-2'-deoxyguanosin (8-OH-dG), myeloperoxidase (MPO); inflammation: interleukin-6 (IL-6), C-reactive protein (CRP), growth differentiation factor-15 (GDF-15), transforming growth factor-B (TGF-B), placental growth factor (PGF); cardiac damage: cardiac troponin-I (TnI), cystatin-C.

Methods

Study population

A detailed description of the WHI study has been published elsewhere.[51] In summary, the WHI is a nationally representative, prospective cohort study focused on preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. The WHI has two main components: a randomized Clinical Trial (CT) and an Observational Study (OS). A total of 161,808 women aged 50-79 were enrolled at 40 clinical centers nationwide beginning in 1993. All women were followed through 2005 with the option of extended follow-up through 2015.[51] A sub-cohort of cancer survivors, the Life and Longevity After Cancer (LILAC), was established in 2013.[52] A primary goal of LILAC was to collect information on cancer treatment and outcomes in women diagnosed with cancer. Women with no prior cancer diagnosis at WHI enrollment were eligible for LILAC if they had a confirmed cancer diagnosis during WHI follow-up in the CT or OS.

This study included participants in the WHI OS or CT who were enrolled in LILAC or in Medicare at the time of their cancer diagnosis and had treatment data available. In addition, women were eligible for this study if they 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. Women were ineligible if they 1) had an adjudicated MACE/HF outcome prior to breast cancer, 2) were diagnosed with metastatic disease or were missing stage, 3) or 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 inclusion and exclusion criteria for this study.

Case selection

Cases were defined as having an incident, physician-adjudicated MACE or HF event defined as coronary heart disease (CHD), which includes MI and CHD death, CVD death not classified as CHD, HF, or stroke 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.[53] 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. Cause of death was determined through linkage with the National Death Index. CHD, stroke, and CVD death events were adjudicated on all participants through 2010, whereas HF was adjudicated through 2005.

CHD was defined as having an acute MI requiring hospitalization, a silent MI, or events requiring hospitalization along with coronary revascularization procedures such as coronary artery bypass or percutaneous transluminal coronary angioplasty. Both definite and probably MIs were included and were classified using an algorithm that consisted of a combination of data including medical history, electrocardiogram readings, and cardiac enzymes.[53]

CVD death includes all deaths associated with definition or possible CHD, deaths associated with stroke or pulmonary embolism, and other CVD deaths not related to CHD. CHD deaths specifically were defined as death with an underlying cause of CHD with one or more of the following: hospitalization for MI within 28 days before death, previous MI or angina, death resulting from a procedure related to coronary artery disease, or a death certificate indicating CHD as the underlying cause of death.

Stroke and HF were included as outcomes in the WHI if they required hospitalization. Stroke was defined as having a rapid onset of persistent neurologic deficit attributed to an obstruction or rupture of the brain arterial system without evidence for other cause and supported by imaging studies.[53] HF was included as an outcome if a participant was hospitalized with signs and symptoms consistent with HF, plus objective documentation consistent with HF including pulmonary edema by chest x-ray or poor ventricular function by imaging studies. A physician diagnosis of HF and receipt of medical treatment were also considered as a HF outcome.

Control selection & matching

Controls were defined as those who 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, respectively. Controls were 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. Age group was matched exactly but frequency matching was used for the other criteria. 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 3 corresponding controls, however each had at least one matched control.

Exposures

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

The WHI has detailed protocols regarding specimen collection, handling, preparation and storage.[51] All WHI staff were trained in standardized methods of specimen acquisition and processing to minimize variation and ensure accuracy of biomarker results. In summary, serum was separated from blood samples within one hour after collection and all samples were maintained at 4 degrees Celsius. After centrifugation, samples were separated into 0.25 ml aliquots and placed into a -80-degree Celsius freezer within 2 hours of collection for future use.

All biomarkers were measured using commercially available kits. All biomarkers except IL-6 and TnI were measured using enzyme-linked immunosorbent assays (ELISA). TnI and IL-6 were measured using ProQuantum RT-PCR kits. All assays were conducted in the University of Washington School of Nursing Office for Nursing Research laboratory. All assays were tested and analyzed following manufacturer's protocols. Samples measured using ELISAs were tested in duplicates, whereas those measured by RT-PCR kits were tested in triplicates, and the average was used in the analysis. For samples that were below the detectable limit, we used a value that is halfway between zero and the 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. Smoking is measured as pack-years and was estimated based on the total number of cigarettes smoked per day and the duration of reported years of smoking. Physical activity is calculated as the number of metabolic-equivalent hours per week (MET-hours/week). This determined based on the type and duration of a variety of physical activities classified as mild, moderate, or strenuous. METs are assigned to each type of activity based on the Compendium of Physical Activities. Alcohol consumption is measured as the number of alcoholic servings per week based on self-reported consumption of standard servings of beer (12 oz), liquor (1.5 oz), and wine (6 oz). 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. Medication use was recorded at multiple time points during WHI follow-up. We were interested in whether a person was taking cardiac medications specifically calcium channel blockers, beta-blockers, ACE inhibitors, or angiotensin receptor blockers. Participants were asked to bring in prescription pill bottles and the name, strength, and dose of each medication was entered into a database. Lastly, cancer characteristics such as stage, chemotherapy use, and laterality of breast cancer were recorded from medical records. For variables measured at multiple time points, the value closest, but most proximal to the pre-cancer biomarker 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 calculated using both means with standard deviations and medians with IQR stratified by case status. 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.

Given that variables were matched loosely based on frequency, unconditional logistic regression was used to evaluate the associations between post-cancer diagnostic biomarkers (8-OH-dG, TnI, CRP, IL-6, MPO, GDF-15, TGF-B, cystatin-C, and PIGF) and risk of MACE or HF.[54, 55] 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 previously.[56] Given the non-normal distribution of the biomarkers, this ratio was log transformed to base 2. A ratio was chosen, as opposed to absolute change values, in order to log transform the data to account for non-normality. Each unit difference in the biomarker ratio represents a doubling in value compared to pre-cancer. 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). Models for TnI and PGF additionally adjusted for pre-cancer biomarker concentrations. Confounders were selected *a priori* based on the relationship of each variable with both the exposures and outcome. Matching variables were included if they are known to be associated with the exposure.[55] Multivariable

models were adjusted for age (5-year categories), income (< $34,000, $35,000 - $74,999, > $75,000), waist circumference (cm), smoking (pack-years), physical activity (total MET-minutes/week), cancer stage (local/regional vs. distant), 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) explored if post-diagnosis biomarkers were individually associated with the risk of each type of outcome (i.e., CVD death, coronary heart disease death, stroke) separately, 2) repeated the proposed analyses in only LILAC participants with abstracted treatment data adjusted for and without chemotherapy, and 3) performed a stratified analysis based on the time from breast cancer to serum collection. For sensitivity analysis #1, unadjusted models were created for each biomarker for each individual outcome. For sensitivity analysis #2, 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 models adjusted for chemotherapy (yes vs. no). For sensitivity analysis #3, 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. 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.

All analyses were conducted using R Version 3.6.1 (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 WHI enrollment was 69.2 (5.5). The majority of participants self-identified as non-Hispanic White (92%) and reported an annual income of < $75,000 (71.8%). More than half of participants were diagnosed with right-sided (60.6%) and regional (77.9%) breast cancer. Approximately 75% were enrolled in LILAC (Table 1). Of the 55 cases, 15 initial events were classified as CHD, 10 as stroke, and 22 as CVD death. The median (IQR) time from the pre-cancer serum collection to breast cancer diagnosis was 665 (301, 958) days and the median (IQR) time from breast cancer diagnosis to the post-cancer serum collection was 494 (272, 869) days.

When comparing cases and controls, cases were more likely to be on beta-blockers (p=0.01) and calcium channel blockers (p=0.039) (Table 1). There were no other significant differences; however, there was a suggestion that cases, on average, smoked more, had higher waist circumferences, were diagnosed with hypertension at a higher rate. There was nearly an equal distribution of all matching variables.

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 these biomarkers, 119 and 126 participants had undetectable concentrations for PGF for pre- and post-cancer time points, respectively. For TnI, 110 and 109 participants had undetectable concentrations for pre- and post-cancer time points, respectively. When comparing pre- or post-cancer serum biomarkers between cases and controls, there were no significant differences (Table 2). 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.

Logistic regression results

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

Sensitivity analyses

When exploring the association of 8-OH-dG with each cardiac outcome, the strongest association was for stroke, albeit not significant (Supplemental Table 2). Besides IL-6, there were no significant interactions when stratified by timing of the biomarker in relation to breast cancer diagnosis (Supplemental Table 3). When IL-6 was measured within 1 year of breast cancer, there was a suggestion that a higher IL-6 biomarker ratio was associated with a higher risk of MACE or HF; however, when measured further from breast cancer diagnosis the associations suggest the opposite relationship. Additionally, although not significant, there was a pattern for most biomarkers where point estimates were elevated within 1 year of breast cancer, and then weakened when measured further out. Lastly, 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).

Discussion:

This is the first study, to our knowledge, to examine the associations of biomarkers with long-term cardiac endpoints in breast cancer survivors. Additionally, this study focused on breast

cancer survivors treated with radiation, which has received less attention compared to chemotherapy. The findings from this study demonstrate that 8-OH-dG, a marker of oxidative stress and DNA damage, is associated with the risk 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 heart failure[57], cardiovascular disease[58], and cancer[59]. Reactive oxygen species oxidize nuclear and mitochondrial DNA damaging DNA and creating 8-OHdG. The majority of research on the effects of cancer treatments on oxidative stress has been done in murine models. 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[60, 61] and one study showed continued elevation of 8-OH-dG DNA adducts 5 weeks after the last treatment.[60] 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.[60, 62] 8-OH-dG has also been examined in the context of occupational health in workers exposed to low doses of radiation. Hospital staff in the radiology departments had consistently elevated levels of serum 8-OH-dG.[63] However, this is the first study to examine 8-OH-dG as a marker of radiation-induced cardiovascular disease in breast cancer survivors. Our findings suggest that post-cancer 8-OH-dG concentrations may be independently associated with long-term cardiac outcomes even after adjustment for relevant confounders such as age, waist circumference, smoking, physical activity, and cardiac medications.

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.[64-68] Although studies examining biomarkers related to radiation-induced cardiac outcomes are limited, a prior study also found no association between post-radiation TnI concentrations and changes in cardiac function as measured by LVEF in breast cancer patients.[64] Thus, this suggests that TnI may not be a valuable biomarker for assessing cardiac dysfunction in breast cancer survivors treated with radiation, especially long-term cardiac outcomes. This is in contrast with studies examining the impact of chemotherapy on cardiac troponin concentrations, which have reported higher troponin concentrations to be associated with an elevated risk of cardiac dysfunction defined as changes in LVEF.[69-71] 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 is similar to previous studies examining the impact of radiation on troponin levels.[65, 68] However, we cannot eliminate the possibility that insufficient statistical power prevented us from detecting an effect.

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 LVEF-defined cardiac function in lung and lymphoma patients treated with contemporary radiation and long-term LVEF changes in breast cancer patients treated with doxorubicin and traztuzamab.[56, 64] While GDF-15 and PGF were associated with cardiac dysfunction in lung and lymphoma patients, no associations were found in breast cancer patients.[64] This was likely attributed to the lower mean heart doses of radiation in breast cancer patients compared to lung and lymphoma patients. However, the participants in this prior study received contemporary radiation, which minimizes doses of radiation to the heart, with a

mean heart dose of 1.3 Gy. While we are unable to obtain the radiation dose, the majority of women in our study received radiation between 1993 – 1998. Thus, they most likely received higher doses of radiation to the heart based on common practices at that time.[72] 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. MPO has been identified as a robust biomarker to predict chemotherapy-induced cardiotoxicity.[56, 69, 71] However, we did not find a signification association in our study. To our knowledge, 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 that exist between the current study and prior studies examining PGF, GDF-15, and MPO and treatment-induced cardiotoxicity. First, the prior studies defined their outcome as cardiac dysfunction in terms of LVEF changes. While heart failure and cardiomyopathy are included in the outcomes of RICVD, RICVD also can manifest as pericardial disease, myocardial fibrosis, coronary artery disease, valvular disease, and arrhythmias.[8] The outcome in the current study focused on clinical cardiovascular outcomes. Additionally, the length of follow-up time to outcome in the current study is substantially longer than previous studies. In the previous study by Demissel et al. (2020), the maximum follow-up time from baseline was 3.7 years, whereas in the current study the median time from breast cancer to first MACE or HF outcome was 7.9 years. Thus, these biomarkers may be more sensitive to LVEF changes that occur shortly after treatment but may not be predictive of long-term cardiac outcomes.

Our study has important limitations. Given this study was observational, there is the possibility of residual confounding. However, given the sample size and rigorous collection of

comprehensive data, 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, thus these results should be interpreted with caution and these findings should be validated in a larger sample size. Despite including all eligible cases, the sample size was small given the limited availability of eligible participants with serum available and the fact that radiation treatment documentation was only collected on a subset of the WHI. Additionally, we conducted a nested case-control study, which provides support for the role of 8-OH-dG in the development of RICVD; however, prospective studies are needed that analyze serum specimens within a shorter timeframe after radiation.

While we are ultimately interested in biomarkers measured after radiation, we did not have radiation end dates available in this data. Thus, we are not able to definitively know the biomarkers in this study were measured after radiation treatment had ended. In this sample, radiation started a median time of 87 days after breast cancer. As radiation treatment typically lasts about 6 weeks, we estimate that approximately 92% had biospecimens collected after radiation ended based on the timing of their post-breast cancer serum collection.

The majority of women in this study received radiation in the 1990s, when cardiac doses were higher, 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 completely eliminated. Reports still suggest elevated CVD risk with contemporary radiation.[47, 73] This study should be repeated in the context of contemporary radiation to determine if the associations with 8-OH-dG are still present.

We included women in this analysis if they had radiation treatment that was identified either through medical record abstraction, Medicare data, or self-report. Thus, it is possible that treatment data was misclassified given we included those who self-reported radiation. However, comparing the two data ascertainment methods (i.e., medical record abstraction/Medicare claims data vs. self-report), the percent agreement was 98% in the eligible sample. This corresponds to a kappa of 0.953 (95% CI: 0.907, 0.999). Given the high degree of agreement, the chance of misclassifying receipt of treatment is minimized. Additionally, based on our matching procedures, the majority of our sample had data collected through medical record abstraction or Medicare claims data.

In this study we defined RICVD as a composite outcome of CHD, CVD death, and stroke. Radiation is thought to produce cardiotoxic effects through microvascular and macrovascular damage leading to cardiac ischemia and myocardial infarction[47, 49]. Given the pathologies associated with RICVD, our composite endpoint captures a comprehensive outcome of events that occur with radiation[13]. While the pathophysiology is heterogeneous, inflammatory and oxidative processes appear to be common in both ischemic vascular effects and cardiomyocyte damage.[12-14, 48, 74] However, based on the unadjusted analyses stratified by the individual cardiac outcomes, it appears there may be differences in the risk estimates between each outcome depending on the biomarker. Thus, future studies should target specific cardiac outcomes.

Despite these limitations, our study has many strengths. This study focused on biomarkers related to 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.[2, 64, 65, 75-78] This study also focused on long-term

cardiac outcomes, rather than changes in LVEF, which are associated with substantial cardiac morbidity and mortality. Cardiac outcomes were also physician-adjudicated in the WHI minimizing the possibility of misclassification.

Conclusions

In breast cancer survivors treated with radiation, higher concentrations of 8-OH-dG were significantly associated with an elevated risk of RICVD. This suggests that oxidative DNA damage may be a putative pathway for RICVD. However, given the small sample size, this study should be repeated in larger samples to confirm these findings. Biomarkers may be a useful strategy to improve the identification and prediction of RICVD in cancer survivors. 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.

Table 1. Baseline (i.e., pre-cancer) characteristics stratified by case status

	Overall (N = 213)	Controls (N = 158)	Cases (N = 55)	p-value
Age at Diagnosis (mean(SD))	69.2 (5.5)	69.2 (5.5)	69.5 (5.5)	0.658
Age at WHI Enrollment (mean(SD))	66.9 (5.5)	66.9 (5.6)	67.0 (5.5)	0.851
Age at WHI Enrollment (5yrs) (n (%))				0.991
55 – 59	28 (13.1)	21 (13.3)	7 (12.7)	
60 – 64	32 (15.0)	24 (15.2)	8 (14.5)	
65 – 69	74 (34.7)	55 (34.8)	19 (34.5)	
70 – 74	70 (32.9)	52 (32.9)	18 (32.7)	
75 - 79	9 (4.2)	6 (3.8)	3 (5.5)	
Race/Ethnicity (n (%))				0.643
Non-Hispanic White	196 (92.0)	147 (93.0)	49 (89.1)	
Non-Hispanic Black	6 (2.8)	4 (2.5)	2 (3.6)	
Other*	9 (4.2)	7 (4.4)	4 (7.3)	
Income (n (%))				0.925
< $34,999	66 (31.0)	47 (29.7)	19 (34.5)	
$35,000 - $74,999	87 (40.8)	68 (43.0)	19 (34.5)	
$75,000 - $99,000	19 (8.9)	13 (8.2)	6 (10.9)	
> $100,000	30 (14.1)	22 (13.9)	8 (14.5)	
Smoking (pack-years) (mean (SD))	11.6 (18.4)	11.3 (18.3)	12.4 (18.6)	0.723
BMI (kg/m^2) (mean (SD))	27.3 (6.5)	27.2 (6.8)	27.7 (5.8)	0.676
Waist circumference (cm) (mean (SD))	84.0 (12.6)	83.1 (12.0)	86.7 (14.11)	0.080
Physical Activity (MET-hours/week) (mean (SD))	16.2 (15.3)	15.5 (14.9)	18.3 (16.2)	0.248
Alcohol (servings/week) (mean (SD))	3.3 (6.5)	3.25 (5.0)	3.6 (9.6)	0.738
Cancer stage (n (%))				0.235
Local	1 (0.5)	0 (0.0)	1 (1.8)	
Regional	166 (77.9)	124 (78.5)	42 (76.4)	
Distant	46 (21.6)	34 (21.5)	12 (21.8)	
Laterality (n (%))				0.921
Right	129 (60.6)	96 (60.8)	33 (60.0)	
Left	84 (39.4)	62 (39.2)	22 (40.0)	
Hx of HTN (n (%))	7 (3.3)	3 (1.9)	4 (7.3)	0.137
Cardiac Medications (n (%))	30 (14.1)	16 (10.1)	14 (25.5)	0.005
Enrolled in LILAC (n (%))	163 (76.5)	121 (76.6)	42 (76.4)	0.999

Treatment Source (n (%))				0.738
Abstraction/Medicare	181 (85.0)	133 (84.2)	48 (87.3)	
Self-Report	32 (15.0)	25 (15.8)	7 (12.7)	
Time-point of pre-cancer serum (n (%))				0.083
Baseline	199 (93.4)	151 (95.6)	48 (87.3)	
Year 3	9 (4.2)	4 (2.5)	5 (9.1)	
Year 6	5 (2.3)	3 (1.9)	2 (3.6)	

Significant at level: *0.05, **0.01, ***0.001

Missing meds: 1.3% of controls and 3.6% of cases

Table 2. Distribution of Pre- and Post-Cancer Biomarkers Stratified by Case Status

	Pre-Cancer					Post-Cancer				
	Control		Case			Control		Case		
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value
8-OH-dG (pg/mL)	10375 (3110.5)	9817 (8348, 11683)	10274 (2560.9)	10651 (8520, 11623)	0.735	10439.7 (2162.4)	9972 (8560, 11857)	10697.2 (2844.3)	10571, 8952, 12176)	0.434
TnI (pg/mL)	8.0 (24.3)	0.2 (0.2, 4.0)	4.8 (13.6)	0.2 (0.2, 2.4)	0.608	6.2 (19.8)	0.2 (0.2, 2.8)	3.7 (8.8)	0.2 (0.2, 1.9)	0.672
PGF (pg/mL)	338.3 (876.8)	1.0 (1.0, 91.0)	114.2 (263.3)	2.6 (1.0, 86.0)	0.67	362.6 (910.5)	1.0 (1.0, 131.4)	149.0 (452.8)	1.0 (1.0, 29.2)	0.336
TGF-B (pg/mL)	30648 (12070.4)	29211 (22337, 37408)	30186 (9518.5)	28837 (23725, 35923)	0.813	27811 (11793)	24880 (20122, 33532)	26814 (9067)	25919 (19647, 31662)	0.989
CRP (mg/L)	9.91 (15.8)	5.8 (1.9, 12.8)	9.6 (7.9)	8.4 (3.0, 13.4)	0.194	6.4 (9.7)	3.3 (1.5, 7.0)	5.8 (6.9)	3.1 (1.4, 7.0)	0.954
IL-6 (pg/mL)	7.7 (13.3)	4.0 (2.7, 7.2)	5.5 (4.1)	4.4 (3.3, 6.6)	0.513	8.9 (17.0)	4.8 (3.3, 7.9)	5.9 (3.5)	5.2 (3.7, 7.0)	0.770
Cystatin-C (ng/mL)	989.5 (287.1)	1047.7 (863.3, 1203.8)	1108.2 (281.7)	1074.6 (950.8, 1235.3)	0.085	1108.3 (292.5)	1080.8 (905.7, 1265.1)	1180.5 (330.2)	1148.7 (962.4, 1356.0)	0.164
MPO (pg/mL)	310.6 (301.7)	203.7 (126.2, 362.6)	296.3 (227.4)	240.1 (127.6, 396.3)	0.708	292.6 (318.2)	181.2 (100.4, 377.8)	293.4 (280.2)	190.3 (119.2, 356.2)	0.571
GDF-15 (pg/mL)	1418.5 (784.9)	1274.5 (998.6, 1585.1)	1362.2 (557.2)	1272.6 (989.5, 1529.7)	0.989	1810.7 (978.8)	1611.9 (1232.0, 2134.0)	1744.7 (634.2)	1667.8 (1250.6, 2054.3)	0.866

*Comparisons (control vs. case) are made with Wilcoxon Rank Test

Table 3. Logistic regression for associations between biomarkers and MACE/HF

	Age-Adjusted[2]		Adjusted[3]	
	OR[1] (95% CI)	p-value	OR[1] (95% CI)	p-value
Cystatin-C	0.99 (0.35, 2.81)	0.987	1.49 (0.44, 5.00)	0.522
8-OH-dG	1.03 (0.84, 1.25)	0.809	3.04 (1.01, 9.21)	0.047*
IL-6	0.97 (0.68, 1.40)	0.885	0.89 (0.57, 1.38)	0.602
CRP	0.88 (0.71, 1.09)	0.251	0.82 (0.62, 1.07)	0.144
GDF-15	1.10 (0.54, 2.26)	0.786	0.95 (0.38, 2.40)	0.921
TGF-B	0.85 (0.49, 1.49)	0.575	0.92 (0.51, 1.68)	0.795
MPO	0.98 (0.77, 1.25)	0.870	1.05 (0.79, 1.39)	0.758
TnI [4]	1.05 (0.51, 2.19)	0.89	1.12 (0.48, 2.61)	0.790
PGF [4]	1.13 (0.44, 2.89)	0.795	1.04 (0.35, 3.10)	0.951

[1] Each biomarker corresponds to 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

[2] Adjusted for age (5-year categories)

[3] Models 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/regional vs. distant), cardiac medications (yes/no)

[4] Categories defined as above vs. below (reference) detection; adjusted for pre-biomarker

Supplementary Material.

Supplemental Table 1. Assay kit specifications and performance measures

Assay	**Manufacturer (cat. no.)**	**Intra-assay CV**	**Inter-assay CV**	**Limit of Detection**
Interleukin 6 (IL-6) (pg/mL)	Invitrogen (A35573)	6.6	11.5	0.05
C-reactive protein (CRP) (pg/mL)	Invitrogen (KHA0031)	3.5	14.9	10
Cystatin-C (pg/mL)	Invitrogen (BMS2279)	2.2	8.9	6.9
8-hydroxy-2'-deoxyguanosin (8-OH-dG) (pg/mL)	Invitrogen (EIADNAD)	5.6	10.4	50.9
Troponin I (pg/mL)	Invitrogen (A46074)	9.9	13.3	0.32
Myeloperoxidase (MPO) (ng/mL)	R&D Systems (DMYE00B)	3.8	7.7	0.062
Growth differentiation factor (GDF-15) (pg/mL)	Invitrogen (EHGDF15)	4.4	6.1	2
Transforming growth factor-B (TGF-B) (pg/mL)	Invitrogen (BMS249)	2.0	9.4	8.6
Placental growth factor (PGF) (pg/mL)	Invitrogen (EHPGF)	5.9	10.0	2

Supplemental Table 2. Age-adjusted logistic regression for post-cancer biomarker by CVD outcome

	CHD (N = 15)		CVD Death (N = 22)		Stroke (N = 10)	
	OR[1,2] (95% CI)	p-value	OR[1,2] (95% CI)	p-value	OR[1,2] (95% CI)	p-value
Cystatin-C	1.03 (0.09, 11.63)	0.98	1.49 (0.26, 7.58)	0.70	0.09 (0.01, 5.40)	0.25
8-OH-dG	2.59 (0.27, 24.6)	0.407	3.09 (0.59, 16.25)	0.18	5.38 (0.33, 84.2)	0.23
IL-6	0.48 (0.19, 1.22)	0.12	0.70 (0.37, 1.30)	0.26	1.14 (0.58, 2.20)	0.71
CRP	0.54 (0.32, 0.91)	0.021	0.76 (0.55, 1.06)	0.10	0.82 (0.51, 1.33)	0.419
GDF-15	2.45 (0.55, 11.02)	0.242	0.19 (0.05, 0.97)	0.045	0.55 (0.05, 5.71)	0.61
TGF-B	0.83 (0.37, 1.84)	0.65	0.51 (0.17, 1.54)	0.23	0.49 (0.07, 3.42)	0.48
MPO	0.87 (0.48, 1.56)	0.63	1.10 (0.72, 1.66)	0.67	0.94 (0.54, 1.63)	0.81
TnI [3]	0.96 (0.71, 1.30)	0.27	1.11 (0.34, 3.69)	0.85	0.98 (0.20, 4.85)	0.98
PGF [3]	2.64 (0.46, 15.24)	0.28	0.59 (0.11, 3.07)	0.53	0.83 (0.04, 17.66)	0.90

[1] Each biomarker corresponds to 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

[2] Adjusted for age (5-years categories)

[3] Categories defined as above vs. below (reference) detection; adjusted for pre-biomarker

Supplemental Table 3. Associations of biomarkers and MACE/HF stratified by time from breast cancer to post-cancer serum collection

	< 1 year (N = 76)		1 – 2 years (N = 66)		> 2 years (N = 71)		
	OR[1] (95% CI)	p-value	OR[1] (95% CI)	p-value	OR (95% CI)	p-value	p-int[3]
CRP	1.10 (0.70,1.73)	0.674	0.62 (0.37, 1.03)	0.065	0.69 (0.40, 1.18)	0.174	0.18
Cystatin-C	1.98 (0.33, 11.9)	0.457	1.20 (0.13, 11.1)	0.87	0.87 (0.04, 17.0)	0.928	0.88
8-OH-dG	2.68 (0.45, 16.0)	0.278	3.58 (0.57, 22.5)	0.173	2.92 (0.36, 23.9)	0.319	0.97
IL-6	1.54 (0.82, 2.90)	0.178	0.37 (0.13, 1.10)	0.051	0.63 (0.25, 1.55)	0.314	0.036
GDF-15	1.61 (0.45, 5.74)	0.461	0.36 (0.05, 2.40)	0.29	0.76 (0.12, 4.87)	0.773	0.41
TGF-B	1.13 (0.57, 2.21)	0.727	0.83 (0.24,2.91)	0.769	0.58 (0.16, 2.16)	0.415	0.65
MPO	1.10 (0.70, 1.70)	0.683	1.10 (0.65, 1.85)	0.733	0.94 (0.55, 1.59)	0.807	0.88
TnI [4]	1.18 (0.33, 4.22)	0.798	2.0 (0.51, 7.91)	0.322	0.55 (0.13, 2.30)	0.41	0.38
PGF [4]	1.18 (0.33, 4.22)	0.798	2.0 (0.51, 7.91)	0.322	0.55 (0.13, 2.30)	0.41	0.38

[1] Each biomarker corresponds to 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

[2] Models adjusted for age (years), income (< $34,999, $35,000 - $74,999, >$75,000), waist circumference (cm), smoking (pack-years), physical activity (total MET-minutes/week), cancer stage (local/regional vs. distant), cardiac medications (yes/no)

[3] Overall interaction between categories tested using the likelihood ratio test

[4] Categories defined as above vs. below (reference) detection; adjusted for pre-biomarker

Supplemental Table 4. Logistic regression for associations between biomarkers and MACE/HF (LILAC w/ and w/out treatment) (N = 131)

	Age-Adjusted (w/out treatment)		Age-Adjusted (w/treatment)	
Post-Cancer Biomarkers	OR[1,2] (95% CI)	p-value	OR[1,3] (95% CI)	p-value
Cystatin-C	0.72 (0.21, 2.47)	0.60	0.71 (2.21, 2.46)	0.59
8-OH-dG	3.30 (0.81, 13.39)	0.095	3.27 (0.81, 13.32)	0.098
IL-6	1.05 (0.68, 1.61)	0.84	1.04 (0.67, 1.60)	0.86
CRP	0.90 (0.70, 1.17)	0.432	0.90 (0.70, 1.16)	0.431
GDF-15	0.68 (0.29, 1.60)	0.38	0.65 (0.27, 1.57)	0.34
TGF-B	0.70 (0.33, 1.46	0.332	0.69 (0.32, 1.47)	0.355
MPO	1.02 (0.75, 1.38)	0.90	1.02 (0.74, 1.39)	0.88
TnI [4]	0.65 (0.25, 1.68)	0.38	0.66 (0.25, 1.71)	0.39
PGF [4]	1.14 (0.38, 3.50)	0.81	1.15 (0.38, 3.50)	0.81

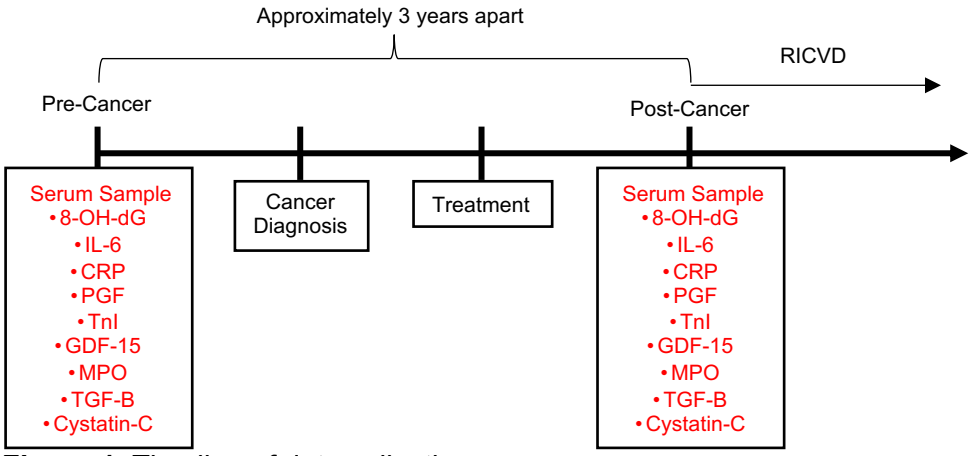
[1] Each biomarker corresponds to the $\log_2$ ratio of post-cancer relative to pre-cancer concentration; each unit difference in the biomarker ratio corresponds to a doubling in value compared to pre-cancer

[2] Modeled adjusted for age (5-year categories)

[3] Modeled adjusted for age (5-year categories) and chemotherapy (yes/no)

[4] Categories defined as above vs. below (reference) detection; adjusted for pre-biomarker

Supplemental Figure 1. Timeline of data collection



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CHAPTER 3

Associations of biomarkers and radiation-induced fatigue in breast cancer survivors

Abstract

Background: Radiation-induced fatigue (RIF) is common in breast cancer (BC) survivors and can last years after treatment. Despite the known physiological consequences of radiation, information on biomarkers of RIF is limited. Therefore, we examined the longitudinal association between serum biomarkers and post-BC fatigue in BC survivors treated with radiation: [oxidative stress] 8-hydroxyguanosine (8-OH-dG), myeloperoxidase (MPO); [inflammation] interleukin-6 (IL-6), C-reactive protein (CRP), growth differentiation factor-15 (GDF-15), placental growth factor (PGF), transforming growth factor-beta (TGF-B); [cardiac damage] cystatin-C, troponin-I (TnI).

Methods: In a secondary analysis, Women's Health Initiative (WHI) participants diagnosed with breast cancer (stages I-III) without prior cardiovascular disease and had pre-and post-BC serum samples drawn three years apart with fatigue measured using the Short-Form 36 (SF-36) vitality subscale at the same times. Biomarkers were analyzed using enzyme-linked immunosorbent assays or RT-qPCR. Higher SF-36 vitality scores correspond to lesser fatigue. Weighted linear regression adjusted for relevant demographic, lifestyle, and psychosocial factors, as well as pre-cancer fatigue. Each biomarker was modeled as the post-BC to pre-BC ratio and log transformed to base 2, thus, beta corresponds to a doubling in value compared to pre-BC.

Results: A total of 180 women with a mean (SD) age of 67.0 (5.5) were included. The mean (SD) vitality score was 66.2 (17.2) and 59.7 (19.7) pre- and post-BC, respectively. The median (IQR) time between pre-BC serum collection to BC was 1.9 (0.8, 2.6) years and 1.4 (0.7, 2.3) years between BC and the post-BC serum collection. After adjustment, a higher biomarker ratio of cystatin-C, IL-6, and GDF-15 were all associated with a lower SF-36 vitality score (i.e.,

higher fatigue) (Table). As an example, for a 2-fold difference in the cystatin-C biomarker ratio, the SF-36 vitality score was lower by 7.31 points (95% CI: -14.2, -0.45).

Conclusions: Inflammatory and cardiac damage biomarkers are associated with RIF in BC survivors. Biomarkers could be measured in clinical practice or assessed in risk prediction models to help identify patients at high risk for RIF.

Introduction

Due in part to improvements in cancer treatments, survival rates for breast cancer have improved and, as such, there are an estimated 3.8 million breast cancer survivors in the United States as of 2021.[1] Radiation therapy is administered to approximately 50% of breast cancer patients due to its demonstrated role in improved survival.[2,3-5] However, despite survival 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.[3, 27, 28]

Biological predictors of radiation-induced fatigue are limited. This is likely due to the multiple mechanisms possibly contributing to fatigue and substantial variability between individuals. Leading hypotheses explaining fatigue involve inflammation, as studies have shown associations between increased inflammatory markers and fatigue in breast cancer patients receiving radiation.[3, 31, 39, 40] Oxidative stress has also been proposed as an underlying mechanism explaining fatigue, yet this has been investigated minimally in cancer survivors.[37, 38] However, these mechanisms may not be sufficient to predict fatigue given the multiple factors contributing to its development. Specifically, research has shown the potential for radiation to damage the heart.[7] Cardiac dysfunction has been associated with reductions in cardiorespiratory fitness, exercise intolerance, and fatigue.[42] In particular, radiation in the treatment of cancers such as breast are more likely to cause cardiac damage given the proximity of exposure to the heart. Therefore, the investigation of a cardiac damage biomarkers in the study of fatigue will provide valuable insight on additional, treatment-specific mechanisms of fatigue.

Thus, there is a need to disentangle the multiple potential mechanisms leading to fatigue in order to improve its assessment, prevention, and treatment. Elucidation of these mechanisms

would guide the development of bio-behavioral interventions to prevent or reduce fatigue in breast cancer survivors. Additionally, multiple factors may influence the relationship between biomarkers and fatigue including treatment characteristics, concurrent treatment, comorbidities, and lifestyle factors.[3] Therefore, studies investigating fatigue must thoughtfully consider the relationships between biomarkers and fatigue while accounting for patient-level characteristics by including detailed data on these multiple factors.

The purpose of this study is to examine biomarkers of oxidative stress, cardiac damage, and inflammation in the development of fatigue in breast cancer survivors treated with radiation in the Women's Health Initiative (WHI). Specifically, we examined the following serum biomarkers: a) oxidative stress: 8-OH-dG, myeloperoxidase (MPO), b) inflammation: interleukin-6 (IL-6), C-reactive protein (CRP), growth differentiation factor-15 (GDF-15), placental growth factor (PGF), c) cardiac damage: cystatin-C, troponin-I (TnI), and d) fibrosis: transforming growth factor-beta (TGF-B).

Methods

Study Population & Design

We conducted a secondary analysis of data collected as part of an ancillary case-control study conducted within the WHI. Detailed descriptions of the design and recruitment of the WHI has been published elsewhere.[51] 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 (CT) and an Observational Study (OS). Data was collected in the main study until 2005.[51] At the end of the main study, women were asked to participate in follow-up extension studies for an additional 5 years of follow-up through 2010 and again through 2015. 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 cancer during WHI follow-up.[52]

This study is built off an original case-control study within the WHI to investigate biomarkers associated with radiation-induced cardiovascular disease (RICVD). Eligible women were those diagnosed with incident invasive breast cancer and treated with radiation, for whom radiation treatment was documented. Through LILAC, radiation was documented either through medical record abstraction or Medicare, or by self-report on the LILAC baseline questionnaire. Women not enrolled in LILAC were included if they were diagnosed with cancer at age 65 or older and were enrolled in Medicare. Incident breast cancer was the first invasive cancer (stages I-III) adjudicated during WHI follow-up. This study required pre- and post-breast cancer diagnosis serum samples drawn three follow-up years apart with the breast cancer diagnosis occurring in between the two blood collections. Women were excluded if they 1) had metastatic disease or missing stage, 2) had an adjudicated or self-reported CVD outcome before the second blood draw, or 3) self-reported history of breast, lung, lymphoma, Hodgkin's, or thyroid cancers at WHI baseline (because previous radiation for these cancers could increase the risk of future RICVD). In the original case-control study, RICVD cases were matched to non-RICVD controls 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). This study included a total of 213 women (55 RICVD cases and 158 non-RICVD controls).

We assessed whether post-cancer diagnosis biomarker data was associated with fatigue post-cancer diagnosis in these women using inverse probability weighting to account for the

selection into the original case-control study (Supplemental Figure 1). For this specific analysis, we 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 this analysis.

Outcome

The main outcome in this analysis was post-breast cancer fatigue, which was measured at the same time as the second biomarker collection (i.e., post-cancer diagnosis biomarker) (Supplemental Figure 1). Fatigue was measured in the WHI using the Short-Form (SF)-36 vitality subscale. Participants were asked during the last four weeks how often they: a) felt full of pep, b) had a lot of energy, c) felt worn out, and d) felt tired. Individual questions were scored from 1 to 6, with 1 representing experiencing the feeling all the time, and a 6 representing experiencing the feeling none of the time. The total index score ranges from 0-100, with higher scores indicating less fatigue. Fatigue has been phenotypically characterized with the SF-36 using a cutoff of 50, with scores < 50 being "fatigued" and scores ≥ 50 being "non-fatigued".[36] Internal consistency of the SF-36 is high with alpha coefficients ranging from 0.89 – 0.91 in cancer populations.[79, 80]

Exposure

The exposures for this study were post-cancer diagnosis biomarker concentrations of 8-OH-dG (pg/mL), IL-6 (pg/mL), CRP (mg/L), TnI (pg/mL), MPO (ng/mL), GDF-15 (pg/mL), TGF-B (pg/mL), cystatin-C (ng/mL), and PGF (pg/mL).

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 one hour after collection. Samples were maintained at 4 degrees Celsius during handling. After separation,

samples were separated into 0.25 ml aliquots and placed into a -80-degree Celsius freezer within 2 hours of collection for future use.

All biomarkers were measured using commercially available assay kits (Supplemental Table 1). All biomarkers, except IL-6 and Troponin-I, 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 CV < 10% and inter-assay CV < 15% (Supplemental Table 1).

Additional variables

Demographic information on age at WHI enrollment, education, and self-identified race/ethnicity were collected from WHI baseline questionnaires.

Additional information on potential confounders such as pre-cancer fatigue, sleep disturbance, depression, pain, physical function, and alcohol use were collected from self-report questionnaires. Fatigue, depression, 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 (IRS). The IRS 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. Pack-years were estimated based on self-report of the number of years a participant smoked, and the number of cigarettes smoked per day on average. Alcohol consumption was measured 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 is measured by the number of total-metabolic equivalents hours per week (MET-hours/week). Specific details of how physical activity was calculated in the WHI has been published elsewhere.[81] In summary, participants were to self-report the frequency and duration of a variety of physical activities. Each activity is assigned METs based on the intensity of the activity based on the Compendium of Physical Activities.[82]

Physical measurements including BMI (kg/m^2) and waist circumference (cm) were measured in-person at baseline clinic visits by trained WHI personnel.

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. Cancer stage is available on all participants as this was an inclusion criterion for the original case-control study. 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

Baseline characteristics were compared between those with and without fatigue using a cut point of 50 as described above. 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-test and chi-square test, 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: 8-OH-dG, IL-6, CRP, TnI, MPO, GDF-15, TGF-B, cystatin-C, and PGF), with the dependent variable of post-cancer diagnosis fatigue. 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 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 if they are associated with both the exposure and outcome. 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 MET-hours/week), alcohol consumption (servings per week), cancer stage (local/regional, distant), depression, physical

function, pain, sleep disturbance, and pre-cancer biomarker concentrations. 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. Since all eligible controls were selected as part of the original case-control study, each case has a weight of 1. The sampling fractions for controls are presented in Supplemental Table 2. Confidence intervals were calculated using robust standard errors to account for the correlation among weighted observations as described previously.[83]

Two pre-specified 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. Of the eligible sample, 135 were enrolled in LILAC where treatment data was primarily ascertained; of those, 105 had treatment data on chemotherapy available either through medical record abstraction or Medicare data. Thus, 105 participants were available for this sensitivity analysis. 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 conducted a stratified analysis using this variable. To test whether there were any differences in the beta coefficients among the three groups, an interaction term was included 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. 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) 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, depression, 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 these biomarkers, 102 and 106 participants had undetectable concentrations for PGF 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, IL-6, and cystatin-C (Table 2). For post-cancer serum

biomarkers, participants with fatigue had significantly higher concentrations of CRP compared to those without fatigue. When comparing differences in concentrations between pre- and post-cancer biomarkers, participants without fatigue had higher concentrations of cystatin-C ($p=0.04$) and GDF-15 ($p<0.001$) and lower concentrations of TGF-B ($p=0.02$) and CRP ($p<0.001$) post-cancer. Participants with fatigue had higher concentrations of GDF-15 ($p=0.001$) and lower concentrations of CRP ($p=0.01$) and MPO ($p=0.01$) post-cancer.

Weighted linear regression for association of post-cancer biomarkers on fatigue scores

After multivariable adjustment, higher concentrations of cystatin-C, IL-6, and GDF-15 were all associated with a lower SF-36 vitality score indicating higher fatigue. 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.

Sensitivity analyses

We explored whether chemotherapy was a substantial confounder in the association between post-cancer biomarkers and fatigue. We repeated the main analysis with chemotherapy included in the models in the 105 participants in LILAC with chemotherapy treatment data available. With adjustment for chemotherapy, this restricted analysis similarly shows higher IL-6 concentrations to be associated with greater fatigue (Supplemental Table 3). However, significant associations emerge such that higher MPO and TNI scores are associated with lesser fatigue (Supplemental Table 3).

To determine 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 (Table 4). Higher CRP concentrations 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 higher levels of cystatin-C, IL-6, and GDF-15 to be associated with higher fatigue in breast cancer survivors treated with radiation, after adjustment for relevant lifestyle, demographic, and psychosocial characteristics. No main associations were found for CRP, 8-OH-dG, PGF, MPO, or TnI. It appears that for CRP, the timing of the serum collection in relation to breast cancer diagnosis influenced the association with fatigue. Higher levels of CRP were significantly associated with greater fatigue if measured 1 – 2 years after breast cancer diagnosis.

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. While radiation therapy produces local effects to damage cancer cells, radiation has been shown to result in an increase in inflammatory circulatory markers which are thought to drive fatigue. 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.[30-32] Increases in pro-inflammatory markers are thought to contribute to fatigue by activating a cascade of events leading to hypothalamic-pituitary-adrenal (HPA) axis disruption, serotonin dysregulation, circadian rhythm disruption, vagal afferent nerve activation, and neuroendocrine impairment.[31] However, limited research has examined the role of inflammatory biomarkers in breast cancer survivors treated

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.[84, 85] The findings from this current study provide support for the role of IL-6 in radiation-induced fatigue as higher levels of IL-6 were significantly associated with greater fatigue in this sample. Possible explanations for the lack of association in the previous two studies could be related to small sample size which ranged from 28 – 147 participants. To our knowledge, the current analysis is the largest study to examine biomarkers of radiation-induced fatigue with a sample size of 180 participants. Additionally, in this current study, data was available for a comprehensive set of potential confounding variables including smoking, pain, physical function, alcohol consumption, and physical activity, which were not included in previous studies.

IL-6 was a significant predictor of fatigue even if measured 2 or more years after breast cancer. While studies examining the role of biomarkers in fatigue have measured biomarkers during or shortly after radiation treatment, our study provides support that biomarkers, including IL-6, are able to distinguish levels of fatigue years after treatment. The modulation of IL-6 in response to radiation has been studied quite extensively in the literature. Studies have shown significant differences in serum IL-6 between breast cancer cases and controls up to 5 years post-diagnosis.[45, 86] Thus, IL-6 may be a valuable biomarker to characterize long-term fatigue that can occur years after treatment in breast cancer survivors.

This study also examined novel biomarkers, which have yet to be examined in fatigue in breast cancer survivors. 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 as measured by creatinine clearance and glomerular filtration rate.[87, 88] This suggests that chemotherapy may influence cystatin-C levels through other pathways. One possible pathway is through cardiac dysfunction. While this has not been explored in cancer survivors, serum cystatin-C has recently been used in epidemiological studies to examine cardiovascular disease (CVD) and has been shown to be a predictive marker for heart failure in older adults without accompanying changes in renal function.[25, 89] Fatigue is a commonly reported symptom in patients with heart failure and CVD due to cardiac dysfunction[41]. Radiation can cause systolic heart failure (heart failure with reduced left ventricular ejection fraction (LVEF)) and diastolic heart failure (heart failure with preserved LVEF). Reductions in LVEF can directly cause fatigue symptoms and diastolic heart failure has been associated with reductions in cardiorespiratory fitness, exercise intolerance, and fatigue.[42]

Likewise, GDF-15 has been implicated in cardiac dysfunction in cancer survivors. In a longitudinal study of 78 women, increased serum concentrations of GDF-15 were associated with reduced LVEF over the course of 15 months after chemotherapy initiation.[56] GDF-15 remained elevated in cancer survivors at least 15 months after treatment, which could explain the strong association in this population as biomarkers were measured on average 1.4 years after breast cancer.[56] The findings from cystatin-C and GDF-15 may be indicative of an underlying pathophysiologic process, which is contributing to fatigue symptoms such as CVD or heart failure. Given the participants in this analysis had no physician-adjudicated diagnosis of CVD prior to breast cancer, these changes are likely reflective of cancer treatment rather than an underlying cardiac disease pre-cancer.

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. Fatigue is a commonly reported symptom in patients with cachexia and studies have shown a direct association between a loss of skeletal muscle mass and fatigue symptoms in patients with advanced cancer.[90, 91] Preclinical models have found significant associations between higher GDF-15 concentrations and loss of muscle mass, and recent studies blocking circulating GDF-15 were able to reverse weight and lean mass loss in mice.[92, 93] Thus, the results of GDF-15 in this study could be reflective of fatigue associated with muscle loss, although body composition data are not available to examine in this study. Future studies should look at the association of GDF-15 and fatigue along with longitudinal changes in body composition, specifically muscle mass.

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 radiation-induced fatigue in breast cancer survivors. Biomarker studies are usually expensive to conduct, thus case-control study designs are typically implemented to only analyze serum specimens on a limited group of participants. However, these studies are typically designed to examine risk associations with one outcome. Methods have been developed to allow for analyses of case-control data in order to take full advantage of the data collected and examine secondary outcomes while taking into account the sampling design of the original case-control study.[94-96] While there are a variety of methods, inverse probability weighting (IPW) is a robust method when sampling probabilities are known. This method uses sampling weights to create data, which resembles a random sampling design with respect to the secondary outcome (i.e., fatigue in this study). Given that the original case-control study sampled from the WHI, a cohort study, sampling probabilities are easily accessible. Although the efficiency of this method is limited, IPW is robust against sampling bias.[94]

Another strength is that we examined a variety of biomarkers, including novel biomarkers, which allows an in-depth assessment of possible mechanisms associated with radiation-induced fatigue. While prior research, and the results from this study, support the role of inflammation in radiation-induced fatigue, there may be other possible mechanisms of fatigue such as underlying cardiac damage or body composition changes. Future research is needed to validate these novel biomarkers and possible mechanistic pathways in other breast cancer populations.

Despite these strengths, the study has limitations. As this is an observational study with post-cancer biomarkers and fatigue measured at the same time point, reverse causation is possible because a temporal order cannot be established. To counteract this, we adjusted for pre-cancer biomarkers and fatigue. While we adjusted for a variety of potential confounders including demographics, lifestyle factors, psychosocial factors, and clinical measurements, there is the possibility of residual confounding. The original case-control study excluded women with diagnosed CVD 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 (Supplemental Table 4). Main differences between these groups are the sample in this current study identify more as non-Hispanic White, were more educated, had smaller waist circumferences, and engaged in more physical activity. These differences are likely reflective of excluding participants with CVD prior to breast cancer given these are all risk factors for CVD. Nevertheless, excluding participants with CVD prior to breast cancer is important for examining cardiac damage biomarkers and fatigue as any changes in biomarkers are likely due to cancer treatment rather than pre-clinical CVD. Future prospective studies should be conducted to validate findings from this study.

Another limitation is that fatigue was measured using the SF-36, which was not created

specifically for cancer-related fatigue. However, it is commonly used as a measure of fatigue in cancer survivors.[79, 97] Convergent validity of the SF-36 with fatigue measures validated in cancer populations show strong correlation including the Fatigue Symptoms Inventory (r = -0.75) [98], Multidimensional Fatigue Symptom Inventory [80] (r = -0.74), and the Piper Fatigue Score (r = -0.74).[99] Furthermore, previous studies have used the SF-36 to characterize fatigue and have been able to detect biomarker differences in fatigued vs. non-fatigued cancer survivors using a 50 point cutoff.[35, 36, 45, 46]

Despite using high-sensitivity biomarker assays, we had a high percentage of participants with PGF and TnI concentrations below detection. Thus, the results for these biomarkers should be interpreted with caution. This could be related to the sample characteristics as these were post-menopausal women. Prior studies have had difficulty measuring serum PGF in post-menopausal women.[100] However, our results are in line with other studies which report TnI being below detection in approximately 50% of participants.[101]

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 are associated with radiation-induced fatigue in cancer survivors. While inflammatory pathways have been studied frequently, results from this study suggest mechanisms of fatigue are likely multifactorial and may also be indicative of underlying pathological processes related to cardiac

dysfunction and body composition changes. This study focused on radiation-induced fatigue in breast cancers, which is an understudied area. Biomarkers, such as IL-6, cystatin-C, PGF, and GDF-15, could be measured in clinical practice or included in risk prediction models to identify patients at high risk for radiation-induced fatigue. Early identification of individuals at risk for radiation-induced fatigue could lead to reductions in fatigue by targeting biobehavioral interventions to those at highest risk identified by specific biological pathways.

Table 1. Baseline (i.e., pre-cancer) characteristics stratified by fatigue scores

	Overall (N = 180)	Non-Fatigued[1] (N = 130)	Fatigued[1] (N = 50)	p-value
Age at Diagnosis (mean (SD))	69.1 (5.5)	68.6 (5.4)	70.3 (5.5)	0.067
Age at WHI Enrollment (mean (SD))	67.0 (5.5)	66.7 (5.4)	67.9 (5.8)	0.185
Age at WHI Enrollment (5yrs) (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*	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)	
Smoking (pack-years) (mean (SD))	11.6 (18.7)	11.6 (18.6)	11.6 (19.2)	0.988
BMI (kg/m^2) (mean (SD))	27.3 (6.8)	26.6 (6.3)	29.0 (7.6)	0.038*
Waist circumference (cm) (mean (SD))		82.1 (11.5)	86.0 (13.7)	0.062
Physical Activity (MET-hours/week) (mean (SD))	16.3 (15.3)	17.7 (16.5)	12.8 (11.0)	0.058
Alcohol (servings/week) (mean (SD))	3.5 (6.9)	3.8 (7.8)	2.8 (3.7)	0.386
Cancer stage				0.823
Local	1 (0.6)	1 (0.8)	0 (0.0)	
Regional	140 (77.8)	101 (77.7)	39 (78.0)	
Distant	39 (21.7)	28 (21.5)	11 (22.0)	
Pain	75.2 (23.0)	79.8 (20.9)	63.5 (24.3)	< 0.001***
Depression	80.1 (14.7)	81.9 (14.2)	75.3 (14.8)	0.006**
Pre-cancer 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*
Enrolled in LILAC	135 (75.0)	97 (74.6)	49 (98.0)	1.00
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[2]	29 (27.6)	21 (24.7)	11 (34.3)	0.305

Significant at level: *0.05, **0.01, ***0.001

[1] Non-fatigued: SF-36 >= 50, fatigued SF-36 < 50

[2] Total number of participants in LILAC with chemotherapy data available is 105

Table 2. Distribution of Pre- and Post-Cancer Biomarkers Stratified by Fatigue

	Pre-Cancer					Post-Cancer				
	Non-Fatigued (N = 130)		Fatigued (N = 50)			Non-Fatigued (N = 130)		Fatigued (N = 50)		
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value[1]	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value[1]
8-OH-dG (pg/mL)	10014 (2520)	9735 (8248, 11404)	10666 (3689)	9816 (8704, 12921)	0.5	10260 (2520)	9942 (8633, 11738)	10468 (3689)	10145 (8142, 11869)	0.9
TnI (pg/mL)	6.27 (19.7)	0.2 (0.2, 4.0)	11.2 (30.9)	0.5 (0.2, 4.7)	0.4	6.02 (19.7)	0.4 (0.2, 2.3)	5.51 (30.9)	0.2 (0.2, 2.5)	0.8
PGF (pg/mL)	292 (819)	1.0 (1.0, 53)	303 (751)	7.0 (1.0, 303)	0.2	314 (819)	1.0 (1.0, 95)	387 (7510)	6.0 (1.0, 92)	0.2
TGF-B (pg/mL)	29848 (11896)	27958 (22294, 35356)	31180 (12262)	29937 (22607, 37475)	0.6	27123 (11896)	24582 (19272, 33532)	27383 (12262)	24899 (20122, 30004)	0.7
CRP (mg/L)	8.0 (12.8)	5.1 (1.8, 10.5)	13.7 (16.3)	9.1 (3.7, 16.7)	0.002**	4.8 (12.8)	2.6 (1.2, 5.7)	8.5 (16.3)	4.4 (2.1, 9.3)	0.01**
IL-6 (pg/mL)	7.2 (13.2)	4.1 (2.6, 6.9)	9.3 (11.0)	5.7 (3.4, 9.7)	0.03*	7.64 (13.2)	5.0 (3.3, 7.5)	11.3 (11.0)	5.9 (3.6, 10.0)	0.1
Cystatin-C (ng/mL)	1027 (300)	992 (810, 1184)	1148 (287)	1103 (898, 1376)	0.01**	1099 (300)	1054 (872, 1248)	1176 (287)	1096 (968, 1440)	0.08
MPO (ng/mL)	300 (297)	206 (127, 318)	334 (277)	255 (133, 469)	0.3	288.5 (297)	195 (106, 363)	208.9 (277)	165 (83, 270)	0.1
GDF-15 (pg/mL)	1404 (853)	1234 (929, 1498)	1398 (498)	1351 (971, 1791)	0.2	1737 (853)	1545 (1201, 1933)	1875 (498)	1842 (1300, 2256)	0.08

[1]Comparisons (control vs. case) are made with Wilcoxon Rank Test

Significant at level: *0.05, **0.01, ***0.001

Table 3. Linear regression results for association of post-cancer biomarker on continuous fatigue scores

	Age-adjusted[2] (N = 181)		Multivariable Adjusted[3] (N = 159)	
	β[1] (95% CI)	p-value	β[1] (95% CI)	p-value
Cystatin-C	5.52 (-4.97, 16.0)	0.302	-7.31 (-14.2, -0.45)	0.037*
8-OH-dG	0.84 (-7.87, 9.54)	0.851	-4.13 (-12.3, 4.06)	0.323
IL-6	-1.15 (-5.17, 2.88)	0.577	-4.45 (-7.62, -1.29)	0.006**
CRP	1.01 (-1.53, 3.55)	0.435	-0.10 (-2.05, 1.85)	0.920
GDF-15	-6.69 (-13.6, 0.25)	0.059	-6.67 (-12.3, -0.99)	0.021*
TGF-B	2.87 (-3.33, 9.07)	0.365	2.75 (-1.84, 7.35)	0.240
MPO	1.98 (-1.10, 5.07)	0.208	1.92 (-0.16, 4.01)	0.079
TnI [4]	0.33 (-8.67, 9.32)	0.943	2.72 (-4.01, 9.45)	0.428
PGF [4]	-10.7 (-20.6, -0.86)	0.033	-5.74 (-15.4, 3.87)	0.242

Significant at level: *0.05, **0.01, ***0.001

[1] Biomarker ratios (post/pre) were log transformed to base 2; β corresponds to 2-fold difference in biomarker ratio

[2] Models adjusted for age (5-year categories)

[3] Models adjusted for age (5-year categories), education (HS/GED or <, > HS – Bachelor's, > Bachelor's), BMI (kg/m^2), smoking (pack-years), physical activity (total MET-minutes/week), alcohol (servings per week), cancer stage (local/regional, distant), depression, physical function, pain, sleep disturbance

[4] Categories defined as above vs. below (reference) detection; additionally, adjusted for pre-biomarker

Table 4. Linear regression results stratified by timing of post-cancer biomarker collection

	< 1 year (N = 66)		1 – 2 years (N = 57)		> 2 years (N = 58)		
	β[1,2] (95% CI)	p-value	β[1,2] (95% CI)	p-value	β[1,2] (95% CI)	p-value	p-int[3]
CRP	3.88 (1.28, 6.49)	0.004**	-4.74 (-7.82, -1.66)	0.003**	-1.76 (-4.84, 1.32)	0.263	< 0.001***
Cystatin-C	-8.64 (-18.3, 0.97)	0.078	-6.67 (-16.8, 3.48)	0.198	-7.28 (-26.2, 11.7)	0.452	0.960
8-OH-dG	-1.54 (-9.59, 6.52)	0.708	-3.10 (-18.8, 12.7)	0.70	-8.85 (-22.4, 4.68)	0.20	0.620
IL-6	-0.90 (-6.13, 4.33)	0.736	-3.53 (-8.48, 1.43)	0.163	-9.39 (-15.6, -3.14)	0.003	0.160
GDF-15	-1.85 (-10.3, 6.63)	0.670	-11.0 (21.7, -0.17)	0.047*	-4.96 (-15.4, 5.44)	0.350	0.40
TGF-B	2.23 (-3.90, 8.36)	0.476	0.16 (-9.64, 9.96)	0.975	5.14 (-6.56, 15.8)	0.346	0.80
MPO	3.25 (0.14, 6.35)	0.04*	3.11 (-0.61, 6.82)	0.101	-1.57 (-4.88, 1.74)	0.351	0.076
TnI [4]	-3.42 (-13.0, 6.19)	0.486	4.5 (0.85, 8,15)	0.010*	-0.84 (-11.8, 10.1)	0.881	0.050
PGF [4]	-9.58 (-19.9, 0.77)	0.070	5.42 (-6.98, 17.8)	0.391	-8.86 (-22.6, 4.84)	0.205	0.066

Significant at level: *0.05, **0.01, ***0.001

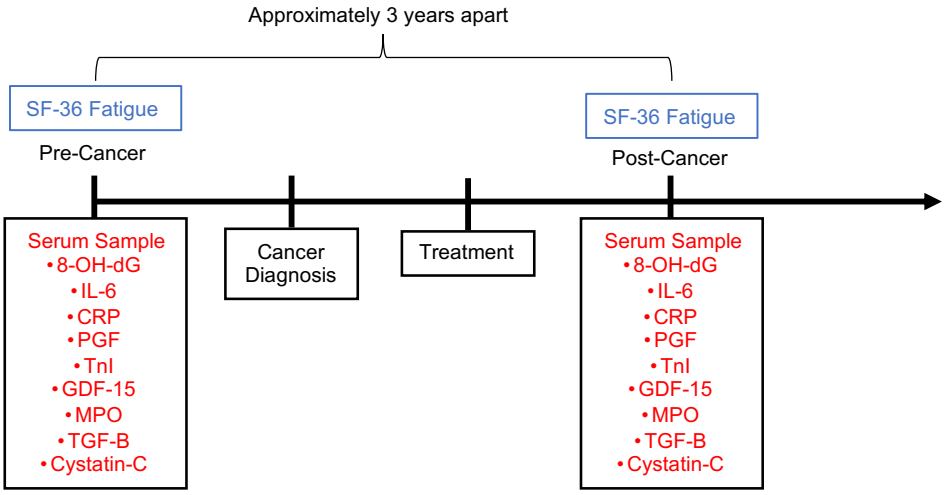
[1] Biomarker ratios (post/pre) were log transformed to base 2; β corresponds to 2-fold difference in biomarker ratio

[2] Models adjusted for age (5-year categories), education (HS/GED or <, > HS – Bachelor's, > Bachelor's), BMI (kg/m^2), smoking (pack-years), physical activity (total MET-minutes/week), alcohol (servings per week), cancer stage (local/regional, distant), depression, physical function, pain, sleep disturbance

[3] Calculated using the likelihood ratio test

[4] Categories defined as above vs. below (reference) detection; additionally, adjusted for pre-biomarker

Supplemental Files



Supplemental Figure 1. Timeline of data Collection

Supplemental Table 1. Assay kit specifications and performance measures

Assay	Manufacturer (cat. no.)	Intra-assay CV	Inter-assay CV	Limit of Detection (LOD)
Interleukin 6 (IL-6) (pg/mL)	Invitrogen (A35573)	6.6	11.5	0.05
C-reactive protein (CRP) (pg/mL)	Invitrogen (KHA0031)	3.5	14.9	10
Cystatin-C (pg/mL)	Invitrogen (BMS2279)	2.2	8.9	6.9
8-hydroxy-2'-deoxyguanosin (8-OH-dG) (pg/mL)	Invitrogen (EIADNAD)	5.6	10.4	50.9
Troponin I (pg/mL)	Invitrogen (A46074)	9.9	13.3	0.32
Myeloperoxidase (MPO) (ng/mL)	R&D Systems (DMYE00B)	3.8	7.7	0.062
Growth differentiation factor (GDF-15) (pg/mL)	Invitrogen (EHGDF15)	4.4	6.1	2
Transforming growth factor-B (TGF-B) (pg/mL)	Invitrogen (BMS249)	2.0	9.4	8.6
Placental growth factor (PGF) (pg/mL)	Invitrogen (EHPGF)	5.9	10.0	2

Supplemental Table 2. Stratified sampling fractions for controls for inverse probability weighting

	LILAC Yes						LILAC No					
	Sampling Fractions						Sampling Fractions					
	Abstraction/Medicare			Abstraction/Medicare			Abstraction/Medicare					
	Blood draw time-point prior to breast cancer			Blood draw time-point prior to breast cancer			Blood draw time-point prior to breast cancer			Blood draw time-point prior to breast cancer		
Age	Baseline	Year 3	Year 6	Baseline	Year 3	Year 6	Baseline	Year 3	Year 6	Baseline	Year 3	Year 6
50 – 54	0	0	0	0	0	0	0	0	0	0	0	0
55 – 59	0.53	0.4	1.0	1	0	0	0	0	0	0	0	0
60 – 64	0.59	0.5	1	0.38	0	0	0	0	0	0	0	0
65 – 69	0.51	0	0.5	0.11	0	0	0.79	0	0	0	0	0
70 – 74	0.97	1	0	0.16	0	0	0.67	0	0	0	0	0
75 – 79	0.18	0	0	0.5	0	0	0	0	0	0	0	0

Supplemental Table 3. Linear regression results for association of post-cancer biomarker on continuous fatigue scores in LILAC[1]

	Adjusted[3] (N = 95)		Adjusted[4] (N = 95)	
Post-Cancer Biomarkers	β^2 (95% CI)	p-value	β^2 (95% CI)	p-value
Cystatin-C	-8.63 (-17.2, -0.10)	0.048*	-7.06 (-12.2, 2.06)	0.129
8-OH-dG	1.77 (-8.59, 12.1)	0.738	1.77 (-8.59, 12.1)	0.738
IL-6	-4.31 (-8.27, -0.39)	0.033*	-4.05 (-7.99, -0.12)	0.044
CRP	-0.39 (-3.31, 2.53)	0.794	0.91 (-1.70, 3.51)	0.495
GDF-15	-2.75 (-10.7, 5.18)	0.497	-2.81 (-10.0, 4.39)	0.444
TGF-B	3.58 (-1.78, 8.94)	0.190	3.40 (-2.51, 9.31)	0.259
MPO	4.95 (2.74, 7.15)	< 0.001***	4.48 (2.31, 6.66)	<0.001***
TnI [5]	1.65 (0.47, 2.83)	0.006	1.33 (0.08, 2.57)	0.037*
PGF [5]	-0.76 (-1.98, 0.46)	0.223	-0.87 (-2.10, 0.37)	0.168

Significant at level: *0.05, **0.01, ***0.001

[1] N = 105 in LILAC with chemotherapy data

[2] Biomarker ratios (post/pre) were log transformed to base 2; β corresponds to 2-fold difference in biomarker ratio

[3] Models adjusted for age (5-year categories), education (HS/GED or <, > HS – Bachelor's, > Bachelor's), BMI (kg/m^2), smoking (pack-years), physical activity (total MET-minutes/week), alcohol (servings per week), cancer stage (local/regional, distant), depression, physical function, pain, sleep disturbance

[4] Models adjusted for age (5-year categories), education (HS/GED or <, > HS – Bachelor's, > Bachelor's), BMI (kg/m^2), smoking (pack-years), physical activity (total MET-minutes/week), alcohol (servings per week), cancer stage (local/regional, distant), depression, physical function, pain, sleep disturbance, chemotherapy (yes/no)

[5] Categories defined as above vs. below (reference) detection; additionally, adjusted for pre-biomarker

Supplemental Table 4. Comparison between study sample with overall breast cancer population in the WHI and LILAC

	Overall (N = 180)	**Overall WHI (Breast) (N = 11130)**	**LILAC (Breast) (N = 5792)**
Age at Diagnosis (mean (SD))	69.1 (5.5)	71.7 (7.91)	71.6 (7.9)
Age at WHI Enrollment (mean (SD))	67.0 (5.5)	62.8 (6.9)	62.2 (6.8)
Race/Ethnicity (n (%))			
Non-Hispanic White	169 (93.9)	9740 (87.5)	5344 (89.5)
Non-Hispanic Black	4 (2.2)	749 (6.7)	322 (5.4)
Other*	7 (3.9)	618 (5.6)	296 (5.0)
Education (n (%))			
High School/GED or less	20 (11.1)	1998 (18.0)	951 (15.9)
> High School – Bachelor's	87 (48.3)	5397 (48.5)	2866 (48.0)
> Bachelor's	73 (40.6)	3658 (32.9)	2116 (35.4)
Smoking (pack-years) (mean (SD))	11.6 (18.7)	10.73 (19.1)	10.47 (18.6)
BMI (kg/m^2) (mean (SD))	27.3 (6.8)	28.5 (6.0)	28.4 (6.0)
Waist circumference (cm) (mean (SD))	83.2 (12.2)	86.8 (13.6)	86.2 (13.6)
Physical Activity (MET-hours/week) (mean (SD))	16.3 (15.3)	12.5 (13.3)	13.0 (13.6)
Alcohol (servings/week) (mean (SD))	3.5 (6.9)	1.9 (4.0)	2.0 (4.2)
Cancer stage			
Local	1 (0.6)	123 (1.1)	49 (0.8)
Regional	140 (77.8)	7492 (67.3)	4380 (73.3)
Distant	39 (21.7)	2258 (20.3)	1378 (23.1)
Pain (mean (SD))	75.2 (23.0)	75.3 (22.7)	76.3 (21.8)
Depression (mean (SD))	80.1 (14.7)	79.7 (13.8)	80.3 (13.4)
Pre-cancer fatigue (mean (SD))	66.3 (17.2)	63.9 (18.7)	65.0 (18.2)
Physical function (mean (SD))	82.6 (17.4)	82.3 (18.8)	83.7 (17.9)
Sleep disturbance (mean (SD))	7.1 (4.3)	6.5 (4.4)	6.3 (4.3)
Enrolled in LILAC (n (%))	135 (75.0)	5792 (53.7)	-
WHI CT (n (%))	6 (3.3)	4603 (41.4)	2731 (45.7)

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CHAPTER 4

Associations between Physical and Mental-Health Related Quality of Life and Sleep Disturbance and CVD Risk in Post-Menopausal Breast Cancer Survivors

Abstract

Background: Lower health-related quality of life (HRQoL) and greater sleep disturbance (SD) are associated with an increased risk of cardiovascular disease (CVD) in the general population. However, these associations may differ in breast cancer (BC) survivors who experience competing risks from cancer and its associated treatment. We examined whether physical or mental HRQoL (including individual subscales) or SD were associated with CVD risk in BC survivors.

Methods: We conducted a longitudinal analysis in the Women's Health Initiative (WHI) of post-menopausal women free of prevalent CVD at baseline and diagnosed with invasive BC during study follow-up from 1993 – 2010. CVD was defined as physician-adjudicated coronary heart disease or stroke. Physical and mental HRQoL, measured by the SF-36 Physical and Mental Component Scores (PCS and MCS, respectively), and SD, measured by the WHI Insomnia Rating Scale (IRS), were recorded post-BC diagnosis. Higher PCS and MCS scores indicate better HRQoL while higher IRS scores indicate worse SD. Time-dependent Cox proportional hazards models were used starting at BC diagnosis until 2010 or censoring adjusted for relevant confounders most proximal to or at BC diagnosis.

Results: In 2,884 BC survivors, 157 developed CVD during a median follow-up of 9.5 years. After adjustment, higher PCS scores were significantly associated with a lower risk of CVD (Table). For a 5-unit difference, the risk of CVD was 9% lower for those with higher PCS scores (95% CI: 0.83, 0.99). Of the SF-36 sub-scales, higher scores on vitality, physical functioning, and general health were significantly associated with a lower risk of CVD.

Conclusion: In BC survivors, we found that better physical HRQoL was associated with lower CVD risk. While further research is needed to replicate these findings, our results support

existing recommendations that women with breast cancer adopt health behaviors that promote physical health.

Introduction

As survival rates have increased for women with breast cancer (BC), there are now 3.8 million BC survivors in the United States as of 2021[1]. While advancements in cancer treatment have contributed to improvements in BC mortality, BC survivors are at an increased risk of cardiovascular disease (CVD) morbidity and mortality long-term [10]. While this has been largely attributed to the direct cardiotoxic effects of cancer treatment, these therapies may also affect CVD risk indirectly via health-related quality of life (HRQoL). Cancer treatments are known to cause adverse effects and symptoms such as increased fatigue, pain, sleep disturbance, depression, and reduced physical function, all of which can impact a person's HRQoL [102, 103]. HRQoL is a broad, multidimensional concept used to describe a person's self-perceived health status, which includes an assessment of physical and emotional well-being [104].

HRQoL is increasingly being recognized as a predictor of CVD risk in the general population [105-108]. Previous studies have shown individual components that are included in the assessment of HRQOL, such as fatigue, pain, sleep disturbance, depression, and physical function are linked with an increased risk of a variety of CVD events [109-113]. In particular, one study within the Women's Health Initiative (WHI) Observational Study (OS) found that participants with low self-reported physical HRQoL scores, as measured by the Rand Short-Form (SF)-36 Physical Component Summary (PCS), were at a 2-fold higher risk of coronary heart disease (CHD) and stroke compared to those with higher scores. This study also examined associations with mental HRQoL (using the SF-36 Mental Component Summary [MCS]), though no significant associations were found [112].

However, these relationships have not been explored in BC survivors. Mechanistically, cancer treatments contribute to inflammation and oxidative stress, which are known, in part, to

lead to the development of CVD. Likewise, inflammation and oxidative stress are associated with fatigue, sleep disturbance, depression, pain, and reduced physical function [114-116]. Additionally, cancer itself leads to a higher inflammatory state with BC patients reporting high levels of symptoms prior to ever receiving treatment [117]. Therefore, it is possible that BC is associated with CVD risk through symptom and HRQoL pathways. However, these associations have yet to be examined in a cancer survivorship population, whom are at a known risk of both poor HRQoL measures and symptoms as well as CVD.

Thus, the purpose of this study was to examine the association of post-cancer physical and mental HRQoL, as measured by SF-36 PCS and MCS scores, and sleep disturbance, as measured by the WHI Insomnia Rating Scale (IRS), on CVD risk in post-menopausal women diagnosed with BC. We additionally explored whether individual subscales of physical and mental HRQoL were associated with CVD risk.

Methods

Study population.

The Women's Health Initiative (WHI) is a population-based prospective cohort study of 161,808 post-menopausal women enrolled between 1993-1998 and followed initially through 2005 [51]. Women aged 50 to 79 were enrolled at 40 clinical centers nationwide. Women were enrolled in either one or more overlapping clinical trials (CTs) or an observational study (OS). At the end of the main study in 2005, women were asked to participate in an extension study with follow-up through 2010. All women provided consent prior to data collection.

The Life and Longevity After Cancer (LILAC) study is a cancer survivorship sub-cohort study within the WHI [52]. A primary goal of LILAC was to retrospectively collect information on cancer treatment in women diagnosed with cancer. Women were eligible for LILAC if they had a

confirmed cancer diagnosis during WHI follow-up in the CT or OS but were free of cancer at WHI baseline. Enrollment for LILAC began in 2013 [52].

For this analysis, we conducted a secondary longitudinal analysis (Supplemental Figure 1). The primary analysis was conducted using the Observational Study (OS) and Clinical Trial (CT) cohorts through 2010. Women diagnosed with an incident, invasive stage I-IV BC diagnosis through 2010 were eligible for this analysis (n = 8,066). Women were excluded if they 1) had an adjudicated CVD outcome prior to BC (n = 178) or 2) did not have a documented MCS, PCS, or WHI IRS score between BC and follow-up (n = 5,004) resulting in a final sample size of 2,884 women (Figure 1).

Outcome.

The primary outcome of interest was an incident, physician-adjudicated CVD event defined as having coronary heart disease (CHD), which includes myocardial infarction (MI) or coronary death, or stroke. CVD events were adjudicated on all participants through the extension study ending in 2010.

WHI cardiac adjudication methods have been in described in detail elsewhere [53]. 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. Cause of death, used in the definition for CHD, was determined through linkage with the National Death Index.

CHD was defined as having an acute MI requiring hospitalization, a silent MI, or CHD death. Both definite and probable MIs were included and were classified using an algorithm that consisted of a combination of data including medical history, electrocardiogram readings, and cardiac enzymes. CHD death was defined as death with an underlying cause of CHD with one or

more of the following: hospitalization for MI within 28 days before death, previous MI or angina, death resulting from a procedure related to coronary artery disease, or a death certificate indicating CHD as the underlying cause of death [53].

Stroke was defined as having a rapid onset of persistent neurologic deficit attributed to an obstruction or rupture of the brain arterial system without evidence for other cause and supported by imaging studies [53].

Exposures.

Three primary exposures were of interest: PCS (physical HRQoL), MCS (mental HRQoL), and WHI IRS (sleep disturbance). The SF-36 and WHI IRS were measured at multiple time points throughout the WHI main study. In the OS, both the SF-36 and WHI IRS were measured at baseline and year 3 of follow-up and in the CT were measured at baseline and years 3, 6, & 9 of follow-up on a subset of participants.

The SF-36 consists of 8 subscales with scores ranging from 0 to 100 with higher scores indicating a more favorable health state: (1) physical functioning [PF], (2) general health perceptions [GH], (3) bodily pain [BP], (4) vitality [VT] (i.e., energy/fatigue), (5) role limitations due to physical health [RP], (6) mental health (i.e. depression) [MH], (7) role limitations due to emotional problems [RE], and (8) social functioning [SF]. As described elsewhere, the MCS and PCS were created using principal components methods [118]. While the MCS and PCS are calculated using scores from all 8 subscales, different weights (i.e., factor scoring coefficients) are applied to each of the subscale scores using an established algorithm [118]. As such, the MH, RE, SF, and VT subscales correlate higher with the MCS, whereas the PF, RP, BP, and GH subscales correlate higher with the PCS. (Supplementary Material). MCS and PCS were considered missing if a participant was missing any of the 8 subscales.

Sleep disturbance was measured using the WHI IRS, which is composed of five questions. These questions assessed frequency of insomnia symptoms over the past four weeks including sleep latency, sleep maintenance, early morning awakening, and average sleep quality. Total IRS scores range from 0-20 with higher scores indicating greater sleep disturbance. A score of 9 has been validated to classify clinically meaningful sleep disturbance [119].

Additional variables.

Demographic information on age, self-identified race/ethnicity, and education were collected on WHI baseline questionnaires. Additional information on potential confounders, such as smoking, physical activity, and alcohol consumption were collected via self-report questionnaires. Physical activity was measured as total metabolic equivalent-minutes per week (MET-minutes/week) calculated from questionnaires as previously described [81]. In summary, women were asked to self-report the frequency, duration, and speed of walking as well as the frequency and duration of physical activities classified as either mild, moderate, or strenuous physical activity. METs were assigned to different intensities of physical activity based on the Compendium of Physical Activities [82]. Alcohol consumption was recorded as the number of alcoholic servings per week. Participants were asked the frequency and quantity of drinking alcohol beverages including beer, wine, and liquor. BMI was measured at clinic visits by trained WHI staff. Medications were collected as part of the WHI clinic visits. Participants were instructed to bring all current medications in their original containers, both prescription and nonprescription, to the baseline and follow-up WHI clinic visits. For this analysis, we were interested in antihypertensive medication use, which includes ACE inhibitors, angiotensin receptor antagonists (ARBs), calcium channel blockers, and beta-blockers. Current smoking status, physical activity, alcohol consumption, BMI, and medication use were measured at multiple timepoints throughout

WHI follow-up. As such, data from the timepoint closest, but prior to BC diagnosis, was used. Lastly, cancer characteristics, such as age at diagnosis, stage at diagnosis, and cancer treatments, including receipt of chemotherapy and radiation, were collected from medical history records and adjudicated outcomes.

Statistical Analysis.

Normality of continuous variables was assessed visually. Baseline (i.e., pre-cancer) characteristics of the sample are reported using means and standard deviations or frequencies and proportions for continuous and categorical variables, respectively. Bivariate statistics employed t-tests and chi-square tests for continuous and categorical variables, respectively, to compare tertiles of PCS and MCS, and compare those with a WHI IRS score with a cut point of 9 as previously described. Median and interquartile range (IQR) were calculated for follow-up time.

Incidence rates (cases/1,000 person-years) and 95% confidence intervals were calculated for CVD for the entire cohort and by categories of PCS, MCS, and WHI IRS scores. The cumulative incidence of CVD for each exposure (i.e., post-cancer MCS, PCS, and WHI IRS) was estimated using time-dependent cumulative incidence curves. Time to incident CVD was defined as the number of days since BC diagnosis. Participants without a CVD outcome were censored at time of last follow-up through 2010.

Multivariable-adjusted time-dependent Cox proportional hazards (PH) models were used to examine the association between PCS, MCS, or WHI IRS scores and risk of incident CVD. MCS, PCS, and WHI IRS scores were modeled as time-varying exposures in separate models using all scores calculated between BC diagnosis and CVD outcome or censoring. This means that the score closest to CVD or censoring was used. To reduce the possibility of reverse causation, exposures within 6 months of the outcome or censoring were excluded. MCS, PCS,

and SF-36 subscale scores were modeled as continuous variables, whereas IRS scores were dichotomized using a cut point of 9 as described previously. For MCS, PCS, and SF-36 subscale scores, beta coefficients correspond to a 5-unit difference. This was chosen as a 5-point change in SF-36 scores has been considered a minimally clinically important difference in prior studies in breast cancer cohorts.[120, 121] Potential covariates were decided *a priori* if they were known risk factors for both the exposure (PCS, MCS, or WHI IRS) and CVD risk. All models were adjusted for age at diagnosis (years), education (HS or GED, > HS – Bachelor's, > Bachelor's), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Other), baseline QoL measure, cancer stage (local, regional, distant), BMI (kg/m^2), physical activity (MET-min/week), alcohol (servings/week), current smoking (yes/no), antihypertensive meds (yes/no). For models examining PCS, MCS was additionally adjusted for and vice versa. Models for WHI IRS were adjusted for both PCS and MCS. A complete case analysis was performed, meaning only those with complete data on exposures and confounders were included. Overall, rates of missing data are minimal with no variable having missing data in more than 1% of participants.

We conducted an exploratory analysis to see which, if any, individual SF-36 subscales scores, were associated with risk of CVD. We additionally conducted a sensitivity analysis within the LILAC cohort to determine if treatment was a significant confounder. For this sensitivity analysis, women were included if they were in LILAC and had treatment data available, resulting in a sample size of 1,662 participants. The main analysis was repeated in this cohort with and without treatment in the models to determine if the addition of treatment influenced the HR estimates.

The proportional hazards assumption was confirmed using Schoenfeld residuals. A two-sided p-value of 0.05 was used to determine statistical significance. All analyses were performed using R Version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of study sample

A total of 2,884 women with a mean (SD) age at diagnosis of 67.3 (6.94) were followed for a median (IQR) of 9.5 (7.0, 11.7) years after BC diagnosis. Overall, the majority of women identified as non-Hispanic white, had at least a bachelor's degree, were non-smokers, and were diagnosed with local BC (Table 1). The mean (SD) baseline PCS, MCS, and sleep disturbance scores were 48.0 (9.9), 53.7 (8.5), and 6.5 (4.3), respectively (Table 1). The median (IQR) time from BC to next measurement of PCS, MCS, or sleep disturbance was 2.3 (1.0, 4.2) years, whereas the median (IQR) time from PCS, MCS, or sleep disturbance to CVD or censoring was 5.8 (5.5, 9.2) years.

Comparison of baseline characteristics by categories of PCS, MCS, and WHI IRS scores

When comparing tertiles of PCS, those in the lowest tertile were more likely to be older, have a higher BMI, be less physically active, consume less alcohol per week, and report greater sleep disturbance (Table 2). Similar results were found when comparing tertiles of MCS with a few differences. Those in the lowest MCS tertile were also more likely to be have a higher BMI, be less physically active, and report greater sleep disturbance (Supplemental Table 1). An association was also found that those in the lowest MCS tertiles were more likely to identify as non-Hispanic black or other. However, those in the lowest MCS tertile were more likely to be younger, which is in contrast with PCS. Approximately, 9.5% of the sample had an IRS score ≥ 9, indicating clinically-relevant sleep disturbance. Those with sleep disturbance were also more

likely to be older, have higher BMI, report less physical activity, and have lower MCS and PCS scores (Supplemental Table 2).

Cumulative incidence curves and incidence rates

During follow-up, there were 157 CVD outcomes with an overall incidence rate of 5.9 (95% CI: 5.0, 6.8) events per 1,000 person-years. The incidence rate of CVD decreased with higher PCS tertiles, with a significant difference when comparing participants in the 1st and 3rd PCS tertiles (Table 3). A similar association is seen when examining the unadjusted cumulative incidence curve for PCS (Figure 2). However, when examining incidence rates by MCS tertiles, participants in the 3rd tertile had a higher incidence rate of CVD; however, this was not statistically different when compared to the 1st or 2nd tertiles (Table 3). This is supported in the cumulative incidence curve, which shows the cumulative incidence of CVD to be highest for those in the 1st MCS quartile until approximately 4,000 days of follow-up when the curve for the 3rd quartile crosses over (Figure 2). Lastly, participants with an IRS score ≥ 9 had a higher incidence of CVD compared to those with a score < 9; however, this difference was not statistically significant (Table 3).

Adjusted Cox PH analysis

In multivariable-adjusted Cox PH models, higher PCS scores were significantly associated with a lower risk of CVD. For a 5-point difference in PCS scores, the risk of CVD was 9% lower for those with higher compared to those with lower scores [HR = 0.91 (95% CI: 0.82, 0.99); p = 0.049] (Table 4). There was no association found between MCS scores or IRS scores and risk of CVD.

Exploratory and sensitivity analyses

When examining individual sub-scales of the SF-36, higher scores on vitality (i.e., fatigue), physical functioning, and general health were all significantly associated with a lower risk of CVD (Table 4). For each 5-point increase in vitality, physical functioning, and general health subscale scores, the risk of CVD was decreased by 4% [HR = 0.96 (95% CI: 0.92, 0.99)], 6% [HR = 0.94 (95% CI: 0.91, 0.97)] and 6% [HR = 0.94 (95% CI: 0.90, 0.98)], respectively, compared to those with lower subscale scores. In the subset of 1,662 participants with treatment data available, the addition of treatment to the models in the LILAC cohort did not alter the HRs, thus, treatment does not appear to be a significant confounder (Supplemental Tables 4 & 5).

Discussion

This study is the first, to our knowledge, to investigate whether physical and mental HRQoL and sleep disturbance are independently associated with the risk of CVD in BC survivors. Overall, this study found that lower PCS scores (i.e., a measure of physical HRQoL) were a significant predictor of CVD in BC survivors after adjustment for multiple potential confounders such as age, BMI, smoking, and physical activity. Additionally, this association appears to be largely driven by physical function and perceptions of general health, which are two SF-36 subscales that are more heavily weighted in the calculation for PCS. By contrast, we found no significant association between either MCS scores or sleep disturbance with CVD risk. While MCS scores were not significant, lower vitality sub scores (i.e., a measure of energy/fatigue) was a significant predictor of CVD despite being a component of MCS.

The findings from this study are supported by research in non-cancer populations which have shown that poor physical HRQoL is a significant predictor of CVD and CVD-related mortality [105-108, 122-124]. The mechanism by which PCS scores influence CVD risk is unclear. It has been suggested that changes in PCS are related to the presence of subclinical CVD disease

that have not yet been reported or noticed. To account for the potential for reverse causation, we excluded PCS scores within 6 months of the outcome or censoring to minimize the risk of reverse causation. However, the median time between PCS measurement and overall follow-up time was over 5.8 years with 75% of participants exceeding 5.5 years. We also excluded participants with an adjudicated CVD outcome prior to BC. Additionally, potential events for CVD adjudication were assessed at least yearly, thus it is unlikely outcomes of interest were missed. To account for the potential that participants had cardiac disease that did not develop into MI/CHD or stroke, we adjusted for cardiac medications including ACE inhibitors, calcium channel blockers, beta blockers, and ARBs. Another potential explanation is that individuals with lower PCS scores are less likely to engage in healthy lifestyle habits, such as physical activity (as supported by our data), which is known to reduce the risk of CVD [125]. Prior studies have found a dose-response relationship between physical activity and SF-36 PCS scores in participants with CVD [126]. However, we found significant associations between PCS scores and CVD despite adjustment for physical activity.

Other hypotheses that provide a potential explanation for the association between PCS and CVD risk include inflammatory pathways. The PCS score is largely calculated by four subscales of the SF-36 including physical functioning, perceptions of general health, physical limitations due to physical function, and pain. Prior studies have shown that poorer physical HRQoL, physical function, and perceived health are associated with higher circulating level of inflammatory cytokines [127-130]. These findings are also true in otherwise healthy individuals without evidence of clinical disease [130]. This is important for BC survivors as cancer treatments produce an inflammatory response, and thus, BC survivors may be more susceptible to lower physical HRQoL [131]. However, it is unclear if physical HRQoL is indicator of an inflammatory

state or if it is a driver of inflammation. Additionally, prior studies have found significant associations between self-rated physical health (i.e., a component of the PCS) and CVD risk and mortality even after adjustment for inflammatory biomarkers [132-134]. This suggests physical HRQoL may independently contribute to CVD risk above and beyond the role of inflammation. Future studies are needed to disentangle the mechanisms contributing to this association and examine physical HRQoL as a potential mediator between inflammation and CVD risk.

In this study, we did not find evidence to support either MCS scores or sleep disturbance were risk factors for CVD in BC survivors. In non-cancer populations, studies have also found weak or null associations when examining MCS scores as a risk factor for CVD [105, 123, 124]. While consistent with previous reports, this finding is interesting as mental illnesses, such as depression and anxiety, have been highly associated with CVD risk [135, 136]. However, depression is thought to contribute to CVD risk through pathways that extend beyond QoL leading to physical consequences such as disturbances in the autonomic nervous system, the HPA axis, and inflammatory pathways. The MCS is a broad measure of mental health, which incorporates measures on social functioning, energy/fatigue, emotional well-being, and physical limitations related to mental health. Thus, the MCS may not be capturing clinical depression or anxiety, which are more closely associated with CVD risk. Future studies should use tools designed specifically to measure depression and anxiety, such as the Patient Health Questionnaire (PHQ-9) or Generalized Anxiety Disorder Scale (GAD-7).

The lack of association between sleep disturbance and CVD risk found in this study is inconsistent with previous literature, which supports that sleep disturbances, including insomnia, sleep duration, and sleep loss, are associated with CVD risk [137-139]. While this could indicate the WHI IRS is not sufficiently sensitive to accurately capture insomnia, this is unlikely as a

previous study in the WHI OS found that participants with a WHI IRS score ≥ 9 had a significantly higher risk of incident CHD [138]. Potential explanations for the lack of association in this study could be that in the context of BC, sleep disturbance is not a strong enough risk factor for CVD when adjusting for traditional CVD risk factors and cancer characteristics. Although not statistically significant, the hazard ratio indicates a higher risk of CVD with higher WHI IRS scores. Thus, a large sample size is likely needed to detect a smaller effect size related to sleep disturbance.

When examining individual subscales, we found energy/fatigue, general health perception, and physical functioning to be significantly associated with incident CVD. However, these results should be interpreted with caution as these subscales are highly correlated with one another. These findings provide further support that measures of self-rated health and fatigue are associated with CVD risk [110, 132, 140-144]. Similar to the mechanisms linking self-rated health to CVD, fatigue is also hypothesized to contribute to CVD through inflammatory pathways in addition to numerous metabolic and hemodynamic processes [140]. Physical function has also been linked to CVD risk through physical activity and exercise capacity. Studies have shown BC survivors with lower physical functional capacity have reduced exercise capacity, which is associated with an increased risk of CVD [145, 146]. This may also be linked to inflammation as exercise interventions have been shown to reduce circulating inflammatory biomarkers [147]. Further research has shown that anti-inflammatory medications given to patients with symptomatic HF improved exercise capacity when compared to placebo controls providing further evidence of the role inflammation plays in exercise capacity and physical function [148]. However, it is difficult to separate the effects of each of these subscales as they are highly related to one another. For instance, fatigue has been shown to be a predictor of overall general health in

older adults and is related physical function [141]. Future studies are needed to examine the effects of these constructs in relation to CVD risk using additional measurements.

As this is an observational study, residual confounding is a potential limitation, and we cannot assume a causal interpretation of these results. However, we attempted to minimize confounding by excluding participants with an adjudicated CVD outcome prior to BC and by adjusting for variety of potential confounders including demographics, lifestyle factors, BMI, cardiac medications, and cancer characteristics. While we do not have inflammatory biomarker data to include in our models as a potential confounder, we controlled for variables that are likely to contribute to inflammation. A strong contributor of systemic inflammation after BC is cancer treatment [149]. In our sensitivity analysis within the LILAC cohort, cancer treatment did not appear to be a confounder in the relationship between PCS and risk of CVD; thus, we do not feel the exclusion of cancer treatment in our main analysis impacted our results. We also adjusted for physical activity, BMI, depression, and smoking, which are all predictors of inflammation in BC survivors [116, 150]. Lastly, there was a relatively long gap between cancer diagnosis and the assessment of PCS, MCS, and IRS scores with a median time of 2.3 years. However, the length of time between cancer diagnosis and treatment completion can take 12 months or more. Despite not having treatment data available on all participants, it is likely that there is sufficient follow-up time to ensure participants completed their cancer treatment. Additionally, cancer treatment can have a long-lasting impact on many subscale measures, such as fatigue and depression, with BC survivors reporting effects years after treatment has ended [151]. Thus, these findings could provide insight into the effects of long-term effects of cancer and its treatment on CVD risk.

Despite these limitations, this study has many strengths. This is the first study, to our knowledge, to examine the role of physical and mental HRQoL in CVD risk in BC survivors.

The WHI has a large sample size of BC survivors who were followed over 10 years of post-BC follow-up in this study. Given the large sample size and long-term follow-up, this study is adequately powered to examine cardiac outcomes that typically occur 10 or more years after cancer treatment. CVD was also physician-adjudicated, which reduces the possibility of misclassification of the outcome. Lastly, PCS, MCS, and WHI IRS scores were measured at multiple time points throughout the WHI follow-up allowing us to examine post-cancer measures.

Conclusion

In this study, our findings suggest that physical HRQoL is associated with the risk of CVD in BC survivors; however, no associations were found for mental HRQoL or sleep disturbance. Our findings related to physical HRQoL are aligned with previous reports in cancer-free population. The implications of these findings are important for both clinicians and researchers. First, our findings highlight the importance of assessing HRQoL measures in order to improve the screening of CVD risk in BC survivors. In particular, physical HRQoL measures may indicate somatic changes without overt disease and, thus, may be an indicator for clinicians to intervene. The SF-36 is a quick and easy measurement tool that could be implemented in clinical settings to aid in the prediction of CVD risk in addition to traditional cardiac risk factors. Additionally, studies using a shorter QoL measure, the SF-12, to calculate PCS scores have found similar results in non-cancer populations.[105] Second, our findings support the importance of promoting strategies to improve physical HRQoL. Future studies should examine whether the addition of PCS scores improves the risk prediction models of CVD in breast cancer survivors beyond that of traditional risk factors. Further research is also needed to examine the influence of inflammation on physical HRQoL and CVD risk and examine the potential mediating effects of

physical HRQoL in BC survivors. Longitudinal studies should also be conducted to examine how changes in physical HRQoL scores over time influence CVD risk.

Table 1. Baseline characteristics of overall sample

	Overall (N = 2,884)
Age at Diagnosis (mean (SD))	67.3 (6.94)
Race/Ethnicity (n (%))	
Non-Hispanic White	2,534 (87.9)
Non-Hispanic Black	192 (6.7)
Other*	152 (5.3)
NA	6 (0.2)
Education (n (%))	
< HS	92 (3.2)
HS or GED	427 (14.8)
> HS – Bachelor's	1,407 (48.8)
> Bachelor's	936 (32.5)
NA	22 (0.8)
Smoking (%)	
Current smoker	161 (5.6)
NA	12 (0.4)
BMI (kg/m^2) (mean (SD))	28.5 (6.0)
Physical Activity (MET-hours/week) (mean (SD))	12.11 (13.5)
Alcohol (servings/week) (mean (SD))	2.43 (4.9)
Antihypertensive medications (n (%))	184 (6.4)
Cancer stage	
Local	2181 (75.6)
Regional	659 (22.9)
Distant	17 (0.6)
NA	27 (0.9)
PCS (mean (SD))	48.0 (9.9)
MCS (mean (SD))	53.7 (8.5)
Sleep Disturbance (mean (SD))	6.5 (4.3)

*****Includes Asian or Pacific Islander, American Indian or Alaskan Native, Hispanic/Latino, or "other" referring to a category not listed

Table 2. Association of PCS Tertiles with baseline characteristics

	PCS			
	Tertile 1 (N = 910) (<46.5)	**Tertile 2 (N = 910) (46.5 – 53.9)**	**Tertile 3 (N = 909) (>53.9)**	**p-value**
Age at Diagnosis (mean (SD))	68.6 (6.8)	67.4 (6.9)	65.7 (6.8)	< 0.001
Race/Ethnicity (n (%))				0.186
Non-Hispanic White	794 (87.3)	800 (87.9)	819 (90.1)	
Non-Hispanic Black	69 (7.60)	57 (6.3)	41 (4.5)	
Other*	45 (4.9)	50 (5.5)	48 (5.3)	
NA	2 (0.2)	3 (0.3)	1 (0.1)	
Education (n (%))				< 0.001
< HS	43 (4.7)	16 (1.8)	16 (1.8)	
HS or GED	159 (17.5)	132 (14.5)	118 (13.0)	
> HS – Bachelor's	459 (50.4)	442 (48.6)	432 (47.5)	
> Bachelor's	246 (27.0)	310 (34.1)	337 (37.1)	
NA	3 (0.3)	10 (1.1)	6 (0.7)	
Smoking (n (%))				0.080
Current smoker	48 (5.3)	54 (5.9)	48 (5.3)	
NA	8 (0.9)	2 (0.2)	1 (0.1)	
BMI (kg/m^2) (mean (SD))	30.3 (6.40)	28.5 (5.7)	26.7 (5.3)	< 0.001
Physical Activity (MET-hours/week) (mean (SD))	8.8 (11.1)	13.0 (13.6)	14.7 (14.8)	< 0.001
Alcohol (servings/week) (mean (SD))	2.1 (5.5)	2.6 (4.8)	2.7 (4.6)	0.029
Antihypertensive medications (n (%))	77 (8.5)	51 (5.5)	49 (5.4)	0.012
Cancer stage				0.399
Local	685 (75.3)	708 (77.5)	669 (73.6)	
Regional	212 (23.3)	189 (20.8)	223 (24.5)	
Distant	4 (0.4)	6 (0.7)	7 (0.8)	
NA	9 (1.0)	7 (0.8)	10 (1.1)	
PCS (mean (SD))	36.4 (7.7)	50.7 (2.1)	56.9 (2.35)	
MCS (mean (SD))	54.2 (9.0)	54.8 (6.8)	52.1 (9.3)	< 0.001
Sleep Disturbance (mean (SD))	7.6 (4.4)	6.09 (4.0)	5.8 (4.2)	< 0.001

*****Includes Asian or Pacific Islander, American Indian or Alaskan Native, Hispanic/Latino, or "other" referring to a category not listed

Table 3. Incidence rates (per 1,000 person-years) of CVD by MCS, PCS, and IRS categories

	1st Quartile	**2nd Quartile**	**3rd Quartile**
PCS	7.9 (6.1, 10.1)	5.9 (4.4, 7.7)	3.4 (2.2, 4.8)*
MCS	5.3 (3.9, 7.1)	5.1 (3.7, 6.8)	6.7 (5.1, 8.7)
	< 9	**>= 9**	
IRS	5.4 (4.4, 6.5)	7.0 (5.3, 9.2)	

*Significant difference between 1st and 3rd quartile

Table 4. Cox PH models for association of quality-of-life measures and risk of CVD (N = 2,884)

	Events	HR[1] (95% CI)	p-value
MCS [2,3,7]	117	0.98 (0.87, 1.09)	0.715
PCS [2,4,7]	117	0.91 (0.83, 0.99)	0.049
Sleep Disturbance[6,8]	121	1.27 (0.84, 1.92)	0.252
SF-36 Subscales [6,7]			
MCS			
Vitality (i.e., fatigue)	131	0.96 (0.92, 0.99)	0.027
Emotional well-being (i.e., depression)	130	0.98 (0.92, 1.04)	0.506
Social functioning	134	0.98 (0.94, 1.01)	0.135
Role limitations due to emotional well-being	132	1.00 (0.97, 1.02)	0.626
PCS			
Physical function	131	0.94 (0.91, 0.97)	0.001
Role limitations due to emotional physical health	131	0.98 (0.96, 1.00)	0.060
Pain	133	0.98 (0.95, 1.01)	0.214
General health	134	0.94 (0.90, 0.98)	0.015

[1] HR represents a 5-unit difference

[2] Adjusted for age at diagnosis (years), education (HS or GED, > HS – Bachelor's, > Bachelor's), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Other), baseline QoL measure[5], cancer stage (local, regional, distant), BMI (kg/m^2), physical activity (MET-min/week), alcohol (servings/week), smoking (yes/no), antihypertensive meds (yes/no)

[3] Additionally adjusted for baseline PCS

[4] Additionally adjusted for baseline MCS

[5] Additionally adjusted for baseline PCS & MCS

[6] Each model is adjusted for the baseline variable according to the exposure

[7] Higher PCS & MCS and subscales scores correspond to a greater health state

[8] IRS dichotomized using cut point of 9; < 9 serves as the reference category

Figure 1. Sample selection flow chart

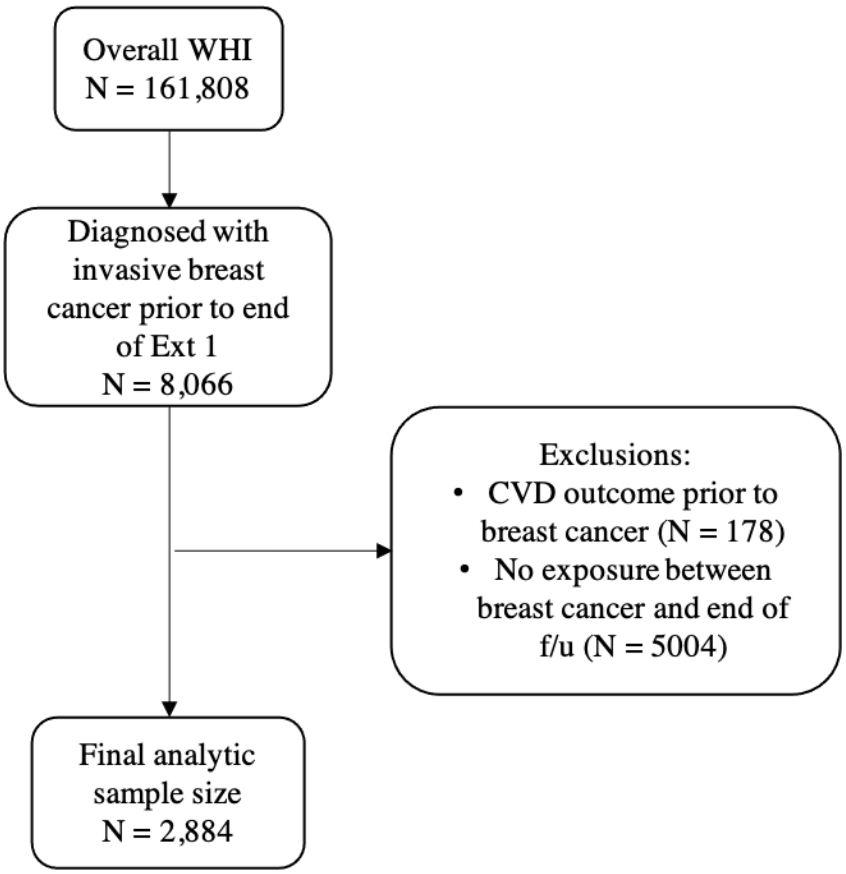
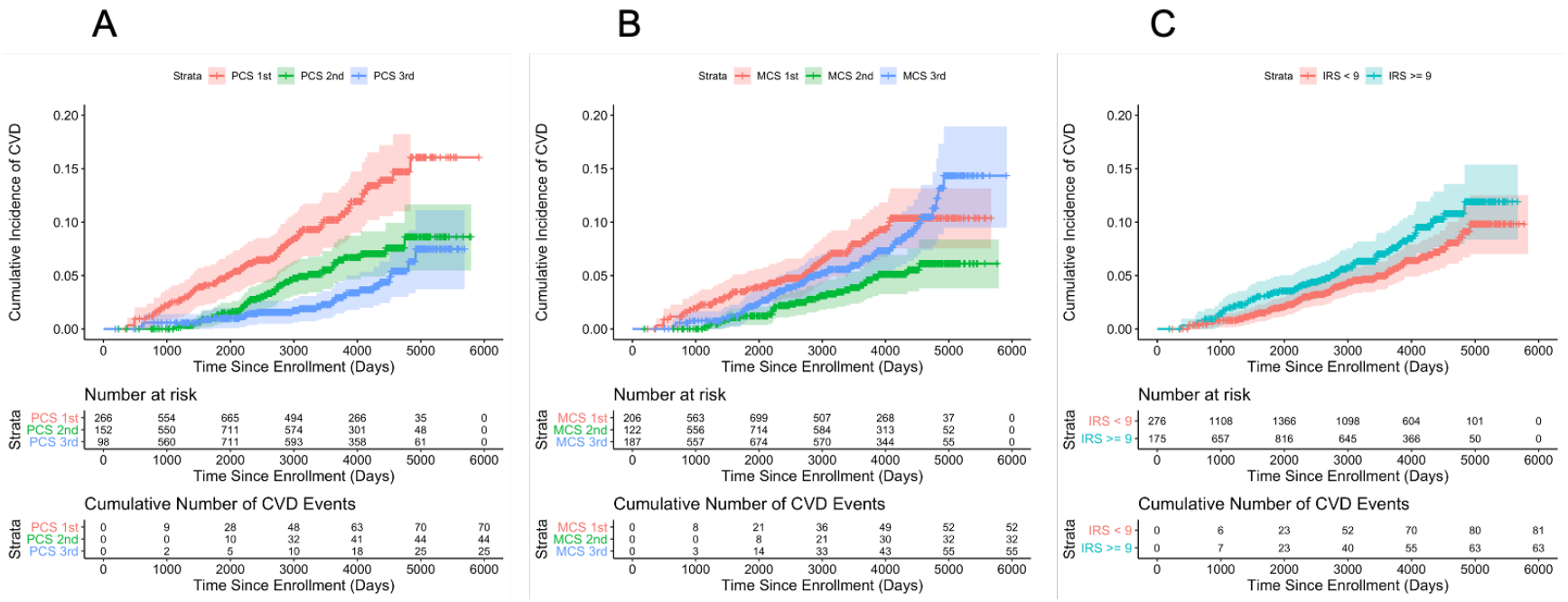


Figure 2. Unadjusted Time-Dependent Cumulative Incidence Curves of CVD by A) PCS tertiles, B) MCS tertiles, and C) IRS categories.



Supplementary Material

Scoring of MCS and PCS. The MCS and PCS are scored using norm-based methods using means, standard deviations, and factor score coefficients derived from the general U.S. population. After calculating the individual subscales, each scale is standardized using a z-score transformation and means and standard deviation of each subscale from the general U.S. population. The z-score is determined by subtraction the subscale mean from the general U.S. population from the calculated score then dividing by the standard deviation from the general U.S. population. Next the eight z-scores are multiplied by the factor scoring coefficients for each subscale. This is done for both the PCS and MCS factor scoring coefficients. The products are then summed and then transformed to norm-based scoring by multiplying the sum by 10 and adding the resulting product to 50. The final score has a mean of 50 and standard deviation of 10 for the general U.S. population.

			Factor Coefficients	
SF-36 Subscale	Mean	SD	PCS	MCS
PF	84.5	22.9	0.42402	-0.22999
RP	81.2	33.8	0.35119	-0.12329
BP	75.5	23.6	0.31754	-0.09731
GH	72.2	20.2	0.24954	-0.01571
VT	61.1	20.9	0.02877	0.23534
SF	83.6	22.4	-0.00753	0.26876
RE	81.3	33.0	-0.19206	0.43407
MH	74.8	18.0	-0.22069	0.48581)

Supplementary Table 1. Association of MCS Tertiles with baseline characteristics

	MCS			
	Tertile 1 (N = 910) (<53.4)	**Tertile 2 (N = 910) (53.4 – 58.2)**	**Tertile 3 (N = 909) (> 58.2)**	**p-value**
Age at Diagnosis (mean(SD))	66.3 (7.1)	66.7 (6.6)	68.6 (6.9)	< 0.001
Race/Ethnicity (n (%))				0.044
Non-Hispanic White	781 (85.8)	815 (89.6)	817 (89.9)	
Non-Hispanic Black	72 (7.9)	45 (4.9)	50 (5.5)	
Other*	54 (5.9)	47 (5.2)	42 (4.6)	
NA	3 (0.3)	3 (0.3)	0 (0.0)	
Education (n (%))				< 0.001
< HS	36 (4.0)	12 (1.3)	27 (3.0)	
HS or GED	138 (15.2)	139 (15.3)	132 (14.5)	
> HS – Bachelor's	476 (52.3)	424 (46.6)	433 (47.6)	
> Bachelor's	251 (27.6)	330 (36.3)	312 (34.3)	
NA	9 (1.0)	5 (0.5)	5 (0.6)	
Smoking (%)				0.002
Current smoker	71 (7.8)	37 (4.1)	42 (4.6)	
NA	6 (0.7)	3 (0.3)	1 (0.1)	
BMI (kg/m^2) (mean (SD))	28.8 (6.2)	27.8 (5.3)	28.9 (5.3)	< 0.001
Physical Activity (MET-hours/week) (mean (SD))	10.5 (12.0)	13.0 (13.6)	13.0 (14.5)	< 0.001
Alcohol (servings/week) (mean (SD))	2.2 (5.4)	2.7 (5.0)	2.5 (4.5)	0.154
Antihypertensive medications (n (%))	63 (6.9)	62 (6.8)	52 (5.7)	0.515
Cancer stage				0.519
Local	678 (74.5)	696 (76.5)	688 (75.7)	
Regional	215 (23.6)	204 (22.4)	205 (22.6)	
Distant	4 (0.4)	6 (0.7)	7 (0.8)	
NA	13 (1.4)	4 (0.4)	9 (1.0)	
PCS (mean (SD))	48.6 (10.2)	49.6 (8.9)	45.8 (10.3)	< 0.001
MCS (mean (SD))	44.4 (8.3)	56.1 (1.2)	60.6 (2.2)	
Sleep Disturbance (mean (SD))	8.2 (4.6)	6.2 (4.0)	5.1 (3.7)	< 0.001

*****Includes Asian or Pacific Islander, American Indian or Alaskan Native, Hispanic/Latino, or "other" referring to a category not listed

Supplementary Table 2. Association of sleep disturbance with baseline characteristics

	Sleep Disturbance		
	< 9 (N = 2,011)	**>= 9 (N = 731)**	**p-value**
Age at Diagnosis (mean(SD))	67.0 (7.0)	67.7 (6.7)	0.018
Race/Ethnicity (n (%))			0.332
Non-Hispanic White	1,758 (87.4)	731 (89.7)	
Non-Hispanic Black	137 (6.8)	45 (5.5)	
Other*	113 (5.6)	37 (4.5)	
NA	3 (0.1)	2 (0.2)	
Education (n (%))			< 0.001
< HS	51 (2.5)	35 (4.3)	
HS or GED	280 (13.9)	140 (17.2)	
> HS – Bachelor's	956 (47.5)	431 (52.9)	
> Bachelor's	710 (35.4)	201 (24.7)	
NA	14 (0.7)	8 (1.0)	
Smoking (%)			0.473
Current smoker	111 (5.5)	44 (5.4)	
NA	6 (0.3)	5 (0.6)	
BMI (kg/m^2) (mean (SD))	28.3 (5.8)	29.1 (6.4)	0.001
Physical Activity (MET-hours/week) (mean (SD))	12.5 (13.5)	11.2 (13.4)	0.019
Alcohol (servings/week) (mean (SD))	2.4 (4.6)	2.5 (5.6)	0.843
Antihypertensive medications (n (%))	116 (5.8)	61 (8.3)	0.015
Cancer stage			0.172
Local	1546 (76.6)	596 (73.0)	
Regional	437 (21.7)	203 (24.9)	
Distant	9 (0.4)	8 (1.0)	
NA	19 (0.9)	7 (0.9)	
PCS (mean (SD))	49.0 (9.1)	45.7 (8.9)	< 0.001
MCS (mean (SD))	55.0 (7.3)	50.4 (1.2)	< 0.001
Sleep Disturbance (mean (SD))	4.3 (2.4)	12.0 (4.0)	

*****Includes Asian or Pacific Islander, American Indian or Alaskan Native, Hispanic/Latino, or "other" referring to a category not listed

Supplementary Table 3. Global test for Schoenfeld residuals for fully adjusted Cox PH models

Model	**Global p-value**
Composite	
MCS	0.81
PCS	0.95
Sleep Disturbance	0.79
SF-36 Subscales	
Fatigue	0.77
Emotional well-being	0.52
Physical function	0.84
Social function	0.76
Emotional limitations	0.69
Physical limitations	0.64
Pain	0.75
General health	0.67

Supplemental Table 4. Sensitivity analysis in LILAC (with treatment) [7]

Continuous[1]	**Events**	**HR** (95% CI)**	**p-value**
Composite			
MCS [2,3]	44	0.93 (0.58, 1.28)	0.703
PCS [2,4]	44	0.84 (0.56, 1.12)	0.308
Sleep Disturbance[6]	48	0.81 (0.20, 1.42)	0.588
SF-36 Subscales			
Fatigue	53	1.00 (0.83, 1.16)	0.960
Emotional well-being	51	0.96 (0.74, 1.15)	0.616
Physical function	52	1.01 (0.85, 1.16)	0.940
Social function	53	0.94 (0.82, 1.01)	0.103
Emotional limitations	53	0.99 (0.91, 1.08)	0.893
Physical limitations	51	0.96 (0.89, 1.04)	0.335
Pain	54	0.92 (0.82, 1.02)	0.148
General health	52	0.83 (0.69, 0.99)	0.052

[1] HR represent a 10-unit difference

[3] Adjusted for age at diagnosis (years), education (HS or GED, > HS – Bachelor's, > Bachelor's), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Other), baseline QoL measure[5], cancer stage (local, regional, distant), BMI (kg/m^2), physical activity (MET-min/week), alcohol (servings/week), smoking (yes/no), antihypertensive meds (yes/no), chemotherapy (yes/no), radiation (yes/no)

[3] Additionally adjusted for baseline PCS

[4] Additionally adjusted for baseline MCS

[5] Each model is adjusted for the baseline variable according to the exposure

[6] Additionally adjusted for baseline PCS & MCS

[7] N = 1,662; 64% of these have treatment data

Supplemental Table 5. Sensitivity analysis in LILAC (without treatment) [7]

Continuous[1]	**Events**	**HR** (95% CI)**	**p-value**
Composite			
MCS [2,3]	76	0.90 (0.63, 1.18)	0.516
PCS [2,4]	76	0.87 (0.65, 1.09)	0.269
Sleep Disturbance[6]	77	1.22 (0.50, 1.94)	0.508
SF-36 Subscales			
Fatigue	84	0.97 (0.84, 1.1=09)	0.592
Emotional well-being	82	0.92 (0.76, 1.08)	0.322
Physical function	83	0.98 (0.86, 1.10)	0.743
Social function	84	0.94 (0.85, 1.02)	0.129
Emotional limitations	84	1.00 (0.93, 1.07)	0.985
Physical limitations	83	0.99 (0.94, 1.05)	0.834
Pain	85	0.92 (0.85, 1.00)	0.057
General health	83	0.83 (0.72, 0.94)	0.006

[1] HR represent a 10-unit difference

[3] Adjusted for age at diagnosis (years), education (HS or GED, > HS – Bachelor's, > Bachelor's), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Other), baseline QoL measure[5], cancer stage (local, regional, distant), BMI (kg/m^2), physical activity (MET-min/week), alcohol (servings/week), smoking (yes/no), antihypertensive meds (yes/no)

[3] Additionally adjusted for baseline PCS

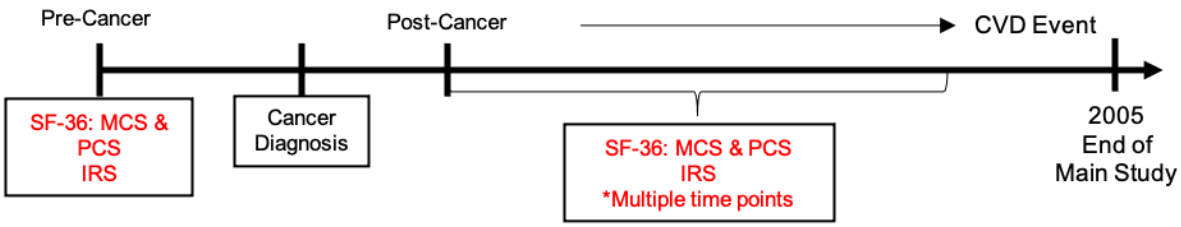
[4] Additionally adjusted for baseline MCS

[5] Each model is adjusted for the baseline variable according to the exposure

[6] Additionally adjusted for baseline PCS & MCS

[7] N = 1,662

Supplemental Figure 1. Timeline of data collection



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CHAPTER 5:

Conclusion

Due to improved survival rates and an aging population, cancer is increasingly being recognized as a chronic condition. As a result of this, cancer survivors are now living with a multitude of adverse effects of cancer treatments, such as cardiovascular disease (CVD) and debilitating symptoms. While research has shown an increased risk of CVD and symptoms in cancer survivors, less is known about who is at greatest risk and how to best manage their cancer treatment in order to prevent or minimize adverse effects, including symptoms. In part, this is due to the complex pathophysiology of treatment-induced CVD and symptoms that has yet to be fully explicated. The use of biomarkers and omics-based measures can help to elucidate these pathophysiologic mechanisms. The field of cancer survivorship could benefit from a precision health approach, which takes into account a person's unique background and combination of comorbidities, genes/biomarkers, environment, clinical, and lifestyle factors in order to inform prediction and prevention strategies. The goal of precision health is to go beyond treatment of disease, but rather focus on prediction and prevention to optimize health.

This dissertation supports the long-term goal towards a greater understanding of the biological mechanisms contributing to CVD and fatigue in cancer survivors in order to guide the development of biobehavioral interventions and health promotion strategies that prevent or reduce these adverse effects. This dissertation is significant as it investigated biomarkers potentially associated with radiation-induced CVD (RICVD) and fatigue (RIF). The results of this dissertation provide support for the role of oxidative DNA damage in the pathophysiology of RICVD, as higher concentrations of 8-OH-dG were associated with RICVD in breast cancer survivors. Additionally, the findings from this dissertation found that higher concentrations of inflammatory (i.e., IL-6, GDF-15) and cardiac damage (cystatin-C) biomarkers were associated with greater RIF in breast cancer survivors. This is the first study to our knowledge to examine

biomarkers of cardiac damage in relation to fatigue in breast cancer survivors. The pathophysiology of RIF is likely multifactorial and may also be indicative of underlying pathological processes related to cardiac dysfunction.

While biomarkers are one aspect of precision health, this work has the potential to provide information on biomarkers that can be used in future risk prediction models. Next steps could translate this biological information into clinical risk models, in conjunction with other known risk factors, to improve the risk prediction of CVD and fatigue in breast cancer survivors. Early identification of individuals at risk for RIC and RIF is beneficial as this line of work could lead to reductions in RICVD and RIF by targeting interventions to those at highest risk. Additionally, a better understanding of the biological mechanisms of RICVD and RIF would help investigators develop interventions to target the specific pathways.

In addition to biomarkers, precision health includes an assessment of clinical and lifestyle risk factors. Findings from this dissertation suggest that physical health-related quality of life (HRQoL) is associated with CVD risk in breast cancer survivors; however, no associations were found for mental HRQoL or sleep disturbance. This is the first study to examine HRQoL as a risk factor for CVD in breast cancer survivors. These findings related to physical HRQoL are aligned with previous reports in cancer-free populations. The implications of these findings are important for both clinicians and researchers. First, these findings highlight the importance of assessing QoL measures in order to help identify breast cancer survivors who may be at a greater risk of CVD. In particular, physical HRQoL measures may indicate somatic changes without overt disease and, thus, may be an indicator for clinicians to intervene. Measuring PCS is a quick and easy measurement tool that could be implemented in clinical settings to aid in the prediction of CVD risk in addition to traditional cardiac risk factors and/or biomarkers. In addition, these

findings highlight the importance implementing strategies to promote physical HRQoL in breast cancer survivors.