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Impaired Cardiorespiratory Fitness Following Thoracic Radiotherapy

Justin M. Canada
Virginia Commonwealth University

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Impaired Cardiorespiratory Fitness Following Thoracic Radiotherapy

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

Justin McNair Canada, MS, RCEP
Bachelor of Science, Old Dominion University, 1997
Master of Science, Virginia Commonwealth University, 2012

Dissertation Advisor: Antonio Abbate, MD, PhD
Vice-Chairman, Division of Cardiology

Virginia Commonwealth University
Richmond, Virginia

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Abstract

IMPAIRED CARDIORESPIRATORY FITNESS FOLLOWING THORACIC RADIOTHERAPY

Justin McNair Canada, PhD, RCEP

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Dissertation Adviser: Antonio Abbate, MD, PhD
Department of Internal Medicine, Division of Cardiology

Cancer (CA) is the second leading cause of death in the United States preceded only by cardiovascular disease (CVD). Over the past 30 years, the 5-year survival rate for all cancers combined has increased by more than 20%. This improved survival rate is due to early diagnosis and advances in treatment involving a multimodality treatment approach that includes radiotherapy [RT] with about half of all CA patients receiving some type of RT sometime during the course of their treatment. Cardiotoxicity is one of the most important adverse reactions of RT and leads to a meaningful risk of CVD-related morbidity and mortality. Radiotherapy-related cardiotoxicity is a heterogeneous clinical syndrome characterized by symptoms related to impaired cardiac function due

to radiation-injury to one or more cardiac structures. Furthermore, the relative risk of CVD increases with increasing incidental radiation dose to the heart.

There is not a unified consensus on the definition of CA-related cardiotoxicity although most trials have focused on changes in resting systolic function, and/or development of cardiac symptoms. Commonly used tools to assess cardiac function are insensitive to minor injury hence subtle changes may go unnoticed for many years. Cardiotoxicity definitions should include a dynamic functional assessment of the CV system. This may allow detection of latent CV abnormalities before the precipitous decline of resting myocardial function or the development of CV symptomology that may impact quality of life.

Cardiopulmonary exercise testing (CPET) including measurement of peak oxygen consumption (VO_2) is the gold standard for the assessment of cardiorespiratory fitness (CRF). Cardiorespiratory fitness is a strong, independent predictor of mortality, CVD-related mortality, HF-related morbidity and mortality, CA-related mortality and may be involved in the pathophysiologic link between anti-CA related treatments and the increased risk of late CVD events. Emerging evidence indicates CRF may be reduced in CA survivors and have utility to detect subclinical cardiotoxicity, but this has not been evaluated in CA survivors treated with RT with significant heart involvement. This dissertation consists of one literature review and one comprehensive paper that will examine the ability of CPET to detect subclinical cardiotoxicity.

Chapter 1

Introduction

Cancer (CA) is the second leading cause of death in the United States (U.S.) with an estimated 1,688,780 new diagnoses expected in the U.S. this year which is the equivalent of 4,600 new cases each day.(1) Breast CA is the most common CA type in women involving 252,710 new cases representing 30 percent (%) of all CA in women.(2) This translates to an annual incidence of new cases of breast CA of 124.9 per 100,000 women per year.(3)

Despite breast CA being the most common type, the most common cause of CA-related deaths are cancers of the lung/bronchus representing 25% and 27% of all estimated deaths for women and men, respectively.(3) However, over the past 30 years, the 5-year survival rate for all CA combined has increased by more than 20% at similar rates between both sexes. In fact, the 5-year survival rate of women with breast CA is now approaching 90%.(2) Currently, there are more than 3.1 million breast CA survivors in the U.S.(4) The improved survival rate is due to early diagnosis and advances in treatment of involving a multimodality treatment approach that involves surgery, systemic therapy (chemotherapy, targeted-therapy, or endocrine therapy [ET]), and radiotherapy [RT]. The multimodality treatment of CA although shown to improve CA-specific recurrence and mortality is offset with an increased risk of non-CA related

morbidity/mortality primarily due to increased cardiovascular disease-related (CVD) events.(5)

Cardiotoxicity, a general term used to describe "toxicity that affects the heart", is one of the most important adverse reactions of systemic therapy and RT and leads to a meaningful risk of CVD-related morbidity and mortality.(6, 7) Cytotoxic agents, targeted therapies, and incidental exposure of the heart to irradiation can all negatively affect the CV system and increase CVD risk.(8, 9) This CVD risk is further pronounced in the setting of combination therapy whereby systemic agents are used in combination or coupled with RT.(10) The reason is that many of these agents reach targets in the microenvironment that do not affect only the cancerous tumor. The improving survival of patients, particularly breast-CA patients (the largest cohort of CA survivors), justifies the use of this multimodality treatment approach, but strategies must be introduced to detect, offset and monitor this CVD risk.

The purpose of this review is to describe current anti-CA treatments, identify those with known cardiotoxic side effects, discuss the proposed mechanisms linking anti-CA treatments with cardiotoxicity, and review the current detection methods used to identify cardiotoxicity in the CA patient. This review will primarily focus on the breast CA patient due to their over-representation for both CA diagnosis and survivorship. Furthermore, the role of anti-CA treatments on cardiorespiratory fitness (CRF) and its determinants is discussed highlighting its potential link to cardiotoxicity. Finally, the measurement of CRF variables using cardiopulmonary exercise testing (CPET) is reviewed with an emphasis on the potential ability to detect cardiotoxicity in the CA patient.

I. Anti-Cancer Treatments

The American Joint Committee on Cancer (AJCC) has developed a system based on clinical and pathologic features to classify patients with CA, define prognosis, and determine the best treatment approaches.(11) This staging system characterizes patients based on primary tumor (T) size, lymph node (N) involvement, and observance of metastasis (M) to classify patients based on the extent of disease and the impact of treatments. Tumor size (T) is graded on a 0–4 scale with higher numbers indicating larger size and/or invasion into adjacent structures. Node (N) involvement is graded on a 0-3 scale based upon CA spread to lymph nodes and the number of nodes involved. Metastasis is classified as 0 (no) or 1 (yes) if the CA has spread to other organs. Cancers are also staged (0–IV) with higher stages indicating larger tumors or the extent of spread according to pathological characteristics based upon tumor size and spread to lymph nodes or other organs. These systems allow application of evidence-based treatments based on CA subtype and can be used to gauge treatment success with the goal of a complete response. Complete response is defined as the absence of invasive carcinoma in the breast and axillary nodes.(11) Specific to breast CA, treatment is also based on the following clinical and pathological features: menopausal status and patient age, stage of disease, grade of the primary tumor, hormone status (estrogen receptor (ER+/-) and progesterone receptor (PR+/-) expression), human epidermal growth factor type 2 receptor (HER2 +/-) expression, and histologic type.(2)

Surgery is considered standard treatment for early, localized, or operable breast CA and may include breast-conserving surgery (BCS) referred to as lumpectomy or

modified radical mastectomy involving removal of the entire breast. In patients with stages I, II, and T3N1 disease the initial management is surgical resection.(12) Breast-conserving surgery coupled with adjuvant RT provides comparable outcomes in terms of disease-specific survival as compared with mastectomy and confers improved quality of life thus it is considered the standard of care for early stage breast CA.(13, 14) Breast conservation therapy (lumpectomy and RT) provides survival equivalent to mastectomy, preserves cosmetic appearance, while providing a similar low risk of CA recurrence in the treated breast.(15)

More than 50% of breast CA patients receive RT as part of their treatment.(16) Radiotherapy can be used alone with curative intent or, more often, is coupled with surgery and systemic therapy based on tumor characteristics. When used after BCS, RT reduces the risk of local recurrence (LR) by as much as 70%. A recent meta-analysis of the Early Breast Cancer Trialists Collaborative Group (EBCTCG) in 2011, including 10,801 women with a median follow-up period of 9.5 years concluded RT proportionally reduced the rate of LR or distant metastases over the first 10-years by about half (relative risk = 0.52) and proportionally reduced the rate of breast CA-related death by approximately one-sixth.(17)

Chemotherapy are systemic agents given as neo-adjuvant therapy (prior to primary scheduled therapy; i.e. surgery) or as adjuvant therapy (after primary therapy) consisting of multiple cycles of polychemotherapy to reduce the risk of breast CA recurrence and provide an additional disease-specific survival benefit.(18) Chemotherapy used neo-adjuvantly or adjuvantly is used in the treatment of approximately 38% of all breast CA survivors although is used in the majority of other

CA types.(3) The most common drugs used for breast CA chemo include anthracyclines, taxanes, flouropyrimidines (5-fluorouracil), cyclophosphamide, and carboplatin.(19) However, taxane-based and anthracycline-based regimes have shown to be superior to cyclophosphamide, 5-fluorouracil, and nonanthracycline-based regimens.(18) For breast CA patients who warrant chemo, multiple cycles of adjuvant chemo including taxanes and anthracyclines are considered “gold-standard” and as such are part of the standard regimen for most patients with node-positive and high-risk node-negative tumors.(20) This benefit of chemo has also been realized in women with hormone receptor +/- status regardless of age or menopausal status.(21) However, not all patients need chemo as the differences in the absolute risk of recurrence is small in patients with small CA or ER+ CA that also receive adjuvant ET.(22)

Hormone receptor status appears to be an important predictor of derived-benefit from chemo. The ER is present in about 70% of invasive breast CA and 80% of ductal carcinoma in situ (DCIS) tumors.(23) Targeted ET with the use of ER modulators or aromatase inhibitors (AI) in post-menopausal women may reduce LR following BCS and prevent development of new primary breast CA in the contralateral breast.(24) Tumors that are ER- benefit substantially from chemo added to ET whereas ER+ tumors do not glean as much benefit from the addition of chemo on top of ET.(25)

The human epidermal growth factor type-2 receptor (HER2) (found in 20% of invasive breast CA) historically has been linked to a higher risk of recurrence, relative resistance to ET due to lower levels of ER expression, and resistance to cyclophosphamide/methotrexate/5-fluorouracil (CMF)-based chemotherapies.(26) However, in 2005 the reports of five randomized trials examining the utility of a targeted

therapy using a humanized monoclonal antibody (Trastuzumab) against the HER2 protein for HER2 overexpressing breast CA demonstrated significant improvements in disease-free survival (DFS)(50% average risk reduction) and overall survival (OS).(27–30) This led to the standardization of Trastuzumab as a treatment for HER2+ breast CA.

Chemotherapy

Anthracyclines

Anthracyclines (ACT) are anti-CA compounds derived from Streptomyces bacteria that are delivered intravenously (IV) and enter cells through passive diffusion. They bind to proteasomes in the cytoplasm and are then translocated into the cell nucleus. Proteasomes are predominantly located in the nucleus of neoplastic and normal proliferative cells. Once ACT enter the nucleus they disassociate from the proteasome and bind to deoxyribonucleic acid (DNA).(31) In addition, by binding to proteasomes, ACT inhibit protease activity leading to inhibition of protein degradation, accumulation of misfolded proteins, and thus induction of apoptosis. The mechanism of action appears to be multifactorial including cell DNA intercalation, interaction with DNA binding proteins, induction of apoptosis, formation of reactive oxygen species (ROS), and anti-angiogenic mechanisms.(32–35) The major drugs used in this class of agents for breast CA include Doxorubicin (brand names: Adriamycin, Doxil), Epirubicin (brand name: Ellence), Daunorubicin (brand names: Cerubidine, DaunoXome), and Mitoxantrone (brand name: Novantrone).(36)

Taxanes

Taxanes are a class of diterpenes first extracted from the bark of Pacific yew trees. Taxanes inhibit cell proliferation by blocking mitotic activity through their actions on microtubules leading to polymerization, mitotic metaphase inhibition, and spindle microtubule rearrangement.(37) Paclitaxel (brand name: Taxol), Docetaxel (brand name: Taxotere) are the major taxanes used for the treatment of breast CA.(38) The Cancer and Leukemia Group B 9344 report was the first to demonstrate the addition of sequential Paclitaxel therapy improved DFS and OS in comparison to cyclophosphamide-doxorubicin (AC) chemotherapy.(39) To date, the optimal dosing regimen appears to be treatment of 4-cycles every 2-weeks in sequential order following ACT/alkylating agent therapy for reducing breast CA recurrence.(40)

5-Fluorouracil

The fluoropyrimidine, Fluorouracil or 5-fluorouracil (5-FU) works as an antimetabolite to prevent cell proliferation. It primarily inhibits the enzyme thymidylate synthase (TS) blocking the thymidine formation required for DNA synthesis.(41) Fluorouracil (brand name: Adrucil) is a pyrimidine analog that interferes with DNA and RNA synthesis by mimicking the building blocks necessary for synthesis. It can be used as a single agent but is most commonly administered via IV in combination with other chemotherapy regimens.

Cyclophosphamide

Cyclophosphamide is a synthetic alkylating agent chemically related to the nitrogen mustards with antineoplastic and immunosuppressive activities. It is the most widely used alkylating agent and has antineoplastic activity in a variety of tumors.(42) In the liver, cyclophosphamide requires activation by cytochrome P-450 and is then

converted to the active metabolites aldophosphamide and phosphoramide mustard, which bind to DNA, thereby inhibiting DNA replication and initiating cell death. Cyclophosphamide is routinely used in combination with other systemic agents and usually administered via IV in divided doses relative to bodyweight.

Carboplatin

Cis-diamminecyclobutanedicarboxylate platinum or Carboplatin contains a platinum atom complexed with two ammonia groups and a cyclobutane-dicarboxyl residue. It is activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups such as guanine-cytosine-rich sites in DNA, thereby inducing intra-strand and inter-strand DNA cross-links, as well as DNA-protein cross-links. These carboplatin-induced DNA and protein effects result in apoptosis and cell growth inhibition.(43) Carboplatin (brand name: Paraplatin) is usually administered as a rapid IV infusion over 30-minutes. In HER2+ breast CA, platinum-based agents exhibit a synergistic cytotoxic effect when coupled with anti-HER2 monoclonal antibodies.(44)

Targeted Therapy

Targeted therapy is the use of agents that target specific changes in CA cell types whereas chemotherapy agents exert their neoplastic effects irrespective of cell type. Overexpression of the HER2 receptor protein is present in about one out of five women with breast CA and is associated with an aggressive subtype that leads to a poor prognosis. Targeting HER2 expression inhibits epidermal growth factors/ HER2 ligand receptor activity and disrupts the phosphorylation of intracellular tyrosine kinases

that regulate cell growth and survival. Trastuzumab (brand name: Herceptin) was the first HER2-targeted therapy approved by the Food and Drug Administration (FDA).(45) The adjuvant use of Trastuzumab is only known to be effective in tumors with aberrant expression of HER2 proteins.(46) Initially approved for use in advanced metastatic breast CA, subsequent studies have demonstrated a reduced risk in CA recurrence (9.5% decrease) and improved OS (3% improvement) in early-stage breast CA following surgery.(47) However, all the trials to date showing benefit have utilized Trastuzumab in combination with varying chemotherapy regimens. Optimal treatment appears to be for twelve months and can be delivered concurrently or sequentially following chemotherapy.(20, 28)

Pertuzumab, another humanized monoclonal antibody that targets different extracellular regions of the HER2 tyrosine kinase receptor and blocks HER2 dimerization is FDA approved in combination with Trastuzumab and Docetaxel for the treatment of HER2+ metastatic breast CA and for neo-adjuvant use prior to surgery in HER2+ breast CA. (48)

Endocrine Therapy

Endocrine therapy works in breast CA by inhibiting the effects of estrogen and progesterone on CA cell growth. They work by inhibiting the body's ability to produce hormones or by interfering with the hormones effects on breast CA cells.(49)

Estrogen Receptor Modulators

Tamoxifen, an estrogen receptor modulator, works by blocking estrogen stimulation of breast CA cells, inhibiting translocation and nuclear binding of the ER. This binding inhibits transcriptional activation of estrogen-responsive genes. Tamoxifen is the only FDA-approved hormonal agent for the prevention of premenopausal breast CA, treatment of DCIS, and the treatment of post-surgical ER+ breast CA.(50, 51) The EBCTCG overview on the use of adjuvant Tamoxifen demonstrated administration for five-years reduced the annual rate of breast CA recurrence by 41% with a 34% reduction in the annual death rate for women with ER+ breast CA.(51)

Aromatase Inhibitors

Following menopause, the synthesis of ovarian hormones ceases, but estrogen production continues by conversion of androgens by aromatase. Aromatase is the enzyme complex involved in the final step of estrogen synthesis by the conversion of androgens. Aromatase inhibitors (AI) block the actions of aromatase resulting in estrogen depletion and are used for the treatment of estrogen-responsive breast CA in postmenopausal women. The American Society of Clinical Oncology (ASCO) guidelines on adjuvant ET recommend AI treatment in postmenopausal women as either initial therapy or as adjunctive sequential therapy following Tamoxifen.(52) The addition of AI in the treatment of postmenopausal ER+ breast CA women results in a modest improvement in DFS.(53)

Radiation therapy (radiotherapy)

Radiation therapy is administered to cells either in the form of photons (x-rays and gamma rays) or particles (protons, neutrons, and electrons). When photons or particles interact with biological materials, ionization takes place. Ionization is the process by which an atom or a molecule acquires a negative or positive charge by gaining or losing electrons to form ions. Ionizations can directly interact with either subcellular structures or water within the cell generating free-radicals. The direct effect of radiation is the absorbance of its energy by DNA in chromosomes leading to ionizations that induce damage including base damage, single-strand breaks, and double-stranded breaks. Free-radicals generated by radiation interact with other molecules that possess an unpaired electron and molecules without unpaired electrons in their outer-shell and can remove a hydrogen molecule from the DNA to cause damage. Radiation can induce DNA damage at three primary points during the cell cycle.(12)

Briefly, the cycle of eukaryotic cells can be separated into four discrete phases: the mitotic (M) phase of the cycle corresponds to mitosis, which is usually followed by cytokinesis. This phase is followed by the gap 1 (G_1) phase, which corresponds to the interval gap between mitosis and initiation of DNA replication. During G_1, the cell is metabolically active and continuously grows but does not replicate its DNA. The G_1 phase is followed by the synthesis (S) phase, during which DNA replication takes place. The completion of DNA synthesis is followed by the gap 2 (G_2) phase, during which cell growth continues and proteins are synthesized in preparation for mitosis. The

cell cycle must progress in a specific order and has checkpoint genes that do not allow progression to the next event until earlier events are complete.(54)

Radiation-induced DNA damage can occur at the border between G_1/ S phase, intra-S phase, and the border between the G_2 phase and mitosis. Cells with intact checkpoints that have sustained DNA damage become arrested at the next checkpoint in the cell cycle. The G_1/S phase and intra-S phase checkpoints inhibit the replication of damaged DNA. The G_2 phase checkpoint inhibits cells from entering mitosis with damaged DNA that is transmitted to its progeny.(49)

In addition to its direct effects on DNA, radiation also affects cellular membranes. Ionizing radiation activates membrane receptor pathways such as epidermal growth factor (EGFR) and transforming growth factor beta (TGF-β) that promote DNA damage repair and/or cell proliferation.(55)

The goal of RT is to deliver enough ionizing radiation to the tumor site which can result in absorbed dose. Most patients treated with RT, receive high-energy, external beam photon therapy. The split-dose repair studies of Elkind et al. have formed the basis of fractionated radiotherapy wherein a dose is delivered in fractions.(56) When RT is delivered in fractions as opposed to a single dose it prolongs cell survival or tumor growth delay. The phase of the cell cycle at the time of RT influences the cell's inherent sensitivity to RT. Cells synchronized in late G1/ early S and G2/M phases are most sensitive, whereas cells in the G1 and mid to late S phases are most resistant to RT. If cells are given a short time interval between doses, they move from a resistant portion of the cell cycle to a more sensitive phase enhancing the tumor response to fractionated RT while this response is somewhat protracted in normal tissue. This concept of re-

assortment is also utilized with systemic agents thus making cells more sensitive to treatment when used in combination with fractionated RT.

External beam photon treatments require high energy (usually 6 to 20 megavolts) beams with sufficient fluence to penetrate tissue and reach the tumor. To preserve normal tissue and maximize tumor dose received, beams are arranged to enter the patient from multiple directions and to intersect at the center of the tumor. Computerized treatment planning systems using x-ray or computed tomography (CT) to develop patient-specific anatomic models with beam-specific dose deposition properties to select beam angles, shapes, and intensities to meet prescribed treatments. Beyond the ability to define the primary target volume for the tumor these treatment planning systems allow characterization of the dose administered to normal tissues.(49)

Radiation doses are calculated to maximize tumor control without producing unacceptable toxicity. The dose of RT required depends on the tumor type, volume of tumor cells, and the use of RT-modifying agents such as chemo. Dose is quantified in Gray (Gy) units defined as the absorption of one joule of radiation energy per kilogram of matter. The effectiveness of a dose of radiation depends on the fraction given with each treatment as well as the time required to complete the course of RT. Standard fractionation for RT is defined as 1.8 to 2.25 Gy per fraction per day with a total dose of whole breast RT of 45 to 54 Gy in the adjuvant setting. A boost or supplementary irradiation whereby a 10 to 16 Gy boost to the tumor bed region is also commonly used and provides an additional reduced risk of recurrence in the ipsilateral breast.(57)

II. Cardiotoxicity of Anti-Cancer Therapies

There are many recognized adverse cardiovascular (CV) effects of anti-CA therapies including heart failure (HF), myocardial ischemia/infarction (MI), hypertension (HTN), thromboembolism (VTE), and arrhythmias.(58) Cardiotoxicity related to anti-CA treatment is important to recognize as it may have a significant impact on the overall prognosis and survival of CA patients. Furthermore, it is likely to remain a significant challenge due to the aging of the population of patients with CA and the introduction of new CA therapies. The risk of cardiotoxicity needs to be balanced with the benefit of evidenced-based therapies to eradicate the CA. Early cardiotoxicity can affect a patient's ability to complete CA treatments while late toxicity may impact CVD mortality in the CA survivor.

The National Cancer Institute proposes the use of the Common Terminology Criteria for Adverse Events to define left-ventricular (LV) dysfunction and HF based on severity into grades 1 to 5.(59) Grade 1 is defined as asymptomatic with elevations in cardiac biomarkers or cardiovascular imaging abnormalities, Grades 2-3 include HF symptoms at mild and moderate exertion. Grade 4 includes severe HF symptoms requiring hemodynamic support and finally, Grade 5 indicates death. The FDA defines ACT-induced cardiotoxicity as >20% decrease in left-ventricular ejection fraction (LVEF) when baseline LVEF is normal and >10% when baseline LVEF is not normal.(60)

There is not a unified consensus on the definition of CA-related cardiotoxicity although most trials have focused on changes in resting systolic function, namely LVEF and/or development of HF symptoms.(61, 62) However, systemic therapies and RT are

known to affect the entire CV system not just resting LVEF. There is a need to expand the definition of cardiotoxicity to include direct effects on cardiac structure, diastolic function, conduction abnormalities, vascular function, hemodynamics, coagulability, and the reserve capacity of the CV system to stress. Cardiotoxicity definitions should include a dynamic functional assessment of the CV system in addition to measures of resting myocardial function. This may allow detection of latent CV abnormalities before the precipitous decline of resting myocardial function or the development of CV symptomology that may impact quality of life.(63, 64)

Cardiotoxicity risk is potentiated by pre-existing CVD risk factors and combinations of systemic agents with or without RT.(65) Advanced age, smoking, sedentarism, obesity, diabetes mellitus, dyslipidemia, HTN, and prior history of CVD are all associated with heightened risk. The “multiple-hit” hypothesis proposed by Jones and colleagues infers that at the time of diagnosis breast CA patients already have an increased risk of developing CVD which is further heightened by the anti-CA treatment.(66) Cardiotoxicity risk factors associated with CA-related treatments include: mediastinal RT, systemic cytotoxic agents, ET, and targeted therapies.(65) The next section of this review will discuss the cardiotoxicity of anti-CA therapies employed in the treatment of the CA patient.

Cardiotoxicity due to Chemotherapy

Anthracyclines

Anthracyclines have long been known to cause LV dysfunction and HF with an incidence in the range of 5-23% of patients.(67) The risk of cardiotoxicity is proportional to the cumulative ACT exposure(68), however, the CA response rate is proportional to the increased ACT dose, thus creating a conundrum.(69) Cardiotoxicity from ACT is heightened when the cumulative dose surpasses 300 milligrams per meter squared (mg/m^2) of body surface area.(70) There is a 5% risk of developing HF with cumulative doses of $400mg/m^2$ and increases to >25% at doses of $700\ mg/m^2$. In the U.S., the combination of polychemotherapy (doxorubicin at $60mg/m^2$ and cyclophosphamide at $600\ mg/m^2$) in four cycles (total doxorubicin dose at $240\ mg/m^2$) is commonly employed as treatment for early-stage breast-CA. The risk of symptomatic HF is relatively rare at this cumulative dose of $240\ mg/m^2$, but asymptomatic CV dysfunction is frequently observed, and the incidence of late occurring LV systolic dysfunction is not completely known.(71)

The mechanisms of ACT cardiotoxicity are not completely understood although the leading hypothesis is that ACTs increase ROS emission within the mitochondria of cardiac myocytes.(72) In this oxidative stress model of cardiotoxicity, ROS causes protein/ nucleic acid/ lipid oxidation and leads to cell death/ dysfunction.

Topoisomerase inhibition by ACTs also appears to be important in the development of cardiotoxicity. Topoisomerases are essential enzymes required for DNA transcription, replication, or recombination and are expressed in two isoenzymes (Top2α and Top2β) in humans. The Top2α enzyme demonstrates high levels of

expression in rapidly proliferating cells. The Top2β enzyme is predominantly expressed in quiescent cells such as myocytes.(73) Inhibition of topoisomerases may be a beneficial effect of ACTs in high-expression Top2α cells, but may lead to cardiotoxicity in predominant Top2β cells. Mice with deletion of cardiomyocyte Top2β genes are protected from doxorubicin-induced cardiotoxity.(74)

Cardiac progenitor cell loss and dysfunction may also be a mechanism of ACT cardiotoxicity.(75) In an animal model of pediatric mice, exposed to doxorubicin at levels below acute cardiotoxicity ranges, impaired vascular development with decreased coronary branching and reduced capillary density upon examination during adulthood was demonstrated.(76) The adult doxorubicin mice when subjected to myocardial ischemia developed worse ischemic cardiomyopathy and HF and a reduced ability to increase capillary density in the infarct border zone. Furthermore, the adult doxorubicin mice demonstrated increased sensitivity to physical stress from high-volume swimming with increased cardiac hypertrophy and LV dilatation.(76)

Anthracycline-induced cardiotoxicity can be grouped into 3 categories by its temporal relationship: acute, early, late. Acute cardiotoxicity occurs during infusion or within one-week of therapy. The acute cardiotoxicity incidence is low (<1%), can include pericarditis and arrhythmias, and usually resolves with discontinuation of therapy. Early cardiotoxicity occurs within 3-12 months of treatment with a peak onset of symptoms of HF at three-months following completion of therapy. Late cardiotoxicity occurs one to several years following treatment where patients may be asymptomatic initially and then develop HF symptoms sometimes even decades after the ACT treatment.(77)

Taxanes

Arrhythmias are the most common cardiac abnormality observed with the use of taxanes.(78) Asymptomatic bradycardia is observed in up to 30% of patients taking Paclitaxel with only 0.1% suffering from serious bradycardias.(78) Taxanes also interfere with the metabolism and excretion of ACTs and increase the risk ACT cardiotoxicity particularly at higher cumulative ACT doses.(79) Taxane treatment with Epirubicin may be less cardiotoxic compared with Doxorubicin.(80) Docetaxel is associated with an incidence of 2.3-8% for the development of LV dysfunction.(81)

Fluoropyrimidines

Fluorouracil is associated with an incidence of cardiotoxicity ranging from 1%-7.6%.(82) The most common manifestations appear to be ischemic in nature including angina and electrocardiogram (ECG) changes that appear more frequently in those with underlying CVD.(83) A systematic review of the pathophysiology of 5-FU cardiotoxicity demonstrated evidence of: interstitial fibrosis, inflammation in the myocardium, hemorrhagic infarction, endothelial damage, increased myocardial energy metabolism, depletion of high-energy phosphates, increased superoxide anion levels, reduced antioxidant capacity, arterial vasoconstriction, alterations in red blood cell (RBC) structure, and increased platelet aggregation/ fibrin formation.(84) Cardiotoxicity usually occurs early during treatment and is more common at higher doses and with continuous infusions.(85)

Cyclophosphamide

Cyclophosphamide therapy is associated with pericardial effusions, pericarditis, and HF which occurs in 7-28% of patients.(60) The risk appears to be dose related (>150 milligram per kilogram [mg/kg]) and usually occurs within 1 to 10 days of the first dose.(60) Like other systemic agents, additional risk factors include combination with ACT and/or RT.(86)

Carboplatin

Vascular toxicity is one of the most important late consequences of platinum-based chemotherapy.(87) Cisplatin, another platinum analog is associated with an accelerated risk of CVD in men with testicular CA.(88) Mechanistically, Cisplatin is associated with mitochondrial membrane depolarization, ultrastructural abnormalities of the mitochondria, activation of the endoplasmic reticulum stress response, increased Caspase-3 activity, and increased apoptosis.(89) Carboplatin, is preferred over Cisplatin in breast CA due to its lower toxicity profile.(90) To date, platinum-based non-ACT regimens in clinical trials have not demonstrated a significant signal for the development of LV dysfunction.(91)

Targeted Therapies – Trastuzumab

The HER2/neu oncogene encodes a transmembrane tyrosine kinase receptor and shares a very similar structure to the epidermal growth factor receptor. The HER2 gene is involved in embryonic heart development and in the adult, is involved in cardio-protection.(92) The HER2 signaling is involved in growth, survival, and inhibition of

apoptosis in cardiac myocytes. In situations of biomechanical stress, a ligand growth factor named neureregulin binds to HER2 to activate the phosphatidylinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cell survival pathways.(93) Trastuzumab, a humanized monoclonal antibody for the HER2 receptor disrupts signaling between neureregulin and the HER2 receptor. This may trigger a decline in myocardial function because of its effects on cardiomyocyte neureregulin-HER2 receptor function. It is believed that exposure to Trastuzumab results in a loss of contractility due to cellular stunning rather than cardiomyocyte death.(94)

Breast CA patients taking HER2-antagonists also experience increased levels of norepinephrine with concomitant increases in blood pressure (BP) and heart rate (HR).(95) Additionally, pre-clinical studies demonstrate beta (β)-adrenergic receptor activity is linked to HER2 expression.(96) Furthermore, trastuzumab triggers mitochondria oxidative stress and induces the expression and activation of pro-apoptotic proteins. This causes mitochondrial damage, opening of the mitochondrial permeability transition pore, and induction of cell death pathways.(97) The incidence of symptomatic HF with Trastuzumab monotherapy is approximately 4% although can be as high as 27% with the concurrent use of ACTs.(45) The reduction in LV dysfunction is considered reversible with the cessation of therapy.(47)

Endocrine Therapy

Tamoxifen use is associated with an overall beneficial effect on lipid profiles, however long-term clinical trials data have failed to show this translates into a CV benefit.(98) In fact, the risk of VTE events, and stroke although rare has been shown to

be increased with the use of tamoxifen over placebo or AI.(99) Anastrozole, an AI with long-term safety data available has been associated with fewer thromboembolic and/or cerebrovascular events compared with Tamoxifen, but no significant difference in CV events.(100)

Radiotherapy

Radiation-induced heart disease (RIHD) is a heterogeneous clinical syndrome characterized by symptoms related to an impaired cardiac function (diastole and/or systole) related to radiation-injury to one or more cardiac structures (myocardium, pericardium, valves, coronary arteries). RIHD may present acutely during treatment in the form of acute radiation myocarditis, but more commonly develops over the long-term leading to leading to a restrictive cardiomyopathy.(101) Radiotherapy is associated with macrovascular, microvascular, endothelial dysfunction, valvular dysfunction, atherosclerosis, myocardial fibrosis, and pericardial disease.(102)

The damage from RT causes cellular vasodilation, platelet aggregation, increased vascular permeability, and secretion of adhesion molecules and growth factors from injured endothelium prompting activation of the acute inflammatory response. Inflammatory cells secrete pro-fibrotic cytokines which convert fibroblasts to myofibroblasts that stimulate excessive extra-cellular matrix (ECM) formation, this accumulation of ECM leads to fibrosis.(103)

In a population-based case-control study of incident HF in female breast CA patients who underwent contemporary RT, HF with preserved ejection fraction (HFpEF) defined as a LVEF ≥ 50% with HF symptoms was the most predominant HF

phenotype.(104) The relative risk of HFpEF increased with increasing mean cardiac radiation dose (MCRD), and risk of HF was higher in those with prior history of CVD or atrial arrhythmias.

Late-onset RIHD occurs at a median of 10–15 years after exposure although the increased risk starts within the first 5 years and persists at least until the 3rd decade.(102, 105) The risk of RIHD is magnified by higher dose, delivery technique, younger age at the start of RT, longer duration since exposure, use of adjuvant chemotherapy, pre-existing CVD, and pre-existing CVD risk factors.(102) A large meta-analysis involving 289,109 women revealed those who underwent RT for left (L)-sided vs. right (R)-sided breast CA had a higher risk of CV death and this was more apparent with prolonged follow-up (≥15 years).(106)

Radiotherapy dose to the heart can vary considerably with mean doses of 1-2 Gy for R-breast disease, but as much as 10 Gy for treatment of the L-breast.(107) To account for this variability, a population-based case-control study of women with invasive breast CA who underwent external-beam RT was undertaken to determine the risk of CVD considering an individual patient's RT dose and the presence of CVD risk factors present during treatment.(105) In women exposed to a range of 0.03 to 27.72 Gy (MCRD = 4.9 Gy) using conventional or modern RT techniques the risk of major CV events increased linearly with MCRD to the heart. Cardiovascular disease risk increased 7.4% per Gy of mean heart dose with no discernible threshold dose below which CV risk did not exist.(105)

The heart dose of RT in the CA patient has decreased over time with the use of modern RT techniques.(108) This includes the application of 3-dimensional CT

treatment planning for accurate heart dose and volume calculation, cardiac shielding, reduced heart dose per fraction (<2.0 Gy/day), reducing total heart dose (<30 Gy), breath-holding techniques, and the use of intensity-modulated RT.(109)

III. Methods to Detect Cardiotoxicity in the Patient with Cancer

Methods to detect cardiotoxicity in the CA patient include multi-modality cardiac imaging to assess ventricular function, cardiac-specific biomarkers, and exercise testing while taking into account the patients' intrinsic CV risk factor profile.(8) Chemotherapy-related cardiac dysfunction (CTRCD) was first described in the late 1960's based upon the presence of HF symptoms following the introduction of ACT for the treatment of CA.(110) The usefulness of LVEF by non-invasive cardiac imaging to detect cardiotoxicity was first reported in 1981 and initiated an era of using symptomology and assessment of LV function to monitor cardiotoxicity in the CA patient.(111) The evaluation of LVEF has emerged as the most widely used strategy for detecting changes in cardiac function during CA treatments. Resting LVEF, however, only provides a snap-shot of cardiac function, is dependent upon preload and HR, and is not prognostic in patients with preserved LVEF (>50%).(112) Impairments can also occur in diastolic relaxation and filling following CA therapy despite a preserved LVEF.(113)

Radionuclide Imaging

A multi-gated acquisition (MUGA) scan provides a cinematographic (cine) image of the beating heart by using a radioactive tracer that emits gamma rays that is injected into the blood with a gamma camera to detect the radiation released by the heart. It has historically been used to calculate the LVEF, define clinical cardiotoxicity, and risk stratify patients undergoing chemotherapy.(114) Guidelines for the use of MUGA to detect an asymptomatic decline in LVEF were developed to guide ACT treatment using

an LVEF decrease of ≥10% to indicate cardiotoxicity.(114) The advantages of MUGA for the assessment of LVEF are its high reproducibility, low variability, few technical limitations, and its widespread use in clinical practice.(115) It outperforms two-dimensional echocardiography (2DE) with respect to accuracy and reproducibility of LVEF measurements.(116) The primary disadvantage of MUGA is incidental radiation exposure.(117) Furthermore, MUGA has significant variability in measurements of LV diastolic function, is non-informative on valvular or pericardial disease, and requires the use of supine bicycle exercise to measure LV functional reserve which is not readily available in standard clinical practice.(118)

Echocardiography

Echocardiography (echo) is the cornerstone of cardiac imaging due to its widespread availability, safety, ease of repeatability, and lack of radiation exposure. It uses high-frequency ultrasound waves from a transducer to create images of the heart. In addition to its ability to determine cardiac dimensions, it also allows a comprehensive assessment of systolic and diastolic function at rest and with exercise, cardiac valves, the aorta, and the pericardium in the patient.(119)

The most commonly used parameter for estimating LV function with echo is the LVEF. The recommendations for chamber quantification from the American Society of Echocardiography and European Association of Echocardiography have established an LVEF ≥ 55% as normal with a reference range of 53 – 73% using the modified biplane Simpson's technique with 2DE.(120, 121) Changes in loading conditions are frequent during chemotherapy and may affect the LVEF due to volume expansion with IV

administration or volume contraction due to vomiting or diarrhea. However, the incorporation of contrast, stress, three-dimensional, speckle-tracking, and tissue Doppler imaging echocardiography improve its clinical predictive value.

Although LVEF is a strong predictor of cardiac outcomes in the general population, 2DE often fails to detect small changes in LV systolic function.(122) A study by Thavendiranathan et al., concluded 2DE to be reliable in the detection of 10% differences in LVEF in CA patients undergoing chemo.(123) A drop in LVEF of 10% is highly significant and may be irreversible suggesting more sensitive parameters of LV function would be useful.(124) This lack of sensitivity has led to the increasing use of speckle-tracking echo and Doppler imaging to detect subtle changes in myocardial function.

Speckle tracking or strain-imaging utilizes the movement of the coherent ultrasound backscatter speckle pattern within echo images to assess myocardial strain throughout the cardiac cycle. The ventricular myocardium simultaneously shortens during systole in the longitudinal and circumferential planes and thickens in the radial plane, with reciprocal changes in diastole. Strain imaging allows for assessment of myocardial shortening and lengthening throughout the cardiac cycle by assessing regional myocardial strain and strain rate. Strain is defined as the change in length of a segment of myocardium relative to its resting length and is expressed as a %; strain rate is the rate of this deformation. Global longitudinal strain (GLS) is the preferred marker of myocardial deformation for the early detection of sub-clinical LV dysfunction.(125)

In 81 breast CA patients treated with chemo who were followed for 15-months with quarterly echo, the GLS after the completion of ACT predicted subsequent CTRCD.(126) Erven et al. demonstrated the ability of strain to detect deficits in breast CA patients undergoing RT wherein L-sided patients demonstrated strain and strain rate reductions after RT that was dose dependent with abnormalities in segments exposed to >3Gy.(127)

Diastolic dysfunction often precedes changes in systolic dysfunction in patients receiving anti-CA therapies.(128) An early reduction in the mitral annular early diastolic velocity (e') has been repeatedly observed in patients receiving ACT chemotherapy and appears to predict future decline in systolic function.(113)

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (CMR) is very accurate for the calculation of LV mass, volumes, and LVEF(129) and when coupled with late-gadolinium contrast-enhancement (LGE) is considered the gold-standard for the determination of myocardial tissue structure.(130) The use of CMR may identify a higher prevalence of myocardial injury/scarring in the CA patient and has higher intra- and inter-observer reproducibility compared with echocardiography.(131) It also allows characterization of myocardial edema, inflammation, and fibrosis thus permitting detection of early and late cardiotoxicity in the CA patient.(132) The benefit of CMR is that it is non-invasive, does not involve radiation, and demonstrates high-resolution through high contrast to noise ratios providing enhanced discrimination between endocardial and epicardial borders.(133) Disadvantages include lack of widespread availability, higher cost, and

contraindications involving ferromagnetic devices (i.e. pacemakers, defibrillators, breast tissue expanders) and claustrophobia.(125)

In CMR, the magnetic fields affect the hydrogen nuclei in the body, which act like miniature magnets. Gradients created by additional coils in the scanner cause a spatially related difference in how these hydrogen nuclei are affected. Generated radiofrequency (RF) pulses can then be used to manipulate the hydrogen nuclei in select planes of any predetermined location and size. Owing to their magnetic properties, the hydrogen nuclei that are affected by the RF pulse will give off an electromagnetic signal that can be detected, transformed, and displayed as a 2- or 3-dimensional image. Gating of images by HR is achieved by ECG leads placed on the patient. Cine imaging provides moving images of the heart and surrounding structures.

Cardiac-specific Biomarkers

Cardiotoxicity in the CA survivor defined by LVEF is not sensitive to detect late declines in heart function and the presence of a normal LVEF does not exclude the possibility of cardiac dysfunction.(48) Measurement of cardiac biomarkers can be a valid diagnostic tool for early diagnosis, assessment, and monitoring of cardiotoxicity. They can be easily repeated, are minimally invasive, allow early diagnosis, are relatively low cost, and do not predispose to incidental irradiation.(124) There may also be benefit from combining circulating biomarkers with the results of imaging modalities.(134)

Markers of Myocardial Injury – Cardiac-specific Troponins

Cardiac troponin (cTn) is the recommended biomarker for acute cardiac injury and elevations correlate with clinical severity, mortality, and cardiotoxicity from anti-CA therapies.(135) Cardiac troponin I (cTnI) and troponin T (cTnT) are the cardiac isoforms of regulatory proteins involved in muscle contraction thus when they are released into the circulation are highly specific for myocardial damage.(136) These two isoforms arise from the same circumstances (i.e. cardiac injury), but vary in concentration, and show differences in diagnostic accuracy.(137) Abnormally elevated levels of circulating cTn are found in HF patients without obvious myocardial ischemia or the absence of CAD suggesting ongoing cardiomyocyte injury or necrosis.(138)

Studied most extensively in ACT-induced cardiotoxicity, cTn identifies patients at risk of future cardiotoxicity, have high negative predictive value to identify those at low risk of toxicity, and strongly correlate with changes in LVEF.(139, 140) In L-sided breast CA patients undergoing RT who were chemotherapy-naive, cTnT increased in a significant proportion of patients (21%) after RT, and correlated with whole heart dose and LV chamber dose.(141) Recently, highly sensitive cTn assays have been developed that can measure to an order of magnitude lower than previously possible.(142) This allows detection of some cTn level in most individuals likely due to cardiomyocyte turnover and may allow detection of subclinical cardiac dysfunction.(142)

Markers of Left Ventricular Wall Stress - Natriuretic Peptides

Natriuretic peptides are secreted by the heart and produced in response to ventricular wall stress from pressure or volume overload.(143) The natriuretic peptides,

B-type natriuretic peptide (BNP) and its amino-terminal fragment precursor N-terminal pro-brain natriuretic peptide (NTproBNP) are recommended for the diagnosis and risk stratification of patients with HF although NTproBNP may be more useful to recognize early subclinical cardiac dysfunction.(144, 145) In the primary care setting involving patients without HF, NTproBNP levels can also discern between the presence of LV systolic dysfunction using a cut-off value of 125 picograms per milliliter (pg/mL).(146) It must be noted however that NTproBNP varies with age, gender, and renal function.(147)

In CA patients, natriuretic peptides generally correlate with increased risk of subsequent cardiotoxicity and elevated NTproBNP raises concern for elevated filling pressures, but professional organizations encourage further study on their utility before standard recommendations can be made.(125) However, it has been shown that NTproBNP levels are higher after RT for L-sided breast CA compared with non-RT matched controls and that NTproBNP correlates with heart volume and %volume of heart receiving higher doses.(148) It is noteworthy, in this same study cTnI levels did not significantly change following RT and remained below the cut-off threshold.

Emerging Novel Biomarkers

The American College of Cardiology Foundation/ American Heart Association guidelines for the management of HF give a Class IIb rating for the measurement of biomarkers of myocardial injury/stress or fibrosis and identify Galectin-3 for added risk stratification in the chronic HF patient.(145) Galectin-3, a β-galactoside–binding lectin member of the galectin family is also a marker of the inflammatory response in HF. Its

expression is increased in activated macrophages and is involved in pathological remodeling leading to fibroblast proliferation and collagen deposition.(149)

C-reactive protein (CRP) is an acute phase reactant synthesized by hepatocytes in response to pro-inflammatory cytokines and is part of the innate immune response.(150) In addition to being a non-specific marker of an inflammatory process, CRP plays a key role in the inflammatory process of atherosclerosis.(151) High-sensitivity C-reactive protein (hsCRP) allows detection of subclinical inflammation and is associated with worsening hemodynamics and outcomes in heart failure.(152) In the prediction of cardiotoxicity, work has shown correlations between hsCRP levels and later development of cardiomyopathy in patients treated with targeted therapies (i.e. Trastuzumab).(153)

Together, these novel biomarkers with HF risk status properties and mechanisms similar to established anti-CA cardiotoxic mechanisms would seem to be viable candidates to quantify the incidence of late cardiotoxicity, but to date their utility is equivocal.(134)

Stress testing

Stress testing can elicit CV and pulmonary abnormalities not present at rest and allows the quantification of functional reserve through the use of physical stress (i.e. exercise) or pharmacologic stress (i.e. sympathomimetic agents).(154) Exercise ECG testing has been used for over 60 years to provoke and identify myocardial ischemia, but over the last several decades been increasingly applied to assess CV risk.(155) When coupled with imaging modalities such as echo or perfusion studies it can provide

even greater diagnostic accuracy.(154) Exercise capacity and assessment of cardiac contractile reserve are independent predictors beyond coronary anatomy and LVEF.(156, 157) The potential role of exercise capacity to diagnose CA-related toxicity is discussed in section IV of this review.

In a study comparing the incidence and distribution of CAD after L-sided versus R-sided RT following BCS for early-stage breast CA (12-years post-RT), the L-sided group demonstrated a significantly higher prevalence of stress abnormalities (59% vs. 8%) using stress echo or perfusion studies.(158) Furthermore, the L-sided abnormalities were predominantly (70%) in the L-anterior descending artery region illuminating the importance of RT techniques.(158)

Stress echocardiography has shown usefulness in the detection and prognosis of stable CAD in patients with an intermediate or high pre-test probability for CAD who underwent chemotherapy regimens associated with ischemia (i.e. 5-FU).(159) Kearney et al. demonstrated the utility of stress echo using strain to detect subclinical LV dysfunction in long-term (36 ±10 years) CA survivors following prior ACT exposure (11±8 years post-treatment).(160) Similarly, Khouri et al. showed the superiority of exercise 2DE to detect subclinical cardiotoxicity not apparent with resting 2DE in breast CA patients undergoing adjuvant therapy.(161)

IV. Cardiorespiratory Fitness in Breast Cancer Patients Who Have Undergone Anti-Cancer Treatments.

Cardiorespiratory fitness (CRF) is a strong and independent predictor of both all-cause and CVD-related mortality wherein high-levels confer protection.(162, 163) Furthermore, it is a predictor of breast CA-specific mortality as well as the risk of a breast CA diagnosis.(164, 165) Finally, it appears to be reduced in breast CA survivors.(166)

Cardiorespiratory fitness is defined as the ability of the circulatory, respiratory, vascular, and muscular systems to supply oxygen (O_2) during sustained physical activity. It is typically expressed as maximal oxygen uptake (VO_2 max) obtained during progressive maximal dynamic exercise. Maximal VO_2 implies the observance of a plateau in O_2 uptake values which is rarely observed in clinical practice thus the term peak VO_2 is often used as a surrogate.(167)

Cytotoxic anti-CA therapies are associated with fatigue, exercise intolerance, cardiomyopathies, and skeletal muscle myopathies that can occur with active treatment and persist in the post-treatment period.(168) This can be due to pain, emotional distress, anemia, weight gain, sedentarism, sleep disturbances, nutritional deficits, decreased functional status, medication side-effects, and comorbidities.(169)

An analysis by Peel et al. developed normative values for peak VO_2 in breast CA patients.(170) They identified 27 clinical trials involving a total of 1,856 females (mean age=52 years) directly measuring peak VO_2 in the pre- or post-adjuvant setting. Adjuvant therapy included chemotherapy in 78% (mostly ACT), RT in 56%, and ET in

33% of patients. The mean peak VO_2 prior to adjuvant therapy was 24.6 ml•kg^{-1}•min^{-1}, whereas the mean peak VO_2 post-adjuvant therapy was 22.2 ml•kg^{-1}•min^{-1}. This equates to a post-adjuvant reduction in VO_2 of -2.4 ml•kg^{-1}•min^{-1}or 10% lower. This was accompanied by a mean post-adjuvant BMI increase of 2.6 kg/m^2. Linear meta-regression analysis of BMI and age with peak VO_2 did not provide evidence of an association (P>0.05). Compared with reference values the pre-adjuvant VO_2 values were significantly lower (17%) than that of healthy, sedentary women (29.7 ml•kg^{-1}•min^{-1}) or 83% of predicted (%pred.) (P=0.007). In the post-adjuvant setting, pVO_2 was 25% lower (75%pred.) (P<0.001) compared to healthy, sedentary values. For comparison, the mean VO_2 of a typical 50-year-old breast CA patient (22.6 ml•kg^{-1}•min^{-1}) was similar to the VO_2 of a healthy 60-year-old sedentary woman (~22.7 ml•kg^{-1}•min^{-1}).

Jones et al. evaluated VO_2 across the entire breast CA treatment continuum and assessed its significance in metastatic disease.(171) A total of 248 women (mean age of 55±8 years) underwent CPET. Patients were divided into four cross-sectional treatment cohorts: 1) pre-adjuvant (n=20), 2) during adjuvant (n=46), 3) post-adjuvant (mean time=27 months, n=130), 4) during adjuvant-therapy with metastatic disease (n=52). In the post-adjuvant cohort, RT was part of treatment in 102 (78%) patients. The mean peak VO_2 was 17.8±4.3 ml•kg^{-1}•min^{-1} (73% pred.). As expected there was a significant difference between peak VO_2 values observed between the different cohorts with the metastatic disease cohort displaying the lowest peak VO_2 values (16.3±3.5 ml•kg^{-1}•min^{-1}). In the metastatic cohort, peak VO_2 in absolute values (L•min^{-1}) held prognostic utility when comparing those <1.09 L•min^{-1} wherein the adjusted hazard ratio for death was 0.32 (95%CI, 0.16-0.67; P=0.002) for a peak VO_2 >1.09 L•min^{-1}.

Germane to this review, individuals in the post-adjuvant setting had a mean VO_2 of 18.4±4.1 ml•kg-^{1}•min^{-1} (78% pred.). This was in the setting of a normal LVEF (62%), normal peak HR (96% pred. max HR), and normal hemoglobin levels (13.5 g/dL).

1) Central Factors associated with CRF

Maximal VO_2 is considered the metric that defines the limits of the cardiopulmonary system. It is defined by the Fick equation as the product of cardiac output (CO) and arteriovenous oxygen content difference (a-vO_2 diff). Cardiac output (HR x stroke volume (SV)) is regarded as the primary determinant of CRF(172) thus any sequelae of anti-CA treatment that impacts chronicity, contractility, preload, or afterload could adversely affect CO and ultimately CRF.

When considering that the primary analysis of cardiotoxicity in patients receiving anti-CA therapies revolves around the measurement of LV systolic function to diagnose HF, it is important to recognize more than half of HF patients have HFpEF.(173) Breast CA survivors also share a number of HFpEF risk factors (female, older age, HTN, obesity, sedentary lifestyle).(174)

Khouri et al. assessed CRF, as well as CO (using 2DE) at rest and immediate post-exercise in 57 women with early-stage breast CA (age=51 years; time post-chemotherapy=26 months; LVEF=55%) and sex-matched healthy controls. Peak VO_2 was 20% lower in the breast CA cohort with no significant difference between groups for maximal HR. Post-exercise SV and cardiac index were significantly lower although post-exercise left-ventricular end-diastolic volume (LVEDV), LVEF, and LVEF reserve were

not significantly different between groups. Cardiac index reserve was significantly related to peak VO_2.(161)

Koelwyn et al. evaluated LV volumes and ventricular-arterial coupling and VO_2 during cycle exercise, using 2DE, in 30 older BC patients (age= 61 years; time post-chemotherapy= 6.5±3.6 years, 77% underwent RT; LVEF=60%) and 30 age-matched controls. Peak VO_2, sub-maximal exercise LVEDV, SV, and effective arterial elastance were not different between groups. However, sub-maximal exercise LVEF was significantly lower secondary to decreased end-systolic elastance (an indirect measure of LV contractility).(175) These studies insinuate a CV limitation in CRF despite a normal LVEF.

2) Peripheral Factors associated with CRF

The benefit of CRF assessment is that it provides a global assessment of the components that transport O_2 from the atmosphere into the mitochondria termed the O_2 cascade. Any therapy that affects components of the Fick equation (CO or a-vO_2 diff) will reduce CRF. Although CO is considered the primary determinant of CRF, anti-CA therapies are known to cause vasculature injury, pulmonary dysfunction, anemia, and skeletal muscle dysfunction.(66)

Beckman et al. evaluated the effect of RT on endothelium-dependent vasodilation in 16 breast CA patients (>3 years post-RT) compared with healthy controls. Using vascular ultrasonography and flow-mediated (FMD) endothelium-dependent (FMED) and endothelium-independent vasodilation (FMEI) techniques, FMED vasodilation was significantly impaired in the irradiated axillary arteries compared

with the contralateral, non-irradiated arteries and also compared with healthy control arteries. Conversely, the FMEI vasodilation was greater in the irradiated arteries compared with the contra-lateral arteries and controls.(176)

Conversely, Jones et al. examined brachial artery FMD in 26 HER2+ breast CA patients (age=48 years; time post-chemo=20 months; 65% underwent RT, LVEF=64%) and 10 healthy controls. The brachial artery FMD (FMED and FMEI) was not significantly different between groups (all P>0.1), and not related to peak VO_2 (P>0.5) although pVO2 was significantly inversely related to BNP (R=-0.53,P=0.006).(174) Koelwyn et al. extended these findings by demonstrating that brachial artery FMD, carotid-femoral and carotid-radial pulse wave velocity, and carotid compliance were not significantly different between breast CA and healthy controls.(175) This suggests large conduit artery endothelial function and arterial stiffness are not impaired in breast CA patients.

Reactive oxygen species, mitochondrial dysfunction, and inflammatory processes have been purported in the literature to be involved in chemotherapy-induced skeletal muscle dysfunction.(177) In an animal model, with adult mice injected with a single dose of cyclophosphamide, treadmill running time was decreased and mitochondrial function (maximal ATP production, phosphocreatine to ATP ratio) remained persistently below baseline following exposure at 6 weeks.(178)

Decreased CRF may also be the result of peripheral muscle weakness as peak VO_2 is related to leg strength in older BC patients.(179) A majority of the O_2 consumed during exercise occurs in the active skeletal muscle thus a decline in peak VO_2 in CA may be due to a reduction in the quantity or quality of skeletal muscle. Villasenor et al.

showed that sarcopenia is prevalent and an independent predictor of prognosis in older breast CA.(180) Finally, Toth et al. demonstrated that before or during CA treatment in 19 CA patients (6=breast), muscle fiber cross-sectional area for both slow-twitch myosin heavy chain (MHC) I and fast-twitch MHC IIA was reduced (~20%) and correlated with functional capacity.(181)

V. Cardiopulmonary Exercise Testing in Cancer Patients Who Have Undergone Anti-Cancer Treatments.

Cardiopulmonary exercise testing is most extensively used in the CA literature to determine eligibility for surgery and post-operative prognosis as it applies to lung CA surgery.(182) Specific to clinical oncology, in 2008 Jones et al. performed a systematic review of formal CPET for adults with CA.(183) Using the recommendations for CPET from the American Thoracic Society/American College of Chest Physicians they attempted to quantify the quality of CPET results in the literature performed on adult CA patients. Their results suggest the reporting of CPET methods and data do not comply with national and international quality standards and they provide recommendations to improve consistency of data reporting and methodology for exercise-oncology researchers and clinicians caring for the adult oncology patient.

Using relevant terms, they identified 90 citations that met inclusion criteria. These 90 studies included 5,179 adults and were dichotomized into two groups: 1) performed CPET solely for quantification of CRF and 2) CPET performed as part of an intervention study. By and large, most tests were performed on women and assessed patients with breast or lung CA either during or after treatment.

Peak VO_2 was the most commonly reported exercise variable. In regards to effort performance, 28/90 (31%) reported peak HR and only 11/90 (12%) reported the peak respiratory exchange ratio. This has major implications if one is trying to determine the robustness of peak exercise variables or assess the efficacy of an intervention.

In regards to assessment of the exercise response, 6/90 (7%) reported the ventilatory threshold, 3/90 (3%) reported the peak O_2 pulse, 11/90 (12%) reported symptoms for test termination, and only 14/90 (16%) reported some metric of ventilation. These variables provide both prognostic and pertinent information to detect the physiologic or possibly pathophysiologic limitations to exercise.(167) Furthermore, they may be sensitive to change resulting from a therapeutic intervention.(184)

Conclusions

In conclusion, CA-associated cardiotoxicity is an important concern in the growing population of survivors mostly consisting of breast CA patients. Multi-modality treatments are improving outcomes yet this may come at the expense of increased late CV risk. Current CV detection methods are based mostly on resting measures of LV systolic function. There is a need to expand this cardio-detection armamentarium to include measures of functional reserve such as CRF. The literature to date, although limited, indicates significantly reduced CRF following anti-CA therapies. The data is stronger for the adverse effects of anti-CA chemotherapeutic regimens, whereas the effects of RT with heart involvement, a recognized risk factor for CVD, on CRF has not been systematically examined. Incorporation of CPET into the assessment of patients who have received radiation to the chest may help understand the short- and long-term consequences and enhance detection of toxicity related to this form of anti-CA therapy. Enhanced detection will likely improve the quality of clinical care and provide insight to the mechanisms contributing to morbidity/mortality in the chest CA patient.

Chapter 2: Impaired Cardiorespiratory Fitness Following Thoracic Radiotherapy

ABSTRACT

Introduction: The risk of cardiotoxicity is one of the most detrimental adverse reactions of radiotherapy (RT) and leads to a significantly increased risk of cardiovascular disease (CVD) mortality and morbidity. RT induces a cardiomyopathy in a dose-dependent manner that leads to impairment in cardiac diastolic and systolic function. Clinical presentation of cardiotoxicity after RT is often delayed several years where the cardiac reserve is severely impaired and patients show signs of heart failure. In animal models the injury to the heart, however, starts immediately during RT where subtle structural and functional changes in the heart are evident early after RT. In the current study we sought to determine whether patients who had received RT to the chest demonstrated exercise intolerance, a marker of impaired cardiac reserve, due to impaired cardiac function.

Methods: We enrolled 30 patients 2.0 (0.6-3.8) years after completion of RT to the chest for the treatment of cancer (CA) with the radiation field involving at least 10% of heart volume receiving at least 5 Gray (Gy) of radiation. Patients underwent cardiopulmonary exercise testing, stress echocardiography, cardiac magnetic resonance imaging, and biomarkers assessment. Exercise intolerance was defined as a reduction of peak oxygen consumption (VO_2) <83% of predicted.

Results: The overall cohort was predominantly Caucasian (n=20 [67%]), mostly female (n=18 [60%]) with a median age of 63 (57-67) years. The peak VO_2 was 16.9 (14.4-20.8) $mL \cdot kg^{-1} \cdot min^{-1}$ or 62% (52-89%) of predicted, reflecting a peak VO_2 <90% predicted in >75% of cases. The mean cardiac radiation dose for the entire cohort was 5.6 (3.7-17.8) Gy and demonstrated a significant inverse association with peak VO_2 (R=-0.380, P=0.04). Multivariate regression revealed the diastolic functional reserve index (DFRI) measured as the velocity of the mitral annulus at tissue Doppler (e') at rest multiplied by the change in e' with exercise ($e'_{rest} \bullet \Delta e'_{exercise}$ - β=0.765, P<0.01) and N-terminal pro-brain natriuretic peptide (NTproBNP) serum levels (β =-0.389, P=0.04) were both independent predictors of peak VO_2.

Conclusions: Patients with CA who received radiation therapy to the chest involving the heart show a dose-dependent impairment in cardiorespiratory fitness (peak VO_2) associated with a reduced cardiac diastolic reserve (DFRI) and markers of myocardial strain due to elevated filling pressures (NTproBNP levels).

INTRODUCTION

Cancer (CA) is the second leading cause of death in the United States (U.S.) preceded only by cardiovascular disease (CVD).(1) Over the past 30 years, the 5-year survival rate for all cancers combined has increased by more than 20% at similar rates between both sexes.(185) This improved survival rate is due to early diagnosis and advances in treatment involving a multimodality treatment approach that involves surgery, systemic therapy, and radiotherapy [RT] with about half of all CA patients receiving some type of RT sometime during the course of their treatment.(4, 185)

Cardiotoxicity, a general term used to describe "toxicity that affects the heart", is one of the most important adverse reactions of RT and leads to a meaningful risk of CVD-related morbidity and mortality.(6, 7) The improvement in survival rate means there are a greater number of CA patients living with the potential adverse effects of these anti-CA therapies such as RT. Cardiotoxicity related to RT is important to recognize as it may have a significant impact on the overall prognosis and survival of CA patients where the CA-related benefits of RT may be offset by an increased risk of CVD events.

Radiotherapy-related cardiotoxicity is a heterogeneous clinical syndrome characterized by symptoms related to impaired cardiac function due to radiation-injury to one or more cardiac structures. Radiotherapy-related cardiotoxicity may present acutely during treatment in the form of acute radiation myocarditis, which is rare, and more commonly develops over the long-term leading to a restrictive-type of cardiomyopathy.(101)

In a population-based case-control study of incident heart failure (HF) in female breast CA patients (the most common CA subtype) who underwent contemporary RT,

HF with preserved ejection fraction (HFpEF) defined as a left-ventricular ejection fraction (LVEF) ≥ 50% with HF symptoms was the most predominant HF phenotype.(104) Furthermore, the relative risk of HFpEF increases with increasing mean cardiac radiation dose (MCRD).(104)

There is not a unified consensus on the definition of CA-related cardiotoxicity although most trials have focused on changes in resting systolic function, namely LVEF and/or development of HF symptoms.(61, 62) Commonly used tools to assess cardiac function (i.e. LVEF) are notoriously insensitive to minor injury, and hence subtle changes may go unnoticed for many years.(122) When considering that cardiotoxicity revolves around the measurement of LV systolic function to diagnose HF, it is important to recognize more than half of all HF patients have HFpEF.(173) Based on the prevalence of HFpEF, a greater need for dynamic functional assessment of the CV system in addition to measures of resting myocardial function may be warranted in defining cardiotoxicity. This may allow detection of latent CV abnormalities before the precipitous decline of resting myocardial function or the development of CV symptomology that may impact quality of life.(64)

Cardiopulmonary exercise testing (CPET) including measurement of peak oxygen consumption (VO_2) is considered the gold standard for the assessment of cardiorespiratory fitness (CRF).(186) Cardiorespiratory fitness is a strong, independent predictor of mortality, CVD-related mortality, HF-related morbidity and mortality, CA-related mortality and may be involved in the pathophysiologic link between anti-CA related treatments and the increased risk of late CVD morbidity and/or mortality.(66, 163, 164, 187) Emerging evidence indicates CRF may be reduced in CA survivors.(170)

To date, no one has examined the contribution of contemporary RT on CRF in patients with CA. Moreover, the degree of reduction in CRF, the primary limitation *(i.e. heart vs. lungs)*, the determination of a dose-response (MCRD-ΔVO_2) relationship, and the mechanistic link attributable to specific anti-CA therapies such as RT are all unexplored.

The purpose of this pilot project was to evaluate CRF with an emphasis on peak VO_2 and its determinants in a subset of CA patients who had previously undergone RT involving a significant dose to the heart. The hypothesis of this study is that patients with CA who have previously undergone RT with radiation dose to the heart have impaired cardiorespiratory fitness, measured as a reduction in peak oxygen consumption (VO_2), mainly due to abnormal cardiac function, in a dose-dependent manner. The ability to demonstrate a significant relationship between exercise capacity, cardiac dysfunction, and RT regimen may signal the importance of CRF assessment in establishing latent cardiotoxicity.

METHODS

We designed a single-center pilot prospective study in patients who had previously undergone irradiation to the chest including a clinically-significant radiation dose to the heart to obtain a cross-sectional assessment of RT cardiotoxicity.

Potential subjects were identified during their routine clinical visits within the radiation oncology department at Virginia Commonwealth University (VCU) Medical Center according to the following inclusion and exclusion criteria:

Inclusion criteria:

- Previous thoracic radiotherapy to the chest;
- minimum radiation dose to the heart of at least 5 Gray (Gy) involving at least 10 percent (%) of the heart volume

Exclusion criteria:

- Inability to provide informed consent;
- age <18 years;
- contraindication to magnetic resonance imaging (MRI) with gadolinium contrast use (including, but not limited to implantable cardioverter defibrillator or pacemaker [not compatible with MRI] or moderate to severe renal impairment [glomerular filtration rate <60 mL/min/1.73 m^2]);
- pregnancy or breastfeeding;
- inability to perform treadmill exercise testing;
- prior history of significant cardiac disease (including prior myocardial infarction, HF, myocarditis, pericarditis, left ventricular hypertrophy, cardiomyopathy, pericardial effusion).

This study was approved by the VCU institutional review board (HM#20006724) prior to commencement and all subjects underwent informed consent prior to study procedures. Clinical data was extracted from the patient medical record. Cancer staging was based upon the American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th edition.(188) Cancers are staged (0–IV) with higher stages indicating larger tumors or the extent of spread to lymph nodes or other organs.

Radiation dose calculation was performed based on a volumetric computed tomography (CT) data set obtained during a treatment planning session. A single well-experienced radiation oncologist performed quantification of total radiation dose and volume of heart and lung exposed. Using dedicated treatment planning software (Pinnacle, Koninklijke Philips N.V.), the heart and lungs were manually contoured on each CT slice generating 3D structures. After radiation beam definition and target dose calculation, heart and lung dose was determined as maximum, minimum and mean dose (MCRD, MLRD) to the whole organ volume as well as using dose-volume histograms to generate %volumes of the heart receiving at least 5 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy, respectively (**Figure 1**).

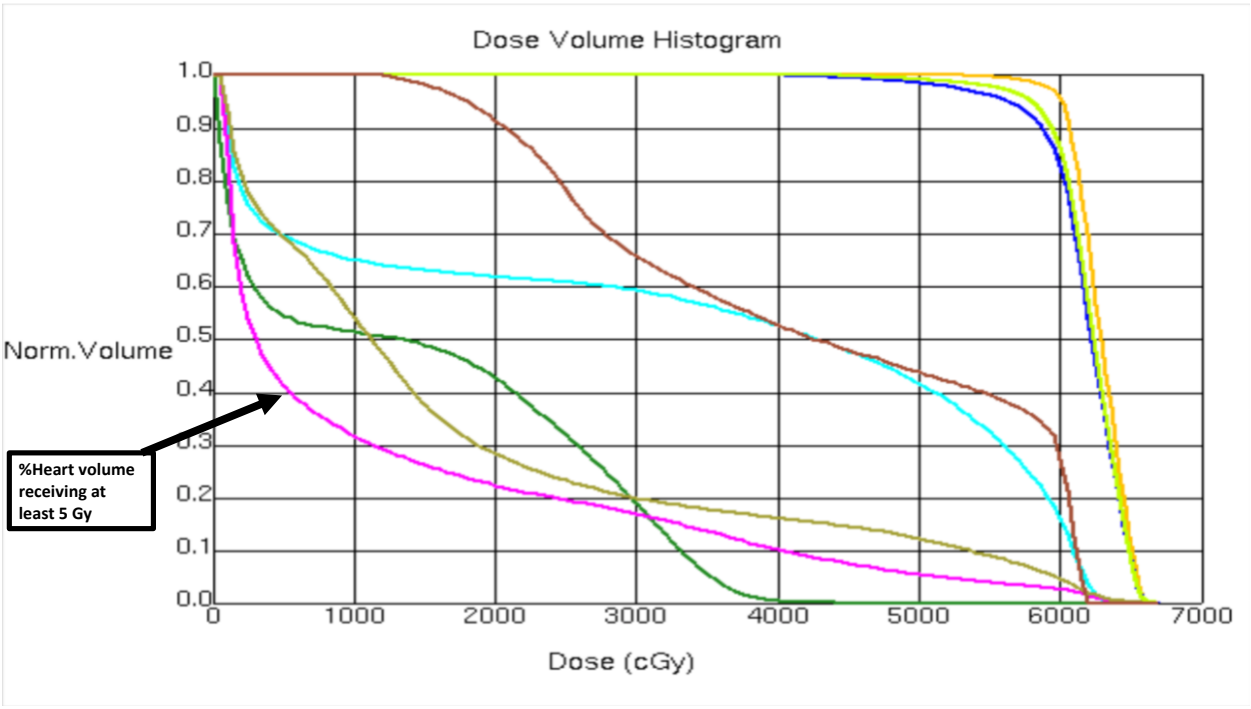

Figure 1. Example of a Dose-Volume Histogram to determine heart exposure during radiotherapy treatment.
Abbreviations: Gy=Gray units; cGy= centigray.

Patients were evaluated for signs of cardiotoxicity through the use of the following procedures:

- Cardiopulmonary exercise testing (CPET)
- Transthoracic Doppler echocardiography at rest and with exercise
- Cardiac-specific blood-based biomarker analysis
- Cardiac magnetic resonance (CMR) imaging with delayed-gadolinium enhancement (LGE).

Additionally, all subjects underwent assessment of anthropometrics, physical activity participation, and completed a health-related quality of life (HRQOL) questionnaire to further characterize the cohort.

Cardiopulmonary Exercise Testing

A physician supervised, symptom-limited CPET was administered to all subjects by a clinical exercise physiologist using a conservative incremental ramping treadmill protocol wherein the speed and grade increased by approximately 0.3 estimated metabolic equivalents every 30 seconds.(189) Ventilatory gas-analysis was performed pre-, during, and post-exercise using a metabolic cart (Parvomedics, Sandy, UT) to measure ventilation (V_E), VO_2, and carbon dioxide production (VCO_2). Prior to each test, the O_2 and CO_2 sensors of the metabolic cart were calibrated using gases of known O_2, nitrogen, and CO_2 concentrations and the flow sensor was calibrated using a standard 3-Liter syringe.

Contraindications to testing and test termination criteria were based upon established American Heart Association/American College of Cardiology guidelines for

exercise testing.(154) All subjects were instructed to follow standard pre-exercise test procedures as outlined by the American Thoracic Society/American College of Chest Physicians and evaluated by a physician prior to testing.(186) This included instructions to arrive in a fasting state, abstain from smoking at least 8 hours before testing, continuation of current medications, no exercise the day of testing, and to wear appropriate exercise attire. Subjects were briefed regarding the exercise protocol and encouraged to exercise to volitional fatigue. Twelve-lead ECG monitoring was conducted at baseline, throughout the test, and at least 5-minutes into the recovery period to assess heart rate (HR) and rhythm. Presence of exercise-induced atrial/ventricular arrhythmias (atrial fibrillation, ventricular ectopy ≥6/minute) and/or ST-T wave segment changes (≥1-mm horizontal or downsloping depression) indicative of myocardial ischemia were considered abnormal consistent with international guidelines.(154) Peak HR in beats per minute (bpm) was indexed to the age-predicted maximal HR to give a percentage using the commonly used equation: 220-age.(190) Chronotropic response to exercise was determined from the chronotropic index (CI), which is the difference between the peak HR and the resting HR relative to the metabolic requirement of exercise (peak VO_2 minus resting VO_2). A CI <0.80 without beta-blockade and ≤0.62 with beta-blockade was considered indicative of chronotropic incompetence and considered an abnormal response.(191, 192) Blood pressure was measured at rest, every two minutes during exercise, and into recovery using an automated exercise-compatible sphygmomanometer (Tango+, SunTech Medical, Morrisville, NC).

During CPET, V_E was determined using a pneumotachometer, and expired gases were sampled to continuously measure VO_2 and VCO_2. The average value for VO_2 during the last 30 seconds of exercise was used to define peak VO_2 expressed in both absolute values ($mL \cdot min^{-1}$) and relative to bodyweight ($mL \cdot kg^{-1} \cdot min^{-1}$). Percent of predicted normal peak VO_2 was calculated according to the prediction equations of Wasserman and colleagues.(193) The peak respiratory (VCO_2/VO_2) exchange ratio (RER) coinciding with the peak VO_2 was used to quantify subject effort. Typically, an RER ≥1.1 is regarded as criterion of an excellent maximal cardiopulmonary effort.(194) However, in the clinical setting an RER ≥1.1 is often not attained although the prognostic utility of peak VO_2 is retained leading to the acceptance of lower thresholds of >1.05 as good or ≥1.0 as acceptable effort.(167, 195–197) A RER<1.0 was used to reflect submaximal effort and/or a non-cardiac reason for stopping in the absence of any hemodynamic or electrocardiographic abnormalities.(154) A peak VO_2 <83% of predicted values was used to identify an abnormal aerobic exercise capacity or exercise intolerance.(193)

The peak oxygen pulse (O_2 pulse) was defined as the ratio between peak VO_2 ($mLO_2 \cdot min^{-1}$) and peak HR in units of mL/beat. Percent predicted O_2 pulse was defined as the percentage of the predicted value achieved by dividing the predicted peak VO_2 by age-predicted peak HR. An O_2 pulse ≤85% of predicted was considered abnormal based on the findings of Oliviera and colleagues.(198)

The ventilatory anaerobic threshold (VAT) was calculated using the dual-methods criteria wherein the V-slope and the ventilatory equivalents methods were employed.(199) The V-slope method was graphically determined by departure of the

VO_2 from a line of identity drawn through a plot of VCO_2 versus VO_2. The ventilatory equivalents method was determined from graphical and averaged tabular data as the point wherein a systematic increase in the ventilatory equivalent for oxygen (V_E/VO_2) occurs without an increase in the ventilatory equivalent for carbon dioxide (V_E/VCO_2).(186) A VAT less than the lower 95% confidence limits for the ratio of predicted VAT to predicted peak VO_2 indicated abnormality.(193)

Ten second averaged V_E and VCO_2 data, from the initiation of exercise to peak, were inserted into spreadsheet software (Microsoft Excel, Microsoft Corp., Bellevue, WA) to calculate the minute ventilation/carbon dioxide production (V_E/VCO_2) slope via least squares linear regression. Additionally, the V_E/VCO_2 slope was indexed to the peak VO_2 to normalize ventilatory efficiency to exercise capacity.(200) The oxygen uptake efficiency slope (OUES) was determined from the linear relation of $\dot{V}O_2$ versus the logarithmic transformation of V_E during exercise, i.e., $\dot{V}O_2$ = a log10 VE + b, where 'a' is the OUES and 'b' is the intercept.(201) The %-predicted OUES was calculated by comparing the observed with the reference values put forth by Sun et al.(202) A %-predicted OUES of <89% of predicted was considered indicative of an abnormal CV limitation as proposed by Barron et al.(203) The oxygen uptake efficiency plateau (OUEP) was calculated as the 90-second average of the highest consecutive measurements of VO_2 ($mL \cdot min^{-1}$)/V_E ($L \cdot min^{-1}$) during the exercise period.(202)

A normal CV limitation to exercise was defined as a peak VO_2 ≥83% of predicted values in the setting of an RER ≥1.0 with a peak HR ≥85% of age-predicted maximal HR.(167, 193, 204) A priori an abnormal cardiovascular response to exercise was defined as exercise intolerance (peak VO_2 <83%) in the presence of any one of the

following observances in the absence of a pulmonary limitation to exercise and a peak RER ≥1.0: 1) VAT <95% confidence limits for the ratio of predicted VAT to predicted peak VO_2(193); 2) Chronotropic index (CI) <0.80 or ≤0.62 with beta-blockade(191, 192); 3) OUES <89% of predicted(203); 4) Peak O_2 pulse <85% of predicted.(198) This would indicate subclinical cardiac dysfunction related directly to undergoing RT treatment and provide a means for early detection of latent heart disease. Inability to detect an abnormal cardiac limitation to exercise posits that cardiovascular dysfunction is not what's driving the exercise intolerance rather it is due to pulmonary limitations, deconditioning or excess body habitus, or that CPET variables may be insensitive to detect early cardiovascular dysfunction. A peripheral limitation to exercise was defined as a peak VO_2 <83% with an RER <1.0 in the absence of any cardiovascular or pulmonary abnormalities.

Pulmonary Function Testing

All subjects underwent standard spirometry prior to exercise including performance of forced vital capacity (FVC), forced expiratory volume in one second (FEV1), the mean forced expiratory flow between 25% and 75% of the FVC ($FEF_{25-75\%}$), peak expiratory flow (PEF), and the directly-measured maximal voluntary ventilation (MVV) maneuver according to American Thoracic Society standards.(205) The presence and severity of airflow limitation was assessed according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.(206) Peak exercise V_E was compared to the MVV to assess ventilatory reserve with a peak V_E/MVV ratio of >0.80 indicating a pulmonary limitation to exercise.(207) Similarly, forehead pulse oximetry

was employed throughout exercise to estimate arterial hemoglobin oxygen saturation. Oxygen saturation values <95% at rest or >5% decrease with exercise were considered abnormal and indicative of a pulmonary limitation to exercise.(207)

Doppler Echocardiography

Standard two-dimensional transthoracic echocardiography was performed to measure left and right ventricular and atrial dimensions, left and right ventricular systolic function, stroke volume (SV), left-ventricular ejection fraction (LVEF), transmitral flow velocities [Early (E), Late (A), E/A ratio, and E wave deceleration time (DT)], tissue Doppler-derived early diastolic mitral annular velocity (e′), and longitudinal systolic strain (s′) measured at tissue Doppler averaged between lateral and septal according to the recommendations of the American Society of Echocardiography.(120, 208, 209) All echocardiographic measurements and analysis were performed by trained cardiologists.

Doppler derived cardiac output (CO) was estimated by measuring flow across the left ventricular outflow tract (LVOT) which is determined by the velocity time integral (VTI) of the Doppler signal directed across the LVOT (LVOT VTI) multiplied by the HR.(210) The LVOT VTI is used to estimate SV since it reflects the column of blood which moves through the LVOT during systole, per the following equation: SV = LVOT VTI x Cross Sectional Area (CSA) of the LVOT.(210) Since estimation of the CSA of the LVOT represents a potential source of significant error the LVOT VTI alone has been suggested as a reasonable surrogate for CO measurement.(211)

The E/e’ ratio was calculated as an estimate of LV filling pressures.(212) The e′ velocity was indexed by the DT to obtain a measure (e′/DT) that reflected both the delay

in relaxation (DT) and the peak velocity in diastolic filling (e′).(213) A higher e′ and shorter DT would reflect better myocardial relaxation, whereas reduced e′ or prolonged DT would each reflect impaired relaxation with an additive value.

Stress echocardiography was also performed to assess the LVOT VTI-derived CO (VTI_{CO}), E, lateral e′ and E/e′ ratio at peak exercise by having the patient sit down immediately post-exercise and obtaining an apical view in < 1-minute. The interval changes in VTI_{CO}, e′ and E/e′ were calculated ($\Delta VTI_{CO_{exercise}}$, $\Delta e'_{exercise}$, $\Delta E/e'_{exercise}$), respectively. The diastolic functional reserve index (DFRI) was defined as the product of $e'_{rest} \bullet \Delta e'_{exercise}$.(214)

Cardiac-specific Blood-based Biomarker Assessment

A blood sample was obtained prior to any study procedures and before exercise to measure the following biomarkers: 1) high-sensitivity cardiac troponin I (hs-cTnI); 2) high-sensitivity cardiac troponin T (hs-cTnT); 3) N-terminal pro-brain natriuretic peptides (NTproBNP); 4) galectin-3 (Gal-3); 5) high-sensitivity C-reactive protein (hsCRP). The hs-cTnI and hs-cTnT plasma samples were collected in K2-Ethylenediaminetetraacetic acid (EDTA) tubes (Becton, Dickinson and Co., Franklin Lakes, NJ), centrifuged, frozen and shipped to Hamilton Health Sciences Research Laboratory (Hamilton, ON). The NTproBNP, Gal-3, and hsCRP plasma samples were collected in K2-EDTA tubes, centrifuged, and sent to a local laboratory (True Health Diagnostics, Richmond, VA).

The hs-cTnI was determined using the Abbott ARCHITECT (Abbott Laboratories, Abbott Park, IL) high-sensitivity troponin I immunoassay. The Abbott hs-cTnI assay is a chemiluminescent microparticle immunoassay for the quantitative determination of the

cTnI in human plasma and serum. The Abbott hs-cTnI assay reportable range is 1 – 50,000 nanograms per liter (ng/L) with a lower reportable limit of <1 ng/L.(142) The 99th percentile limit of the distribution of values in a reference population for males = 14.0 ng/L females = 11.1 ng/L, all-subjects = 13.6 ng/L, and at these concentrations the assay coefficient of variation (CV) is 5.0%.(142)

The hs-cTnT was determined using the Roche Elecsys Troponin T Gen 5 STAT (Roche Diagnostics, Indianapolis, IN) electrochemiluminescence immunoassay with an analytical range from 3 to 10,000 ng/L. The 99th percentile limit of the distribution of values in a reference U.S. population is 19 ng/L for both genders, 14 ng/L for females and 22 ng/L for males with a CV of <4%.(215)

The NTproBNP was determined using the Roche Elecsys proBNP (Roche Diagnostics, Indianapolis, IN) immunoassay for the in vitro quantitative determination of N-terminal pro-brain natriuretic peptide in human serum and plasma. It has a measuring range of 5.00-35,000 pg/mL with a limit of detection (LOD) of <5.00 pg/mL. The NTproBNP is a marker of myocardial strain/stretch and a surrogate for HF that has been found to correlate with radiation dose to the heart in left-sided breast CA patients after RT(148) and in patients with lung CA.(216) An NTproBNP <125 pg/mL is considered normal and effectively rules out the presence of LV dysfunction.(146)

Galectin-3 a novel mediator of HF development and progression(145) was measured with the Abbott ARCHITECT Galectin-3 assay (Abbott Laboratories, Abbott Park, IL) a chemiluminescent microparticle immunoassay for the quantitative determination of galectin-3 in human serum and EDTA plasma on the ARCHITECT i System. Galectin-3 is a galactoside-binding lectin expressed by macrophages during

phagocytosis and linked to the development of myocardial fibrosis.(217) An elevated Gal-3 level (≥17.8 ng/mL) is indicative of increased cardiovascular risk.(218)

High-sensitivity C-reactive protein is the prototypical inflammatory biomarker, and associated with worsening hemodynamics and outcomes in heart failure.(152) The hsCRP was determined using an ultrasensitive latex-enhanced immunoassay (Siemens Healthcare, Elangen, HR).(219) It has an analytical range of 0.175 to 20 mg/L with a CV of < 10%. A low hsCRP level (<1 mg/L) is associated with a low cardiovascular risk.(220)

Cardiac Magnetic Resonance Imaging

Cardiac Magnetic Resonance imaging was performed on a Siemens Aera 1.5 Tesla scanner (Siemens Healthcare, Elangen, HR) following a clinical assessment, measurement of renal function, a pregnancy test (if indicated), and completion of an MRI safety checklist. All studies were interpreted by a single experienced cardiovascular radiologist. For CMR, selected MRI sequences including cardiac dimensions (volumes and mass), systolic and diastolic function, and gadolinium-contrast application was obtained. Cardiac Magnetic Resonance imaging is the gold-standard for the assessment of ventricular function and volumes.(221) Delayed gadolinium enhancement imaging allows detection of myocardial fibrosis and scar and provides good diagnostic and prognostic value in cardiovascular diseases.(222) Areas of late-gadolinium enhancement (LGE) were considered a marker of myocardial injury.

The time-1 (T1) relaxation is a measure in milliseconds of how quickly the net magnetization vector recovers to its ground state static magnetic field.(223) The concept of T1 mapping refers to pixelwise illustrations of absolute T1 relaxation times

on a map. Native T1 values are determined primarily by edema and an increase in interstitial space and myocardial T1 (native T1 myo) is prolonged in the presence of extracellular volume (ECV) expansion.(224) In gadolinium-enhanced T1 mapping (post-contrast T1 myo), contrast is distributed throughout the extracellular space and shorten T1 relaxation times of myocardium proportional to the concentration of contrast-agent.(225)

The calculation of the ECV fraction requires measurements of myocardial and blood T1 before and after contrast administration along with the patient's hematocrit value according to the following formula:

$$ECV = (1 - \text{hematocrit}) \frac{\frac{1}{\text{post-contrast T1 myo}} - \frac{1}{\text{native T1 myo}}}{\frac{1}{\text{post-contrast T1 blood}} - \frac{1}{\text{native T1 blood}}}$$

Estimation of the ECV fraction was used to quantify diffuse myocardial injury.(226)

Anthropometrics Assessment

Body composition was assessed pre-exercise via body mass index (BMI), waist and hip circumferences, and single-frequency bioelectrical impedance analysis (BIA) (Quantum II, RJL Systems, Inc., Clinton Township, MI) by experienced technicians.

Body mass index was utilized to assess weight relative to height and calculated by the equation: BMI = kg/m^2. Overweight was defined as a BMI of 25.0-29.9 kg/m^2 and BMI ≥30.0 kg/m^2 as obese.(227) Waist (above the iliac crest) and hip (maximal circumference of hip/proximal thigh, just below gluteal fold) circumferences were

obtained to characterize body fat distribution with a flexible tape measure on the skin surface in duplicate.(204)

Bioelectrical impedance analysis has been validated as a measure of body adiposity when compared to reference methods such as dual X-ray absorptiometry.(228) Measurements were obtained prior to exercise in a fasted state with subjects on their current medications. Resistance and reactance (Xc) was calculated at a 50-kHz frequency at controlled room temperature with subjects placed in a supine position with arms and legs abducted approximately 45° to each other. Source electrodes were placed proximal to the metacarpophalangeal joint on the dorsal surfaces of the right hand and distal to the transverse arch on the superior surface of the right foot. Sensor electrodes were placed at the midpoint between the styloid processes and between the medial and lateral malleolus on the right ankle. Reactance and Xc were recorded to the nearest ohm and imputed into predictive equations to calculate fat mass (FM), fat-free mass (FFM), and total body water.(228) Fat mass and FFM was then indexed to height in meters squared. Percent body fat was calculated using FM and bodyweight in kilograms.

Quality of Life and Physical Activity Questionnaires

Two questionnaires were used to assess cancer-specific HRQOL and current levels of physical activity. The Functional Assessment of Cancer Therapy – General 7-item version (FACT-G7) is a validated HRQOL questionnaire with a scoring range of 0-28 and a mean value of 18.04 ± 4.97 in healthy individuals wherein higher scores indicate better HRQOL that can be used with any tumor type.(229)

The International Physical Activity Questionnaire (IPAQ) – Short form is a validated instrument to assess physical activity levels in adults.(230) The IPAQ assesses subjective physical activity participation in the form of walking, moderate-intensity, and vigorous-intensity activities weighted by energy requirements defined in metabolic equivalents (METS) taking into account frequency and duration to provide a volume of physical activity defined as MET-min/week.(230) Both questionnaires were administered by trained personnel prior to study procedures.

Statistical Analysis

The objective of this pilot cross-sectional study was to determine the prevalence of exercise intolerance after chest irradiation, if exercise intolerance was related to markers of cardiac function, and if the "cardiac dose" – the amount of radiation the heart is exposed to – correlated with injury or dysfunction.

Being the first study addressing the correlation of cardiac radiation dose with such parameters, it was not possible to estimate the sample size needed for statistical purposes. Given the design of this pilot study (single-cohort), the statistical analysis consisted primarily of descriptive statistics. A sample size of at least 29 subjects was considered to be required for a correlation coefficient >0.50 to demonstrate a moderate relationship between variables of interest with a power of 80% (α=0.05) while 20 subjects would provide a power of >95% for a correlation coefficient >0.70 (α=0.05) reflecting a strong relationship. Continuous data are reported as median and interquartile range (IQR) or absolute range for potential deviation from a Gaussian distribution. Discrete variables are reported as a number and percentage. The

nonparametric Kruskal-Wallis test was used for comparisons between groups. The Chi square test was used to compare nominal level variables. Univariate analysis between CPET variables, cardiac biomarkers, and echo and CMR parameters was performed using the Spearman rank correlation coefficient test.

Multivariate analysis using a linear regression model was performed using a stepwise approach including those variables associated with $p<0.05$ at univariate analysis from pre-specified cardiac, pulmonary, and body composition parameters to determine which predictor variables best explain peak VO_2. Significant univariate predictors were assessed for multicollinearity prior to placement in the multivariate model. An additional correction for type of CA and for use of anthracyclines was performed by a mixed model of multivariate analysis using a General Linear Model. Statistical analysis was performed using SPSS version 24.0 (IBM Corp, Armonk, NY).

RESULTS

Thirty subjects were enrolled between August 2016 - November 2017. During this time period 106 potential subjects were screened for study inclusion of which 76 were not enrolled for the following reasons:

1) 37 (35%) did not meet protocol minimum RT heart dose requirement;

2) 27 (25%) were not interested;

3) 5 (5%) failed to show up for their appointment;

4) 5 (5%) had contraindications to undergo MRI;

5) 2 (2%) had contraindication to undergo CPET.

Table 1 describes the clinical characteristics of the enrolled subjects which included 15 (50%) subjects who received RT for lung CA, 10 (33%) for breast CA, 2 (7%) for esophageal CA, 1 (3%) for Hodgkin's lymphoma, 1 (3%) for a desmoid tumor, and 1 (3%) for Castleman's disease. The overall cohort was predominantly Caucasian (n=20 [67%]), mostly female (n=18 [60%]) with a median age of 63 (57-67) years. The median time since CA diagnosis was 2.6 years with a total range of 0.3 - 29.0 years.

Table 1: Clinical Characteristics of the Cohort.

Variable	**Entire Cohort (N=30)**
Cancer type	
Lung	15 (50%)
Breast	10 (33%)
Esophageal	2 (7%)
Hodgkin's Lymphoma	1 (3%)
Other diseases	
Desmoid Tumor	1 (3%)
Castleman's Disease	1 (3%)
Caucasian	20 (67%)
Female	18 (60%)
Age (years)	63 (57-67)
Time since Cancer Diagnosis (years)	2.6 (0.3-29.0)*
Time since completion of Chemotherapy (years)	1.7 (0-21.8)*
Prior chemotherapy	26 (87%)
Anthracycline-based chemotherapy	7 (24%)
Time since completion of Radiotherapy (years)	2.0 (0.1-28.7)*

Date are listed as median and (interquartile range), or total range*, or n (%).

Non-small cell carcinoma was the primary lung CA type (13/15 [87%]) with the remaining 2 having small-cell carcinoma. The breast CA cohort (n=10) consisted of seven (70%) with left-sided disease and three (30%) with right-sided disease. Invasive ductal carcinoma was overwhelmingly the most common breast CA type (n=9/10 or 90%) followed by one patient with an invasive lobular carcinoma. The prevalence of hormone receptor and HER2 status of the breast CA cohort was as follows: ER+ = 8/10 (80%), PR+ = 7/10 (70%), HER2+ = 2/10 (20%). **Table 2** provides a detailed breakdown of CA stage by diagnosis (breast CA or lung CA and other diseases).

Table 2: Cancer Stage of Study Participants.

Stage	Breast CA n=10	Lung CA or other diseases n=20
IA	1 (10%)	1 (5%)
IB	1 (10%)	
II	1 (10%)	
IIA		1 (5%)
IIB	1 (10%)	1 (5%)
IIIA	3 (30%)	10 (50%)
IIIB	2 (20%)	4 (20%)
IIIC	1 (10%)	
Unknown		3 (15%)

Fifteen (50%) of all patients (breast CA =10 (100%), lung CA or other diseases = 5 (25%) had previously undergone surgery. Twenty-six (87%) of all patients (breast CA = 9 (90%), lung CA or other diseases = 17 (85%) had previously undergone neo-adjuvant, adjuvant, or concurrent chemotherapy. Specifically, seven (70%) of the breast CA patients underwent neo-adjuvant chemotherapy, and 2 (20%) underwent adjuvant chemotherapy. Twelve (60%) of the lung CA or other disease patients underwent concurrent chemoradiation followed by 3 (15%) who underwent adjuvant chemotherapy, and 2 (10%) who underwent neo-adjuvant chemotherapy. Seven (24%) of the total cohort underwent regimens including anthracyclines which included 6/7 breast CA patients and one patient with Hodgkin's lymphoma. **Table 3** lists the types of chemotherapy, frequency of use, and average doses of the cohort. Seven (70%) of the breast CA patients were on concomitant hormonal therapy at the time of evaluation. Time since completion of chemo was 1.7 years with a total range of 0.1-28.7 years.

Table 3: Chemotherapy Regimens of the Cohort.

Chemotherapy Type	n (%)	Dose
Taxol	18 (60%)	483 ± 289 mg/m^2
Carboplatin	13 (43%)	863 ± 636 mg
Cyclophosphamide	8 (27%)	2540 ± 523 mg/m^2
Doxorubicin	7 (23%)	234 ± 44 mg/m^2
Cisplatin	5 (17%)	271 ± 82 mg
Etoposide	4 (13%)	597 ± 248 mg/m^2
Pemetrexed	2 (7%)	1500 ± 707 mg/m^2
Imatinib	1 (3%)	300 mg
Rituximab	1 (3%)	375 mg/m^2 every 3-months
Nivolumab	1 (3%)	2240 mg
Trastuzumab	1 (3%)	104 mg/kg
Pertuzumab	1 (3%)	1260 mg/kg
Vinblastine	1 (3%)	36 mg/m^2
Dacarbazine	1 (3%)	2250 mg/m^2
Bleomycin	1 (3%)	60 u/m^2

Data are listed as n (%) and mean $\pm$ standard deviation.
Abbreviations: mg/m^2=milligrams per meter squared.

All patients had previously undergone neo-adjuvant/adjuvant or concurrent RT with a median duration of 2.0 years with an absolute range of 0.1 - 28.7 years since end of RT treatment. Seventeen (85%) of the lung CA and other diseases group underwent primary RT, 2 (10%) underwent adjuvant RT followed by 1 (5%) who underwent neo-adjuvant RT. The median number of RT fractions was 30 (range = 4-35) with a median of 2.0 (range = 1.5-12.0) Gy per fraction for a prescribed RT dose of 60.0 (range = 30.4-70.0) Gy. Five of these subjects also had additional previous RT treatments (#fractions = 4 [range = 4-20], Gy per fraction = 12.0 [range = 1.8-12.0] Gy, total prescribed dose = 48.0 [range = 32.0-60.0]) Gy.

The median number of RT fractions for the breast CA subjects was 32 (range = 16-33) with a median of 2.0 (range = 1.8-2.7) Gy per fraction for a prescribed RT dose of 60.2 (range = 42.6-66.0) Gy. One of the breast CA patients also had additional

previous RT treatments (#fractions = 28, Gy per fraction = 1.8 Gy, total prescribed dose = 50.4 Gy).

The MCRD and MLRD, reflective of the dose contributions from all RT treatments for each patient, for the entire cohort was 5.6 (3.7-17.8) and 9.4 (6.4-14.5) Gy, respectively. Specific to CA type, the MCRD for the lung CA and other diseases was 12.4 (range = 3.1-42.0) Gy and the MLRD was 12.7 (range = 3.3-21.5) Gy. The MCRD for breast CA patients was 3.7 (range = 1.9-5.5) Gy while the MLRD was 6.9 (range = 0.5-14.7) Gy, respectively. **Table 4** lists the MCRD, MLRD, mean %heart and lung volumes that received at least 5 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy, respectively. When separating the CA types (breast vs. lung CA and other diseases) there was a significant difference in MCRD, and the %heart volume receiving at least 5 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy (all P's≤0.02) with the lung Ca and other diseases subjects receiving higher heart doses than breast CA patients.

Table 4: Heart & Lung Radiotherapy Volumes.

	Entire Cohort N=30	Breast CA n=10	Lung Ca and Other Diseases n=20	P-value
Heart Volumes				
MCRD	5.6 (3.7-17.8)	3.7 (2.8-4.3)	12.4 (5.5-24.9)	<0.001
V5 Gy	39.5 (15.8-80.5)	13.5 (11.5-30.0)	62.0 (36.5-87.2)	<0.001
V10 Gy	19.3 (8.8-67.3)	8.2 (2.8-9.0)	40.0 (18.0-73.8)	<0.001
V20 Gy	7.0 (1.2-35.0)	1.6 (0.8-3.5)	24.0 (7.0-60.3)	<0.01
V30 Gy	2.5 (0-15.0)	0.1 (0-2.3)	5.1 (0.1-26.5)	0.02
V40 Gy	1.0 (0-7.8)	0 (0-1.0)	2.5 (0-12.8)	0.01
V50 Gy	0 (0-3.0)	0 (0-0)	0.5 (0-5.5)	<0.01
Lung Volumes				
MLRD	9.4 (6.4-14.5)	6.9 (5.7-10.4)	12.7 (7.6-16.6)	0.06
V5 Gy	42.0 (25.7-60.0)	27.4 (17.0-39.7)	54.0 (29.0-66.5)	0.01
V10 Gy	28.7 (20.0-36.8)	20.5 (13.3-29.9)	33.0 (23.5-43.5)	0.02
V20 Gy	17.0 (11.3-25.8)	13.6 (11.3-20.1)	18.5 (10.8-26.5)	0.28
V30 Gy	11.9 (5.5-18.0)	9.8 (7.0-13.5)	13.0 (4.0-18.5)	0.53
V40 Gy	7.0 (3.0-11.0)	5.7 (3.5-9.0)	10.0 (2.8-14.0)	0.46
V50 Gy	2.0 (1.0-6.8)	1.2 (0-2.0)	4.0 (1.0-9.5)	0.03

Values are listed as median and (interquartile range).
Abbreviations: MCRD=mean cardiac radiation dose; V=percent volume of the heart; Gy=Gray units; MLRD=mean lung radiation dose.

Cardiovascular Risk and Comorbidity Status

Hypertension was the primary established CVD risk factor (n=17 [57%]) followed by hypercholesterolemia. **Table 5** lists the prevalence of CVD risk factors and cardiovascular medication use amongst the group. Lung disease was the most common non-CVD-related comorbidity present in 17 (57%) individuals. All subjects were Eastern Cooperative Oncology Group (ECOG) status 0-1 with a mean Karnofsky grade of 90±10.

Table 5: Prevalence of Established Cardiovascular Disease Risk Factors and Cardiovascular Medication Usage.

Cardiovascular Disease Risk Factors	**N (%)**
Hypertension	17 (57%)
Diabetes Mellitus-Type II	7 (23%)
Hypercholesterolemia	14 (47%)
Early Family History of Cardiovascular Disease	9 (30%)
Current Smoker	6 (20%)
Obesity (Body Mass Index > 30)	10 (33%)
Sedentary Lifestyle	12 (40%)
Cardiovascular Medications	
Beta-blockers	5 (17%)
Angiotensin blockers	6 (20%)
Aldosterone inhibitors	2 (7%)
Statins	10 (33%)
Calcium channel blockers	5 (17%)
Diuretics	12 (40%)
Thiazide diuretics	7/12 (58%)
Loop diuretics	5/12 (42%)
Anti-platelets	13 (43%)

Anthropometrics Assessment

Nineteen (63%) subjects met BMI criteria for overweight (9 [30%]) or obesity (10 [33%]). Anthropometrics of the group are detailed in **Table 6**.

Table 6: Anthropometrics of the Cohort.

Variable	**Entire Cohort (N=30)**
Weight (kg)	76.1 (62.2-85.2)
Body Mass Index (kg/m^2)	27.1 (23.6-30.6)
Waist Circumference (cm)	96 (84-106)
Waist/Hip Ratio	0.86 (0.83-0.95)
Fat Mass %	33 (23-40)
Fat Mass (kg)	23.1 (15.3-33.1)
Fat Mass Index	8.7 (5.3-12.1)
Fat-Free Mass %	66 (60-77)
Fat-Free Mass (kg)	51.0 (44.2-60.1)
Fat-Free Mass Index	18.4 (16.6-20.2)

Data are listed as median and (interquartile range).

Abbreviations: kg=kilograms; kg/m^2=kilograms per meter squared; cm=centimeters.

Cardiopulmonary Exercise Testing (CPET)

The peak VO_2 for the entire cohort was 1376 (1057-1552) $mL \cdot min^{-1}$, normalized to bodyweight was 16.9 (14.4-20.8) $mL \cdot kg^{-1} \cdot min^{-1}$ or moderately reduced at 62 (52-89) % of predicted values based upon age/gender/anthropometrics-based normative values. **Table 7** provides a comprehensive summary of the CPET variables analyzed with this study. As expected peak VO_2 was inversely correlated with age (R=-0.401, P=0.031). Peak VO_2 was not significantly different with regards to gender (P=0.116) or race (P=0.556). However, it was significantly higher (P=0.008) in the breast CA cohort compared with the lung CA and other diseases (21.0 [17.8-23.6] $mL \cdot kg^{-1} \cdot min^{-1}$ versus 16.0 [13.0-18.6] $mL \cdot kg^{-1} \cdot min^{-1}$) or 93 (77-98) %predicted versus 54 (48-68) %predicted. Peak VO_2 was not significantly different when comparing those who underwent chemotherapy of any type (P=0.66) versus those who did not undergo chemotherapy. Peak VO_2 was significantly higher (P=0.021) in those who underwent ACT regimens (22.0 [16.2-23.2] versus 16.6 [14.4-19.6] $mL \cdot kg^{-1} \cdot min^{-1}$) although it did not correlate with anthracycline dose when treated as a continuous variable (R=0.535, P>0.2).

The median peak RER was 1.02 (0.95-1.09) with 16/30 (53%) achieving an RER ≥ 1.0, 12/30 (40%) reaching an RER ≥ 1.05, and 12/30 (40%) reaching an RER ≥ 1.10, respectively. The primary reason for test termination was dyspnea (43%) followed by fatigue (30%) with 27% stopping for other reasons (musculoskeletal limitations, lightheaded/dizziness).

Table 7: Cardiopulmonary Exercise Test Variables.

CPET Variables	**Entire Cohort**
Absolute Peak VO_2 ($mL \cdot min^{-1}$)	1376 (1057-1552)
Percent-predicted Absolute Peak VO_2 (%)	62 (51-98)
Relative VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	16.9 (14.4-20.8)
Percent-predicted Relative VO_2 (%)	62 (52-89)
Relative Peak VO_2 <83% predicted	22 (73%)
METS	4.8 (4.1-5.9)
Oxygen Pulse (mL/beat)	9.2 (7.5-10.7)
Percent-predicted Oxygen Pulse (%)	82 (66-96)
Oxygen Pulse <85% predicted	13 (45%)
Ventilatory Anaerobic Threshold ($mL \cdot min^{-1}$)	1040 (842-1234)
Percent-predicted VAT (%)	53 (44-70)
Ventilatory Anaerobic Threshold ($mL \cdot kg^{-1} \cdot min^{-1}$)	13.5 (11.4-14.7)
Percent-predicted VAT (%)	54 (43-59)
VE/VCO_2 Slope	32.4 (27.9-35.5)
$VE/VCO_2/VO_2$ ratio	1.69 (1.40-2.48)
OUES	1.58 (1.38-1.97)
OUEP	37 (30.5-42.0)
Peak RER	1.02 (0.95-1.09)
Exercise Time (minutes)	9.5 (7.9-12.0)
Resting Heart Rate (bpm)	73 (68-86)
Maximal Heart Rate (bpm)	150 (122-164)
Percent-predicted APMHR (%)	91 (79-100)
Chronotropic Index	1.10(0.92-1.39)
Heart Rate Recovery-1 (bpm)	20 (13-26)
Resting Systolic BP (mmHg)	124 (111-143)
Resting Diastolic BP (mmHg)	70 (63-81)
Max Systolic BP (mmHg)	174 (155-190)
Max Diastolic BP (mmHg)	70 (70-80)
Rate-Pressure Product (Systolic mmHg x HR)	24.0 (19.7-29.8)
Resting SPO_2 (%)	99 (98-100)
Exercise SPO_2 (%)	97 (95-99)
Δ $SPO_{2\ exercise}$	1 (0-4)
VE/MVV ratio	0.67 (0.54-0.78)
Breathing reserve (Liters)	23.0 (12.1-41.5)
Peak Minute Ventilation ($L \cdot min^{-1}$)	47.7 (42.2-53.1)
Peak Respiratory Rate ($breaths \cdot min^{-1}$)	36 (31-42)
Peak Tidal Volume (Liters)	1.26 (1.03-1.62)

Data are listed as median and (interquartile range) or n (%).

Abbreviations: VO_2=oxygen consumption; METS=metabolic equivalents; VAT=ventilatory anaerobic threshold; VE/VCO_2=minute ventilation to carbon dioxide production; OUES=oxygen uptake efficiency slope; OUEP=oxygen uptake efficiency plateau; RER=respiratory exchange ratio; APMHR=age-predicted maximal heart rate, SPO_2=oxygen saturation; VE/MVV=peak minute ventilation/maximal voluntary ventilation ratio.

Pulmonary Function Results

The majority of the cohort (n=16 [53%]) did not show evidence of any significant airflow limitation while the remaining subjects were graded according to GOLD criteria as follows: Grade 1 (Mild) = 2 (7%); Grade 2 (Moderate) = 7 (23%); Grade 3 (Severe) = 4 (13%); Grade 4 (Very Severe) = 1 (3%). Presence of airflow limitation was not identified in the breast CA patients and was predominantly confined to those with lung CA (n=13/14 [93%] of the remaining cohort). **Table 8** provides a detailed assessment of spirometry values for the entire cohort.

Table 8: Pulmonary Function Results of the Cohort.

Variables	**Entire Cohort (N=30)**
Forced Vital Capacity (Liters)	2.80 (2.39-3.40)
FVC%	83 (74-97)
Forced Expiratory Volume 1-second (Liters)	2.01 (1.50-2.46)
FEV1%	75 (55-95)
FEV1/FVC ratio	0.72 (0.57-0.79)
Forced Expiratory Flow 25-75%	1.33 (0.85-2.26)
FEF%	51 (31-95)
Peak Expiratory Flow (L/Sec)	4.61 (3.29-5.80)
PEF%	67 (46-98)
Direct MVV (Liters per minute)	72.4 (54.2-95.6)
MVV%	78 (45-92)

Data are listed as median and (interquartile range).

Abbreviations: FVC=Forced vital capacity; FEV1=Forced expiratory volume in 1-second; FEF=Forced expiratory flow; PEF=Peak expiratory flow; MVV=Maximal voluntary ventilation.

Doppler Echocardiography

Two-dimensional echocardiography revealed half of the subjects (n=15 [50%]) had an LVEF (52 (47-60)%) less than the lower limit of the normal reference range (53-73%, mean±2-standard deviations [SD]). Using European Society of Cardiology (ESC) criteria(231): two patients had a reduced LVEF (<40%), seven had a mid-range LVEF

(≥40% - 49%) with the remaining having LVEF's ≥50%. Thirteen (43%) patients met criteria for diastolic dysfunction based upon ESC recommendations defined as at least two of the following to be present: functional alterations of - E/e'>13; or mean e' <9 cm/s; or structural alterations of - left-atrial volume index (LAVI) >34 mL/m^2; or left-ventricular hypertrophy.(231) Furthermore, when using the ***ESC Diagnostic algorithm for a diagnosis of heart failure of non-acute onset*** and assigning exercise intolerance as a typical symptom of HF with the exposition to cardiotoxic drug/radiation as an assessment of HF probability the prevalence of HFpEF was 21% in the cohort evidenced by a NTproBNP ≥125 pg/mL and the aforementioned echo diastolic dysfunction criteria with a preserved LVEF (≥50%). **Table 9** provides a detailed summary of the Doppler echocardiographic variables of the entire cohort. In univariate analysis, the echo-derived resting e' (R=-0.562, P=0.024), and stress echo Δ $VTI_{CO\ exercise}$ (R=-0.521, P=0.046) both correlated with the MCRD.

Table 9: Echocardio-Doppler Parameters.

Variables	Entire Cohort (N=30)
Left-ventricular ejection fraction (%)	52 (47-60)
LVEDV (mL)	83 (70-102)
LVESV (mL)	41 (30-51)
Stroke Volume (mL)	44 (36-52)
LVEDV Index (mL)	45 (39-56)
LVESV Index (mL)	22 (16-26)
Stroke Volume Index (mL/m^2)	23 (19-29)
E (cm/s)	74.1 (62.3-87.3)
A (cm/s)	86.5 (72.5-94.2)
E/A ratio	0.89 (0.72-1.03)
LAVI (mL/m^2)	21.2 (17.1-28.1)
e' (cm/s)	8.0 (7.1-9.6)
s' (cm/s)	7.9 (7.1-8.7)
a' (cm/s)	10.3 (8.2-11.4)
Deceleration time (ms)	215 (172-244)
E/e'	8.9 (7.0-12.8)
e'/DT	0.039 (0.031-0.046)
Exercise E (cm/s)	100 (76-123)
Exercise e' (cm/s)	10.7 (7.8-14.9)
Δ $e'_{exercise}$ (cm/s)	1.3 (-0.5-5.3)
Exercise E/e'	7.9 (7.0-13.5)
Δ $E/e'_{exercise}$	0.4 (-2.8-1.9)
DFRI ($e'_{rest} \bullet \Delta e'_{exercise}$)	12.5 (-3.8-48.7)
LVOT VTI – Rest (cm)	16.6 (14.4-20.4)
LVOT VTI – Exercise (cm)	19.5 (17.5-25.0)
Δ $LVOT\ VTI_{exercise}$	3.1 (2.1-5.9)

Data are listed median and (interquartile range).

Abbreviations: LVEDV=left-ventricular end-diastolic volume; LVESV=left-ventricular end-systolic volume; E=early transmitral velocity; A=late transmitral velocity; LAVI=left-atrial volume index; e'= early diastolic mitral annular velocity; s'=longitudinal systolic strain; a'=late diastolic myocardial velocity; E/e'=ratio of early transmitral velocity to early diastolic mitral annular velocity; e'/DT=ratio of early diastolic mitral annular velocity to deceleration time; DFRI= diastolic functional reserve index; cm/s=centimeters per second; ms=milliseconds; LVOT VTI=left-ventricular outflow tract velocity time integral.

Cardiac-specific Blood-based Biomarker Assessment

Table 10 indicates the proportion of the cohort with abnormal responses for each blood-based biomarker and the median values for each. Elevated hsCRP and NTproBNP was noted in over half of all subjects. The cardiac troponins hs-cTnI and hs-

cTnT were detected in 28/30 (93%) and 29/30 (unable to detect hs-cTnT in 1-subject due to hemolysis) (97%) of all subjects, respectively.

Table 10: Cardiac-specific Blood-based Biomarkers.

Biomarker	Abnormal Response	Values
NTproBNP (pg/mL)	18 (60%)	187 (51-310)
hsCRP (mg/L)	19 (63%)	2.9 (1.5-6.2)
Galectin-3 (ng/mL)	10 (33%)	15.0 (13.3-18.8)
hs-cTnT (ng/L)	5 (17%)	9.05 (5.28-12.79)
hs-cTnI (ng/L)	3 (10%)	3.00 (2.00-6.50)

Legend: Date are listed as n (%) or median and (interquartile range). Abnormal response was defined as: NTproBNP ≥125 pg/mL, hsCRP >2 mg/L, Galectin-3 >17.8 ng/mL, hs-cTnT >22ng/L-Male and >14ng/L-Female, hs-cTnI >14ng/L-Male and >11.1ng/L-Female.

Abbreviations: NTproBNP=N-terminal pro-brain natriuretic peptide; pg/mL=picograms per milliliter; hsCRP=high-sensitivity C-reactive protein; mg/L=milligrams per liter; hs-cTnT=high-sensitivity cardiac troponin T; ng/L=nanograms per liter; hs-cTnI=high-sensitivity cardiac troponin I; ng/mL=nanograms per milliliter.

Health-Related Quality of Life and Physical Activity Questionnaires

The median FACT-G7 was within a normal range with a score 20 (15.0-23.5). FACT-G7 scores did not significantly correlate with peak VO_2, echo parameters, cardiac biomarkers, or CMR variables (all P's>0.06). The median IPAQ score was 792 (330-1689) MET-min/week and the distribution of physical activity (PA) according to IPAQ categories was as follows: Category-1 (Inactive) = 40%, Category-2 (Minimally Active) = 40%, Category-3 (Highly Activity) = 20%. The IPAQ-derived MET-min/week as a continuous variable or PA categories did not correlate with peak VO_2 (R=0.207, P=0.282) and (R=0.145, P=0.452), respectively. However, the FACT-G7 score did demonstrate a significant positive relationship with the IPAQ-derived volume of PA (R=0.423, P=0.02).

Cardiac Magnetic Resonance Imaging

Using CMR assessment of LVEF: 8 (27%) had an LVEF below the lower limit of normal (<57%, mean ± 2SD) and 2 (7%) had an LVEF greater than the upper limit of normal (>77%, mean ± 2SD).(232) The CMR LVEF, ECV, left-ventricular end-diastolic volume (LVEDV), left-ventricular end-systolic volume (LVESV), SV, SV index (SVI), LGE, or myocardial T1 mapping (pre- and post-contrast) did not correlate with MCRD (all R's<0.31, P's>0.12) or peak VO_2 (all R's<0.3, P's>0.08). **Table 11** provides a detailed description of the CMR variables of interest.

Table 11: Cardiac Magnetic Resonance Imaging Parameters.

Variables	**Entire Cohort (N=30)**
Left-ventricular ejection fraction (%)	64 (53-74)
LVEDV (mL)	117.2 (93.6-136.6)
LVESV (mL)	40.5 (31.1-62.0)
LV Stroke Volume (mL)	68.2 (54.9-80.6)
LV Stroke Volume Index (mL/m^2)	38.0 (31.0-43.8)
Presence of late-gadolinium enhancement	12 (41%)
Myocardial T1 Mapping (ms)	1030 (1016-1067)
Post-contrast myocardial T1 Mapping (ms)	442 (416-466)
Extracellular Volume (%)	26.9 (24.8-29.2)

Data are listed as median and (interquartile range) or n (%).

Abbreviations: LVEDV=left-ventricular end-diastolic volume; LVESV=left-ventricular end-systolic volume; mL=milliliter; mL/m^2=milliliters per meter squared; ms=milliseconds.

Limitation to Exercise

Normal aerobic exercise capacity was observed in 8 (27%) subjects evidenced as a peak VO_2 above 83% of predicted per age, sex, height, and weight. In the remaining 22 (73%) of subjects with a reduced peak VO_2 <83% of predicted, 9 (30%) demonstrated a predominant CV limitation, 8 (27%) a pulmonary limitation, and 5 (17%) a non-cardiopulmonary or indeterminate limitation to exercise. **Table 12** details the

comparison of groups based upon aerobic exercise capacity and the predominant limitation to exercise. There were significant differences between the groups in CA type (x^2=[3, n=30] 16.7, P<0.01), peak VO_2 (P<0.01), FEV1 (P<0.01), and MCRD (P=0.03).

Between group comparisons using Bonferroni correction of continuous variables revealed peak VO_2 was significantly higher in those with normal exercise capacity relative to those with a predominant pulmonary limitation (P<0.01) or those with a predominant cardiac limitation to exercise (P<0.01). The FEV1 was significantly higher in the normal aerobic exercise capacity group compared to those with a pulmonary limitation (P<0.01). Furthermore, the MCRD was lower in those with normal aerobic exercise capacity compared to those with a pulmonary limitation (P=0.05).

Table 12: Comparison of groups based upon limitation to exercise.

	Normal Exercise Capacity (n=8)	CV Limitation (n=9)	Pulmonary Limitation (n=8)	Indeterminate Limitation (n=5)	P-value
Age (years)	59.5 (49.5-67.5)	59.0 (49.0-65.0)	63.5 (57.5-72.3)	64.0 (61.5)	0.42
Female	8 (100%)	4 (44%)	3 (38%)	3 (60%)	0.05
Breast CA	7 (88%)	1 (11%)	0 (0%)	2 (40%)	**<0.01**
Peak VO_2	22.8 (20.5-25.0)	16.0 (13.7-17.6)	15.5 (11.7-18.8)	16.5 (13.7-18.5)	**<0.01**
BMI (kg/m^2)	27.0 (22.9-30.6)	25.1 (19.7-30.2)	26.3 (23.6-28.0)	36.9 (29.5-46.2)	0.08
FEV1 (Liters)	2.35 (2.10-2.72)	2.11 (1.49-2.53)	1.12 (0.87-1.86)	1.90 (1.74-2.59)	**<0.01**
MRI LVEF (%)	65 (63-75)	59 (53-68)	60 (46-73)	73 (54-78)	0.34
Chemotherapy	7 (88%)	9 (100%)	5 (63%)	5 (100%)	0.11
MCRD (Gy)	3.5 (2.7-5.1)	10.3 (4.0-22.6)	12.3 (5.2-26.5)	10.7 (4.4-20.3)	**0.03**
Time since RT (years)	1.5 (0.7-2.5)	1.8 (0.4-4.0)	5.2 (1.7-7.8)	0.5 (0.2-2.6)	0.16
Hypertension	4 (50%	6 (67%)	3 (38%)	4 (80%)	0.44
Dyslipidemia	4 (50%)	5 (56%)	4 (50%)	4 (50%)	0.42
Diabetes	3 (38%)	1 (11%)	1 (13%)	3 (38%)	0.63
Obesity	3 (38%)	3 (33%)	1 (13%)	3 (60%)	0.37
Smoking	0 (0%)	1 (11%)	4 (50%)	1 (20%)	0.08
MET-min/week	1689 (1064-4467)	462 (132-983)	662 (26-924)	2523 (468-8025)	0.09

Data are listed as median and (interquartile range) or n (%).

Abbreviations: CA=cancer; VO_2=oxygen consumption; BMI=body mass index; FEV1=forced expiratory volume in 1-second; MRI=magnetic resonance imaging; LVEF=left-ventricular ejection fraction; MCRD=mean cardiac radiation dose; Gy=Gray units; RT=radiotherapy; MET=metabolic equivalent.

Predictors of Peak Oxygen Uptake

An assessment of pre-specified physiologic predictors of peak VO_2 listed in **Table 13** at univariate analysis revealed significant associations with RT, cardiac, body composition, and ventilatory parameters and includes the variables retained in a

multivariate analysis model. The MCRD demonstrated a significant inverse association **(Figure 2)** with peak VO_2 (R=-0.380, P=0.04), but total prescribed radiation dose or MLRD did not reveal a significant relationship.

The cardiac parameters e', E/e', ΔE/e' $_{exercise}$, e'/DT ratio, DFRI, $\Delta VTI_{CO\ exercise}$, NTproBNP, hs-cTnI, and hs-cTnT were all significantly associated with peak VO_2 in the entire cohort. The waist/hip ratio was the only body composition parameter that demonstrated a significant correlation with peak VO_2 (R=-0.431, P=0.03). The ventilatory parameters FVC, FEV1, FEV1/FVC, MVV, and ΔSPO2 with exercise were all associated with peak VO_2 at univariate analysis.

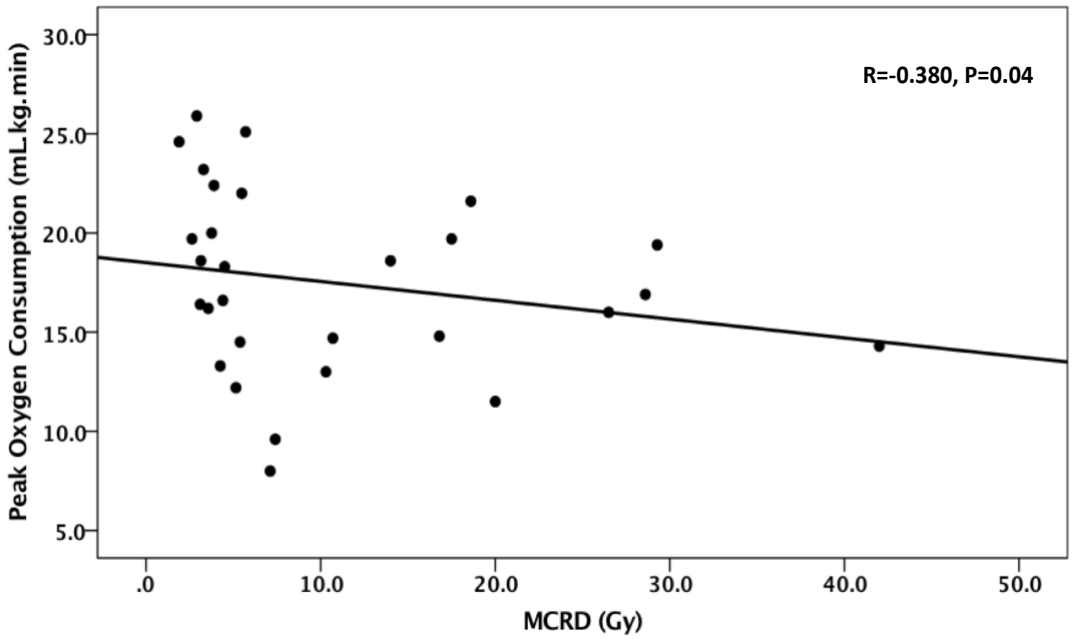

Figure 2. Relationship of peak VO_2 to the mean cardiac radiation dose.
Abbreviations: VO_2=oxygen consumption; MCRD=mean cardiac radiation dose; Gy=Gray units.

Pre-specified cardiac, pulmonary, and body composition parameters that demonstrated a significant relationship to peak VO_2 ($P<0.05$) were entered into a stepwise multivariate regression model that revealed the DFRI and NTproBNP were both independent predictors of peak VO_2 in the entire cohort ($R^2=0.73$, $P<0.01$; DFRI - $ß=0.765$, $P<0.01$; NTproBNP - $ß=-0.389$, $P=0.04$).

The DFRI and NTproBNP were then entered into a general linear model with the addition of two potential categorical predictors: 1) CA-type (breast vs. lung and other diseases) and 2) use of anthracycline-based chemotherapy (yes or no). This resulted in the loss of NTproBNP as an independent predictor ($P=0.06$), but the DFRI remained strongly associated with peak VO_2 ($R^2=0.74$, $P<0.01$).

The DFRI reflects the velocity of myocardial relaxation at rest and with exercise. The strong association between DFRI and peak VO_2 supports a central role of impaired left ventricular diastolic reserve in the pathophysiology of radiation-induced exercise intolerance.

Table 13: Multivariate Analysis of Predictors of Peak VO_2 for the Entire Cohort.

Variable	R-value	Univariate P-value	Multivariate P-value
Radiotherapy Parameters			
Total Prescribed Dose (Gy)	0.610	0.76	
MCRD (Gy)	-0.380	**0.04**	0.64
MLRD (Gy)	0.123	0.54	
Cardiac Parameters			
MRI LVEF (%)	0.050	0.80	
ECV (%)	-0.177	0.39	
Rest e'	0.494	**<0.01**	0.76
E/e'	-0.552	**<0.01**	0.31
Δ e'$_{exercise}$	0.644	**<0.01**	0.41
Exercise e'	0.574	**0.02**	0.58
Exercise E/e'	-0.487	0.08	
Δ E/e'$_{exercise}$	-0.329	0.21	
e'/DT ratio	0.427	**0.02**	0.58
DFRI	0.693	**<0.01**	**<0.01**
Exercise VTI_{CO}	0.200	0.46	
Δ $VTI_{CO\ exercise}$	0.614	**0.02**	0.86
NTproBNP	-0.590	**<0.01**	**0.04**
hs-cTnI	-0.515	**<0.01**	0.66
hs-cTnT	-0.550	**<0.01**	0.57
Galectin-3	-0.279	0.14	
hsCRP	-0.301	0.11	
Body Composition Parameters			
Weight (kg)	-0.157	0.42	
BMI	-0.051	0.80	
Waist Circumference	-0.368	0.06	
W/H Ratio	-0.431	**0.03**	0.82
Fat Mass%	-0.008	0.97	
Fat Mass Index	-0.013	0.95	
Fat-Free Mass%	-0.005	0.98	
Fat-Free Mass Index	-0.186	0.33	
Ventilatory Parameters			
FVC	0.469	**0.01**	0.54
FEV1	0.673	**<0.01**	0.16
FEV1/FVC	0.550	**<0.01**	0.21
Direct MVV	0.600	**<0.01**	0.07
Δ $SPO_{2\ exercise}$	-0.429	**0.02**	0.70

Abbreviations: Gy=Gray; MCRD=mean cardiac radiation dose; MLRD=mean lung radiation dose; LVEF=left-ventricular ejection fraction; e'=early diastolic mitral annular velocity; E/e'=ratio of early transmitral velocity to early diastolic mitral annular velocity; Δ=delta; e'/DT=ratio of early diastolic mitral annular velocity to deceleration time; DFRI= diastolic functional reserve index; VTI_{CO} =left-ventricular outflow tract velocity time integral cardiac output; NTproBNP=N-terminal pro-brain natriuretic peptide; hs-cTnT=high-sensitivity cardiac troponin T; hs-cTnI=high-sensitivity cardiac troponin I; hsCRP=high-sensitivity C-reactive protein; kg=kilograms; BMI=body mass index; W/H=waist/hip; FVC=Forced vital capacity; FEV1=Forced expiratory volume in 1-second; MVV=Maximal voluntary ventilation; SPO_2=oxygen saturation.

Predictors of peak VO_2 according to primary cause of limitation.

Dividing the cohort by primary exercise limitation (cardiac vs. pulmonary vs. other) and examining the predictors of peak VO_2 in each group revealed the relationship between MCRD and peak VO_2 was further strengthened **(Figure 3)** (R=-0.569, P=0.02) in those with a primary cardiac limitation to exercise. Doppler-derived stress echo diastolic parameters (∆e' with exercise, exercise e', DFRI), cardiac-specific biomarkers (NTproBNP, hs-cTnT), and pulmonary function (FEV1, MVV) were also univariate predictors of peak VO_2 **(Table 14)**. None of the body composition parameters correlated with peak VO_2 in those with a cardiac limitation to exercise. A multivariate stepwise regression model created using significant univariate predictors (P<0.05) revealed that the variable: exercise e' was an independent predictor of peak VO_2 (R=0.785, P=0.01). Exercise e' reflects the velocity of myocardial relaxation with exercise. Also, in this subgroup, the strong association between diastolic reserve and peak VO_2 support its central role of in the pathophysiology of radiation-induced exercise intolerance.

Those limited predominantly by a pulmonary limitation to exercise also demonstrated significant associations between peak VO_2 and Doppler-derived rest/stress echo diastolic parameters (e', E/e', $\Delta e'_{exercise}$, exercise e', exercise E/e', $\Delta E/e'_{exercise}$, DFRI, exercise VTI_{CO}, Δ $VTI_{CO\ exercise}$), cardiac-specific biomarkers (hs-cTnI, Gal-3), and pulmonary function (MVV, $\Delta SPO2_{exercise}$) parameters **(Table 15)**. None of the body composition parameters correlated with peak VO_2 in those with a primary pulmonary limitation to exercise. In a multivariate model, only the echo-Doppler diastolic parameter, exercise E/e' reflective of the increase in LV filling pressure with exercise was an independent predictor of peak VO_2 (R=1.00, P<0.001). Also, in this subgroup with

pulmonary limitation, the strong inverse association between diastolic function and peak VO_2 support its central role of in the pathophysiology of radiation-induced exercise intolerance.

In those with an indeterminate or non-cardiopulmonary limitation to exercise only the Doppler-derived echo diastolic parameter: resting E/e' and hsCRP were univariately associated with peak VO_2. However, due to the small size of the sample (n=5) multivariate analysis was not performed.

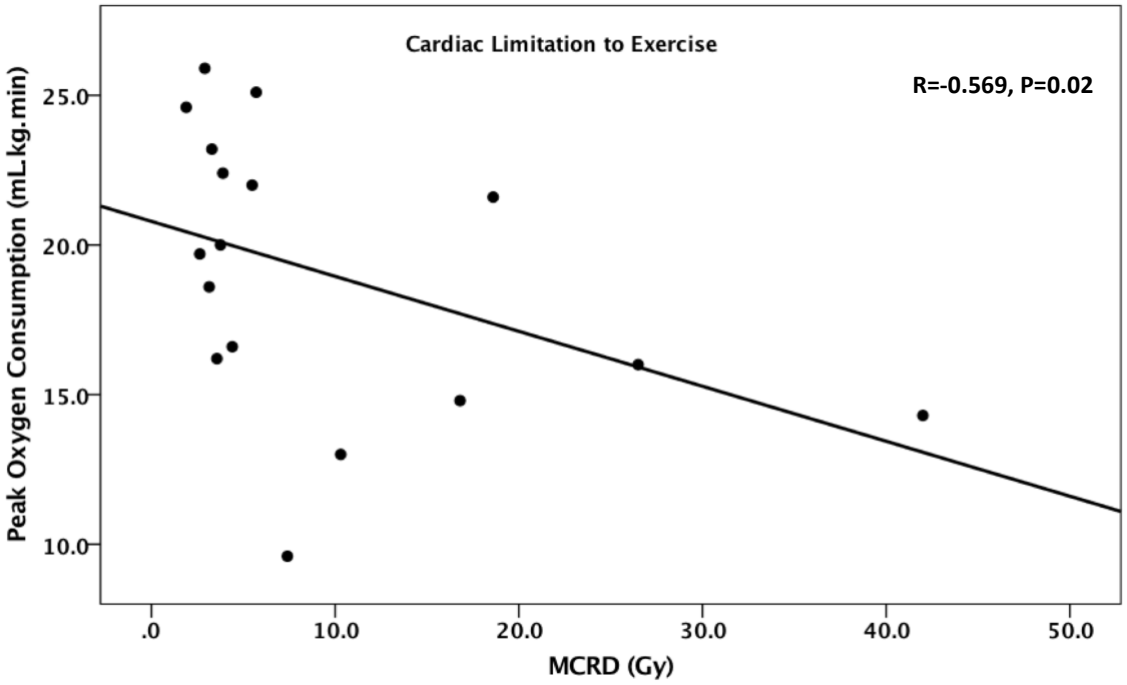

Figure 3. Relationship of peak VO_2 to the mean cardiac radiation dose in individuals with a predominant cardiac limitation to exercise.
Abbreviations: VO_2=oxygen consumption; MCRD=mean cardiac radiation dose; Gy=Gray units.

Exploratory Analysis: Relationship of Other CPET Variables to Mean Cardiac Radiation Dose.

In addition to peak VO_2, numerous other CPET-derived variables correlated with radiation doses.

Peak VO_2 in absolute values ($mL \cdot min^{-1}$) moderately correlated with MCRD (R=-0.432, P=0.019), V5Gy (R=-0.434, P=0.019), and V10Gy (R=-0.470, P=0.010). Percent-predicted peak VO_2 demonstrated a significant inverse relationship with MCRD (R=-0.471, P=0.010), V5Gy (R=-0.453, P=0.014), V10Gy (R=-0.489, P=0.007), V20Gy (R=-0.413, P=0.026, and V40Gy (R=-0.369, P=0.045).

The peak %-predicted O_2 pulse was inversely associated with all RT doses: MCRD (R=-0.505, P=0.005), V5Gy (R=-0.514, P=0.004), V10Gy (R=-0.561, P=0.002), V20Gy (R=-0.452, P=0.014), V30Gy (R=-0.476, P=0.009), V40Gy (R=-0.536, P=0.003), and V50Gy (R=-0.420, P=0.023).

The OUEP and %-predicted OUEP also demonstrated significant inverse associations with MCRD ([R=-0.419, P=0.024], V5Gy [R=-0.463, P=0.012], V10Gy [R=-0.465, P=0.011] and %-predicted OUEP: MCRD [R=-0.429, P=0.020], V5Gy [R=-0.477, P=0.009], V10Gy [R=-0.466, P=0.011]), respectively.

The standard exercise test variables maximal HR and the rate-pressure product (RPP) (Max HR x Max systolic BP) inversely correlated with MCRD ([R=-0.441, P=0.017], [R=-0.486, P=0.008], V5Gy [R=-0.451, P=0.014], [R=-0.420, P=0.023], and V10Gy [R=-0.500, P=0.006], [R=-0.467, P=-0.011]), RPP only: V30Gy [R=-0.377 P=0.044], V40Gy [R=-0.394, P=0.035, V50Gy [R=-0.515, P=0.004),respectively.

Collectively, including peak VO_2, the %-predicted peak O_2 pulse showed the strongest correlations with MCRD and %heart volume exposed to RT dose. Interestingly, other key CPET variables(167) including the VE/VCO_2 slope (R=0.259, P=0.175), exercise time (R=-0.352, P=0.061), HRR-1' (R=-0.127, P=0.513), and the OUES (R=0.048, P=0.807) did not correlate with MCRD.

Table 14: Multivariate Analysis by Cardiac Limitation to Exercise.

Variable	R-Value	Univariate P-value	Multivariate P-value
Radiotherapy Parameters			
Total Prescribed Dose (Gy)	0.209	0.42	
MCRD (Gy)	-0.569	**0.02**	
MLRD (Gy)	0.017	0.95	
Cardiac Parameters			
MRI LVEF (%)	0.049	0.85	
ECV (%)	-0.129	0.65	
Rest e'	0.407	0.13	
E/e'	-0.482	0.06	
Δ $e'_{exercise}$	0.700	**0.04**	
Exercise e'	0.693	**0.03**	**0.01**
Exercise E/e'	-0.576	0.08	
Δ $E/e'_{exercise}$	-0.139	0.70	
e'/DT ratio	0.344	0.19	
DFRI	0.783	**0.01**	
Exercise VTI_{CO}	0.103	0.78	
Δ $VTI_{CO\ exercise}$	0.633	0.07	
NTproBNP	-0.601	**0.01**	
hs-cTnI	-0.309	0.23	
hs-cTnT	-0.598	**0.01**	
Galectin-3	0.174	0.50	
hsCRP	-0.243	0.35	
Body Composition Parameters			
Weight (kg)	-0.135	0.61	
BMI	-0.059	0.82	
Waist Circumference	-0.374	0.17	
W/H Ratio	-0.364	0.18	
Fat Mass%	-0.022	0.93	
Fat Mass Index	-0.056	0.83	
Fat Free Mass%	0.054	0.84	
Fat Free Mass Index	-0.199	0.45	
Ventilatory Parameters			
FVC	0.401	0.11	
FEV1	0.628	**<0.01**	
FEV1/FVC Ratio	0.400	0.11	
Direct MVV	0.488	**<0.05**	
Δ $SPO_{2exercise}$	-0.230	0.37	

All abbreviations are the same as Table 13.

Table 15: Multivariate Analysis by Pulmonary Limitation to Exercise.

Variable	R-Value	Univariate P-value	Multivariate P-value
Radiotherapy Parameters			
Total Prescribed Dose (Gy)	0.000	1.00	
MCRD (Gy)	0.357	0.39	
MLRD (Gy)	0.638	0.17	
Cardiac Parameters			
MRI LVEF (%)	-0.321	0.48	
ECV (%)	0.143	0.76	
Rest e'	0.714	**<0.05**	
E/e'	-0.762	**0.03**	
Δ e'$_{exercise}$	1.000	**<0.01**	
Exercise e'	1.000	**<0.01**	
Exercise E/e'	-1.000	**<0.01**	**<0.001**
Δ E/e'$_{exercise}$	-1.000	**<0.01**	
e'/DT ratio	0.381	0.35	
DFRI	1.000	**<0.01**	
Exercise VTI$_{CO}$	-1.000	**<0.01**	
Δ VTI$_{CO\ exercise}$	1.000	**<0.01**	
NTproBNP	-0.476	0.23	
hs-cTnI	-0.719	**<0.05**	
hs-cTnT	-0.381	0.35	
Galectin-3	-0.833	**0.01**	
hsCRP	-0.381	0.35	
Body Composition Parameters			
Weight (kg)	0.143	0.74	
BMI	0.143	0.74	
Waist Circumference	-0.238	0.57	
W/H Ratio	-0.143	0.74	
Fat Mass%	-0.190	0.65	
Fat Mass Index	-0.190	0.65	
Fat Free Mass%	0.190	0.65	
Fat Free Mass Index	0.167	0.69	
Ventilatory Parameters			
FVC	0.548	0.16	
FEV1	0.595	0.12	
FEV1/FVC Ratio	0.690	0.06	
Direct MVV	0.762	**0.03**	
Δ SPO$_{2exercise}$	-0.741	**0.04**	

All abbreviations are the same as Table 13.

DISCUSSION

The results of this pilot study indicate aerobic exercise capacity defined as peak VO_2 is markedly reduced (38% less than predicted normal values) in CA patients who have previously undergone thoracic radiation wherein the heart received significant RT dose. This exercise intolerance is multifactorial but our results indicate this is predominantly due to a cardiac dysfunction in a group of patients without a clinical diagnosis of CVD. The Doppler-stress echo-derived diastolic functional reserve index (DFRI) and the cardiac-biomarker NTproBNP are strong, independent predictors of peak VO_2. Reduced aerobic exercise capacity is inversely associated with multiple indices of abnormal CV function using multiple imaging and biomarker analyses. Furthermore, the MCRD received during RT correlates with aerobic exercise capacity. This indicates CRF is sensitive to detect latent CV abnormalities in this cohort. This confirms the presence of impaired CRF in patients with CA who have previously undergone thoracic radiation wherein the heart received significant RT dose. The results also further show that the impairment in CRF shows a dose-dependent relationship with the radiation dose to the heart, and that impaired CRF is predominantly due to cardiovascular limitations in diastolic function with exercise (impaired diastolic reserve).

Aerobic exercise capacity is reduced in CA survivors whom have previously undergone thoracic radiation wherein the heart received significant RT dose.

The results of the current study demonstrate aerobic exercise capacity is markedly reduced in CA patients who have previously undergone thoracic radiation

wherein the heart received significant RT dose. Exercise capacity in this population has not been previously been characterized to this detail in reference to the RT regimen. The finding of reduced exercise capacity has been previously observed by others in the study of CA survivors who have anti-CA therapies.(166, 170, 174, 233, 234) Jones et al. evaluated peak VO_2 in 47 post-menopausal hormone receptor+ breast CA women who all received anthracycline-based chemotherapy and 98% also underwent RT (mean=47±2.4 Gy) and observed a peak VO_2 of 17.9±4.3 $mL \cdot kg^{-1} \cdot min^{-1}$ or 24% below age-gender matched healthy controls. Significant univariate predictors of peak VO_2 were BMI, glucose, CRP, and insulin. Associations with RT dose and/or MCRD was not reported.(233)

In yet another study Jones et al. evaluated the CV risk profile including CRF in 26 early-stage HER2+ breast CA patients (65% received RT) treated with adjuvant taxane-anthracycline chemotherapy and/or trastuzumab.(174) Peak VO_2 was 19.2 $mL \cdot kg^{-1} \cdot min^{-1}$ and was significantly lower than controls. However, radiotherapy dose and/or MCRD was not reported.

Burnett and colleagues sought to determine the proportion of breast CA survivors (at least 3-months post-chemotherapy or left-chest RT, 80% received anthracycline-based chemotherapy, 40% received RT) with 2 or more CVD risk factors exhibiting a low VO_2 max in 30 patients.(166) The mean VO_2 was 25.4±5.3 $mL \cdot kg^{-1} \cdot min^{-1}$ which was commensurate to the 20^{th} percentile threshold value for age-gender matched normative values with 77% having values below the 20^{th} percentile value. Radiotherapy dose and/or MCRD was not reported.

A study by Adams et al. looked at CV status in 48 Hodgkin's disease survivors who received mediastinal irradiation (40 [27.0-51.7] Gy).(234) The peak VO_2 was significantly reduced (defined as < 20 $mL \cdot kg^{-1} \cdot min^{-1}$) in 30% of survivors. Furthermore, VO_2 max was significantly correlated with increasing fatigue, shortness of breath, and decreased physical component scores on the short-form-36 HRQOL questionnaire. All subjects in this study underwent mediastinal RT, but relationships with dose and/or heart involvement was not reported.

A review by Peel and colleagues developed normative values for peak VO_2 in breast CA patients.(170) They identified 27 clinical trials involving a total of 1,856 females (chemotherapy: n=78%, RT=56%, endocrine therapy= 33%) directly measuring pVO_2 in the pre- or post-adjuvant setting. Radiotherapy dose and/or MCRD was not reported. The mean pVO_2 prior to adjuvant therapy was 24.6 $ml \bullet kg^{-1} \bullet min^{-1}$, whereas the mean pVO_2 post-adjuvant therapy was 22.2 $ml \bullet kg^{-1} \bullet min^{-1}$. This equated to a post-adjuvant reduction in VO_2 of -2.4 $ml \bullet kg^{-1} \bullet min^{-1}$or 10% lower. Compared with reference values the pre-adjuvant VO_2 values were significantly lower (17%) than that of healthy, sedentary women (29.7 $ml \bullet kg^{-1} \bullet min^{-1}$) or 83% of predicted . In the post-adjuvant setting, pVO_2 was 25% lower (75% of predicted) compared to healthy, sedentary values.

A summarization of these prior studies in CA survivors is that reduced CRF is consistently observed in CA patients compared with normative values. Exercise testing occurred at different time points across the CA treatment continuum and has largely been cross-sectional in nature. They were treated predominantly with chemotherapy although a majority also having received adjuvant RT. The reduction in CRF appears to be more pronounced in the post-adjuvant period and is associated with a higher

prevalence of CVD risk factors. Reduced CRF is usually observed in the setting of a normal LVEF. The novelty of the current study lies in its detailed characterization of the exercise response in CA survivors and the contribution of significant RT dose to the heart.

CRF in RT-treated chest CA survivors with significant heart dose is influenced predominantly due to cardiovascular dysfunction.

Cardiorespiratory fitness is determined by the components of the Fick equation where: VO_2 = CO x a-vO_2 difference. In normal healthy individuals and patients with systolic HF, CO is generally regarded as the primary determinant of CRF although this has not been consistently shown in those with HFpEF, the predominant HF phenotype in the breast CA patient.(235–237)

In the aforementioned study by Jones et al. the decreased peak VO_2 in breast CA patients with a preserved LVEF (>50%) using impedance cardiography was due to a reduced CO response attributed to a blunted increase in SV with exercise compared with controls.(233) This was based on the finding that peak HR and a-vO_2 difference was not different between the groups. The finding of a similar a-vO_2 difference, however, may suggest impaired microvascular dysfunction and/or skeletal muscle abnormalities may also limit peak VO_2 as oxygen extraction is directly proportional to muscle oxygen diffusion conductance and inversely related to CO.(238, 239)

Khouri et al. evaluated peak VO_2, LV volumes, and CO using stress 2DE in 57 female breast CA patients treated with doxorubicin-containing adjuvant therapy (79% received RT).(161) Peak VO_2 was 20% lower in patients and stress echo SV and

cardiac index (CI) were lower than controls. Furthermore, the post-stress increase in CI predicted peak VO_2.

A study by Koelwyn et al. evaluated arterial elastance (Ea), end-systolic elastance (Ees), and ventricular-arterial coupling (Ea/Ees) to determine the presence of vascular dysfunction following anthracycline-based chemotherapy.(175) In a cross-sectional design, 30 ER+, HER2- breast CA survivors (77% underwent RT) and 30 age-BMI-activity-matched controls underwent discontinuous CPET on an upright bicycle ergometer with 2DE images obtained at 25%, 50%, and 75% of peak work rate to calculate EDV, ESV, and LVEF. Central and peripheral vascular structure and function was also assessed. No significant differences were noted with resting measures of ventricular-arterial coupling between groups. The exercise Ea response was also not significantly different in survivors compared with controls. However, Ees was significantly reduced in survivors during exercise with a resulting elevated Ea/Ees ratio compared with controls at all exercise stages. Resting measures of LV systolic function were not different between CA survivors and controls, but LVEF was reduced at all three submaximal workloads in the survivor group. No significant differences between groups were found in regards to central and peripheral vascular structure and function. The results of this study indicate impaired ventricular-arterial coupling due to a reduced LV contractility in breast CA survivors at a mid-term follow-up period (>5-years) who were previously treated with anthracyclines with a majority having underwent adjuvant RT.

Conversely, in a small pilot feasibility study (n=14) of comprehensive pulmonary evaluation following thoracic lung RT in childhood CA survivors, De and colleagues

described the prevalence of CPET abnormalities.(240) In 14 subjects (median time since RT=4.8 years, prescribed RT dose=21 Gy, MLRD=11.9 Gy), of which 11 underwent CPET where nine patients demonstrated CPET abnormalities with the majority, seven (64%) being described as having a pulmonary limitation to exercise. However, they did not report CRF metrics, MCRD, or the status of CV function.

These studies allude to seemingly normal cardiac function in CA patients who have undergone anti-CA treatments when referencing resting values but reveal unmasking of cardiac abnormalities when subjected to stress. The majority of studies examining exercise determinants in CA survivors to date have been cardio-centric with most of the emphasis being placed on indices of systolic function.(161, 174, 175) Presently, there is scant information on the contribution of diastolic dysfunction on CRF in CA survivors although breast CA survivors share a number of risk factors associated with HFpEF patients.(66, 104) Heart failure with a preserved ejection fraction is a heterogeneous syndrome although evidence of diastolic dysfunction is a critical component.(231)

In an elegant animal model, Saiki et al. used cardiac radiation exposure to induce diastolic dysfunction with preserved ejection fraction.(241) Male rats were subjected to diffuse cardiac radiation at two different doses (10 and 20 Gy) using adeno-associated virus serotype-9 gene delivery of the rat sodium-iodide symporter gene followed by injection of radioactive Iodine-131 at 10-weeks age, were followed for five-months, and then underwent treadmill exercise testing, echo, hemodynamic catheterization, and tissue harvest. Radiation treated rats had reduced exercise capacity, increased LV diastolic stiffness, impaired myocardial relaxation, elevated filling-pressures, but similar

LVEF compared with controls. Post-hoc analysis showed evidence of a significant inverse linear trend between exercise capacity and radiation dose suggesting a dose-response relationship. Exercise capacity was inversely correlated with mean circulatory filling pressure, positively correlated with microvascular density, and inversely correlated with LV fibrosis. Pathology revealed increased LV fibrosis, mild concentric cardiomyocyte hypertrophy, and reduced microvascular density. This study provides mechanistic insight into pathological link between HFpEF with diastolic dysfunction, RT exposure, and the resulting impairment of exercise capacity.

Cardiac-biomarker NTproBNP and the diastolic functional reserve index (DFRI) are strong, independent determinants of peak VO_2.

Natriuretic peptides (BNP, NTproBNP) are markers of ventricular wall stress and are produced endogenously to counteract the adverse effects of sympathetic nervous system RAAS activation in the presence of cardiac dysfunction.(242) Furthermore, natriuretic peptides are accurate in the diagnosis of HF, improve risk stratification of HF patients, improve patient management, may be helpful to screen for asymptomatic LV dysfunction in high-risk patients, and powerful predictors of outcome in predicting death and hospitalization in HF patients.(243, 244) The inactive amino-terminal portion of pro-BNP, NTproBNP, is secreted in equivalent proportions to BNP, but has a longer half-life and may be more sensitive to detect early stage LV dysfunction.(245) Natriuretic peptides also inversely correlate with peak VO_2 and are sensitive to change with interventions designed to improve CRF .(246–248) This supports the current study wherein NTproBNP was a strong, independent predictor of exercise capacity along with

indices of cardiac dysfunction in chest CA patients who had previously undergone RT with significant heart dose.

The DFRI has previously been shown to predict exercise capacity in individuals with exertional diastolic dysfunction with a decreased DFRI indicating exercise intolerance.(213, 214, 249) The utility of the DFRI is its ability to identify diastolic abnormalities not apparent at rest. The DFRI is the Doppler-echo derived product of $e'_{rest} \bullet \Delta e'_{exercise}$. The early mitral annulus diastolic velocity (e'), a surrogate of myocardial relaxation, demonstrates a strong inverse correlation with the isovolumetric time constant (*tau*) a reference marker of LV relaxation and is less dependent upon preload.(250, 251) During exercise, augmentation of CO is achieved by increases in SV and HR. The tachycardia induced by exercise reduces diastolic filling time and the mitral inflow rate must increase to maintain or increase SV which can be accomplished by faster relaxation.(252) However, with diastolic dysfunction augmentation of relaxation is prevented as the HR increases during exercise.(253) The finding of reduced CRF and its strong association with DFRI in the present indicates impaired relaxation is driving the exercise intolerance.

Mean cardiac radiation dose is inversely associated with CRF.

In the current study multiple imaging modalities and cardiac-biomarkers were utilized with the intent to identify CV abnormalities related to RT dose. Using CMR techniques (LVEF, LGE, ECV), Doppler echocardiography (systolic and diastolic parameters, Doppler spectra), and cardiac-specific blood-based biomarkers only the echo-derived early diastolic mitral annular velocity (e'), and Δ $VTI_{CO\ exercise}$ were able to

demonstrate a significant relationship with the MCRD. On the contrary, multiple CRF variable demonstrated a significant relationship with RT doses. Moreover, this relationship was further strengthened when evaluating RT dose in those with a predominant cardiac limitation to exercise. Although a univariate predictor of peak VO_2 the MCRD was not an independent predictor in multivariate analysis. This indicates MCRD is not directly influencing CRF rather the pathophysiology associated with radiation exposure causes impaired relaxation evidenced by the reduced DFRI leads to exercise intolerance.

In a population-based case-control study of incident HF in female breast CA patients (MCRD=2.5 Gy, mean time post-RT=5.8 years), Saiki and colleagues demonstrated a dose-response relationship between MCRD and the incidence of HF.(104) The odds ratio (95%CI) for HF per log MCRD was 9.1 (3.4–24.4) for any HF, 16.9 (3.9–73.7) for HFpEF, and 3.17 (0.8–13.0) for HFrEF.

In the Saiki et al. animal study, *tau* was linearly related to radiation dose.(241) Rats exposed to 10 Gy demonstrated longer relaxation times compared with controls and rats receiving 20 Gy had even longer relaxation times.

In another animal study by Mezzarroma et al., contractile reserve measured with an isoproteronol challenge decreased in a dose-dependent manner.(254) Mice exposed to two different doses (20 or 14 Gy) experienced a graduated attenuation of %LVEF change in the acute (72-hours) and late (4-months) stages compared to sham controls.

Wang and colleagues demonstrated a linear relationship between MCRD and the risk of cardiac events in lung CA patients who had undergone thoracic RT.(255) In 127 patients with stage III NSCLC (ECOG status 0-1, prescribed RT dose=74 Gy,

MCRD=12.3 Gy, cardiac V5Gy = 36.5%) heart dose and baseline CVD each independently predicted the incidence of cardiac events.

CRF is sensitive to detect subclinical cardiac dysfunction in CA survivors who have received thoracic radiation with heart involvement.

Traditionally, cardiotoxicity of anti-CA treatments has been defined by reductions in the LVEF. The change in LVEF, however, is insensitive to detect subtle declines in CV function and when it declines may manifest in overt HF with disabling symptoms and a poor prognosis.(256, 257) This has led to the active investigation of alternative imaging and blood biomarkers for the detection of early-onset cardiac injury including cardiac troponins, NTproBNP, echo tissue Doppler imaging, and CMR with LGE measurements.(258) Although not yet systematically evaluated to detect cardiotoxicity there have been increasing calls to recognize CPET and the measurement of peak VO_2 as a potential diagnostic tool and/or indicator of anti-CA related cardiac dysfunction.(258, 259) In the present study, peak VO_2 demonstrated a dose-response relationship with RT dose that was strengthened in those with a predominant cardiac limitation to exercise. Peak VO_2, a well-established indicator of prognosis in the cardiac patient(167), demonstrated strong correlations with NTproBNP, hs-cTnI, hs-cTnT, and multiple indices of diastolic function which are themselves known to predict prognosis.(244, 260)

Is CRF a Therapeutic Target to Prevent or Reverse Cardiovascular Morbidity/Mortality in CA Survivors?

Cardiorespiratory fitness is a global assessment of the interconnected responses involving the cardiovascular, pulmonary, skeletal muscle, hematopoietic, and neuropsychological systems to exercise.(186) The direct and indirect effects of anti-CA therapies have the potential to adversely influence all of these systems thus reducing CRF. Exercise training regimens have consistently been shown to have favorable effects on each of these integrated systems.(261) Furthermore, it has been demonstrated to be one of the few effective interventions to improve CRF in HFpEF patients which appears to be the predominant cardiac phenotype in CA patients.(104, 238, 262) An elegant review by Scott et al. recently addressed the efficacy of exercise on cardiovascular toxicity in adult CA survivors.(263) The results indicate that at present the results of exercise training to mitigate cardiotoxicity are encouraging, but limited with most of the evidence coming from observational studies.

Neurohormonal blockade through the use of beta-blockers and RAAS inhibitors have been shown to decrease cardiac troponin and NTproBNP levels.(264) Evidence is accumulating for the use of beta-blockers (ß-blockers) in the prevention of cardiotoxicity.(265) Beta-blocker usage is associated with a lower incidence of HF following anti-CA treatment with anthracyclines and trastuzumab.(266) Animal studies allude to the cardio-protective effects of angiotensin-converting enzyme inhibitors (ACE-I) in anthracycline cardiotoxicity.(267) In an epidemiological cohort study of 142,990 women with breast CA exposure to ß-blockers and ACE-I (defined as a filled prescription for such) resulted in a reduction of cardiotoxicity (adjusted hazard ratio (adj.HR) =0.77 (0.62-0.95) and all-cause mortality (adj.HR=0.79 [0.70-0.90]) compared with the non-exposed (never prescribed ß-blockers/ACE-I) group.(268) Angiotensin-

converting enzyme inhibitors also reduce the effects of radiation-induced nephropathy(269) although the benefit in RIHD has not been demonstrated. The independent predictive value of NTproBNP on peak VO_2 in the current study suggests this may be viable target to improve cardiorespiratory fitness.

Future Directions

The observational nature of this study cannot prove a cause-effect relationship between RT dose to the heart and CRF. The demonstration of a direct cause-effect relationship would require a longitudinal study involving assessment of CRF and cardiac function both before and after administration of anti-CA therapies. Furthermore, a prospective interventional study would be required to ascertain if improvements in CRF translate into improvement of cardiac function and thus reduced CV risk status in this cohort.

Limitations

The primary limitation of this study is its observational single-time point and thus leading to a cross-sectional assessment rather than longitudinal assessment of the disease. Therefore, despite the multiple correlations between cardiac variables, CRF, and RT dose a cause-effect link cannot be proven.

There was significant heterogeneity in CA type and concomitant anti-CA systemic agent utilization investigated with this study. However, the unifying coexistence of a significant cardiac radiation dose threshold, but varying dose amounts based upon CA type and guideline-directed treatments (i.e. lower in breast CA vs. higher in lung CA) may have allowed detection of the observed dose-response relationship.

From a technical standpoint, when ascertaining organ-system limitation to exercise, no specific procedures were performed that directly measured peripheral vascular function or skeletal muscle characteristics both of which are known to contribute to exercise capacity.(270) There is also the potential confounding effects of patient medication use on the exercise response observed in this study.

CONCLUSIONS

Impaired CRF is common in patients with CA who have previously undergone thoracic radiation wherein the heart received significant RT dose. The impairment in CRF shows a dose-dependent relationship with the radiation dose to the heart, and that impaired CRF is predominantly due to cardiovascular limitations in diastolic function with exercise (impaired diastolic reserve). Cardiopulmonary exercise testing is able to detect subclinical cardiotoxicity in chest CA patients treated with thoracic irradiation including a significant heart dose. This study warrants further investigation into radiation-induced exercise intolerance and the efficacy of interventions to improve CRF in this population.

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