Non-Invasive Imaging for the Assessment of Cardiac Dose and Function Following Focused External Beam Irradiation

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Abstract

Technological advances in imaging and radiotherapy have led to significant improvement in the survival rate of breast cancer patients. However, a larger proportion of patients are now exhibiting the less understood, latent effects of incidental cardiac irradiation that occurs during left-sided breast radiotherapy. Here, we examine the utility of four-dimensional computed tomography (4D-CT) for the accurate assessment of cardiac dose; and a hybrid positron emission tomography (PET) magnetic resonance imaging (MRI) system to longitudinally study radiation-induced cardiac effects in a canine model.

Using 4D-CT and deformable dose accumulation, we assessed the variation caused by breathing motion in the estimated dose to the heart, left-ventricle, and left anterior descending artery (LAD) of left-sided breast cancer patients. The LAD showed substantial variation in dose due to breathing. In light of this, we suggest the use of 4D-CT and dose accumulation for future clinical studies looking at the relationship between LAD dose and cardiac toxicity.

Although symptoms of cardiac dysfunction may not manifest clinically for 10-15 years post radiation, PET-MRI can potentially identify earlier changes in cardiac inflammation and perfusion that are typically asymptomatic. Using PET-MRI, the progression of radiation-induced cardiac toxicity was assessed in a large animal model. Five canines were imaged using $^{13}$N-ammonia and $^{18}$F-fluorodeoxyglucose (FDG) PET-MRI to assess changes in myocardial perfusion and inflammation, respectively. All subjects were imaged at baseline, 1 week, 4 weeks, 3 months, 6 months, and 12 months after focused cardiac irradiation. To the best of our knowledge PET has not been previously used to assess cardiac perfusion following irradiation.

The delivered dose to the heart, left ventricle, LAD, and left circumflex artery were comparable to what has been observed during breast radiotherapy. Relative to baseline, a transient increase in myocardial perfusion was observed followed by a gradual return to baseline. However, a persistent increase in FDG uptake was observed throughout the entire left ventricle, including both irradiated and less-irradiated portions of the heart.
In light of these findings, we suggest the use of this imaging approach for future human studies to assess mitigation strategies aimed at minimizing cardiac exposure and long-term toxicity subsequent to left-sided breast irradiation.

Keywords

Radiotherapy, breast cancer, radiation-induced toxicity, cardiac imaging, respiratory motion, four dimensional computed tomography (4D-CT), positron emission tomography (PET), perfusion, inflammation, hybrid imaging.
Co-Authorship Statement

This thesis contains some material from manuscripts previously published in *International Journal of Radiation Oncology*Biology*Physics*. The copyright permissions are provided in Appendix A.

The second chapter of this thesis is adapted from an original research article, “Assessment of Intrafraction Breathing Motion on Left Anterior Descending Artery Dose During Left-Sided Breast Radiation Therapy” published in the *International Journal of Radiation Oncology*Biology*Physics* 2016 (in press; published online: February 12, 2016) by El-Sherif O, Yu E, Xhaferllari I, Gaede S. All authors contributed to the design of the study, the interpretation of the results, and reviewing the manuscript. In addition to the above, I performed the organ delineation, the dosimetric analysis, analyzed the results, and wrote the manuscript.

The third chapter of this thesis is adapted from another original research article to be submitted for publication. The work is entitled, “$^{18}$F-FDG and $^{13}$N-ammonia Cardiac PET Imaging in a canine model of cardiac exposures associated with breast cancer radiotherapy”, by El-Sherif O, Xhaferllari I, Sykes J, Butler J, DeKemp R, Renaud J, Battista J, Wisenberg G, Prato F.S., Gaede S. All authors contributed to the interpretation of the results and reviewing the manuscript. Additionally, I.X. assisted in the radiation treatment planning and delivery process. J.S. and J. B. contributed to the design of the study, provided technical assistance regarding care of the animals, and set-up/operation of the imaging console. R.D. and J.R. designed the software used for tracer kinetic analysis. J.B., G.W., F.S.P., made significant contributions to the design of the study. S.G. made significant contributions to the design of the study and assisted in the radiation treatment planning and delivery process. I contributed to the design of the study, design of the radiation treatment plan, assisted J.S. and J.B. in experimental set-up and operation, analyzed the data, and was the principal author of the manuscript.

The fourth chapter of this thesis is a longitudinal follow-up of the same subjects from chapter 3. The aim of this study was to identify whether the early changes observed in
myocardial perfusion and inflammation (1 month) observed in chapter 3 were sustained for a longer period (out to 1 year). This chapter was co-authored by El-Sherif O, Yin, H, Battista J, Wisenberg G, Prato F.S., Gaede S. All authors contributed to the design of the study, the interpretation of the results, and reviewing the manuscript. Y.H. performed the histological analysis and authored that corresponding section in the methods of this chapter. S.G. assisted in the radiation treatment planning and delivery process. I contributed to the design of the radiation treatment plan, assisted in the experimental set-up and operation, analyzed the data, and wrote the manuscript. This study is expected to be submitted for publication in *Radiotherapy & Oncology* pending a more complete histological analysis of the ex-vivo cardiac samples (performed with assistance from Dr.'s Geoffrey Pickering and Hao Yin).
Acknowledgments

The contents of this thesis cover a myriad of topics from within the fields of radiation biology, radiation physics, radiation therapy, and medical imaging physics. Due to the multidisciplinary nature of this project; its completion would have never been possible without the expertise and active involvement of many individuals from the Departments of Radiation Oncology, Cardiology, Medical Biophysics, Nuclear Medicine, and Pathology.

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I would like to thank the computing team at the London Regional Cancer Program, Carol Johnson and Jeff Kempe for your technical support over the years. I will forever miss our lively discussions around the lunch table. To my former office mates Spencer Martin, Brandon Disher, and Anthony Lausch, I’m thrilled to finally join the three of you on the LRCP all-stars wall of fame.

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List of Abbreviations

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<tr>
<td>$^{13}$NH$_3$</td>
<td>$^{13}$Nitrogen - ammonia</td>
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<tr>
<td>3D-CRT</td>
<td>3 dimensional conformal radiation therapy</td>
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<td>4D-CT</td>
<td>four-dimensional computed tomography</td>
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<td>ABC</td>
<td>active breathing control</td>
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<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<td>BED</td>
<td>biological equivalent dose</td>
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<td>BREL</td>
<td>left-sided breast cancer patient</td>
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<td>CT</td>
<td>computed tomography</td>
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<td>CT-SIM</td>
<td>computed tomography simulation</td>
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<td>DIBH</td>
<td>deep inspiration breath-hold</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DVH</td>
<td>dose volume histogram</td>
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<td>EOE</td>
<td>end of exhalation</td>
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<td>EOI</td>
<td>end of inhalation</td>
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<tr>
<td>FDG</td>
<td>fluorodeoxyglucose</td>
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<td>FH-CT</td>
<td>fast helical computed tomography</td>
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<td>IG</td>
<td>inspiratory gating</td>
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<td>IMRT</td>
<td>intensity modulated radiation therapy</td>
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<td>IV</td>
<td>intravenous</td>
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<td>Abbreviation</td>
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<tr>
<td>LAD</td>
<td>left anterior descending artery</td>
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<td>LAR</td>
<td>lifetime attributable risk</td>
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<tr>
<td>LCX</td>
<td>left circumflex artery</td>
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<tr>
<td>LINAC</td>
<td>linear accelerator</td>
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<tr>
<td>LIST-</td>
<td>individual coincident events are stored along with their timestamps. This mode allows for retrospective adjustments of the number of image frames and duration of each frame.</td>
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<tr>
<td>LV</td>
<td>left ventricle</td>
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<td>midVENT</td>
<td>mid ventilation</td>
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<td>MRAC</td>
<td>magnetic resonance attenuation correction</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>OSEM</td>
<td>ordered subset expectation maximization</td>
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<tr>
<td>PET</td>
<td>positron emission computed tomography</td>
</tr>
<tr>
<td>POP</td>
<td>parallel opposed radiation beam arrangement</td>
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<tr>
<td>RIHD</td>
<td>radiation induced ischemic heart disease</td>
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<td>RICD</td>
<td>radiation induced cardiac disease</td>
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<tr>
<td>RPM</td>
<td>real time position monitoring system</td>
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<tr>
<td>RT</td>
<td>radiation therapy</td>
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<td>SPECT</td>
<td>single photon emission computed tomography</td>
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STATIC-mode
PET

a single image volume or frame is created by grouping all
coincident events together within a preset time interval

SUV

standard uptake value

SUR

standard uptake ratio
Chapter 1

1 Introduction

This thesis deals with an important and prognostically significant side-effect of breast cancer treatment, specifically the cardiac effects on perfusion and inflammation of radiation therapy. We begin with an overview of radiation therapy for cancer treatment followed by a summary of the relevant literature on the topic of radiation induced cardiac toxicity. Brief explanations are provided with regards to the techniques employed to assess these effects in subsequent chapters. This chapter is concluded by highlighting the gaps in knowledge, relevant problems/questions to the area of study, and the hypothesis/aims of the thesis.

1.1 Radiation therapy for the treatment of cancer

In 2015, the Canadian Cancer Society reported approximately 80,000 Canadian deaths due to cancer. Although this is a very sobering statistic, the significant progress made in the last quarter century in diagnosing, understanding, and treating this disease is encouraging. Technological advances in medical imaging systems (1) have improved the ability to diagnose, plan treatments, and monitor response/recurrence in virtually every cancer type. Identification of the exact location of cancer sites, even for moving tumours situated in the lungs, is now possible and routine clinical practice.

After diagnosis, cancer is typically treated through surgery, chemotherapy, and/or radiation therapy. Although this thesis provides less emphasis on surgery and chemotherapy, their integral role in the treatment of cancer should not be underestimated.

Approximately two thirds of cancer patients will be treated with radiation (2). Radiation therapy can be delivered using two approaches 1) external beam radiotherapy, where a radioactive source originates outside the patient or 2) brachytherapy, where the radioactive source is implanted within the patient. Nearly 90% of patients treated with radiation, receive external beam radiotherapy(2), delivered via a linear accelerator (commonly referred to as a LINAC).
1.1.1 Linear Accelerators (LINACs) – General Overview

The linear accelerator (LINAC) is used to accelerate charged particles (typically 4-25 MeV electrons) that can be used to treat superficial tumours. Deeper lesions can also be treated using non-charged particles created after the kinetic energy of the electrons is converted into x-rays. Figure 1-1 is adapted from Podgorsak, et al (3) and highlights the major components of a typical LINAC.

Figure 1-1: A schematic of a typical isocentric medical linear accelerator (panel A) and a photograph of a commercial unit (Varian Medical Systems; panel B). adapted from Podgorsak, 2005 (3)
In general, the forming of the treatment beam of radiation begins with the genesis of “free” electrons. Electrons are liberated from a cathode through a process of thermionic emission and accelerated through a perforated anode into the next major component of the LINAC, the *accelerating waveguide*. Inside the *accelerating waveguide*, the injected electrons interact with high powered radiofrequency fields. In the waveguide electrons can reach velocities greater than 99.9% of the speed of light. These high-energy electrons are then steered, through the use of magnets, towards an x-ray target located in the head of the LINAC. The energy lost by the electrons during the collisions with the target produce high energy x-rays for treatment. Alternatively, the target can be retracted and electrons are allowed to exit the LINAC for electron beam therapy. The above is a very brief overview with regards to the inner workings of the typical medical LINAC, the following references provide a more detailed description(3, 4).

The delivery of radiation therapy via LINACs has been significantly improved over the last 25 years and continues to improve rapidly (5, 6). Medical LINACs in both academic and smaller non-academic cancer centres now have the ability to shape/vary the intensity of the radiation beam and provide on board imaging. Figure 1-2 illustrates the progressive improvements in our ability to conform radiation doses to a tumour. The techniques available to achieve this conformity have been collectively termed intensity modulated radiation therapy (IMRT) and have been made possible through the addition of computer-controlled multi-leaf collimator systems (MLC) in the head of the LINAC. The positions of the MLCs needed to achieve a desired dose distribution is obtained during the radiation treatment planning process through an inverse optimization algorithm(7).

On board, fluoroscopic x-ray imaging and cone-beam computed tomography(8) systems have been primarily used to improve the ability to reproducibly set up patients on the treatment couch. Since RT is typically multi-fractionated, the day to day reproducibility is paramount for successful treatment. Through the use of on-board imaging, set-up uncertainty is now ≤ 5mm (9). Additionally, on board imaging can potentially provide more personalized treatment strategies, commonly referred to as adaptive radiation therapy(10). The goal of adaptive radiation therapy is to quickly and efficiently re-plan
and optimize each patient’s radiation treatment in response to volumetric and/or functional changes in their anatomy observed via imaging during the course of treatment.

Figure 1-2: Prostate cancer radiotherapy 1935–2010. Prostate cancer irradiation is a good example of the improvement of radiotherapy technology over the past decades. By increasing the beam energy and the precision of the targeting, it was possible to escalate the dose to the prostate without exceeding the tolerance dose of healthy tissues; allowing the move from palliative irradiation to curative treatment. Abbreviations: 3D-CRT, 3D conformal radiotherapy; IMRT, intensity modulated radiotherapy; RT, radiotherapy. Reprinted with permission from Nature Review Clinical Oncology; Thariat J, et al. Nat. Rev. Clin. Oncol. 2012;10:52–60.

The ultimate metric for assessing the effectiveness of these advances, is how they translate to improvements in the overall survival of cancer patients. One of the most encouraging examples can be drawn from the significant improvement in the survival rate of breast cancer patients (11) (see Figure 1-3). The five-year, 10-year, and 15-year survival rate for breast cancer is now 89%, 83%, and 78% respectively (11, 12). In addition to the improved conformity of radiotherapy delivery, this cohort of patients has also benefited from technological advances in medical imaging which have led to earlier diagnosis. An important caveat when interpreting these improvements in survival rates is the presence of known bias (~overestimation of 1.25 years) (13). This lead bias is due to the fact that breast cancer patients who have been diagnosed earlier will naturally live longer after diagnosis. The improved survival consequently leads to an increase in the
less understood long-term side-effects of cancer treatment. One clinically significant and heavily debated side effect of breast, lung, and esophageal cancer treatment is radiation-induced cardiac toxicity (RICT)(14). The over-arching focus of this thesis is the negative effects of breast cancer radiotherapy on the heart.


1.2 Left-sided breast cancer radiotherapy

The five year survival statistic of Canadian breast cancer patients is above 80%; however, breast cancer still accounts for nearly 25% of the total yearly female cancer mortalities(15). Although this may seem counter-intuitive, it is explained by the large incidence rate of breast cancer; statistically, 1 in 4 females will be diagnosed with breast cancer in their lifetime(15). Although patients are responding well to treatment, the large volume of diagnosed patients leads to a significant number of mortalities even with cure rates at ~80%.

Breast cancer is typically treated with a combination of surgery, chemotherapy, and/or radiation therapy. The radiotherapy for breast cancer is most commonly delivered using a medical LINAC. The primary goal for breast cancer radiotherapy planning is to deliver
the clinically prescribed radiation dose (~50 Gy) to the entire breast or if following mastectomy, the entire chest wall. The radiation is typically delivered using two sequential radiation beams oriented such that they are tangential to the chest wall and opposed to each other, commonly referred to as tangential parallel-opposed or POP arrangement (see Figure 1-4). A secondary goal of radiotherapy planning is to avoid irradiating the surrounding healthy organs. However, this cannot always be achieved when organs are in close proximity to the treatment site.

Figure 1-4: A transverse computed tomography (CT) image of a left-sided breast cancer patient. The radiation dose distribution from treatment is overlaid onto the left breast. The entire heart, left ventricle only, and left anterior descending artery are contoured in magenta, yellow, and blue respectively. The typical orientation of the tangential parallel opposed beam arrangement is indicated by the red arrows.

For left-sided breast cancer patients, the lung, heart, and major coronary arteries tend to be co-irradiated due their close proximity to the anterior chest wall (Figure 1-4). A recently published systematic review reported the mean dose to the hearts of left-sided breast cancer patients to be 5.4 Gy and as high as ~8Gy if the treatment includes the internal mammary chain(16). Darby et al reported that the rate of a major coronary event
increased linearly by 7.4% per Gy with no apparent threshold below which there is no risk (17). The relative risk of cardiac toxicity due to radiation is also considerably amplified by the presence of pre-existent risk factors such as diabetes, smoking, and chemotherapy (17). Comparisons between left-sided and right sided breast cancer patients in terms of cardiac toxicity are essential in understanding the effects of radiation isolated from the concomitant factors such as chemotherapy and more conventional cardiac risk factors (see Figure 1-5). Correa et al, reported clinically significant heart disease developed in 59% of left-sided breast cancer patients compared to only 8% in right-sided breast cancer patients (18), 70% of which were in the left anterior descending artery (LAD) territory (predominately in the middle and distal segments).

![Figure 1-5: A comparison of left and right sided breast cancer patients regarding the presence of clinically significant coronary artery disease 10 -15 years following radiotherapy treatment. Proportions are obtained from Canadian Cancer Statistics, 2015 and Correa CR, et al. J. Clin. Oncol. 2007(18).](image)

The evidence for the harmful effects of radiation on the heart is supported by numerous studies looking at clinical endpoints such as cardiac mortality and/or myocardial infarction in cancer patients and atomic bomb survivors (14, 16, 17, 19–22). Irradiation of the heart can result in a myriad of known cardiac and vascular pathologies; however, the underlying mechanisms and progression are not yet well understood. The future management and prevention of radiation-induced cardiac toxicity will require close
collaboration between the departments of oncology and cardiology. As a result, a new ‘hybrid’ discipline, termed cardio-oncology is beginning to emerge(23).

1.3 Radiation induced cardiac toxicity: Clinical Endpoints

There are several known clinically significant side-effects to cardiac irradiation. A detailed review of the literature is provided by Gagliardi, et al (14). The most common clinical endpoints that have been associated with cardiac irradiation are; pericarditis, myocardial infarction, valvular disease, congestive heart failure, and coronary artery disease. Some of these endpoints also occur in a high proportion of older patients without cardiac irradiation, making conclusions difficult without the use of non-irradiated control groups. In general, trials comparing irradiated and non-irradiated subjects for the above endpoints have shown relative risks ranging from 1.2 to 3.5 for those treated with radiation (14).

1.3.1 Acute and chronic pericarditis

Pericarditis is inflammation within the thin membrane that encloses the heart, referred to as the pericardium. Patients with pericarditis often suffer from chest pain. Generally, the disease will clear up naturally within a few days to weeks, once the inflammatory process resolves, although the length of the active process will vary depending on the inciting stimulus for inflammation. In rare cases surgery is needed to prevent complications.

A longitudinal analysis report on the incidence of pericarditis in women treated with radiotherapy for breast cancer(22) showed a pericarditis relative incidence ratio of 1.61 between the left-sided vs right-sided breast cancer patients. It should be noted that patients included in the above study were treated in the late 70’s and followed for up to 30 years post-radiotherapy. Current methods for breast irradiation have improved significantly and as a result pericarditis is now an uncommon side effect of breast cancer radiotherapy. Acute pericarditis typically develops a few weeks following high exposure levels to the heart (≥ 36 Gy). These exposure levels are not typically observed in the breast cancer population but can sometimes occur for patients with tumours located within the mediastinum.
1.3.2 Myocardial infarction

Myocardial infarction or more commonly called a heart attack refers to damage or death to a portion of the myocardium. Myocardial damage and/or death are usually the result of an occluded coronary artery preventing the flow of blood to the myocardium.

The incidence ratio for acute myocardial infarction between left-sided and right-sided breast cancer patients is reported as 1.22, with 95% confidence interval between 1.06-1.42(22). As previously mentioned these incidence ratios may be inflated due to higher exposure levels to the heart observed using older treatment technology. Literature using more modern treatment methods are rare due to the latency (>15 years) for myocardial infarction to develop following irradiation. A study of >14,000 long-term childhood cancer survivors receiving radiotherapy (heart doses > 5Gy) have shown a hazard rate of 2.4 for myocardial infarction compared to matched non-irradiated cancer survivors(24).

1.3.3 Valvular Disease

Valvular disease following cardiac irradiation can lead to valve stenosis and/or regurgitation. Valve stenosis refers to a heart valve that does not fully open. This leads to increased load on the heart to pump blood through the narrow opening of the stenosed valve. Valve regurgitation refers to “leaky” valves that do not close properly causing additional strain on the heart to pump blood.

An incidence ratio 1.54 for valvular disease between left-sided and right-sided breast cancer patients has been previously reported(22). Radiation related valvular abnormalities are more commonly observed in Hodgkin’s lymphoma survivors(25) than in breast cancer survivors as Hodgkin’s patients will often receive high dose mediastinal radiation. Valvular disease can be expected following heart doses >25 Gy(26).

1.3.4 Congestive heart failure

Damage to the myocardium most commonly caused by myocardial infarction can lead to reduced ability for the heart to contract/relax; this is referred to as congestive heart failure.
An increased incidence of congestive heart failure (incidence ratio = 7.4) has been observed following cardiac irradiation during the treatment of Hodgkin’s lymphoma(27). Increased risk of congestive heart failure following breast cancer radiotherapy has only been shown for early treatment techniques between 1970 and 1986(28). Heart doses <7Gy do not appear to result in an increased risk of congestive heart failure(29).

1.3.5 Coronary artery disease

Coronary artery disease refers to significant blockage of the coronary arteries leading to a disruption in blood flow to the heart. The source of the blockage is typically referred to as an atherosclerotic plaque. The atherosclerotic process is still not fully understood, Libby, et al provide a comprehensive review of the topic(30).

Harris, et al, showed that left-sided breast cancer patients treated between 1977-1994 had a significantly greater risk of coronary artery disease than right-sided patients(31). Coronary artery disease is believed to be the predominant clinical endpoint following cardiac irradiation(32). Experimental studies provide evidence for two interesting hypotheses; 1) radiation causes an acceleration of the atherosclerotic process(33) and, 2) the resulting atherosclerotic plaques are of a phenotype that is more prone to rupture(33).

The most common theme in the clinical end-points discussed above is that the progression towards them is slow (> 10 years following irradiation). In order to study the progression of radiation induced cardiac toxicity in a timelier manner, sub-clinical endpoints in asymptomatic patients and/or animal models need to be used. In addition to improved understanding of the disease progression, identifying predictive sub-clinical endpoints may open a window for early interventions that can protect breast cancer patients from the life threatening side effects discussed above.

1.4 Radiation induced cardiac toxicity: Subclinical Endpoints

More subtle changes will surely precede the life threatening clinical endpoints mentioned in the previous section. In this thesis, subclinical endpoints refer to these subtle changes if they can be detected non-invasively in humans. Experimental studies in animal models
play a pivotal role in identifying subclinical endpoints that can be used to monitor and then potentially augment radiation treatment to reduce the risk of radiation induced cardiac toxicity. We begin with a brief introduction of the interaction of ionizing radiation on biological tissue; this area of study is commonly referred to as radiobiology. Our focus is not to provide a comprehensive review of radiobiology, but rather we aim to highlight areas most relevant to radiation induced cardiac toxicity. A more complete review of radiobiology is provided by Hall and Giaccia(34).

1.4.1 Radiobiology primer

The study of the effects of ionizing radiation on living organisms began almost immediately after Röntgen’s discovery of the x-ray in 1895. The biological effects observed following irradiation are primarily a result of DNA damage. Ionizing radiation can either directly or indirectly (through the genesis of highly reactive oxygen species) cause breaks in the DNA. Consequently, the cell’s natural repair mechanisms respond to the damaged DNA leading to 1 of 3 potential outcomes: 1) the cell successfully repairs the DNA; 2) the cell cannot repair the damage and responds by initiating apoptosis; 3) the cell incorrectly repairs the DNA resulting in a mutated living cell that may have an altered role within the body. This cascade provided the foundation for the fundamental law of radiobiology, “law of Bergonie and Tribondeau”(35). In general, the law designates organs/cells with low mitotic vs high mitotic activity to be considered radiation-resistant and radiation-sensitive, respectively. Additionally, the law states that radiation sensitivity is inversely proportional to the cell’s differentiation status. The heart and the blood vessels have been shown to be composed of highly differentiated cells with low mitotic activity. Annual cardiomyocyte turnover is less than 1% (36) in comparison to the intestines and lungs which have 100% turnover in just a few days and months, respectively (37). This is counter-intuitive considering the vast amount of epidemiological evidence presented previously with regards to the heart’s apparent sensitivity to radiation, although the clinical effects are generally not observed for several years. As such, the radiobiology of the heart and vasculature follow a less common and less understood cascade towards radiation injury.
1.4.2 Radiobiology of the vasculature

The vasculature, specifically the endothelial cells lining the lumen of blood vessels are considered highly differentiated cells with low mitotic activity. Evidence exists that radiation injury to cells does not just follow the typical DNA repair cascade but may also originate at the cell membrane. Radiation has been shown in vitro and in vivo to initiate the hydrolysis of the cell membrane resulting in cell signaling of apoptosis(38). The same apoptotic signal mediator has also been observed in the heart during myocardial ischemia(39). The effects of radiation on the cellular membrane often results in alterations to the attachment, morphology, and continuity of the endothelial cell monolayer lining the vasculature(40).

The majority of these alterations result in potential subclinical endpoints such as increased vascular permeability, fibrosis, and/or inflammation(41, 42). Changes in vascular permeability of the microcirculation can and has been previously assessed in breast cancer patients through the use of perfusion imaging. However, results from human studies using single photon emission computed tomography (SPECT) following breast cancer radiotherapy are inconclusive. Zellars et al, reports regions of increased and separate regions of decreased perfusion(43). Other studies have shown reductions in cardiac perfusion following radiotherapy in ~50% of left sided breast cancer patients(44, 45). Chung et al, did not observe any perfusion defects 6 months following left-sided breast radiotherapy(46). Lawrence et al reported that the incidence of new perfusion defects 6, 12, 18, and 24 months after radiotherapy was 27%, 29%, 38%, and 42%, respectively(47). Potential technical reasons for disagreement are presented in subsequent sections.

Irradiation of the vascular endothelium has been shown to activate transcription factors that lead to pro-inflammatory, pro-coagulation, and leukocyte adhesion (33, 48–51). These studies in mice have shown that focal cardiac irradiation leads to an acceleration of the atherosclerotic process with plaque phenotypes prone to rupture. Gabriels et al, have shown that high doses to the heart (>16 Gy) induce a cell signaling cascade towards fibrosis, whereas low doses (~2 Gy) trigger an initial “survival response”, encouraging cellular growth, development, and proliferation(49). In mice, increases in endothelial cell
surface markers related to inflammation and proliferation can remain elevated up to 20 weeks post irradiation (49, 52). Through the use of nuclear imaging, myocardial fibrosis and inflammation can also be assessed non-invasively in breast cancer patients following radiotherapy. However, to the best of our knowledge it has not yet been attempted.

In light of the findings above, macrovascular and microvascular functional imaging is a logical target for monitoring the progression of radiation induced cardiac toxicity in humans. Vascular imaging may also play a role in assessing the efficacy of advanced radiation delivery strategies used to mitigate cardiac toxicity.

1.5 Advanced radiation delivery strategies for mitigation

1.5.1 Standard breast cancer radiotherapy

The commonly used setup prior to irradiation is as follows; breast cancer patients are typically set-up in the supine position with arms extended above the head. A breast board is used for patient immobilization. Radio-opaque markers are typically used to help identify clinical boundaries of the targeted breast volume. The typical prescription dose is 50 Gy in 25 fractions. Beam energies are typically between 4 – 10 MV; however, mixed beams, beams with higher energies, and local boosts can be used to ensure sufficient target coverage and minimize the incidence of local recurrence.

The tangential parallel opposed technique for breast irradiation begins with a free-breathing computed tomography scan that is used for treatment simulation. Tangential beams are used to target the breast volume and the beams are angled to minimize the divergence of the beam into the heart. A recently published meta-analysis showed that the mean heart dose using standard tangential fields can vary considerably (2.5 - 11.18 Gy)(53).

Additionally, intensity modulated radiation therapy (IMRT) can be used for treatment. IMRT beams vary in intensity to produce non-uniform fluency, allowing for greater conformity to target structures while reducing the dose to specified avoidance structures. For more degrees of freedom, IMRT can also be delivered in an arc, either with volumetric modulated arc therapy (VMAT) or with Tomotherapy. The previously
mentioned meta-analysis showed that mean heart doses using IMRT can also vary considerably (1.1 - 13.4 Gy)(53).

Several modifications and alternatives to the traditional tangential parallel opposed beam arrangement have been proposed to reduce the amount of heart exposure during breast radiotherapy. The modifications primarily employ respiratory motion management strategies to minimize heart dose during radiotherapy. Additional, proposed alternatives to the standard treatment are brachytherapy, proton radiotherapy, and treating patients in the prone position. Currently there has not been any consensus regarding the “best approach”. The reasons for the lack of consensus and other key findings from the published literature are summarized below.

1.5.2 Respiratory motion management during radiotherapy

Modern day LINACs can be automatically triggered such that the beam of radiation is only delivered at specific portions of a patient's breathing cycle. The three most commonly used respiratory triggers are abdominal strain gauges, video tracking of an external marker, and spirometry(54). The abdominal strain gauge, commonly referred to as the bellows system (Philips Medical Systems, Cleveland, USA) is tightly fastened to a patient’s abdomen, below the diaphragm. A patient’s respiratory trace is obtained by measuring changes in the pressure exerted by the breathing induced motion of diaphragm against the bellows system. Another approach is video monitoring of an external marker secured to the patient’s abdomen. This system is commercially referred to as the real-time position management system (Varian RPM system, Varian Medical Systems, Palo Alto, USA). The motion of the external marker follows the same principle described for the bellows device. The third approach uses a spirometer to obtain a respiratory signal by measuring the volume of air entering and exiting the lungs. This system is commonly referred to as active breathing control (ABC). These systems are commonly used for triggering the LINAC to specific parts of a patient’s breathing cycle. They are also used to reduce respiratory motion artifacts in tomographic imaging, specifically for lung cancer patients. It should be noted that these signals are only surrogates of internal organ motion and require validation as deviations have been reported (55). The application of these systems regarding reducing heart exposure during radiotherapy is principled in that
the heart is typically furthest from the breast during the end of inspiration. By triggering
the beam on at the end of inspiration, the added separation between the breast and heart
leads to a significant reduction in cardiac exposure.

1.5.3 Inspiratory gating and breath-holds during left-sided breast radiotherapy

The use of breath-holds during treatment, specifically deep-inspiration breath-holds have
been shown to significantly reduce the mean heart dose in breast cancer patients by 40%
(56). With regards to the deep inspiration breath-hold techniques, concern exists with
regards to compliance and inefficiency in terms of time needed to train patients prior to
treatment. In light of this, Korreman et al, proposed the use of inspiratory gating while
the patient is freely breathing to avoid the need for patient training (57). Their study has
shown that the dose reduction to the heart and LAD is not statistically different between
deep-inspiration breath-holds vs inspiratory gating during free-breathing. It should be
noted that both breath-hold and gated methods do not account for the contractile motion
of the heart itself. Wang, et al has shown that this motion may be negligible for the heart
but not for the LAD (58). To account for cardiac motion, their study suggests that
treatment plans should maintain at least a 5 mm margin between the posterior edge of the
radiation field and the LAD.

The use of gating and breath-holds consistently results in reduced mean heart dose;
however, a recent clinical trial unexpectedly reported a worsening in cardiac perfusion in
the apex of the left ventricular compared to standard treatment(43). The authors of the
study suggest that although the motion management approaches reduce mean heart dose,
they may cause a repositioning of the heart such that critical sub-structures such as the
LAD may receive higher doses. Thus, although the mean heart dose is often cited, it
may not be an appropriate metric for the assessment of these treatment strategies. Several
studies suggest that doses to the LAD may be more appropriate (18, 59) for predicting
cardiac toxicity. Currently there is no clinical consensus as to a means of stratifying
patients most likely to benefit from gating/breath-hold treatments.
1.5.4 Brachytherapy for the treatment of breast cancer

Brachytherapy refers to a type of radiation treatment in which a source of radiation is placed near or within the area being treated. Brachytherapy has the benefit of being able to deliver high doses of radiation with very rapid fall-off in dose to tissues not immediately adjacent to the source. Placement of the radioactive sources is performed in the operating room with the aid of image guidance. The sources are typically “after-loaded”, meaning that a catheter is first placed at the target position then the sources are automatically advanced through the catheter and loaded into position. The dose distribution for a given source can be altered by changing their positions within the target and/or changing the duration they are loaded (referred to as dwell time). Several brachytherapy approaches have been proposed for early stage breast cancer patients (60–63); however, the most promising results are for the multi-catheter interstitial brachytherapy approach(62, 63). In comparison to standard radiotherapy, brachytherapy can significantly reduce both the maximum dose and the total volume of the heart that is irradiated during treatment(62). More importantly, this reduction in dose to the heart can be achieved without compromises in local control/rate recurrence at least 5-years post treatment(63). The use of brachytherapy for the treatment of early stage breast cancer is commonly referred to as accelerated partial breast irradiation. In addition to the reasons mentioned above, accelerated partial breast irradiation is an attractive alternative to the standard treatment since it can shorten the course of treatment from 3-7 weeks to 2-5 days. Unfortunately, this technique has only shown comparable control rates for early stage breast cancer patients with no lymph node involvement (stages 0, I, and IIa) (63). Accelerated partial breast irradiation can also be delivered intra-operatively (61); however this approach was shown to result in higher proportion of recurrence then the standard technique. Additionally, stereotactic external beam radiotherapy has been used as an attractive alternative for the delivery of accelerated partial breast irradiation as this approach is non-invasive and significantly easier to implement clinically (64). This approach has shown comparable local control to the standard (1.5% recurrence at 5 years); however poor cosmetic outcomes are typically reported with external beam radiotherapy.
1.5.5 Breast cancer treatment in the prone position

Another proposed approach for reducing cardiac irradiation during breast cancer treatment is to treat the patient lying prone on the treatment couch (65–67). With the patient lying in the prone position, more separation can be achieved between the heart and the breast. Kirby, et al has shown that significant reductions in dose to the LAD (mean dose reduction ~6 Gy; max dose reduction ~30 Gy) can be achieved(66). However, generally these reductions are only observed in patients with larger breast volume (>1000 cm³). Furthermore, treatment in the prone position was shown to be detrimental in women with smaller breast volume since in the prone position the separation between the heart and the chest wall is reduced. Conversely, Lymberis, et al reported that most women benefited from the prone treatment position regardless of breast size; however, doses to the LAD were not reported in this study(65). Further study is needed to provide guidelines for ensuring reproducible day to day patient setup prior to widespread adoption in the clinic.

1.5.6 Breast cancer treatment using proton radiotherapy

External beam radiotherapy is typically carried out with LINACs that produce either x-rays or electrons; however, there is considerable interest in the use of heavier particles such as protons for breast cancer radiotherapy in order to reduce the dose to the heart(68–71). Galland-Girodet et al, reported 0 Gy mean dose and only 4 Gy max dose to the heart using protons for accelerated partial breast irradiation (72). Proton radiotherapy is capable of reducing dose deposition to healthy tissues situated distal to the target due to their superior dose deposition characteristics in comparison to photons and electrons. However, similar to photon radiotherapy, high skin dose and poor cosmetic outcomes are often reported (72). The radiotherapy comparative effectiveness trial (RADCOMP) has been funded to compare the effectiveness of proton vs photon radiotherapy for breast patients (NCT02603341). Identifying whether or not protons can reduce cardiac mortality after breast cancer is one of the RADCOMP trial’s primary goals. Proton therapy is largely limited by the considerable amount of capital needed to create a medical proton accelerator. Thus only a few institutions have them for clinical use. Although upfront capital costs are high, reported estimates of the cost per treatment using protons shows
only a 5% increase in comparison to the cost of a 6 week course of standard tangential whole breast irradiation(68).

Currently there is no consensus with regards to how a center should identify which left-sided breast cancer patients are most likely to benefit from these advanced techniques which are generally more resource intensive and time consuming that the standard treatment. Additionally, long-term assessment of these novel strategies is still needed. Long-term follow-up studies are currently in place and are most ideal since radiation induced cardiac toxicity is a latent side-effect (typically 10-15 years post radiation). However, with the result from future studies pending, diagnostic and functional cardiac imaging has been suggested to monitor the evolution and progression of radiation induced cardiac toxicity at earlier time points(73, 74).

1.6 Advanced cardiac imaging of radiation-induced injury

The potential effects of radiation on the heart can be imaged non-invasively through echocardiography, computed tomography (CT), nuclear tomographic imaging, and magnetic resonance imaging (MRI), each with relative strengths in assessing different parameters of radiation-induced injury. Their previous and potential role for studying radiation-induced cardiac toxicity is discussed below.

1.6.1 Echocardiography

Echocardiography can produce both 2-dimensional and 3-dimensional images of the heart. In general, images are produced after an ultrasonic pulse is emitted from an ultrasonic transducer through the specimen to be imaged. Ultrasonic echoes produced at the interfaces between different tissue types are acquired by the transducer and used for image reconstruction. In addition to images, the Doppler Effect can be utilized to obtain functional information such as blood flow and regional strain. Fenster, et al provides a more detailed and technical review of ultrasound imaging(75).

Echocardiography has shown decreased myocardial systolic deformation (strain) in the left sided breast cancer patients as early as 2 months and up to 14 months post irradiation(76, 77). Regional changes were observed compared to baseline measures;
however, they were not statistically correlated with regional dose. Unfortunately, the authors did not provide a patho-physiological mechanism for the observed reduction in myocardial strain. The overall clinical consequences of reductions in myocardial strain due to irradiation require further investigation.

### 1.6.2 Computed Tomography (CT)

Computed tomography imaging can provide high resolution cardiac images (~1mm isotropic voxels) and anatomic information such as the extent of coronary stenosis and functional information such as regional myocardial perfusion. Additionally, CT images can be used to accurately calculate doses from radiotherapy. CT based dose calculations are possible because the image signal intensity is directly related to tissue electron density (78). CT images are acquired by detecting the transmission of kilovoltage (kV) photons as they pass through a sample. Rotating the photon source and detector around the specimen gives multiple projections that can be later reconstructed into a 2-dimensional (2D) image. A 3-dimensional (3D) volume can be obtained by longitudinally advancing the couch of the CT scanner while acquiring projections, commonly referred to as helical CT. Each set of projections is reconstructed into an image “slice” through the sample. For those interested, Johns and Cunningham provide a more comprehensive description of CT imaging (79).

Cardiac CT studies of radiation induced cardiac toxicity are limited to microCT studies in mice (80) and Hodgkins survivors (81, 82). For left-sided breast cancer patients, the role of CT has been primarily reserved for quantifying the delivered dose to the heart and the LAD following standard and more advanced radiotherapy techniques (83, 84).

Calculating the dose to heart, left ventricle, and LAD from standard helical CT imaging is challenging since this technique does not capture the motion of the heart or the LAD. Standard helical CT scanners used for breast radiotherapy planning do not have the temporal resolution to acquire artifact free, real-time images of internal organ motion due to respiration. This also poses a major challenge for treating tumor sites subject to respiratory motion such as the lung or liver. This problem is typically addressed through the use of a longer duration helical CT scan in which the pitch of the couch is slowed
such that multiple images can be acquired at the same slice position. Through the use of a breathing motion surrogate, the images can be retrospectively sorted to specific portions of the breathing cycle. This technique is commonly referred to as 4D-CT imaging (85, 86). However, 4D-CT is not part of the current standard of care for breast cancer patients and only few studies have partially examined its utility for assessing cardiac dose associated with breast radiotherapy (83, 84, 87, 88). The use of 4D-CT imaging also allows for the estimation of dose distributions that have been “blurred/warped” in accordance to the motion of the internal anatomy. This warping of dose is commonly referred to as 4D dose accumulation (89). Unfortunately, adoption of 4D dose accumulation in clinics today is primarily hindered by challenges associated with proper validation of the accuracy of the dose deformation (90).

In comparison to all the other imaging modalities, CT is the most widely available and extensively used in day to day cancer radiotherapy. Thus the emergence of functional cardiac CT perfusion may have a key role in future radiation induced cardiac toxicity trials (91). Quantitative cardiac CT perfusion has been recently validated using microspheres in porcine model of myocardial infaction (92). In comparison to gold-standard measurement (microspheres), CT perfusion is strongly correlated with the gold-standard measurement (R = 0.81), however, the method tends to overestimate perfusion by 20-25% due to beam hardening artifact caused by the contrast agent within the LV.

1.6.3 Nuclear tomographic imaging

Nuclear tomographic imaging has been used extensively to study cardiac pathology and in the routine clinical management of cardiovascular disease (93). In general, nuclear imaging is performed after a radio-isotope is injected into or inhaled by the patient. The radio-isotopes used in nuclear imaging are commonly referred to as radio-tracers. Different tracers tend to be specific to different biochemical pathways that occur within the body. The emitted radio-activity from the tracers can be detected by the imaging system after and/or during its distribution within the body. Tomographic image reconstruction algorithms are used to convert the emitted signal from within the body into images. The pixel intensity of the reconstructed images is proportional to the radio-tracer concentration in that region. The two most commonly used nuclear imaging systems are
single photon emission computed tomography (SPECT) and positron emission tomography (PET). There are many differences between SPECT and PET imaging systems, however, many of these differences are initially rooted in the fact that they use different types of radio-isotopes. In SPECT, the radio-isotopes are gamma-emitters; in PET, the radio-isotopes are positron emitters. A comprehensive description of these techniques is provided by Cherry, et al (94). To date, the use of nuclear imaging for the assessment of radiation-induced cardiac toxicity has been limited to SPECT myocardial perfusion, ejection fraction, and multigated acquisition studies.

1.6.3.1 SPECT imaging of radiation-induced cardiac toxicity

SPECT myocardial perfusion imaging is typically performed using thallium (\(^{201}\)Tl) labelled or technetium (\(^{99m}\)Tc) labelled radio-tracers (95). Both of these tracers become metabolically trapped within the myocardium and their distribution within the heart is proportional to the regional myocardial blood flow. Thallium is an analog to potassium and is actively transported into cells through the sodium potassium pump. Unlike \(^{201}\)Tl, \(^{99m}\)Tc does not have a natural biochemical affinity for the myocardium. For this reason, \(^{99m}\)Tc is bound to a molecule with some cardiac affinity (typically sestamibi or tetrofosmin). Sestamibi and tetrofosmin are positively charged molecules that enter the intracellular space via passive transport and accumulate in the mitochondria of cardiac cells(96).

Numerous studies have reported myocardial perfusion defects following left-sided breast cancer radiotherapy(18, 43, 45–47, 59, 97–99). An often cited finding was the ability to stratify patients most likely to present with perfusion defects by the volume of the heart irradiated with 25 Gy or more (47). Marks et al, studied a cohort of 114 patients treated between 1998 -2001. The patients were followed using SPECT perfusion imaging between 6 to 24 months post radiotherapy. In this study, new perfusion defects occurred in more than half of left-sided breast cancer patients with more than 5% of the heart irradiated by 25 Gy or more(47). These defects were typically observed 1-2 years post radiotherapy, however, in a smaller proportion of patients (< 30%), defects as early as 6 months post irradiation were observed. The perfusion defects tend to be isolated to myocardial regions irradiated by at least 25 Gy. For left-sided breast cancer patients, this
tends to be the area of the heart supplied by the LAD (59). Similar findings are echoed in many of the other SPECT studies, however, not all studies observed cardiac perfusion defects. Sioka et al, compared 28 left and 18 right sided breast cancer patient treated between 1998 – 2010 using SPECT with a median follow-up of 40 months (45). Although the study found a difference between age-matched controls vs breast cancer patients, no differences were observed between left and right-sided patients. This lack of difference may be attributed to the small number of patients in the comparison. A recent study of 32 left-sided breast cancer patients with pre and 1 year follow-up SPECT, did not show any significant differences in perfusion or ejection fraction (46). The discrepancy between the findings from this study and the previous studies may be attributed to the lower mean heart dose or the shorter follow-up (1 year, 2.8 Gy (46) vs 40 months, ~7.7 Gy(45)). It appears that at the lower heart doses observed using modern radiotherapy techniques, SPECT cardiac imaging studies cannot identify a relationship between dose and defects until 3-6 years following treatment (100). This delay impedes our ability to understand the progression of the disease in humans and also requires lengthy studies to assess the benefits of newer treatment strategies and/or techniques.

Currently, SPECT cardiac imaging for the detection of perfusion defects is either assessed qualitatively and/or semi-quantitatively. The use of tracer kinetic modelling and dynamic SPECT imaging is currently an active area of research and can potentially allow for earlier assessment of radiation-induced perfusion defects(101). Although positron emission tomography is not as widely available as SPECT, the implementation of absolute cardiac perfusion quantification is well-established.

1.6.3.2 Cardiac PET imaging

To the best of our knowledge, PET has not been previously used to assess perfusion changes following cardiac irradiation. The use of PET has advantages over SPECT due to its ability to provide the quantification of myocardial perfusion in absolute units of ml/min/g. Perfusion quantification requires quick and accurate measures of the radiotracer concentration within the myocardium. The temporal resolution of PET is typically higher in comparison to conventional SPECT systems due to better count sensitivity. Additionally, the temporal resolution in conventional SPECT systems are limited by the
need to rotate the detectors around the patient. However, newer state of the art SPECT systems can have temporal resolution of ~ 10s, which is similar to PET (102). In terms of accuracy in quantifying tracer concentration, SPECT is limited by the ability to correct for photon attenuation and scatter. Although SPECT attenuation and scatter correction techniques have been validated, they are not as well established as PET attenuation correction methods. Furthermore, both imaging modalities are limited by their poor spatial resolution. This limitation currently hinders the ability to detect non-transmural cardiac injury.

Attenuation refers to the reduction in the intensity of the photons as they traverse the body towards the PET or SPECT detectors. To correct for this loss in intensity, the distance travelled by the photons and the probability of interaction across that same path, commonly referred to as the linear attenuation coefficients are needed. This information can be approximated using CT as the signal intensity in CT is known to be directly proportional to the linear attenuation coefficient. Estimating the attenuation path length travelled by the photons in PET is more straightforward than SPECT.

In SPECT, the nuclear decay of the radio-tracer will result in a single photon emission. However, in PET, the nuclear decay results in the emission of a positron. Upon emission, the positron will become annihilated following collision with an electron. The annihilation of the electron and positron releases two mono-energetic photons travelling in directly opposite directions. PET takes advantage of this process by monitoring the presence of a pair of coincidental detector events. The line traced out between two PET detectors sharing a coincidental event gives an approximation of the total distance travelled by the photons. Since these coincident events do not occur in SPECT, the distance travelled by the photons must be estimated through more complicated approaches described elsewhere (103). Assuming these and other corrections can be made such that the signal is a good approximation to the tracer concentration, tracer kinetic modeling can be used to quantify absolute measures of myocardial perfusion.
1.6.3.3 Tracer kinetic modelling for myocardial perfusion

Three main PET tracers have been utilized for myocardial perfusion quantification; 1) $^{15}$O labelled water, $^{13}$N labelled ammonia, and $^{82}$Rubidium (104–106). Although multiple tracer kinetic models exist for each of these perfusion tracers; our discussion will be limited to the 1-compartment model for $^{13}$N labeled ammonia since this model is used specifically in Chapters 3 and 4 of this thesis (107).

Several key assumptions are needed to simplify the process of estimating perfusion. The first and most important assumption is that the time-varying radio-tracer concentration within the vessel (intravascular space) and outside the vessel (extravascular compartment) can be measured accurately. Additionally, it is assumed that the contrast agent is well-mixed within these compartments such that the concentration is uniform throughout. Figure 1-6 shows a visual representation of the 1-compartment model used for the estimation of $^{13}$N-ammonia perfusion. The single compartment represents a volume of myocardial tissue surrounding the capillaries.

Figure 1-6: Visual representation of the one-compartment model. $K_1$ represents the rate of tracer accumulation within the extravascular compartment, measured in units of ml/min/g. The rate at which the tracer washes out of the compartment is defined as $K_2$, measured in units of min$^{-1}$.

The rate of tracer accumulation within the extravascular compartment is defined as $K_1$, measured in units of ml/min/g. The rate at which the tracer washes out of the compartment is defined as $K_2$, measured in units of min$^{-1}$. The rate of change of tracer concentration within this compartment is described by the following differential equation:
\[
\frac{dc_{tissue}(t)}{dt} = \rho K_1 c_{blood}(t) - K_2 c_{tissue}(t) \tag{1-1}
\]

where \(c_{tissue}\), \(c_{blood}\), and \(\rho\) represent the radio-tracer concentration in the tissue (extravascular compartment), concentration in the blood (intravascular space), and the tissue density respectively. A worked solution to equation 1-1 is provided in Appendix B. The quantities \(c_{tissue}\) and \(c_{blood}\), are measured; the quantities \(K_1\) and \(K_2\) are fitted parameters typically obtained from a least-squares regression algorithm. The quantities \(c_{tissue}\) and \(c_{blood}\) are obtained from calculating the mean pixel intensities within regions of interest in the myocardial tissue and left-ventricular cavity (see Figure 1-7). If the percentage of tracer removed from the blood to the tissue is known, commonly referred to as the extraction fraction; the perfusion can then be calculated from \(K_1\) using equation 2 as originally described by Renkin and Crone (108, 109):

\[
K_1 = E \ast F \tag{1-2}
\]

where, \(E\) and \(F\) represent the tracer extraction fraction and perfusion respectively.

Although PET and SPECT can target many specific patho-physiological processes, myocardial SPECT perfusion has been the most interrogated sub-clinical end-point in radiation induced cardiac toxicity studies. Other sub-clinical end-points have been observed in mice following cardiac irradiation such as an increase in pro-inflammatory cell signals(51, 52). Cardiac inflammation can be imaged using PET in humans and may potentially be a novel sub-clinical marker of radiation-induced cardiac toxicity(110, 111).

1.6.3.4 Cardiac PET imaging of myocardial inflammation

The utility of cardiac PET for imaging the progression of myocardial inflammation following cardiac irradiation is a major component of the third and fourth chapters of this thesis. Here we provide a brief summary of this advanced imaging technique.

Cardiac inflammation can be imaged using a very common PET radio-tracer, fluorodeoxyglucose (\(^{18}\)F-FDG). \(^{18}\)F-FDG is a glucose analog that enters cells in proportion to the rate of glucose metabolism and is trapped in proportion to the rate of
glucose phosphorulation. Many different cells within the body, such as inflammatory cells and cardiomyocytes regularly metabolize glucose. Since glucose uptake is present in both inflammatory cells and cardiomyocytes, distinguishing these two tissues is not possible with $^{18}$F-FDG unless glucose metabolism can be suppressed in one cell type and not the other.

Figure 1-7: Dynamic $^{13}$N-ammonia PET perfusion images that have been overlaid on cardiac MRI for anatomical context. The standard uptake value (SUV) of $^{13}$N-ammonia is quantified in regions of interest within the myocardial left ventricular tissue and blood indicated by purple and yellow rings respectively. These regions of interest act as measured surrogates for the radio-tracer concentration in the tissue (extravascular compartment) and the concentration in the blood (intravascular space) used in the 1-compartment kinetic model.
Unlike inflammatory cells, normal cardiomyocytes favour fatty acid metabolism. Taking advantage of the heart’s preferred utilization of free fatty acids allows us to suppress normal $^{18}$F-FDG uptake by cardiomyocytes. This is achieved through fasting (12-18 hours) followed by lipid administration (via fatty diet or lipid injection) prior to PET imaging (110, 112). Prolonged fasting is used to lower blood insulin and blood glucose levels, causing the heart to switch to fatty acid metabolism. The administration of lipids prior to PET imaging is to provide the “starved” heart with sufficient metabolites to further minimize the heart’s need for glucose and consequently minimizing the uptake of $^{18}$F-FDG. Under these conditions, observed $^{18}$F-FDG uptake in the region of the heart is thought to be related to the metabolism of inflammatory cells and not the cardiomyocytes. Figure 1-8 shows an $^{18}$F-FDG cardiac PET image of the same subject with and without myocardial glucose suppression. Adequate glucose suppression is not always achieved. The results from previous randomized trial indicated that under an optimized protocol suppression can be expected in ~88% of healthy human patients(113).

This approach has been previously used to assess cardiac inflammation associated with non-radiation induced cardiomyopathies such as post myocardial infarction (111) and cardiac sarcoidosis (110). $^{18}$F-FDG PET has been previously used as a non-invasive means to study RIHD after stereotactic lung (114) and esophageal (115, 116) radiotherapy. However, to the best of our knowledge cardiac PET inflammation imaging has not been previously utilized to assess the progression of inflammation related to radiation-induced cardiac toxicity using doses relevant to the breast cancer population.

1.6.4 Magnetic resonance imaging (MRI)

The technological advancements in the last 10-15 years have placed MRI at the forefront of cardiac imaging. Cardiac MRI has a myriad of capabilities such as imaging of myocardial edema, coronary stenosis, myocardial perfusion, fibrosis, and valvular disease. During a single examination, MRI can provide a comprehensive cardiac work-up without delivering ionizing radiation to patients, making it an excellent candidate for longitudinal follow-up studies.
Figure 1-8: Transverse and sagittal image slices of the 18F-FDG standard uptake value (SUV) with (upper panels) and without (lower panels) the cardiac glucose suppression protocol.

MRI is used to produce images that highlight differences in the 1 or more intrinsic magnetic properties of different tissues within the body. In general, an image is produced after a sample is magnetized within a large uniform magnetic field. Once magnetization is complete, tissue types with differing magnetic properties cannot be distinguished from each other until after the next step, commonly referred to as excitation. The excitation step typically consists of interrogating the sample with a short radio-frequency pulse that temporarily “knocks” all the tissue types away from the original magnetized state. After the excitation is complete, the different tissue types return back to the original magnetized state. However, the rate of this return will vary for different tissue types with different magnetic properties. This return to the original magnetized state is commonly referred to as relaxation. Different tissue types tend to have different relaxation rates. This is the key physical distinction between tissues that allows MRI images to possess excellent soft-tissue contrast. The final step is a carefully timed read-out of signal that is produced during relaxation. Timing of the read-out is crucial since it is used to maximize
the contrast between the different tissue types prior to complete relaxation. This process of magnetization, excitation, relaxation, and read-out can be repeated until the desired image quality is achieved. Brown et al provide a more comprehensive discussion of MRI(117).

To the best of our knowledge, only a single full length article has been published where cardiac MRI is used for the study of radiation induced cardiac toxicity in the left-sided breast cancer population(118). Heggemann, et al performed cardiac MRIs of 49 left-sided breast cancer patients at baseline, 6 months, 12 months, and 24 months following adjuvant treatment. Temporary decreases in the contractile function of the heart that resolved 24 months post radiation were observed. No evidence of fibrosis was presented; fibrosis was assessed qualitatively through late gadolinium MRI contrast enhancement. As stated by the authors, a major limitation of their study is low patient accrual due to the extended duration of each imaging session. Numerous functional parameters can be imaged with MRI and the lack of knowledge with regards to radiation-induced cardiac disease makes it difficult to tailor a protocol to detect radiation induced cardiac toxicity. Controlled large animal studies using clinically approved MRI sequences may be beneficial towards the future implementation of MRI imaging protocols with an aim to monitor the progression of radiation induced cardiac toxicity in humans.

For clarity, the information provided in this section is not intended to motivate the use of MRI in the current thesis. The capabilities of MRI for assessing cardiac function were not utilized in this thesis; however, future work may include an investigation of cardiac MRI on the same subjects to assess RIHD.

1.6.5 Hybrid imaging techniques

Each of the imaging modalities discussed above have their inherent strengths and limitations. Often times the strengths of one imaging method tends to be a weakness of the other and vice versa. This leads to an inevitable compromise when choosing which imaging modality is optimal. This problem has motivated the development of many hybrid imaging systems in which two modalities are combined into a single system(119). Arguably the most successful “marriage” between two imaging modalities was the
combination nuclear emission tomography (SPECT or PET) with computed tomography (CT). The successful combination of SPECT with CT and PET with CT has led to a single imaging system with the ability to provide functional information such as perfusion and cellular proliferation overlaid on anatomical cross-sections for context. Additionally, attenuation correction obtained from the CT has significantly improved image quality of SPECT and PET. Today virtually all commercially manufactured PET systems come with a CT. Due to the success of these systems the commercial development of rival PET-MRI and SPECT-MRI hybrid systems has emerged. Unlike CT which is predominately used for anatomical imaging, MRI is capable of both functional and anatomical imaging.

The complementary strengths of PET and MRI are shown in Figure 1-9. In the context of this thesis, PET-MRI represents an attractive approach to understanding and monitoring the progression of radiation-induced cardiac toxicity. As previously mentioned, preclinical studies in mice have shown evidence of inflammation, changes in myocardial perfusion, and transient increases in cellular proliferation(52). In humans, typically one and at most two of the above functional parameters can be assessed in a single session using PET-CT or SPECT-CT. Unlike PET-CT and SPECT-CT, PET-MRI acquires images in true simultaneous fashion; therefore it has the potential to acquire more functional information regarding the heart in a shorter imaging session. Most of the hardware compatibility issues between the PET and MRI appear to have been sorted out(120). However, the biggest limitation of PET-MRI is with regards to attenuation correction for the PET images. Unlike CT, the MRI signal is not related to the linear attenuation coefficients of matter. For example, although bone and lung are quite different in terms of their ability to attenuate photons, they’re highly indistinguishable in the majority of MRI pulse sequences. Due to the importance of this problem, MRI attenuation correction has become a highly active area of research(121–123).

A commonly used approach for attenuation correction in MRI is to segment anatomical areas corresponding to lung, fat, and tissue using a specialized MRI pulse sequence (124). This is followed by a substitution of the original pixel intensities with discrete attenuation coefficient values that are typically observed in lung, fat, and tissue. Validation against
PET-CT attenuation correction for cardiac PET-MRI imaging is supported in the literature(121). Although we are optimistic and excited with regards to this novel imaging platform, a significant amount of research is needed to understand the ultimate role of PET-MRI in cardio-oncology.

Figure 1-9: Radar diagrams of the relative strengths and weaknesses of MRI, PET, and hybrid PET-MRI. This figure is adapted with permission from Shah NJ, et al. J. Magn. Reson. 2013;229:101–115.
1.7 Gaps in knowledge, challenges, questions, and hypotheses

Cardiac side effects in left-sided breast cancer patients after post-operative radiotherapy has become one of the most debated issues in radiation oncology (20, 125, 126). This is because the number of patients at risk is large and many are young. The increased rate of a major coronary event in breast patients is high, 1.32 relative risk of a major coronary event in comparison to age-matched controls (17). The relative risks are even greater in cohorts with pre-existing risk factors such as smoking (1.87) and diabetes (3.23) (17). Fortunately, approaches such as active breathing control, can reduce the mean dose to the heart during treatment. However, the benefits of these approaches and the means in which they are assessed have been recently put into question (43, 126). The widespread clinical adoption of active breathing control and other similar techniques aimed at reducing mean heart doses during radiotherapy are directly impacted by the following problems/challenges.

1. Guidelines for radiotherapy planning left-sided breast cancer are needed to minimize the risk of radiation-induced cardiac toxicity. Currently, such a guideline does not exist since the true relationship between cardiac dose and cardiac toxicity remains uncertain. The risk of a major coronary event is often cited to increase by 7.4% per Gy to the heart (17). However, due to inherent difficulties in estimating the dose to the heart, the uncertainty in this value is likely even higher than what is reported (95 % confidence interval, 2.9% to 14.5%). Thus, further work is needed to provide more accurate assessment of dose to the heart and sub-structures such as the coronary arteries.

2. Since cardiac toxicity following breast radiotherapy is a latent side-effect of treatment (~10-15 years post treatment), recently proposed strategies to mitigate this effect cannot be assessed in a timely manner with current approaches.

The following questions have been formulated with the aim of helping to guide decisions with regards to radiotherapy planning and delivery that can minimize radiation-induced cardiac toxicity:
1. Does respiratory motion impact left-side breast cancer radiotherapy planning in terms of the accuracy of estimating doses to the heart, left-ventricle, and LAD?  
2. Can the effects of heart, left ventricular, and LAD irradiation on myocardial perfusion and inflammation be visualized using PET at radiation dose levels comparable to modern left-sided breast radiotherapy?  
3. Can the effects of cardiac irradiation be visualized using PET at earlier time points in comparison to other previously used imaging modalities?  
4. Is there a spatial relationship between the magnitude of dose and regional changes in cardiac inflammation?  
5. Is there a spatial relationship between the magnitude of dose and regional changes in cardiac perfusion?  
6. Are specific regions within the heart such as the arteries more sensitive to radiation effects than others?  

The answers to the above questions will be instrumental in the design of future clinical studies looking to properly assess strategies for mitigating radiation induced cardiac toxicity following radiotherapy. Lastly, we present two hypotheses: 1) regarding radiation treatment planning of left-sided breast cancer and 2) regarding the assessment cardiac responses to radiation treatment:  

*A significant variation in dose across heart structures is attributable to respiratory-induced motion during left-breast cancer radiotherapy.*  

*A reduction in left ventricular perfusion and an enhancement in inflammation is related to local radiation dose levels within the heart.*  

### 1.8 Thesis outline  

1.8.1 Radiation dose uncertainty associated with motion of the heart, left ventricle, and left anterior descending artery during left-sided breast radiotherapy (Chapter 2)  

The second chapter of this thesis is adapted from an original research article, “Assessment of Intrafraction Breathing Motion on Left Anterior Descending Artery Dose

In this chapter doses to heart, left ventricle, and LAD were calculated for 30 left-sided breast cancer patients receiving radiation therapy. Radiation dose was calculated using the clinical standard free breathing CT scans that do not account for respiratory motion and was compared to calculations where respiratory motion was accounted for. The impact of respiratory motion on heart dose was assessed through 4D-CT imaging and 4D dose accumulation. The findings show that the clinical standard is appropriate for measuring doses to the heart and left ventricle; however, a clinically significant discrepancy exists for the LAD. Preclinical studies have suggested that the dose to the artery may be of equal or greater concern than the dose to the heart. Thus, the results from this study will aid future studies that aim to correlate artery dose to radiation-induced cardiac toxicity. Additionally, to the best of our knowledge this is the largest left-sided breast cancer patient study assessing dose to the heart, left ventricle, and LAD that uses both modern day radiotherapy techniques and accounts for respiratory motion. Results from this study are used in Chapters 3 and Chapter 4 to aid in the design of a canine model of cardiac exposures from left-sided breast radiotherapy.

1.8.2 Early $^{18}$F-FDG and $^{13}$N-ammonia cardiac PET imaging in a canine model following cardiac exposures associated with breast cancer radiotherapy (Chapter 3)

The third chapter of this thesis is adapted from another original research article, “$^{18}$F-FDG and $^{13}$N-ammonia Cardiac PET Imaging in a canine model of cardiac exposures associated with breast cancer radiotherapy”, by El-Sherif O, Xhaferllari I, Sykes J, Butler J, DeKemp R, Renaud J, Battista J, Wisenberg G, Prato F.S., Gaede S. The article has been accepted pending revisions from *Radiotherapy & Oncology*.

In this chapter a canine model of cardiac exposures associated with left-sided breast cancer radiotherapy is presented. The hearts of five canines were imaged using $^{18}$F-FDG (inflammation tracer) and $^{13}$N-ammonia (perfusion tracer) cardiac PET at baseline, 1
week, and 4 weeks following cardiac irradiation. The delivered dose to the heart, left ventricle, and LAD was designed to match the expected biological equivalent dose from a multi-fractionated left sided breast cancer population (data obtained from Chapter 1). The distribution of the dose was intentionally focused on the LAD and anterior apical portion of the left ventricle. This resulted in a dose-gradient within the heart. Although this specific dose gradient is not typically observed in patients, it allowed us to assess the relationship between spatial dose deposition and cardiac injury. The results from this study showed that cardiac PET can identify statistically significant increases in the uptake of $^{18}$F-FDG and $^{13}$N-ammonia, as early as 1 week following cardiac irradiation. A spatial relationship between dose and tracer uptake was not observed; rather the response to radiation was global, affecting regions with higher dose in comparison to regions irradiated at a substantially lower dose similarly.

These novel findings suggest that an increase in myocardial perfusion (beginning ~1 week post irradiation) occurs and is associated with a global inflammatory response (increased FDG uptake). Further work is needed to establish a more concrete connection between these early changes observed using cardiac PET with long-term cardiac toxicity.

1.8.3 The utility of $^{18}$F-FDG and $^{13}$N-ammonia PET for monitoring the progression of cardiac inflammation and perfusion following focused cardiac irradiation in canines (Chapter 4)

The fourth chapter of this thesis is a longitudinal follow-up of the same five subjects from chapter 3. The aim of this study is to identify whether the changes observed in myocardial perfusion and inflammation observed in chapter 3 are acute responses to radiation or persistent. Here, the hearts of five canines were imaged using $^{18}$F-FDG (inflammation tracer) and $^{13}$N-ammonia (perfusion tracer) cardiac PET at 3 months, 6 months, and 12 months following cardiac irradiation.

The results of this study show that $^{18}$F-FDG and $^{13}$N-ammonia PET can be used for the longitudinal assessment of myocardial perfusion and inflammation in association with cardiac external beam irradiation. In response to cardiac irradiation, progressive increases in FDG uptake, a non-invasive means of assessing inflammation were observed and
supported by preliminary ex-vivo histology. A transient increase in myocardial perfusion that returned to baseline levels (in 3 subjects) and below baseline levels (in 2 subjects) was also observed. A global response to radiation was observed affecting regions with high dose and low doses similarly; further validation of this finding through more complete histology is needed. These results provide support for the future use of $^{18}$F-FDG and $^{13}$N-ammonia PET imaging for the longitudinal assessment of previously proposed treatment options aimed at reducing radiation induced cardiac toxicity in humans.

This study is expected to be submitted for publication in *Radiotherapy & Oncology* pending a more comprehensive histological analysis of the ex-vivo cardiac samples. The histology is currently underway.

### 1.8.4 Conclusions and Future Work (Chapter 5)

Here, the key findings from the previous chapters are summarized and potential future related studies are proposed and motivated.
Chapter 2

2 Radiation dose uncertainty associated with motion of the heart, left ventricle, and left anterior descending artery during left-sided breast radiation therapy

Postoperative radiotherapy (RT) is the standard of care for breast cancer patients. Technological advances in medical imaging and RT have led to significant improvement in the survival rate of these patients (127). However, a portion of breast cancer survivors are now exhibiting the latent effects of normal tissue irradiation, namely, radiation-induced ischemic heart disease (RIHD)(125). Due to proximity, left-sided breast cancer (BREL) patients’ hearts are partially irradiated during RT, placing them at a greater risk of RIHD(17, 32, 128).

Tangential intensity modulated radiation therapy (IMRT)(129–131) delivered under free-breathing is widely used for cardiac dose-sparing(132, 133). Recently, motion-management techniques such as, active breathing control (ABC), deep inspiration breath-hold (DIBH), and inspiratory-gating (IG) have been proposed as options for reducing incidental cardiac irradiation(134). Although numerous studies have shown that these motion-management techniques can significantly reduce the dose to the heart, the clinical benefits are still unknown. In a randomized trial, Zellars et al showed that the use of ABC was not significantly associated with the prevention of cardiac perfusion deficits (43). Preclinical studies suggest that the radio-sensitivity of the heart is heterogeneous and that direct radiation exposure to specific regions within the heart, specifically, the major coronary arteries are correlated with the acceleration of the atherosclerotic process(33, 48, 49). In light of these preclinical findings, the typically reported dose estimates for just the heart volume alone may not be sufficient for assessing the efficacy of cardiac dose sparing techniques. Doses to the heart may need to be supplemented with dose estimates for the coronary arteries as well. Unfortunately, due to the impact of respiratory motion, estimating the dose to the coronaries is difficult. Additionally, the standard of care for left-sided breast RT uses a standard fast helical CT (FH-CT) for treatment planning that does not account for internal organ motion during treatment simulation.
To address this concern, the dosimetric impact of patient breathing on heart dose has been previously quantified through the use of 4D-CT (83, 84, 87, 88). However, these studies have not investigated the dosimetric impact on the cardiac sub-structures such as the left ventricle (LV) and the left anterior descending artery (LAD). To better understand the limitations of using standard FH-CT for left breast RT planning we have compared DVH metrics for the heart, LV, and LAD regions obtained from the FH-CT to a 4D dose accumulation.

The purpose of this study is to use 4D-CT imaging to predict the level of uncertainty in cardiac dose estimates of the heart and its sub-structures that arises due to breathing motion during RT. Additionally, the impact of potential errors associated with dose deformation on the 4D dose accumulation within the heart, LV, and LAD is examined.

2.1 Methods and Material

2.1.1 Imaging

Institutional review-board approval was obtained to retrospectively analyse the planning CT data of 30 consecutive patients whom have previously undergone RT for left-sided breast cancer. The study design is outlined in Figure 2-1. As standard of care within our institution, all BREL patients receive both a standard fast-helical CT (FH-CT) and a 4D-CT for RT planning. The FH-CT and 4D-CT were acquired using a 16-slice Philips Brilliance large bore CT scanner (Philip Medical Systems, Milpitas, CA). The Varian RPM system (Varian Medical Systems, Palo Alto, CA) was used to monitor the motion of an external fiducial positioned on the patient during 4D-CT imaging. The fiducial provides a surrogate for the internal organ motion during normal respiration and is used to sort the 4D-CT data into 10 separate breathing phases.

2.1.2 Treatment planning

Treatment plans were created on the FH-CT using the Pinnacle\textsuperscript{3} treatment planning system (Philips Radiation Oncology Systems, Fitchburg, WI). Dose calculations were performed with the collapse cone convolution algorithm and tissue inhomogeniety
correction implemented in Pinnacle\textsuperscript{3}. A Summary of the patient treatment characteristics for this study can be found.
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Appendix B: Worked general solution to equation 1-1

\[ \frac{dC_{tissue}(t)}{dt} = \rho K_1 C_{blood}(t) - K_2 C_{tissue}(t) \]  \hspace{1cm} 1-1

begin by taking the Laplace transform of both sides of equation 1-1

\[ L\left\{ \frac{dC_{tissue}(t)}{dt} \right\} = L\{\rho K_1 C_{blood}(t) - K_2 C_{tissue}(t)\} \]

in the Laplace domain we have the following equation

\[ s\tilde{C}_{tissue}(t) - C_{tissue}(0) = \rho K_1 \tilde{C}_{blood}(t) - K_2 \tilde{C}_{tissue}(t) \] \hspace{1cm} A-1

where \( \tilde{C}_{tissue}(t) \) and \( C_{tissue}(0) \) are the Laplace transform of \( C_{tissue} \) and the initial condition in the time domain respectively. Since the initial condition is equal to zero we can rearrange equation A-1 to solve for \( \tilde{C}_{tissue}(t) \)

\[ \tilde{C}_{tissue}(t) = \frac{\rho K_1 \tilde{C}_{blood}(t)}{s + k_2} \]

\[ \tilde{C}_{tissue}(t) = \rho K_1 \tilde{C}_{blood}(t) \frac{1}{s + k_2} \]

The following equation is obtained by taking the inverse Laplace transform of both sides. Also, recall that multiplication in the Laplace domain is convolution in the time domain.

\[ L^{-1}C_{tissue} = L^{-1}\rho K_1 C_{blood}t \otimes L^{-1}s + k_2 \]

\[ C_{tissue}(t) = \rho K_1 C_{blood}(t) \otimes e^{bt} - K_2 t \]
Figure 2-1: Outline of study design. Abbrev: LV = left ventricle; LAD = left anterior descending artery.
2.1.3  4D dose accumulation

When performing the 4D-dose accumulation, each phase of the 4D-CT was weighted equally. Due to patient variability in breathing, time-weighted dose accumulations may be necessary, specifically when the 4D-CT is reconstructed in amplitude mode (135). However, when a 4D-CT is reconstructed in phase mode, the equally weighted approach has some inherent probability-weighting (135). This can be observed by examining the position of the heart in the 40-70% phases. The 40-70% phases all tend to be close to the end of exhalation from an amplitude perspective (see Figure 2-2). To obtain the accumulated dose distribution, the FH-CT was registered to the 4D-CT then the finalized FH-CT treatment fields and isocenter were transferred to each phase of the 4D-CT. An automatic rigid registration was performed between the FH-CT and 4D-CT minimizing the squared differences of the voxel intensities. The quality of the registration between the FH-CT and 4D-CT phase were verified qualitatively by visually comparing the alignment of the vertebral bodies. The dose was recalculated on each of the phases. The DICOM RT dose files were then exported from Pinnacle to MIMVista (MIM Software Inc., Cleveland, OH, version 6.4.3) to obtain the deformable dose accumulation. The warped doses were accumulated to the 50% phase of the 4D-CT for all patients, the heart, LV, and LAD contours on the 50% phase of the 4D-CT were the ones used in our comparison with the FH-CT.

Figure 2-2: A) Respiratory trace of a representative patient. The 5 coloured boxes outline the amplitude divisions of the RPM trace corresponding to the end of exhalation (EOE), end of inhalation (EOI), preEOE, preEOI, and mid ventilation (midVENT) respiratory amplitudes. (B) The corresponding RPM derived frequency
distribution for each respiratory phase. (C) Amplitude of the craniocaudal centroid position of the heart contour for each 4D-CT phase of a representative patient.

2.1.4 Contouring and dose warping accuracy

To improve the accuracy of our contours, we employed a previously validated cardiac atlas (136). Contours were originally drawn on the FH-CT then propagated to all 10 phases of the 4D-CT through the deformation algorithm within MIMVista (137). Auto-propagated contours were reviewed and edited for any delineation errors. Additionally, to account for dose uncertainty to the LAD due to cardiac motion, all LAD contours were created with a standard 5mm diameter (58).

The mean spatial error of the deformation algorithm has been previously estimated to be 1.5mm (137–139). The sensitivity of the DVH metrics due to the uncertainty in the dose deformation is estimated through rigid translation of the contours in three directions. All contours associated with the dose accumulation were shifted in the anterior-posterior and lateral-medial directions by ±0.75mm, ±1.5mm, ±3.5mm, and ±7.0mm. These shift values are in line with previously reported deformation errors associated with MIMVista (137–139). It should be noted that the max reported error can be up to 20mm, however, a conservative max of 7 mm is considered here with the following justification; Nie et al showed that in the presence of added image noise, <4% of the total voxels can be expected to have deformation errors >7 mm and the max reported error of 20mm are present in less than 1% of the volume (140). Additionally, the 20 mm error may not be relevant here considering this value exceeds the magnitude of total respiratory induced heart motion. Due to the slice thickness being 3mm, superior-inferior translations of ±3.5mm and ±7.0mm were only performed. Variation in dose due to translation is inevitable even without dose warping since many of these contours are in close proximity to high spatial dose gradients. Thus the FH-CT contours were translated along the un-warped dose distribution for comparison. Dose variation is reported in all three directions as the dose difference (mean ± standard error of mean) between the translated contours and the original fixed contours. A paired samples t-test was used to compare means from the shifted 4DCT contours on the warped dose distribution to the shifted FH-CT contours on the un-warped dose.
2.1.5 Statistics and data analysis

Student’s paired t-test ($p$ values $< 0.05$ were considered statistically significant) and Bland Altman plots were used to assess the level of agreement between FH-CT and 4D dose accumulation (141). As suggested by Bland et al, the 95% confidence interval of the difference in the measures is used to illustrate how far apart the dose estimates from 4D dose accumulation and FH-CT are likely to be for most patients. Prior to checking for statistical significance, Shapiro-Wilk tests were performed to verify the normality assumption required by the t-test.

2.2 Results

2.2.1 Delineation errors associated with auto-propagation

All contours were manually contoured on the FH-CT with guidance from a validated cardiac atlas (136). Auto-propagation of these contours from the FH-CT to the 4D-CT required some minor manual editing for the LAD contour only, the heart and LV contours did not have any obvious delineation errors. The auto-propagation produced the following 2 errors with the LAD contour; 1) shrinking of the LAD contour and 2) splitting of the LAD contour into 2 separate ROIs within the same slice. Shrinking of the LAD contour is problematic due to the spatial uncertainty of the LAD caused by cardiac motion (58). These errors were addressed as follows; the LAD contours were expanded to ensure a standard 5mm margin. Split LAD contours were qualitatively judged based on which of the two contours were closest to the expected anatomical position of the LAD. The LAD typically originates from the left main artery and then runs down the interventricular groove, between the left and right ventricles. The contour closest in proximity to this definition is kept and the other is deleted.

2.2.2 Dosimetric comparisons

The irradiated volume of the heart and LV typically exhibited minimal differences when comparing the FH-CT to each phase of the 4D-CT. However, larger differences in irradiated volume were observed in the estimated dose to the LAD region. The LAD region’s potential sensitivity to motion can be visualized in Figure 2-3. The LAD is
typically situated in close proximity to the steep dose-gradients along the beam edges of the tangential fields.

Figure 2-3: A comparison of the fast helical CT (FH-CT), end of exhalation, and end of inspiration radiation dose distributions for a representative patient. The heart, left ventricle (LV), and left anterior descending artery (LAD) are outlined in magenta, yellow, and red respectively. Differences in the irradiated volume and magnitude of the heart, LV, and LAD are outlined by the white arrows for comparison.

Figure 2-4 shows differences between EOI, EOE, and the 4D accumulated DVHs of the heart, LV, and LAD for a representative patient. The comparison of the mean dose difference between FH-CT and 4D accumulated doses for all patients is shown in Figure 2-5. Overall the dose differences were generally minimal for the heart and LV. The average ± 95% confidence interval of 4D accumulated dose and FH-CT differences in mean dose estimates for the heart, LV, and LAD were 0.1 ± 0.5 Gy, 0.0 ± 1.0 Gy, and -0.5 ± 8.7 Gy respectively. The average ± 95% confidence interval of 4D accumulated dose and FH-CT differences in V_{50%} for the heart and LV were 0.8 ± 1 % and 1.0 ± 3% respectively. The FH-CT appears to underestimate for the maximal dose to 0.2 cc of the LAD in comparison to the 4D accumulated dose by 1.3Gy ± 4.4Gy (mean ± SD). The uncertainty in the dose accumulation due to errors in deformation was assessed by comparing dose estimates after a rigid translation of the contours in three directions. Additionally, the spread of the warped dose can be interpreted as the uncertainty in the
mean heart, LV, and LAD dose metrics. The results of this analysis are presented in Table 2-1. Shifting of the heart and LV contours in the medial-lateral and anterior-posterior directions on the warped 4D dose did not result in any statistically significant differences in comparison to the same contour shifts on the un-warped FHCT dose. Superior-inferior shifts of the heart contour resulted in a significant difference, however, the magnitude of this difference is small (warped dose: 0.30Gy ± 0.03; un-warped dose: 0.33Gy ± 0.03 for a 7mm shift). Similarly, 7mm superior-inferior shifts of the LV had statistically significant but likely clinically insignificant differences (warped dose: 0.45Gy ± 0.05; un-warped dose: 0.50Gy ± 0.06) since they are within the patient setup uncertainty of the treatment. For the LAD, medial-lateral, anterior-posterior, and superior-inferior shifts on the warped dose were statistically different from the shifts on the un-warped dose. The largest difference was observed for the medial-lateral shifts of 7mm (warped dose: 4.36Gy ± 0.37; un-warped dose: 4.98Gy ± 0.40).

2.3 Discussion and conclusions

The results of our study show that the mean dose to the heart and LV exhibit small to moderate variations across a typical BREL patient’s breathing cycle, typically less than ±0.5 Gy for the mean heart dose and ±1.0 Gy for the mean LV dose. However, no significant bias was observed between the 4D-CT and FH-CT derived cardiac DVH metrics. This finding is in agreement with several previous publications that also used 4D-CT for the assessment (83, 84, 87, 88). The variation in mean heart dose due to intrafraction motion (movement during imaging/treatment) shown here is on the same order of magnitude as previously reported errors expected due to potential organ delineation inaccuracies, 0.14±0.14 Gy (136). Similar to the heart, the errors due to intrafraction motion of the LV appear to be only slightly larger than inaccuracies due to delineation, ±1.0 Gy vs ±0.3Gy for the LV (136). The heart and LV are relatively large volumes and typically only a small proportion of these volumes are within the region of the dose distribution where the dose-gradient is steep. Thus, movements of the heart and LV in the medial-lateral or superior–inferior directions cause minor variations in the overall mean dose estimates to these structures. However, the heart is an organ composed
of many different tissues that are integral to maintaining functionality. To date, the variation in radio-sensitivity of the various tissues within the heart is poorly understood.

Figure 2-4: Variation between the end of inspiration (EOI), end of exhalation (EOE), and 4D dose accumulation (4D) dose volume histograms (DVH) of a representative patient. Variation in dose to the heart, left ventricle (LV), and left anterior descending artery (LAD). (B) is an inset of (A).
Figure 2-5: Bland Altman plots showing the level of agreement between the 4D dose accumulation 4D-dose and the fast helical CT (FH-CT) mean dose estimates for the heart (A), left ventricle (B), and LAD (C). The blue and red lines indicate the mean difference and 95% confidence interval respectively for all 30 patients.

Table 2-1: differences in mean dose between shifted and un-shifted contours on warped and unwarped dose distributions

<table>
<thead>
<tr>
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<th>Planned</th>
<th>Medial-Lateral Shift</th>
<th>Anterior –Posterior Shift</th>
<th>Superior – Inferior Shift</th>
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<td>unwarped</td>
<td>warped</td>
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<td>Heart</td>
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<td>4.36±0.37</td>
<td>4.98±0.40</td>
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</table>

Tabulated values represent the differences in mean dose between shifted and un-shifted contours (mean ± standard error of mean). Dose differences are reported in absolute units of Gy. Boldfaced values indicate statistically significant differences between shifts on warped doses vs shifts on un-warped doses (p<0.05).
What is known is that the function of arteries and microvasculature within the heart can be compromised through radiation exposure(33). Our use of 4D-CT and 4D-dose accumulation have shown that incorporating the movement of the heart provides added information with regards to the blurring of the dose during radiotherapy. Additionally, we have shown that the area of the heart that may be of most concern, is the LAD, however, further validation in clinical studies is still needed to better understand the relationship between LAD irradiation and RIHD. The use of 4D-CT in potential future work in this area is suggested to obtain more accurate dose estimates to the LAD.

The irradiated volume of the LAD was shown to have a much greater variation due to respiratory induced motion than both the heart and LV; ± 8.7 Gy for the mean LAD dose. To the best of our knowledge this is the first time the impact of intrafraction motion on LAD dose has been reported. A previous study examined the displacement of the LAD and showed the respiratory induced motion can lead to displacements as large as 9mm towards the chest wall(84). These reported LAD displacements are consistent with what was observed in this study. The visibility of the LAD on both FH-CT and 4D-CT is difficult and the accuracy of the contour without the use of IV contrast is a limitation for this study. However, this study shows that errors due to intrafraction motion of the LAD appear to be larger for patients than the uncertainty that arises due to contouring inaccuracies, ± 8.7 Gy vs 2.56Gy(136).

Another source of uncertainty comes from the dose deformation algorithm. However, the 95% confidence intervals for the errors associated with the intrafraction breathing motion of the LAD appear to exceed the errors associated with dose deformation. Dose deformation uncertainty was assessed here using rigid translations of the contours (Table 2-1). The small differences observed between the warped plan and the unwarped plans suggest good agreement between the local dose gradient magnitudes. Shifting the LAD contour by the mean expected error of the deformation algorithm (1.5mm)resulted in an uncertainty in the LAD dose of ~1.5 Gy, less than the variation observed due to intrafraction breathing motion (± 8.7 Gy). The 7mm shifts representing a “conservative max” error of the deformation algorithm resulted in large variations in the estimated LAD dose (5.9 Gy). Previous studies have reported <4% of the total voxels to have
deformation errors of this magnitude(140). Thus, although the likelihood of a 7mm error in deformation appears to be small, inaccuracies in the dose deformation of this magnitude will have a significant impact on reported dose estimates to the LAD. A limitation of this analysis is that the total uncertainty due to deformation is difficult to quantify for small structures in close proximity to high dose gradients such as the LAD. Further investigation of the uncertainty in the dose accumulation for this patient cohort is still warranted.

The concern for dose deposition to the LAD for BREL patients is substantiated by the proportion of these patients presenting with clinically significant coronary stenosis of the LAD and myocardial perfusion defects that are isolated to myocardial segments known to be supplied by the LAD (18, 59, 142, 143).

This study assumes that the respiratory trace of the external surrogate during 4D-CT simulation is reproducible during RT. Although, patient breathing likely varies from day to day, a previous study reported good day to day reproducibility of probability distributions for lung tumour motion(144). We expect similar reproducibility considering the respiratory induced motion of the heart is more subtle than that of a lung tumour.

Although the 4D-CT may provide added information with regards to the motion of the internal anatomy, there is an added risk of radiation-induced malignancies due to the increased dose from the 4D-CT. The effective dose from 4D-CT is approximately 8 times the dose from a conventional FH-CT(145) (~6mSv vs ~48mSv). The lifetime attributable risk (LAR) for fatal cancer induction was estimated in adults to be 33 cases per 100,000 standard chest CT examinations (~0.03%)(146). An approximated LAR from a 4D-CT can be expected to yield 231 more cases per 100,000 (~0.2%). Studies have shown that a reduction in heart dose by 1Gy correlates to a 7.4% decrease in the rate of a major coronary event(17). The 0.2% increased LAR for the induction of a fatal cancer due to the added exposure from 4D-CT can be potentially outweighed by just a small reduction in the heart dose (~0.03Gy to avoid a coronary event or ~1.2 Gy to avoid death due to heart disease). Thus, prior to the adoption of 4D-CT for left-sided breast treatment planning, further work is needed to identify if the added information from the 4D-CT can
be successfully used in the treatment planning process to reduce the dose to the heart, LV, and/or LAD.

In conclusion, we found small variations in the dose to heart and LV due to intrafraction respiratory motion during RT. However, the LAD showed substantial variation in dose due to breathing. Although the respiratory induced variations in LAD dose may not have an immediate clinical impact, we suggest that future clinical studies looking at the relationship between RIHD and LAD dose to consider the use of 4D dose accumulation rather than relying on the static treatment planning methods of the past.
Chapter 3

3 Early $^{18}$F-FDG and $^{13}$N-ammonia PET imaging in a canine model following cardiac exposures associated with breast cancer radiotherapy

*Chapter 3 hypothesis:* A reduction in left ventricular perfusion and an enhancement in inflammation is related to local radiation dose levels within the heart.

Advances in imaging and radiotherapy have led to significant improvement in the survival rate of breast cancer patients (127). However, several recent studies have shown that a large proportion of breast cancer patients show evidence of late radiation induced ischemic heart disease (RIHD) (16, 17, 147) following tangential external beam radiotherapy. Due to the development of new techniques many patients are living longer. Previously latent effects of radiation are now being seen. The relative risk of ischemic heart disease is believed to be proportional to the volume of the heart that is irradiated during therapy (17). To address this concern, some cancer clinics are now using respiratory motion management techniques, such as deep inspiration breath-holding (DIBH), to add greater spatial separation between the heart and the left-breast during radiotherapy (148, 149). The use of DIBH has been shown to reduce the irradiated volume of the heart. Unfortunately, due to the close proximity of the heart to the left breast, the DIBH technique cannot completely eliminate the incidental cardiac irradiation associated with radiotherapy. Reported average doses to the myocardium and the left anterior descending artery (LAD) in patients treated with DIBH can be up to 2Gy and 8Gy, respectively (148). Cancer centers using tangential fields that have not adopted the DIBH technique can see average doses as high as 6 Gy and 32 Gy to the myocardium and LAD, respectively. It is believed that the relative risk of a major coronary event increases by 7.4% per Gy without any evidence of a threshold dose below which there is no added risk (17). The pathogenesis of RICD is not well understood and clinical symptoms do not typically manifest until 10-15 years after radiotherapy. However, a recent study in mice identified significant up-regulation of inflammatory markers expressed on the vascular
endothelium in response to radiation(52), suggesting changes in the microvasculature developing as early as 10 and up to 20 weeks post-irradiation.

$^{18}$F-FDG PET has been previously used as a non-invasive means to study RICD after stereotactic lung(114) and esophageal(115, 116) radiotherapy. Mean doses to the heart after lung and esophageal cancer are typically greater than 20 Gy, higher than what is observed in breast cancer patients. As previously described in Chapter 1, the cardiac injuries observed at high doses (i.e. pericarditis) are different from those observed at lower doses (i.e. ischemia). Due to this difference in the expected radiation induced injury to the heart, extrapolating the results from lung and esophageal studies to set guidelines for left-sided breast cancer radiotherapy is uncertain. Additionally, the majority of pre-clinical studies on this topic have used murine models; however, significant differences have been identified in comparison to human physiology. Further, large animal models have been suggested for easier translation of basic science findings to clinical practice, as they offer a more representative scale model of cardiac human physiology than murine models(150). Here we examine the utility of $^{18}$F-FDG and $^{13}$N-ammonia cardiac PET for the assessment of radiation induced myocardial inflammation and altered perfusion in a canine model. $^{18}$F- FDG and $^{13}$N-ammonia PET have been previously validated as non-invasive means of quantifying cardiac inflammation(111) and myocardial perfusion(151), respectively. $^{18}$F- FDG and $^{13}$N-ammonia PET can potentially quantify regional heterogeneity in inflammation and perfusion concurrently in association with the known patterns of regional dose deposition within the heart. In the current study, changes in the uptake of $^{8}$F-FDG and $^{13}$N-ammonia were quantified at baseline, 1 week post, and 4 weeks post external beam cardiac irradiation. Irradiation was designed to mimic the typical exposure observed during left-sided breast cancer radiotherapy. This study aims to probe the spatial and quantitative relationship between radiation dose-deposition and acute changes in perfusion and associated inflammatory response.
3.1 Materials and methods

3.1.1 Study design

Cardiac perfusion and inflammation imaging on five adult female, bred-for-research hounds (21 - 26 Kg) was performed on a hybrid PET/MRI system (Biograph mMR; Siemens AG) at baseline, 1, and 4 weeks post cardiac irradiation (see Figure 3-1 for details). Animals were anesthetized during imaging/irradiation using propofol (4-6 mg/kg) and maintained with 2% isoflurane. The study was approved by the Animal Care Committee of the Western University (Protocol 2014-005, Appendix D). The choice for 5 subjects was supported by an a priori power analysis (G*power, Version 3.1.9.2). The required number of samples was calculated such that the study would have a statistical power ≥ 0.95, at a significance criterion (α-value) = 0.05. An estimated effect size was derived from literature comparing perfusion estimates in normal volunteers to 5 groups of patients with varying levels of myocardial ischemia(152).

Figure 3-1: An overview of the longitudinal study is shown in panel A. Timing of the baseline, 1 week and 4 week follow-up PET/MRI imaging protocol is shown in panel B. Abbrev. CT-SIM – computed tomography radiotherapy simulation scan; IV – intravenous; MRAC – magnetic resonance attenuation correction.
3.1.2 Radiation treatment planning & delivery

The radiation dose distribution was designed to resemble the typical cardiac exposure delivered during left-sided breast cancer radiotherapy. Dose volume histogram (DVH) metrics were obtained from 30 consecutive clinically treated left-sided breast cases at the London Regional Cancer Program (London, ON Canada). The 95% confidence intervals of the extracted DVH metrics were used as dose constraints for the heart and its substructures in this study. A detailed summary of the included patient treatment
characteristics and the derived dose constraints are provided in
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Appendix B: Worked general solution to equation 1-1

\[ \frac{dc_{tissue}(t)}{dt} = \rho K_1 C_{blood}(t) - K_2 C_{tissue}(t) \]  

begin by taking the Laplace transform of both sides of equation 1-1

\[ L \left\{ \frac{dc_{tissue}(t)}{dt} \right\} = L\{\rho K_1 C_{blood}(t) - K_2 C_{tissue}(t)\} \]

in the Laplace domain we have the following equation

\[ s \tilde{C}_{tissue}(t) - C_{tissue}(0) = \rho K_1 \tilde{C}_{blood}(t) - K_2 \tilde{C}_{tissue}(t) \]  

where \( \tilde{C}_{tissue}(t) \) and \( C_{tissue}(0) \) are the Laplace transform of \( C_{tissue} \) and the initial condition in the time domain respectively. Since the initial condition is equal to zero we can rearrange equation A-1 to solve for \( \tilde{C}_{tissue}(t) \)

\[ \tilde{C}_{tissue}(t) = \frac{\rho K_1 \tilde{C}_{blood}(t)}{s + k_2} \]

\[ \tilde{C}_{tissue}(t) = \rho K_1 \tilde{C}_{blood}(t) \frac{1}{s + k_2} \]

The following equation is obtained by taking the inverse Laplace transform of both sides. Also, recall that multiplication in the Laplace domain is convolution in the time domain.

\[ L^{-1}\{L^{-1}\rho K_1 C_{blood}(t) \otimes L^{-1}s - k_2 \} \]

\[ C_{tissue}(t) = \rho K_1 C_{blood}(t) \otimes e^{-k_2 t} - K_2 t \]
Appendix C and Appendix E respectively. All animals received a standard fast helical CT and a contrast enhanced CT (Discovery VCT; GE Healthcare) for radiation treatment planning. All subjects were ventilated using shallow breaths in order to minimize the need to correct for intrafraction respiratory motion. Contours of the heart, left ventricle (LV), left circumflex (LCX), and LAD were manually delineated on the contrast enhanced CT for improved visualization. Myocardial regions of the left ventricle were further segmented in accordance with the 17 segment model as described by the American Heart Association (AHA)(153). Complete contours were overlaid onto the non-contrast enhanced CT images for radiation treatment planning. Volumetric modulated arc therapy (VMAT) radiation treatment plans were created using the Pinnacle³ treatment planning system (Phillips Radiation Oncology Systems). The treatment plans consisted of two, 180°, 6 MV photon arcs and deliberately focused on to the myocardial regions supplied by the LAD while intentionally avoiding the basal anterolateral portion of the LV and the LCX in order to compare cardiac function in irradiated vs minimally-irradiated segments. Dose was calculated using the adaptive convolve dose algorithm (155–157) implemented in Pinnacle³.

During baseline imaging, tattoos, radio-opaque markers, and wall-mounted lasers were used to aid with re-positioning of the animals on the day of irradiation. Prior to treatment, cone-beam CT and intra-treatment fluoroscopic imaging were used to verify the position of the animal’s hearts during irradiation.

The biological equivalent dose (158) of the multi-fractionated radiation prescriptions was delivered in a single fraction. An $\alpha/\beta$ ratio of 2.5 Gy (159) was used to convert the multi-fractionated scheme (~30 Gy in 25 fractions prescribed to a point within the LAD) to a single fraction delivery (~9 Gy in 1 fraction) (159). All animals were irradiated with a TrueBeam linear accelerator (Varian Medical Systems).

### 3.1.3 Imaging

Cardiac perfusion and inflammation was assessed using $^{13}$N-ammonia and $^{18}$F-FDG PET imaging, respectively (details outlined in Figure 3-1).
Cardiac perfusion was imaged both under pharmacological stress and at rest. Pharmacological stress perfusion was induced in all animals using 3 mg/min intravenous injection of adenosine over 10 minutes. Adenosine was injected prior to the manual bolus injection of $^{13}$N-ammonia (~5 MBq/Kg) followed by a saline flush. Rest perfusion was assessed with a second injection of $^{13}$N-ammonia. The data was acquired in list-mode and retrospectively binned into time periods using the bellows respiratory trigger. The list mode data was grouped into 16 frames: 12x10s, 2x30s, 1x60s, 1x360s. All PET data was reconstructed using an iterative 3D ordered subset expectation maximization algorithm (OSEM) (160) with 3 iterations, 21 subsets, 172x172x127 matrix size, and a 4-mm Gaussian smoothing filter, yielding a voxel size of 2.08x 2.08 x 2.03 mm. The hybrid PET/MRI system corrects for random coincident events using the standard delayed window correction method. For scatter correction, a single scatter iterative algorithm is used. The scatter correction technique was originally described by Watson et al (161). The dead time is measured empirically and applied as a correction to the PET signal after normalization.

Prato et al, (111) previously described and validated an $^{18}$F-FDG PET imaging protocol capable of identifying abnormal accumulation of inflammatory cells within the heart following coronary occlusion. The same imaging protocol is adopted here to study RICD. In order to image cardiac inflammation, the normal myocardial uptake of glucose was suppressed prior to the injection of $^{18}$F-FDG. The suppression of glycolysis was achieved through fasting and an intravenous injection of heparin (2000 IU) immediately followed by a 20% lipid (Intralipid; Baxter Health-care Corp.) infusion (0.25 ml/min/kg; 50 min duration) 20 min prior to the injection of $^{18}$F-FDG. This myocardial suppression protocol was validated before the current study in 3 healthy subjects. The data was acquired in list-mode 60 mins after the injection of $^{18}$F-FDG. A single static frame (20min duration; respiratory triggered) was reconstructed using OSEM. Attenuation was corrected for all PET scans using a 2-point Dixon MR imaging pulse sequence (124, 162), which automatically segments and substitutes discrete attenuation coefficients of lung, adipose tissue, and soft tissue. MR-based attenuation correction for cardiac PET has been previously validated and shown to be strongly correlated with standard CT-based
attenuation correction with narrow Bland-Altman limits of agreement (-8.5% to +12.6%)(23).

3.1.4 Data Analysis

Perfusion and inflammation results were grouped according to the typical canine coronary artery distribution(163). Each of the 17 myocardial regions was classified as being predominately supplied by the LAD, LCX, or both arteries.

Cardiac perfusion was quantified using a one-compartment tracer kinetic model(151) implemented using the semi-automated analysis program, FlowQuant® (University of Ottawa Heart Institute)(164). A unidirectional uptake rate constant, $K_1$, from the arterial blood to the tissue compartment provides a direct estimate of myocardial perfusion (ml/min/g). This assumption has been previously shown to be valid since the tracer extraction for $^{13}$N-ammonia is >92%(151, 165) for flows up to 6 ml/min/g. In general, $K_1$ is obtained from the deconvolution of the imaged-derived tissue tracer concentration curve (obtained from each of the 17 myocardial segments) and the arterial input function (obtained from a region interest within the left ventricular blood pool). The 1-compartment model derivation of the relationship between the measured tracer concentration in the blood, tissue, and the estimated $K_1$ and $K_2$ values is provided in Appendix B. The $K_1$ and $K_2$ value is estimated through an iterative approach minimizing the cost function defined in equation 3-1.

$$C_{tissue}(t) - \rho K_1 C_{blood}(t) \otimes e^{-K_2 t}$$  \hspace{1cm} 3-1

Changes in cardiac inflammation were analyzed as changes in the mean standard uptake value ($\text{SUV}_{\text{mean}}$) and standard uptake ratio (SUR). The definition of the SUV is provided in equation 3-2.

$$\text{SUV} = \frac{\text{Activity in myocardial region (MBq)}/\text{regional volume (ml)}}{\text{Injected activity (Mbq)}/\text{subject weight (g)}}$$  \hspace{1cm} 3-2

The SUR has been previously reported as a means of reducing inter-study variation. The SUR value is obtained by normalizing the $\text{SUV}_{\text{mean}}$ by the SUV within the aorta(166).
SUV\textsubscript{mean} and SUR were computed for all 17 myocardial regions defined by the AHA. For statistical comparison, the 17 myocardial regions were aggregated and grouped based on coronary regions (LAD vs LCX vs Both), axial regions (basal vs mid-ventricle vs apex), and radial regions (lateral wall vs inferior wall vs anterior wall vs septal wall). Table 3-1 proves further details regarding this regional grouping.

**Table 3-1: Grouping of American Heart Association 17 segment model for statistical comparisons**

<table>
<thead>
<tr>
<th>Comparison Group 1</th>
<th>Comparison Group 2</th>
<th>Comparison Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>coronary regions</td>
<td>AHA segment</td>
<td>Axial Regions</td>
</tr>
<tr>
<td>LAD</td>
<td>1, 2, 7, 8, 13</td>
<td>Basal</td>
</tr>
<tr>
<td>LCX</td>
<td>4, 5, 10, 11, 15</td>
<td>Mid-ventricular</td>
</tr>
<tr>
<td>Both</td>
<td>3, 6, 9, 12, 14, 16, 17</td>
<td>Apex</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

All statistical analyses were performed using SPSS 23 (IBM Corp.). Differences in cardiac perfusion and inflammation before and after irradiation and regional differences between myocardial regions supplied by the LAD and LCX were tested using a multivariate two-way repeated measures analysis of variance. Bonferroni post hoc analysis was used to correct for multiple comparisons. Pearson’s product-moment correlation coefficient was used to test the association between regional dose deposition and changes in perfusion/inflammation. All data are reported as mean ± standard error of the mean (SEM) unless otherwise indicated.

### 3.2 Results

The average regional dose, injected dose of all radiotracer, and plasma glucose levels (PGL) prior to imaging are tabulated in
Appendix F. No statistically significant differences were observed between scans for the injected doses of $^{13}$N-ammonia and $^{18}$F-FDG. Although no statistically significant differences were found between PGL, the baseline PGL and PGL at 4 weeks did approach statistical significance ($p = 0.055$). Mean doses delivered to the heart (1.7 Gy), LV (2.7 Gy), LAD (5.5 Gy), and LCX (1.1 Gy) are typical of values observed in left-sided breast cancer radiotherapy patients. Figure 3-2 shows the computed radiation dose distribution superimposed onto the contrast-enhanced CT image for a representative subject. Figure 3-3 shows the DVH’s for the heart, LAD, and LV of a representative subject in comparison with DVH’s of two patients analyzed in Chapter 2.

Figure 3-2: A coronal, contrast-enhanced CT slice (panel A) showing the heart, left ventricle (LV), left anterior descending artery (LAD), and left circumflex artery (LCX) contours on a representative subject (subject 3). The spatial distribution of the radiation dose is shown in panel B. The basal anterolateral portion of the LV and the LCX were intentionally avoided (see panel B) in order to compare cardiac function in irradiated vs non-irradiated segments.

A statistically significant increase in the $^{18}$F-FDG $\text{SUV}_{\text{mean}}$ and SUR was observed post irradiation. Increased cardiac uptake of $^{18}$F-FDG was found throughout the entire LV, including targeted and un-targeted myocardial regions (see Figure 3-4). The change in $\text{SUV}_{\text{mean}}$ and SUR aggregated from all 5 subjects is presented in Figure 3-5. A correlation could not be established between regional dose and differences in uptake of $^{18}$F-FDG relative to baseline.
A statistically significant increase in both rest and stress $^{13}$NH$_3$ perfusion was observed post cardiac irradiation. Static $^{13}$NH$_3$ cardiac PET uptake images are shown for a representative subject (subject 3) in Figure 3-6. Increased stress perfusion was found throughout the entire LV, with no statistically significant difference between regions regardless of regional dose magnitude (see Figure 5). Linear regression analysis between dose and perfusion did not reveal any significant relationships between dose and perfusion effects. The FDG uptake and resting state perfusion appear to progressively increase within the first 4 weeks following cardiac irradiation.

3.3 Discussion

The risk of RICD after left-sided breast radiotherapy is substantiated by several epidemiological studies; however, the early events and progression of the disease still remain unclear. RICD is considered a significant health problem due to the large number of women receiving radiotherapy for breast cancer(17, 32, 127) and surviving to an older age where cardiac issues become evident. This study demonstrated that focal cardiac irradiation to the LAD with heart doses comparable to that of standard breast irradiation techniques results in an early increase in $^{18}$F-FDG uptake and myocardial perfusion. This study also demonstrates that these changes can be detected as early as 1 week post irradiation. It should be noted that collateral cardiac irradiation as a consequence of breast radiotherapy is typically delivered with 30 dose fractions. However, this study was performed using a single irradiation. In this study, the delivered single fraction dose was set using a previously reported $\alpha/\beta$ ratio of 2.5 Gy(159). Evidence exists regarding the validity of applying the linear quadratic model for single fraction deliveries(167), although this matter is still debated (168, 169).
Figure 3-3: Cumulative dose volume histogram comparison between human and canine cohorts for the heart, LAD, and LV.
Figure 3-4: $^{18}$F-FDG SUV PET images of a representative subject (subject 3) at baseline, 1 week and 4 weeks post cardiac irradiation.
Figure 3-5: Changes in 18F-FDG mean standard uptake value (SUV\text{mean}) and standard uptake ratios (SUR) obtained from regions of interest within the left ventricle pre and post cardiac irradiation. Panels along the left and right column represent changes in SUV\text{mean} and SUR respectively. Uptake in panels A and B are grouped by myocardial regions supplied by the left anterior descending (LAD) and/or the left circumflex artery (LCX). Uptake in panels C and D are grouped by basal, middle (Mid) and apical (apex) myocardial regions. Uptake in panels E and F are grouped by inferior, lateral, anterior, and septal myocardial regions. The mean dose delivered is reported in the legend, error bars indicate the standard error of the mean, and statistically significant differences are indicated by horizontal lines spanning the comparison points as \*p<0.05 and \**p<0.01.
Figure 3-6: $^{13}$NH$_3$ standard uptake value (SUV) PET images of a representative subject (subject 3) at baseline, 1 week and 4 weeks post cardiac irradiation. Images are reconstructed from list-mode data acquired 4 - 10 minutes post radiotracer injection.
Figure 3-7: Changes in $^{13}$N-ammonia myocardial perfusion obtained from regions of interest within the left ventricle pre and post cardiac irradiation. Panels along the left (i) and right (ii) columns represent perfusion changes observed at rest and under pharmacological stress respectively. Panels A. i) and ii) are grouped by myocardial regions supplied by the left anterior descending (LAD) and/or the left circumflex (LCX). Panels B. i) and ii) are grouped by basal, middle (Mid) and apical (apex) myocardial regions. Panels C. i) and ii) are grouped by inferior, lateral, anterior, and septal myocardial regions. The estimated dose delivered is reported in the legend, error bars indicate the standard error of the mean, and statistically significant differences are indicated by horizontal lines spanning the comparison points as *$p<0.05$ and **$p<0.01$.

It is believed that the earliest morphological changes to heart after irradiation are related to the microvasculature. Pre-clinical evidence supports the hypothesis that an inflammatory response following cardiac irradiation is a precursor and the mechanism for the evolution of RICD(19). Here we have shown that during the suppression of competing myocardial glucose uptake, a global increase in $^{18}$F-FDG uptake is triggered in response to focused cardiac irradiation. We hypothesize that this increased uptake is indicative of systemic inflammatory response to the radiation. The merits of this imaging approach has been previously shown for assessing inflammation in cardiac sarcoidosis(110) and after myocardial infarction(111). However, the ability to successfully suppress the normal $^{18}$F-FDG physiological uptake by myocardium is difficult to verify and a potential limitation of this technique. Additionally, it has been previously reported that anesthesia through the use of isoflurane may reduce the suppressive effects of fasting and heparin on myocardial glucose utilization in mice(170). Although we cannot be completely certain that full glucose suppression was achieved in this study, we have shown depressed SUV values (Figure 3) at baseline and follow up in comparison to previously reported SUV values without suppression(171). Furthermore, although the imaging protocol was consistent throughout the study, we cannot completely rule out potential for variability in the level of suppression between subjects and/or time points and the spatial heterogeneity of suppression within subjects. Thus, additional tests such as histopathology would be required to confirm the presence of inflammation.
Unfortunately, histopathology confirmation could not be included since subjects were not sacrificed at the conclusion of this acute study.

We observed similar heterogeneity in $^{18}$F-FDG uptake throughout the different planes of the LV compared to findings in previously reported literature(172, 173). Increased uptake was observed in the mid and apical planes compared to the basal plane. No statistically significant variation was found between inferior, lateral, anterior, and septal LV regions. However, the trend in Figure 3-5, panel E is in agreement with the expected spatial variation in uptake(174). $^{18}$F-FDG uptake tends to be higher in the lateral and inferior walls than the anterior wall and septum of normal myocardium. Three early explanations, one technical and two physiological were previously provided for the observed heterogeneity in normal myocardial FDG uptake, however they were refuted. In axial PET images the septal/anterior LV walls appear thinner than the inferior/lateral walls. Consequently, it was reasonably hypothesized that counts would be reduced in the thinner portions of the LV. Additionally, potential variability in regional perfusion and contractile work load of the LV has been previously provided as explanations for the heterogeneous uptake. However, all three explanations were later refuted through the use of $^{11}$C-acetate (myocardial oxygen consumption tracer) and $^{15}$O-water (perfusion tracer) PET imaging(175). Groppler, et al, showed homogeneous myocardial perfusion and clearance of $^{11}$C-acetate from normal myocardium, suggesting that the previous explanations for the heterogeneous uptake of FDG are unlikely. To the best of our knowledge, no further explanations have been provided in the literature. All that we know is that this heterogeneity is commonly observed(172, 174).

No correlation was found between the dose delivered to specific myocardial regions and the change in uptake relative to baseline, i.e. the areas of higher radiation dose did not appear to have greater increases in perfusion or FDG uptake with greater dose. To the best of our knowledge, this dose-independent uniform global inflammatory response at 1 week post irradiation in the heart at dose levels comparable to what is observed in the breast cancer population is a novel finding. The lack of correlation with dose magnitude may be due to the small number of animals used in this study and also by the relatively few myocardial regions with exposures below 1 Gy. Approximately 8% and 1% of the
total number of contoured LV regions received doses less than 1 Gy and 0.5 Gy, respectively. The clinical implication of this finding supports the recent interest in the use of standard heart failure therapies such as ACE inhibitors as a potential strategy to reduce the risk of RICD (176).

Clinical assessments of myocardial perfusion post left-sided breast radiotherapy consistently report reductions in myocardial perfusion at least 6 months after radiotherapy (43, 45, 47, 59, 99, 177–180). However, results from this study show an increase in myocardial perfusion 1 week after cardiac irradiation by approximately 20% relative to baseline. This finding suggests that a transient increase in perfusion (~1 week post irradiation) due to a global inflammatory response may precede reductions in perfusion observed at 6 months. This may develop as a result of late fibrotic changes within either myocardium or epicardial vessels leading to hemodynamically significant stenoses. This increase in perfusion is in line with two pre-clinical studies in murine models (181, 182). Lauk et al (181) and Seeman et al (182) observed increases in endothelial cell proliferation (3 weeks post irradiation) and increases in microvascular density 20 weeks following cardiac irradiation respectively. Additionally, an increase in vascular permeability has been reported in mice, 4 weeks after partial cardiac irradiation (80).

The uncertainty in the quantification of myocardial perfusion using $^{13}$NH$_3$ PET has been previously reported as approximately 10%(104, 105). It is believed to be dominated by variation in cardiac motion, signal noise, errors in compartmental modeling, partial volume effects, spillover from the right ventricular blood pool/adjacent liver activity, and potential errors in image registration between time points. However, the mean increases in perfusion after cardiac irradiation relative to baseline shown here are greater than the expected overall reported uncertainty in perfusion estimation.

It should be noted that the absolute measures of stress perfusion reported here appear to be reduced in comparison to literature values with similar adenosine administration protocols. This was a consistent finding in all animals and it is speculated that it may be related to the dose of adenosine. This prompted an additional stress $^{13}$NH$_3$ PET scan in
two animals with a double dose of adenosine; however, perfusion results at the double
dose still remained blunted. Previous studies have also reported difficulties with the use
of intravenous adenosine infusion in comparison to dipyridamole in the quantification of
myocardial blood flow in canines(151).

A potential limitation of this study is the canine animal model differs in comparison to
human hearts with regards to the increased number of collateral vessels. However, the
impact of this difference on the translation of these findings to humans is not currently
known. Further work using other large animal models such as pigs with reduced
collateral circulation may be needed.

It should be noted that although the study was performed using a hybrid PET/MRI
scanner, all of the techniques presented can also be implemented using PET/CT.

3.4 Conclusion

These early findings post cardiac irradiation echo the previous pre-clinical literature
using mice. However, the results from this study are presented in a large animal model
which more closely resembles the vessel architecture of humans than murine models.

Additionally, the findings are reported using a clinical imaging system and protocol that
can be easily modified for use in human studies. In light of these findings, we suggest the
use of this imaging approach for future human studies attempting to assess the clinical
efficacy of approaches to minimize cardiac exposure during left-sided breast irradiation.
Specifically, the efficacy of techniques aimed at reducing the risk of RIHD such as the
administration of anti-inflammatory medications (ex. ACE-inhibitors) may potentially be
assessed at much earlier time points through the use of \(^{18}\text{F-FDG}\) inflammation and/or
\(^{13}\text{NH}_3\) perfusion PET imaging.
Chapter 4

The utility of $^{18}$F-FDG and $^{13}$N-ammonia PET for monitoring the progression of cardiac inflammation and perfusion following focused cardiac irradiation in canines

Chapter 4 hypothesis: A reduction in left ventricular perfusion and an enhancement in inflammation is related to local radiation dose levels within the heart.

Cancer patients receiving radiotherapy for the treatment of thoracic malignancies such as lung, breast, and esophageal are at risk of radiation-induced cardiovascular disease (RICD). RICD is believed to be dose-dependent and can lead to the following: pericarditis, valvular dysfunction, fibrosis, and/or coronary artery disease (32). Acute pericarditis and valvular dysfunction are less prevalent today due to technological advances in radiotherapy that have led to significant reductions in incidental cardiac exposure. However, the more latent manifestation of RICD, coronary artery disease, is still prevalent in both the “high-dose”(183) and “low-dose”(17) patient populations. A recent clinical trial (183) (RTOG 0617) comparing radiation dose regimens for the treatment of lung cancer, suggests that the benefit of high-dose deposition to lung tumours may be outweighed by the risks associated with increased cardiac irradiation. Furthermore, there is substantial interest in new treatment strategies aimed at reducing cardiac toxicity following left-sided breast irradiation (17, 21, 43, 149, 184). Despite the recognition of RICD as a significant hurdle in improving patient survival, our understanding of the evolution of RICD is limited to preclinical studies in murine models (49, 51, 185). In order to study RICD progression in humans, non-invasive means for assessing cardiac toxicity with sufficient sensitivity to detect subtle changes before they become clinically significant is needed.

Positron emission tomography (PET) imaging coupled with $^{13}$N-ammonia ($^{13}$NH$_3$) and $^{18}$F-fluorodeoxyglucose (FDG) are promising radiotracers for non-invasive, longitudinal assessment of cardiac perfusion and inflammation respectively. The use of $^{13}$NH$_3$ PET has been previously validated and widely considered the gold-standard in the assessment
of cardiac perfusion non-invasively in humans (151, 186). The motivation to assess myocardial perfusion comes from preclinical studies suggesting that radiation injury to the microvasculature precedes RICD (52). Myocardial perfusion has been previously studied in the context of RICD through the use of single photon emission computed tomography (45, 47, 59, 99, 180, 187). SPECT studies suggest that perfusion changes are expected in patients where at least 5% of the left ventricle is within the field (defined by the 50% isodose line). However, to the best of our knowledge a direct correlation between dose magnitude and changes in perfusion has not been previously reported. The use of PET imaging has an advantage over SPECT due to the ability to provide absolute quantification of myocardial perfusion using dynamic data acquisitions and tracer kinetic modelling (188). Quantitative approaches to perfusion assessment have shown to be more sensitive and more specific (189) in identifying myocardial perfusion abnormalities, which is the typical pattern within the first decade after incidental cardiac irradiation during radiotherapy. Additionally, due to the short half-life (~10mins), $^{13}$NH$_3$ may be coupled with FDG PET in a single imaging session. FDG PET has been previously used to assess cardiac inflammation in cardiac sarcoidosis (110) and following myocardial infarction (111). Similar to perfusion abnormalities, preclinical studies have shown evidence of an inflammatory response following cardiac irradiation and preceding RICD (19). Although FDG cardiac PET imaging studies in the context of RICD exist, they are limited to studies where radiation dose to the heart are more relevant to lung and esophageal patients (41, 114–116, 190, 191). Extrapolating the lung and esophageal radiotherapy findings to the breast cancer population is questionable considering the significant differences in mean dose to the heart. Previous to Chapter 3 of this thesis and to the best of our knowledge, FDG PET has not been used to assess cardiac inflammation after exposure levels that are typically observed following left-sided breast radiotherapy.

The purpose of this study was to examine the utility of $^{13}$NH$_3$ and FDG PET in monitoring the evolution of cardiac perfusion and inflammation following cardiac external beam irradiation. Cardiac inflammation and perfusion imaging was repeated over 12 months in a canine model before and following cardiac irradiation. Unlike previous studies, cardiac irradiation was designed to mimic the typical heart exposure observed after left-sided breast radiotherapy. In addition to non-invasive PET imaging, a
preliminary histological analysis of the excised heart from a representative subject was conducted 12 months following cardiac irradiation.

4.1 Materials and methods

4.1.1 Study design

The study design is outlined in Figure 4-1. Five bred-for-research female hounds (21-26 kg) underwent cardiac perfusion and inflammation PET imaging at baseline, and 3 months, 6 months, 12 months following focused cardiac external beam irradiation. At 12 months, the animals were sacrificed and the hearts were excised for histological staining. The study was approved by the Animal Care Committee of the Western University (Protocol 2014-005).

Figure 4-1: The study design and imaging time-points are outlined in panel A. The PET/MRI imaging protocol performed each session is shown in panel B. In addition to the PET/MRI imaging, the baseline imaging session included contrast and non-contrast enhanced computed tomography (CT) imaging for organ delineation and radiation treatment planning, respectively. Abbrev. IV – intravenous; MRAC – magnetic resonance attenuation correction.
4.1.2 Imaging

The cardiac perfusion and inflammation PET imaging protocol (details in Figure 4-1, panel B) was conducted on a hybrid PET-MR scanner (Biograph mMR; Siemens AG). All radiation and imaging sessions were conducted using general anesthesia with propofol for induction and 2% isoflurane to maintain a surgical level of anesthesia. A 2-point Dixon pulse sequence was used for MRI-based attenuation correction. The 2-point Dixon provides bulk substitution of attenuation coefficients for regions identified as fat and water (124, 162). Good agreement between the 2-point Dixon method and CT-based attenuation correction has been previously shown for cardiac PET imaging (162).

Cardiac perfusion was imaged using $^{13}$NH$_3$ with list-mode PET acquisition reconstructed into 16 dynamic frames (12x10s, 2x30s, 1x60s, 1x360s). PET data was reconstructed using an ordered-subset expectation maximization algorithm (OSEM)(160) with 21 subsets, 3 iterations, 4-mm Gaussian smoothing filter, and 172x172x127 matrix size, yielding a voxel size of 2.08x2.08x2.03 mm. Each animal was manually injected with a bolus of ~5 MBq/Kg of $^{13}$NH$_3$ radiotracer followed by a saline flush. In order to estimate the myocardial perfusion reserve (MPR), each animal received two separate $^{13}$NH$_3$ injections, one at resting-state and another during pharmacological stress (following a 3 mg/min intravenous injection of adenosine over 10 minutes). Cardiac perfusion was quantified in units of ml/min/g using a 1-compartment tracer kinetic model (107) implemented using a semi-automatic software package, FlowQuant© (University of Ottawa Heart Institute). Perfusion was quantified for each of 17 left ventricular segments using the American Heart Association(AHA) model (153).

Cardiac inflammation was imaged using FDG PET after the suppression of the normal physiological uptake of glucose by the myocardium. As described previously (111), normal myocardial glucose uptake was suppressed through fasting and an intravenous injection of unfractionated heparin (2000 IU) followed by a 20% lipid (Intralipid; Baxter Health-care Corp.) infusion beginning 20 min prior to the injection of FDG. The mean standard uptake value (SUV) was calculated from a respiratory gated, static PET acquisition acquired 60 min after the injection of FDG. Similar to the perfusion analysis, the SUV was quantified using the AHA 17 segment model.
4.1.3 Radiation delivery

Each animal received a contrast enhanced and non-contrast enhanced fast helical computed tomography scan (Discovery VCT; GE Healthcare) for organ delineation and radiation delivery planning, respectively. Contours of the heart, left ventricle (LV), left anterior descending artery (LAD), and left circumflex artery (LCX) were initially generated on the contrast enhanced CT and then migrated to the registered non-contrast enhanced CT. The non-contrast enhanced images and contours were transferred to a clinical treatment planning system (Pinnacle³; Phillips Radiation Oncology Systems) for radiation treatment planning. The treatment plan was designed to mimic the typical cardiac exposure observed during left-sided breast radiotherapy. Dose-volume histogram (DVH) metrics for the heart, LV, and LAD were obtained from 30 consecutive patients previously treated for left-sided breast cancer. The 95% confidence intervals of the DVH metrics were implemented as constraints in the development of the radiation plan for this study. All animals received volumetric modulated arc therapy (VMAT) plans consisting of two, 180°, 6MV photon arcs that were deliberately focused onto myocardial regions supplied by the LAD. In addition, dose to the LCX and the basal anterolateral portion of the LV was minimized in order to compare cardiac function in irradiated and less-irradiated myocardial segments. The dose distribution was calculated using the adaptive convolve dose calculation algorithm (155, 157, 192).

In patients, radiation is typically delivered in multiple fractions. However, in this study a biological equivalent dose (158) of the multi-fractionated treatment was delivered in a single fraction. The multi-fractionated prescription (~30 Gy in 25 fraction prescribed to a point within the LAD) was converted to a single fraction prescription using an α/β of 2.5 Gy (~9 Gy in 1 fraction) (159). All animals were irradiated using a TrueBeam linear accelerator v1.6 (Varian Medical Systems, Palo Alto, USA). Radio-opaque markers, wall-mounted lasers, and tattoos were used to aid in re-positioning the animal on the accelerator. Image guidance through pre-treatment cone-beam CT and during treatment fluoroscopy were used for additional setup verification.
4.1.4 Histological analysis

A representative subject was selected for histological analysis. All animals were euthanized using an intravenous injection of potassium chloride. The isolated hearts were rinsed with phosphate-buffered saline and fixed through aortic infusion of 10% formalin and then fixed with formalin at room temperature for at least one week. The fixed heart was embedded in 3.5% agar (Sigma A7002) and then sliced into 4.4 mm-thick cross sections. The heart tissues were sampled according to standardized 17 segment myocardial segmentation guideline for tomographic imaging as specified by the American Heart Association (153). The segmented heart tissues were processed and embedded in paraffin. Five-micron paraffin sections from each segment were stained with hematoxylin and eosin for histology. Myocardial inflammation was assessed via immunohistochemistry for CD45, a common leukocyte antigen, on tissue sections. CD45 was immunostained using a rabbit polyclonal CD45 antibody (ab10558, Abcam). Bound antibodies were visualized using ABC reagent and daminobenzidine (Vector Laboratories). The bright-field images were acquired under Olympus BX-51 light microscope. The abundance of CD45-immunoreactive cells was visually quantified in a random high powered (40X) field at the anterior-septal region within the apex of the left ventricle.

4.1.5 Data analysis

Mean values of myocardial perfusion, perfusion reserve, and inflammation were compared to baseline values using a multivariate two-way repeated measures analysis of variance (ANOVA). A bonferroni post hoc test was used to correct for multiple comparisons. Perfusion and SUV values were grouped by anatomical region and also in accordance with the typical canine coronary artery distribution (163). Each myocardial segment was classified as being supplied by the LAD and/or the LCX artery. The relationship between dose deposition and changes in cardiac perfusion/inflammation was also assessed through Pearson’s product-moment correlation coefficient ($R^2$). All statistical tests were performed using SPSS 23 (IBM Corp.) and all values are reported as mean ± standard error of the mean (SEM) unless otherwise indicated.
4.2 Results

The blood plasma glucose levels, injected $^{13}$NH$_3$ activity, and injected FDG activity during all imaging sessions is tabulated in Appendix F. All animals received cardiac external beam irradiation that was deliberately focused onto the LAD. The spatial dose distribution can be visualized for a representative subject in Figure 4-2. The mean radiation dose delivered for the 5 animals to the heart, LV, LAD, and LCX was 1.7±0.1 Gy, 2.7±0.1 Gy, 5.5±0.3 Gy and 1.1±0.2 Gy, respectively. Delivered doses to the heart and substructures for each individual subject are tabulated in Appendix F.

Cardiac inflammation was assessed through the comparison of cardiac FDG uptake at baseline and following cardiac irradiation. The uptake of FDG in the LV myocardium was increased in comparison to baseline for all animals. Figure 4-3, panel A shows the increased FDG PET uptake in the heart, 12 months after cardiac irradiation for a representative subject. The histological images following irradiation from the same subject represented in the PET images is shown in Figure 4-3, panel B. Figure 4-3, panel B show both the standard H and E staining as well as CD45 staining highlighting persistent inflammatory cell activity at 12 months. Hematoxylin and eosin staining did not identify any signs of fibrosis, or morphological damage to the LV wall. Irregular endothelial cell morphology along the inner lumen of some arterioles and arteries were also present.

Figure 4-4 shows a linear increase in SUV over the first 6 months. The increased uptake of FDG relative to baseline was found to be statistically significant at all time points following cardiac irradiation ($p<0.01$). The changes in FDG uptake compared to baseline uptake were global and did not vary in response to the administered radiation dose with no significant correlation found between regional dose and changes in FDG uptake relative to baseline.

Changes in cardiac perfusion were monitored through $^{13}$NH$_3$ dynamic PET imaging coupled with tracer kinetic modeling for absolute quantification of perfusion. An increase in adenosine stress perfusion was observed throughout the entire LV at 3 months and 6
months following cardiac irradiation ($p<0.01$). However, by 12 months the stress perfusion had declined to near baseline levels.

**Figure 4-2:** Transverse, sagittal, and coronal contrast-enhanced CT slices (panel A) showing the heart, left ventricle (LV), LAD, and LCX contours of a representative subject. The radiation dose distribution is overlaid onto the anatomy in panel B. Dose to the basal anterolateral portion of the LV and the LCX artery were intentionally minimized (see panel B) in order to compare cardiac function in irradiated vs less-irradiated segments.

Figure 4-5 and Figure 4-6 show similar changes in perfusion relative to baseline in both areas of higher and lower radiation dose deposition. Similar to the FDG findings, no significant correlation was found between regional dose and changes in perfusion relative to baseline. A statistically significant increase in resting-state perfusion was observed at 6 and 12 months ($p<0.01$). Rest perfusion estimates in basal myocardial regions were higher than in the apex of the left ventricle. Changes in myocardial perfusion reserve relative to baseline were only observed at 3 months post irradiation ($p<0.01$). Unfortunately, rest perfusion estimates at the 12 month time-point for subject 2 were not obtained due to a technical issue during data acquisition.
Figure 4-3: Panel A. Transverse and sagittal image slices of the 18F-FDG standard uptake before and 12 months after cardiac irradiation for a representative subject (subject 3). Panel B. Histological slides from the left ventricular myocardium. Hematoxylin and eosin staining and CD45 antigen immunohistochemistry staining show cellular morphology and the presence of inflammatory cells respectively. CD45 positively stained inflammatory cells (leukocytes) within the myocardium are indicated by red arrows.
Figure 4-4: The temporal progression of $^{18}$F-FDG standard uptake within the left ventricle myocardium. Uptake in panel A is grouped by myocardial regions supplied by the left anterior descending (LAD) and/or the left circumflex (LCX). Uptake in panel B is grouped by inferior, lateral, anterior, and septal myocardial regions. Uptake in panel C is grouped by basal, middle (Mid) and apical (Apex) myocardial regions. The estimated dose delivered is reported in the legend, error bars indicate the standard error of the mean, and statistically significant difference were observed between all time points ($p<0.01$).

Figure 4-5: The delivered radiation dose distribution (panel A) and adenosine stress myocardial perfusion values at baseline and 3 months, 6 months, and 12 months post cardiac irradiation (panel B). Dose and perfusion values are overlaid onto a model of the left ventricle myocardium for a representative subject (subject 3).
Figure 4-6: Changes in $^{13}$N-ammonia myocardial resting-state perfusion (i), adenosine induced stress perfusion (ii), and myocardial perfusion reserve (iii) pre and post cardiac irradiation. Panels A. i), ii), and iii) are grouped by basal, middle (Mid) and apical (apex) myocardial regions. Panels B. i), ii), and iii) are grouped by inferior, lateral, anterior, and septal myocardial regions. The estimated dose delivered is reported in the legend, error bars indicate the standard error of the mean, and statistically significant difference are indicated by horizontal lines spanning the comparison points as *p<0.05 and **p<0.01.

4.3 Discussion

The progressively increasing uptake of FDG in the myocardium is suspected to be due to the infiltration of inflammatory cells in response to cardiac irradiation. This finding is further supported though ex-vivo images of CD45 positively stained cells within the myocardium. A similar pattern of inflammatory response following cardiac irradiation has been previously shown in mice (48, 52, 182). Through the use of flow cytometry, Sievert et al was able to show increases in endothelial cell surface markers related to
inflammation and proliferation that remained elevated up to 20 weeks post irradiation(52). The inflammatory response to cardiac irradiation has been previously associated with structural damage to the myocardium and microcirculation (182); however, hematoxylin and eosin staining of ex-vivo samples here did not result in evidence of structural damage due to radiation. Since structural damage is usually preceded by the inflammatory response, we suspect that this may have been observed in our study had we extended the follow-up period beyond 12 months, even though perfusion, in an absolute sense, was never compromised. Our results parallel the findings of preclinical studies in mice, although our study used a large animal model and a clinical system capable of non-invasive imaging of human subjects. The large animal model such as the canine provides a more representative model of the human cardiac anatomy then mice (150). Further, the canine inflammatory response to myocardial infarction has also been shown to be much closer to humans than the murine model, where an accelerated pattern of inflammation is seen, much faster than observed in patients. Additionally, large animal models facilitate the translation of basic science findings into clinical practice, specifically in the development of diagnostic imaging protocols to be used in human studies. These findings support recent interest in the use of ACE inhibitors for radioprotection of the heart (176) and the potential for FDG PET to assess its efficacy. Although the typical use of ACE inhibitors is related to reducing hypertension, studies have shown potential anti-inflammatory action capable of reducing the reactive oxygen species created during tissue irradiation (193). However, additional work is still needed to provide evidence of a link between cardiac inflammation and future radiation induced cardiac toxicity. This link must be made in order to fully support the use of FDG for assessing the efficacy of ACE inhibitors.

The $^{13}$NH$_3$ PET results show a transient increase in both rest and stress perfusion following cardiac irradiation. To the best of our knowledge, no previous studies have shown increases in quantitative myocardial perfusion following cardiac irradiation. Previous clinical cardiac perfusion studies of left-sided breast cancer patients following cardiac irradiation have mixed results and are limited to qualitative assessment of SPECT images only. Zellars et al showed both regional increases and decreases in myocardial perfusion scores 6 months after radiotherapy(43). Conversely, other studies have shown
reductions in cardiac perfusion following radiotherapy in ~50% of left sided breast cancer patients (45, 97, 100, 187). Chung et al did not observe any clinically significant SPECT perfusion defects 6 months following left-sided breast radiotherapy (46). Lawrence et al reported that the incidence of new perfusion defects 6, 12, 18, and 24 months after radiotherapy was 27%, 29%, 38%, and 42%, respectively (47). In our study, two of the 5 subjects had 12 month stress perfusion estimates below baseline estimates, consistent with the incidence rates reported by Lawrence et al (47). However, the regional changes in perfusion in these two animals were not found to be correlated with the regional dose. After aggregating the results from all subjects, we did not identify statistically significant perfusion defects compared to baseline. The transient increase in perfusion following irradiation shown here is supported by several murine studies that have reported increases in endothelial cell proliferation, increases in microvascular density, and increases in vascular permeability following cardiac irradiation, presumably as an additional paired response associated with both the acute and sub-acute inflammatory response out to 6 months (181, 182).

Increased FDG uptake and myocardial perfusion were observed globally, affecting both irradiated and “less” irradiated regions of the heart. This finding supports the conclusion by Darby et al, suggesting that there may not be a dose threshold for which there is no added risk of ischemic heart disease (17). In this study, approximately 8% and less than 1% of the LV wall received doses below 1 Gy and 0.5 Gy, respectively. We speculate that focal changes in FDG uptake and or perfusion related to dose deposition would require a more highly conformal delivery of radiation and a greater number of subjects than presented here. However, this type of irradiation would be less-representative of the left-sided breast cancer population.

The broad extrapolation of the findings presented from this study to the clinical population is limited by the small number of animals assessed as well as other factors outlined below. Stress perfusion and perfusion reserve values reported here are blunted in comparison to literature values. This finding was consistent for all animals and present in both pre and post cardiac irradiation scans. Suspicion that this may be due to the dosage of adenosine injected for vasodilation prompted an additional experiment in two animals.
at 6 months with twice the dose of adenosine. The increased dose did not result in any significant increases in stress perfusion either in absolute values or perfusion reserve. Previous studies have also reported difficulties inducing a stress response using adenosine infusion in the quantification of myocardial perfusion in canines (151). Lastly, incidental cardiac irradiation following breast cancer radiotherapy is typically delivered in multiple fractions; however, here the biological equivalent dose to the heart was delivered in a single fraction. Literature support exists for the validity of applying the linear quadratic model for single fraction deliveries (167); however this matter is still debated(168, 169). A recent study focusing on stereotactic ablative radiotherapy has suggested the use of a hybridization of the linear quadratic model and the multi-target model to improve estimates of the biological equivalent dose for single fraction deliveries(194). The estimated biological equivalent dose is also sensitive to uncertainty in the accuracy of the alpha/beta reported by Gillete, et al(159). Fowler et al reported changes in BED by ~5% per 1% change in alpha/beta(196). It should be noted that the results from Chapter 2 suggest that uncertainty in the estimated mean dose to the LAD used to calculate the BED greatly exceeds the reported uncertainty due inaccurate estimates for the alpha/beta. Lastly, although the mean dose to the heart, LV, and LAD are comparable to the exposures observed in the breast cancer population; the distribution of the dose is different. In this study, a greater volume of the heart was exposed to low doses of radiation (< 1 Gy) in comparison to what is observed in breast patients treated with standard parallel opposed tangential beams.

Although we consistently observed increases in cardiac uptake of 18F-FDG as a result of irradiation; a more complete histological analysis is needed to be certain that the increase in 18F-FDG post myocardial irradiation is due to cardiac inflammation. However, an inflammatory response to cardiac irradiation is strongly supported by previous studies in mice (48, 50, 51, 182). With regards to the validity of 18F-FDG PET for assessing inflammation, our group recently published a study using the same imaging protocol to assess inflammation following myocardial infarction (111) with correlation of in vivo FDG values with ex vivo measures of Indium labeled white blood cells. As indicated in the methods section of the study by Prato et al, the myocardial suppression protocol used to suppress myocardial glucose uptake was validated in a separate subset of 3 healthy
canines(111). Given that the same protocol was implemented here by the same team and using the same hardware, we are confident that the protocol was implemented correctly and equally reliable. Additionally, these results were consistent for all subjects which were imaged serially on separate days. Also, myocardial suppression was consistent and achieved in all animals at baseline; this is qualitatively supported by the minimal contrast between the LV blood pool and the myocardium on the baseline FDG images (see Figure 4-3) and quantitatively by the very low SUV values in the myocardium, well below the values that are seen in images using glucose clamp techniques to augment myocardial uptake of FDG (see Figure 1-8).

The cumulative dose to the heart from PET and CT imaging has been estimated using previously reported values(197) in conjunction with the average injected tracer activity. for this study to be ~0.2 mGy, 6.5 mGy, and ~6mGy for each $^{13}$N-ammonia, $^{18}$F-FDG, and each CT scan respectively. Thus the total cumulative dose from all 6 imaging sessions is approximately 1cGy which can be considered to have negligible impact on the current findings since it accounts for less than 1% of mean dose delivered to the heart from the medical LINAC.

We reiterate the statement from Chapter 3 that although the study was performed using a hybrid PET/MRI scanner, all of the techniques presented can also be implemented using PET/CT.

### 4.4 Conclusion

The results of this study have demonstrated that $^{13}$NH$_3$ and FDG PET can be used for the longitudinal assessment of myocardial perfusion and inflammation in association with cardiac external beam irradiation. Canine cardiac irradiation was designed to mirror the expected heart exposure associated with left-sided breast radiotherapy. In response to cardiac irradiation, progressive increases in FDG uptake, a non-invasive means of assessing inflammation, was observed and supported by ex-vivo histology in a single representative subject. A transient increase in myocardial stress perfusion that returned to
baseline levels (in 3 subjects) and below baseline levels (in 2 subjects) was observed. These findings alone do not yet provide conclusive support for or against the future use of $^{13}$NH$_3$ and/or FDG PET imaging for the longitudinal assessment of the efficacy of treatment approaches previously proposed for reducing radiotherapy-related radiation induced cardiac toxicity in humans. A further longitudinal study is needed to identify an imaging parameter that is correlated with regional dose magnitudes and/or cardiac outcomes.
Chapter 5

5 Conclusions and Future Work

This chapter begins with a brief summary of the experiments, key results, and conclusions from previous chapters of this thesis. The summary is followed by revisiting the research questions and hypothesis that were initially proposed in the introduction (see section 1.7). A discussion of proposed answers to those research questions and the level of agreement between the initial hypothesis and the thesis results are provided. Lastly, we motivate future clinical and pre-clinical research projects that can further advance the findings of this work. Specifically, future projects that can 1) extend our fundamental understanding of the pathophysiology of radiation-induced cardiac toxicity; and 2) improve the clinical management/prevention of radiation-induced cardiac toxicity for breast cancer patients subjected to radiation therapy.

5.1 Summary of Chapter 2

This chapter provided an assessment of the effects of respiratory-induced cardiac motion on the ability to quantify the dose to the heart, left ventricle, and left anterior descending (LAD) artery during standard breast cancer radiotherapy planning.

In breast cancer radiotherapy, the standard treatment planning approach uses a fast helical CT scan that cannot accurately capture the motion of internal organs caused by breathing. This is a concern for assessing dose to the heart since the anatomical position of the heart is influenced by respiration. In other clinical cases where internal motion amplitude is more pronounced (such as lung tumours) the fast helical CT is not appropriate. Rather, a helical CT scan with a much smaller couch pitch is used to capture multiple slice images over time. This technique is commonly referred to as a 4D-CT scan. The quasi-stalled translation of the couch through the CT allows for multiple ‘snapshot’ images to be acquired at the same axial slice position as a function of time. Additionally, during 4D-CT imaging each axial image slice is tagged according to phase of the breathing cycle during the acquisition. Since multiple images are obtained at each axial slice, it is possible to retrospectively sort the images into multiple 3D volumes at multiple time
points in the breathing cycle. Information regarding the breathing cycle used for sorting is obtained from the motion of an external surrogate attached to the patient’s abdomen and monitored using wall-mounted cameras. Using 4D-CT and a non-rigid image registration algorithm, the planned radiation dose can then be warped in accordance to the motion of the internal anatomy.

To assess the impact of respiratory motion on breast treatment planning, we compared the standard fast helical CT to the 4D-CT scan techniques (with dose warping) for calculating dose levels in of the heart, left ventricle, and LAD in 30 patients.

The results of this study showed minimal impact of respiratory motion on the mean dose to the heart and left ventricle. However, substantial variation in dose due to respiratory induced motion was observed for the left anterior descending artery (95% CI: ± 8.7 Gy). We concluded that the fast helical CT could provide a good approximation for doses to the heart and left ventricle; however, future clinical studies attempting to assess the clinical impact of LAD irradiation should use 4D-CT derived dose estimates for improved accuracy.

The study is unique in that it is the only published article (to the best of our knowledge) assessing the dose uncertainty due to respiratory motion on cardiac substructures (left ventricle and LAD) using the clinical imaging standard (fast helical CT). Previous studies using 4D-CT have quantified dose to the heart as a whole (83, 84, 87, 88). The added value of reporting the variations in left-ventricle and the LAD dose due to respiration is supported in the literature (14). There is now ample mounting evidence that left-sided breast cancer survivors experience a higher risk of radiation induced ischemic heart disease, a disruption of the normal circulatory system of the heart (14, 17, 126). Thus, the importance of dose estimates specifically to the left ventricle and LAD is warranted as these are the main localized sites that include the micro and macro-circulatory systems of the heart. Additionally, our study is the largest known study (30 patients) examining the impact of respiration motion focused on the dose to heart substructures. The conclusions drawn from Ding, et al (87) and Yue, et al (88) are based on a small sample of 6 and 7 left-sided breast cancer patients respectively.
The results from this study were also critical in the design of a clinically-relevant radiation dose distribution for large animal (i.e. canine) studies presented in Chapter 3 and Chapter 4.

5.2 Summary of Chapter 3

This chapter provided early, in-vivo, non-invasive assessment of myocardial perfusion and inflammation following focused cardiac irradiation in a canine model. Myocardial perfusion and inflammation were assessed using $^{13}$N-ammonia and $^{18}$F-FDG positron emission tomography imaging implemented on Canada’s first installation of hybrid PET-MRI scanner. Using information from chapter 2, the delivered dose to the heart was designed to resemble typical cardiac exposures of left-sided breast radiotherapy. The radiation dose distribution was computed and delivered using clinical treatment planning software (Pinnacle$^3$, Phillips) and a state-of-the-art medical linear accelerator (Truebeam, Varian), respectively. Myocardial perfusion and inflammation scans were performed at baseline, 1 week, and 4 weeks following cardiac irradiation. Absolute quantification of perfusion was performed through tracer kinetic modeling. Similar to standard clinical assessments, myocardial perfusion was assessed at both rest and during adenosine-induced vasodilation. To visualize myocardial inflammation using $^{18}$F-FDG PET, image data were acquired after the suppression of normal myocardial glucose metabolism to avoid this confounding factor.

The results of this study showed an increase in both myocardial perfusion and inflammation compared to baseline levels. Changes were observed at 1 week and remained elevated up to 4 weeks. This functional response appeared to be global rather than local, affecting both more intensely irradiated and less-irradiated myocardial segments similarly. This finding is consistent with the observed lack of correlation between local dose levels and changes in perfusion and $^{18}$F-FDG uptake.

To the best of our knowledge, the effects of cardiac irradiation at dose levels typically observed in the left-sided breast cancer population have not been previously studied using PET scans. The current findings are also supported by recently published results in a murine model(52). The majority of pre-clinical studies on radiation induced cardiac
toxicity have used murine models; however, here a large animal model of cardiac exposures is presented instead and the scaling allowed delivery of dose distributions comparable to human therapeutic exposures. The value added through the use of a large animal model provides easier translation of our findings into clinical practice. The large animal model offers a more representatively sized heart than murine models (150). Additionally, all previous nuclear imaging studies on the topic have used SPECT systems with qualitative analysis. Here, using cardiac PET, changes were observed very early as soon as 1 week post-radiation. This early observation is contrary to other imaging studies where changes could not be detected until at least 6 months (typically 18 months) post irradiation (45, 47). These findings are encouraging, however, future work is still needed to assess whether or not these PET findings are precursors to clinical end-points such as radiation-induced ischemia or if they are temporary effects of radiation that will resolve naturally.

5.3 Summary of Chapter 4

The fourth chapter of the thesis is a continuation of the third chapter. The same imaging techniques described in Chapter 3 were used to assess whether the early increases in perfusion and $^{18}$F-FDG uptake are transient or persistent effects of cardiac irradiation. All subjects were re-imaged at 3 months, 6 months, and 12 months using $^{13}$N-ammonia and $^{18}$F-FDG on a hybrid PET-MRI scanner. At 12 months the animals were euthanized and their hearts were excised for ex-vivo histology. One sample subject was selected for haematoxylin/eosin staining and CD45 immunohistochemistry to assess cellular morphology and the presence of inflammatory cells, respectively. A significant number of inflammatory cells were observed. However, histological analysis was preliminary as it was only performed in a single animal. Further analysis is needed to confirm the established presence and relative distribution of inflammatory cells within the left ventricle of the myocardium.

The observed early increase in perfusion (seen in Chapter 3) relative to baseline was seen to be maintained up to 6 months post cardiac irradiation. At 12 months, the myocardial stress perfusion estimates returned to baseline in 3 subjects and dropped below baseline in the remaining 2. A significant correlation could not be found between the magnitude of
perfusion changes and local dose deposition. To the best of our knowledge, early increases in perfusion following cardiac irradiation have not been observed previously. However, past myocardial perfusion studies following cardiac irradiation have been limited to qualitative cardiac SPECT imaging at much later time points, 6 months post irradiation (43, 45, 177, 198). This novel finding is supported by experimental studies in mice showing increases in vascular permeability, increases in vascular endothelial cell proliferation markers, and a transient increase in capillary density within 20 – 40 weeks following cardiac irradiation (19, 125, 182). These fundamental radiobiological mechanisms support and may explain our PET imaging observations at early time points.

Statistically significant increases in $^{18}$F-FDG myocardial uptake at 3 months, 6 months and 12 months post irradiation were observed. Unlike perfusion, the uptake of $^{18}$F-FDG gradually increased with time and does not appear to be resolving to baseline levels. The early increases in $^{18}$F-FDG myocardial uptake (seen in Chapter 3) relative to baseline do not therefore appear to be temporary; this response is persistent in all animals over the course of this 12 month study. This response to cardiac irradiation also appears to be global. A significant correlation could not be found between the magnitude of $^{18}$F-FDG uptake changes and the local dose deposition. These findings are novel, but a more complete histological analysis is still needed to assess whether the lack or correlation is due to a true global inflammatory response or limitations in hardware sensitivity. To the best of our knowledge, a non-invasive $^{18}$F-FDG PET imaging assessment of inflammation in which myocardial glucose metabolism is co-suppressed has not been previously used following cardiac irradiation levels relevant to breast radiotherapy. An inflammatory response to cardiac irradiation has been previously reported using ex-vivo techniques in mice (33, 50, 52). Attempts to correlate regional dose to inflammation is not currently possible in mice due to the small size of the heart. The findings from Chapter 4 are based on a much larger heart specimen and strongly support the clinical use of PET for a more timely assessment of treatment strategies aimed at reducing radiation induced ischemia.

The key results from Chapters 3 and 4 have been combined in Figure 5-1.
Figure 5-1: The temporal progression of $^{18}$F-FDG standard uptake (panel A), $^{13}$N-ammonia rest perfusion (panel B), and stress perfusion (panel C) within the left ventricle myocardium. Results are grouped by myocardial regions supplied by the left anterior descending (LAD) and/or the left circumflex (LCX). The estimated dose delivered is reported in the legend; error bars indicate the standard error of the mean.

5.4 Conclusions

In Chapter 1, the goal of this thesis was motivated with the following previously unanswered research question:

1. Does respiratory motion impact left-side breast cancer radiotherapy planning in terms of the accuracy of estimating doses to the heart, left-ventricle, and left anterior descending artery?

2. Can the effects of heart, left ventricle, and left anterior descending artery irradiation on myocardial perfusion and inflammation be visualized using PET at dose magnitudes comparable to modern left-sided breast radiotherapy?

3. Can the effects of cardiac irradiation be visualized using PET at earlier time points in comparison to other previously used imaging modalities?

4. Is there a spatial relationship between the magnitude of dose and regional changes in cardiac inflammation?
5. Is there a spatial relationship between the magnitude of dose and regional changes in cardiac perfusion?

6. Are specific regions within the heart, such as arteries, more sensitive to radiation effects than others?

**Question 1** was answered in Chapter 2 of this thesis. The answer to this question is “yes” for the LAD and left ventricle but not for the heart. Using detailed 4D-CT scan data we showed that respiratory motion had the most significant dosimetric impact on the LAD (±8.7 Gy). The magnitude of the variations in dose to the heart (±0.5 Gy) and left ventricle (±1.0 Gy) were much smaller. Additionally, the findings in the heart zone may be considered negligible since they are on the same order of magnitude as previously reported uncertainties due inter-observer variations in heart delineation(136). These findings also agree with our first hypothesis: “A significant variation in dose across heart structures is attributable to respiratory-induced motion during left-breast cancer radiotherapy.”

**Question 2** was answered in Chapters 3 and 4 of this thesis. The answer to this question is “yes”. PET can measure the effects of cardiac irradiation on perfusion and inflammation. We have shown statistically significant changes relative to baseline in myocardial perfusion and $^{18}$F-FDG uptake (indicative of inflammation) using PET.

**Question 3** was answered in Chapter 3 of this thesis. The answer to this question is also “yes”. PET can visualize the effects of cardiac irradiation at earlier time points in comparison to previously published more qualitative imaging studies using other modalities such as SPECT. In Chapter 3 we measured a statistically significant increase in both myocardial perfusion and $^{18}$F-FDG PET as early as 1 week post cardiac irradiation. A caveat for this answer is that previous SPECT studies were performed in breast cancer patients not a canine model. Although the canine model is routinely used for cardiac studies, further study is needed to determine whether the temporal response to radiation is different than what is observed in humans. For a definitive answer a humans study using PET is proposed as future work
**Question 4 and Question 5** were answered in Chapters 3 and 4 of this thesis. The answer to this question based solely on the results from this thesis is “no”. Both the perfusion changes and $^{18}$F-FDG uptake changes were not correlated with the spatial dose distribution. The effects of cardiac irradiation appeared to be more global, affecting both more irradiated and less irradiated segments similarly. A global response to focal cardiac injury is not a unique finding to this study. Others have observed considerable inflammatory cell recruitment in myocardium remote from the myocardial infarction(199). Furthermore, there is published evidence in other organs of a remote response in non-irradiated regions adjacent to irradiated regions, this is commonly referred to as the “bystander effect”(200, 201). That being said, it is a possibility that a correlation could be found given a larger sample size of animals; however, this is purely speculative. In addition to a greater sample size, perhaps a steeper dose gradient would yield a greater dose “spread” across myocardial regions for comparison. This may be relevant if $^{18}$F-FDG PET may not have the sensitivity or specificity to detect potentially subtle variations between myocardial regions irradiated by 9 Gy vs 1 Gy. Further study is needed prior to completely ruling out the presence of a relationship between dose magnitude and perfusion/inflammation changes.

In an attempt to answer **Question 6**, the dose distribution used in Chapters 3 and 4 was designed such that cardiac function could be assessed in localized regions where 1) the tissue and supplying artery were both focused with radiation; 2) only the tissue focused but the supplying artery was minimally irradiated; and 3) the tissue and the supplying artery were minimally irradiated. All three regions showed a similar response. Due to the global response to cardiac irradiation, the radio-sensitivity of the left ventricular tissue and the coronary arteries appear to be similar.

Our findings only partially agree with our second hypothesis: “A reduction in left ventricular perfusion and an enhancement in inflammation are related to local radiation dose levels within the heart.”

The observed increase in $^{18}$F-FDG was expected due to previous reports showing increases in inflammation in response to cardiac irradiation(52). The increase in FDG is in
agreement with our hypothesis; however, the result differs from the hypothesis in that it was a systemic increase in FDG as opposed to one which is proportional to the dose magnitude.

To the best of our knowledge the observed transient increase in perfusion has not been previously observed. We suspected that cardiac irradiation would lead to a gradual decline in perfusion. Declines in perfusion are hypothesized to be due to functional damage to the microcirculation supported by previous SPECT perfusion studies(198). Alternatively, the delivered cardiac doses in this study resulted in a reversed response by the microcirculation. Although this was unanticipated, it may partly explain the discrepancy with previously published SPECT perfusion studies. Perhaps the detection of perfusion defects can only occur after the initial “survival response” has subsided. This may explain the lesser incidence of cardiac perfusion defects at 6 months (27%) vs 24 months (42%) post radiotherapy(198). Lastly, the transient increase in perfusion followed by a decrease at 12 months is supported by a recent pre-clinical study in mice showing a transient increase in capillary density (+6-24%) at 20 weeks post radiation preceding a decline at 60 weeks (182).

5.5 Future Work

5.5.1 Clinical PET assessment of radiation induced cardiac toxicity following left-sided breast cancer radiotherapy

A natural continuation of the work presented in this thesis would be an extension to apply the dose planning and imaging techniques to a sample of left sided breast cancer patients. We propose a longitudinal study in humans comparing efficacy of alternatives to standard parallel opposed radiotherapy treatment (alternatives were previously discussed in section 1.5). The use of baseline and 1 week/4 week post radiotherapy PET imaging is proposed to assess cardiac inflammation in these patients. We hypothesize that patients showing reduced inflammation on PET will be less susceptible to future radiation induced ischemia. Unfortunately, this hypothesis cannot be assessed without long-term patient follow-up (10-15 years post radiotherapy).
Since it is well documented that inflammation often precedes tissue fibrosis(42), inflammatory response in a cohort of patients could be counteracted through the use of clinically approved anti-inflammatory medication (ie ACE inhibitors). Although ACE inhibitors are primarily used to treat hypertension, they’ve also been shown to have anti-inflammatory(202) and free radical scavenging properties(193). The use of ACE inhibitors to reduce radiation-induced cardiac damage has shown promise in a recent mouse study(176). Translation of these findings to humans would benefit significantly from a non-invasive means of assessing local cardiac inflammation such as \(^{18}\)F-FDG PET.

5.5.2 Incorporating breathing induced cardiac motion information in left-sided breast cancer radiotherapy planning to reduce cardiac exposures

Many studies have shown that breathing-adapted radiotherapy (respiratory gating or breath-holds) can provide significant reductions in cardiac exposures (56). However, these approaches can significantly increase the complexity and duration of planning and treatment of patients. Treating all breast cancer patients with this approach will likely reduce patient throughput and delay treatments. Furthermore, a recent study has shown that for some patients, breathing-adapted radiotherapy may lead to worse cardiac function than what is observed with the standard treatment approach(142). This problem motivates the search for an approach to quickly stratify patients most likely to benefit from breathing adapted radiotherapy.

Currently, two stratification criteria have been previously published: 1) stratification based on the distance between the heart/LAD to a line segment spanning the sternum and the mid-lateral edge of the body, referred to as mean heart depth(84), and 2) stratification based on a dose-volume threshold (apply breath-holds if the standard treatment plan results in more than 10 cm\(^3\) of the heart to be exposed to 25 Gy or more)(149). Although conceptually simple to implement, the use of the mean heart depth for stratification has not been widely accepted due to its poor correlation with the actual measured dose to the heart and LAD (reported R\(^2\) < 0.12) (84). The dose volume constraint proposed for stratification by Wang, et al is derived from results from a previous study(47). Lawrence,
et al, showed that the incidence of perfusion defects increased significantly (from 10-20% to 50-60%) for patients with >5% of the left ventricle exposed to at least 25 Gy (47). Unfortunately, implementing this dose-volume constraint for stratification requires a complete radiotherapy treatment plan to be created prior to making the decision. Unless centres can automate the task of creating breast radiotherapy plans, this method may significantly increase the treatment planning workload and lead to delays in patient treatments.

A future study where each patient’s breathing-induced cardiac motion is characterized is likely needed to establish better patient selection guidelines. For example, the impact of breathing motion is routinely characterized in lung cancer treatment planning through the use of 4D-CT. In lung cancer radiotherapy planning, the motion derived from the 4D-CT is used to create an internal target volume that encompasses the full range of tumour motion. We have shown in Chapter 2 that 4D-CT can also similarly be used to characterize the dosimetric impact of heart, left ventricle, and LAD motion. The ability to characterize the motion of the heart and LAD, can potentially lead to patient specific motion margins for these organs as well. Future studies may choose to investigate the role of cardiac motion margins created for avoiding the heart, left ventricle, and LAD at all possible phases of the breathing cycle. Without further investigation, the fast helical CT will likely continue being the clinical standard for breast radiotherapy planning and the effects of breathing on heart dose will continue to be ignored until 4D CT is implemented routinely.

5.5.3 Potential improvements in radiation induced cardiac toxicity models

Pre-clinical research is still needed to help us better understand the underlying pathophysiology of radiation induced cardiac toxicity. The relationship between dose magnitude and cardiac injury is still not well understood and raises questions about local and global response outside the irradiated zone. Previous proposals that cite a 7.4% increase in the rate of a major coronary event per 1 Gy dose to the heart may not be relevant today since this relationship was derived from obsolete breast cancer treatment techniques between the year 1958 and 2001 (17). In addition, today’s patients receive
adjuvant therapy, combining radiation with new chemotherapy agents not previously used until the early 2000’s. The relevance of this to radiation-induced cardiac toxicity is motivated by evidence linking chemotherapy with severe cardiac disorders(203–205). Anthracyclines (FDA approval in early 1970’s) and trastuzumab (FDA approval in late 1990’s) are two breast cancer chemotherapy agents that are commonly used and have been shown to negatively impact cardiac function. The effects of anthracyclines on cardiac function are well documented(206–208). Swain, et al, reviewed the literature and concluded that the cumulative percentage of patients with anthracycline-related congestive heart failure was between 5% - 26% (206). Anthracyclines have been shown to cause a buildup of reactive oxygen species that directly damage mitochondrial DNA within cardiomyocytes inducing cell death(209). This effect is similar to radiation-induced oxygen effects and may be compounded by chemotherapy activation. Anthracyclines directly damage cardiomyocytes, however, trastuzumab is believed to indirectly impact the cells through the disruption of the repair process(210). Furthermore, it is believed that these cardio toxic effects are even greater for patients with significant cardiac irradiation(211).

In light of this, future animal models attempting to study the relationship between cardiac toxicity and radiation should also include anthracyclines and trastuzumab. Here, we have shown the utility of PET for assessing cardiac response to radiation alone, however future PET studies should include the combined effects of chemotherapy and radiation.

5.5.4 Cardiac radio-sensitivity and setting clinical guidelines for “safe” exposure levels

It has been previously hypothesized that the radio-sensitivity of the heart is heterogeneous, meaning that some parts of the heart respond differently to radiation exposure than other regions. This heterogeneity in sensitivity has been observed in preclinical studies comparing the effects of coronary artery irradiation versus left ventricle irradiation(15, 18). Coronary artery irradiation has been shown to cause an acceleration of the formation of plaques that are prone to rupture(33). The left ventricle tissue is highly vascularised and irradiation of this structure leads to significant decline in the local functional capillaries(182). Both these effects are not mutually exclusive and
can lead to myocardial ischemia and infarction. Safe dose limits for each of these specific cardiac substructures are not yet known. In light of this, the current guidelines based on a single dose value (i.e. mean heart dose) may be a gross oversimplification.

Both dose magnitude and irradiated volume need to be considered when setting dose tolerance levels for organs. For the heart it is not known whether a low dose delivered to a large volume is less harmful than a high dose to a small volume. It has been hypothesized that the functional integrity of the coronary arteries is analogous to a chain. In other words, these arteries may constitute a radiobiological “serial organ” and the functionality of all of the subunits that make up the artery must be maintained. The radiation tolerance of serial organs such as the spinal cord depends on the magnitude of local dose delivered to a sub-unit than the total irradiated volume. Conversely, parallel organs such as the lungs and kidneys have a considerable functional reserve capacity; meaning they can still function normally even when small fraction of the organ is damaged. The microcirculation of the heart is housed in the left ventricles and has significant functional reserve capacity, supporting the hypotheses that unlike the artery it will behave as a parallel organ. Setting exposure guideline for the heart is therefore complicated because it is functionally dependent on both the major coronary arteries (serial organs) and the left ventricular tissue (parallel organ).

Future studies attempting to assess exposure guidelines for cardiac sub-units such as the coronary arteries and left ventricles should be performed in large animal models not mice. With the large animal models the heart is large enough to allow for more focused irradiation of the arteries and/or left ventricular tissue. Such a study can potentially lead to separate dose limits for the arteries and the left ventricle replacing or augmenting the current use of the total mean heart dose. In summary, we continue to propose that a large animal model should be used to mimic clinically-relevant dose levels and dose distributions giving opportunity to distinguish serial and parallel responses.
5.5.5 Hybrid PET-MRI imaging of radiation induced cardiac toxicity

Although this thesis used a hybrid PET-MRI system to assess cardiac function following radiation exposure, only PET findings were reported and used in our studies. As previously discussed (sections 1.6.5), the MRI compliments the PET system by adding both additional functional imaging options and anatomical context. Also, since PET-MRI systems can acquire images simultaneously, the imaging protocol implemented in Chapter 3 and Chapter 4 can be significantly reduced by assessing perfusion using dynamic contrast enhanced MRI while simultaneously assessing inflammation with $^{18}$F-FDG PET. Decreasing the imaging session duration is important for maintaining compliance when attempting to translate animal research to humans. Furthermore the use of MRI is more suitable for longitudinal human studies due to the reduction in radiation doses to the patient compared with PET imaging alone. Lastly, in addition to assessing perfusion with MRI, the system can determine the potential presence of radiation-induced edema, wall motion abnormalities, and fibrosis. To the best of our knowledge in-vivo assessment of radiation induced cardiac edema and fibrosis has not been fully quantified using MRI. Edema and fibrosis are two additional sub-clinical end points that may be used for assessing radiation induced cardiac toxicity mitigation strategies in humans.

It is hoped that the imaging and dosimetric findings reported in this thesis will assist in developing novel cardiac toxicity reduction strategies that will enhance complication-free survival of breast cancer patients subjected to the benefits of effective adaptive radiotherapy.
6 References


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185. Barjaktarovic Z, Shyla A, Azimzadeh O, *et al.* Ionising radiation induces persistent alterations in the cardiac mitochondrial function of C57BL/6 mice 40 weeks after local


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Appendix B: Worked general solution to equation 1-1

\[
\frac{dC_{tissue}(t)}{dt} = \rho K_1 C_{blood}(t) - K_2 C_{tissue}(t)
\]  

begin by taking the Laplace transform of both sides of equation 1-1

\[
L\left\{\frac{dC_{tissue}(t)}{dt}\right\} = L\{\rho K_1 C_{blood}(t) - K_2 C_{tissue}(t)\}
\]

in the Laplace domain we have the following equation

\[
s\tilde{C}_{tissue}(t) - C_{tissue}(0) = \rho K_1 \tilde{C}_{blood}(t) - K_2 \tilde{C}_{tissue}(t)
\]  

A-1

where \(\tilde{C}_{tissue}(t)\) and \(C_{tissue}(0)\) are the Laplace transform of \(C_{tissue}\) and the initial condition in the time domain respectively. Since the initial condition is equal to zero we can rearrange equation A-1 to solve for \(\tilde{C}_{tissue}(t)\)

\[
\tilde{C}_{tissue}(t) = \frac{\rho K_1 \tilde{C}_{blood}(t)}{s + K_2}
\]

\[
\tilde{C}_{tissue}(t) = \rho K_1 \tilde{C}_{blood}(t) \frac{1}{s + K_2}
\]

The following equation is obtained by taking the inverse Laplace transform of both sides. Also, recall that multiplication in the Laplace domain is convolution in the time domain.

\[
L^{-1}\{\tilde{C}_{tissue}(t)\} = L^{-1}\{\rho K_1 \tilde{C}_{blood}(t)\} \otimes L^{-1}\{\frac{1}{s + K_2}\}
\]

\[
C_{tissue}(t) = \rho K_1 C_{blood}(t) \otimes e^{-K_2 t}
\]
## Appendix C: Summary of patient treatment characteristics

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<td><strong>Whole Breast POP</strong></td>
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<tr>
<td>Standard</td>
<td>50 Gy in 25</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>42.5 Gy in 16</td>
<td>6</td>
</tr>
<tr>
<td>+ Boost</td>
<td>50 Gy in 25; 10 Gy in 5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>42.5 Gy in 16; 10 Gy in 5</td>
<td>4</td>
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<tr>
<td>+ SCLAV</td>
<td>50 Gy in 25</td>
<td>2</td>
</tr>
<tr>
<td>+ Boost; + SCLAV</td>
<td>50 Gy in 25; 10 Gy in 5</td>
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</tr>
<tr>
<td>+ IMC</td>
<td>42.5 Gy in 16</td>
<td>1</td>
</tr>
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<td>+ IMC; + SCLAV</td>
<td>50 Gy in 25</td>
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<td><strong>Chest Wall POP</strong></td>
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<td>+ SCLAV</td>
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<tr>
<td>+ SCLAV; + IMC</td>
<td>50 Gy in 25</td>
<td>2</td>
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</table>

Abbreviations: POP: tangential parallel opposed photon beams; SCLAV: supraclavicular irradiation; IMC: internal mammary chain irradiation (wide tangential parallel opposed photon beams); *all delivered using photons (6MV or 6MV & 18 MV Fields)
Appendix D: Animal Ethics Approval

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Subject: eSirisus Notification - New Protocol Modification Has Been APPROVED 2014-005:1

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From:

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AUP Number: 2014-005
PI Name: Prato, Frank
AUP Title: The Use of Hybrid PET/MR Imaging to Assess Radiation-induced Toxicity: A Canine Pilot Study (Pilot)

Official Notification of AUS Approval: A MODIFICATION to Animal Use Protocol 2014-005 has been approved.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee
Appendix E: Summary of multi-fractionated and BED corrected single fraction radiation dose constraints to the heart obtained from clinical patient data.

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<th>Biological Equivalent Dose (BED)</th>
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<td>Mean</td>
<td>95% CI</td>
<td>mean</td>
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<td>Heart</td>
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<td>[9.2, 27.6]</td>
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<td>28.8</td>
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Abbreviations: CI: confidence interval; V_{50}: volume of organ receiving at least 50% of the prescribed dose; D_{0.4}: maximal dose to 0.4 cm^3 of the organ; LAD: left anterior descending artery. * α/β ratio used for BED calculation was 2.5 Gy (Gillette S, et al. Radiother. Oncol. 1992;23:41–52)
## Appendix F: Summary of experimental details and cardiac dose distribution

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</table>

**Abbreviations:** PGL: plasma glucose level; ¹³N<sub>stress</sub>: ammonia injection during pharmacological stress perfusion; ¹³N<sub>rest</sub>: ammonia injection during rest perfusion; LV: left ventricle; LAD: left anterior descending artery; LCX: left circumflex artery. All data presented as mean ± standard error of mean. Since the radioactive decay follows a Poisson distribution, the expected standard deviation in the individual tracer activities is equal to the square root of the measured activity.
Curriculum Vitae

Name: Omar El-Sherif, M.Sc.

Education

Ph.D. in Medical Biophysics (CAMPEP accredited)
expected 2016
The University of Western Ontario, London, Ontario Canada
- Concentration: Radiation induced ischemic heart disease.
- Distinction: Translational Breast Cancer Research Fellowship (TCBRU).
- Leadership training: emotional intelligence and crucial conversations

M.Sc. in Medical Biophysics
June 2011
The University of Western Ontario, London, Ontario Canada
- Concentration: Cardiac magnetic resonance imaging (MRI)
- Distinction: CIHR-STP Strategic training program: vascular research fellowship
- Technical Training: Siemens IDEA MRI sequence programming certificate

H. BEng. in Biomedical Engineering
April 2008
The University of Guelph, Guelph, Ontario Canada
- graduated with honours

Relevant Work Experience

Clinical Physics
01/2015 - 05/2015 Linear Accelerator Quality Assurance Internship, London Regional Cancer Program. Responsibilities included weekly mechanical, safety, and radiation dosimetry testing on Varian external beam radiotherapy units.
01/2014 - 04/2014

Teaching Experience

09/2011 – Present Teaching Assistant, Medical Biophysics 4th Year Thesis (MedBio 4970), Department of Medical Biophysics, Western University. Responsibilities included lecture, evaluation of assignments, lab reports, thesis reports, thesis presentations, and oral examinations

01/2015 -05/2015 Curriculum Design and Development, Problem Solving (graduate level course), Department of Medical Biophysics, Western University. Responsibilities included application of formal pedagogical concepts in the design of learning objectives, lecture material, planned exercises, and assessment tools for 0.5 credit graduate level course.
01/2015 - 05/2015  **Research Mentor**, (3rd year undergraduate research project): Kevin Li (3rd year student), Department of Medical Biophysics, Western University

**Research**

01/2011 - 06/2011  **Research Assistant**, Robarts Research Institute, Department of Medical Biophysics. (Supervisor: Dr. James White)

01/2008 - 04/2008  **Undergraduate Research Assistant**, University of Guelph, Department of Computer Engineering. (Supervisor: Dr. Stefano Gregory)

### Academic Scholarships

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<td>Ontario Graduate Scholarship - Queen Elizabeth II Graduate Scholarship in Science and Technology</td>
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<td>Western Graduate Research Scholarship</td>
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<td>Canadian Institute of Health Research Strategic Training Program (CIHR-STP): Vascular Research Fellowship</td>
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### Awards

06/15  "Best in Physics" American Association of Physicists in Medicine (AAPM) Annual Meeting: "Hybrid PET-MRI Imaging of Acute Radiation Induced Cardiac Toxicity"

04/15  1st place poster. Cancer Imaging Network of Ontario, Annual Scientific Meeting: "Dosimetric Analysis of Respiratory-Induced Cardiac Intrafraction Motion in Left-sided Breast Cancer Radiotherapy"

10/14  Nominated for Alan C. Groom Award for top oral presentation of the 2014-2015 Department of Medical Biophysics Seminar Series.

07/14  Nominated for the Canadian Organization of Medical Physicists Annual Meeting, J.R. Cunningham young investigator symposium, Banff AB, "Dosimetric Analysis of Respiratory Induced Cardiac Intrafraction Motion in Left-sided Breast Cancer Radiotherapy"
Recognized by Schulich School of Medicine and Dentistry as being within the top 5% of doctoral students.

1st place poster presentation: London Health Research Day: "Heart radiation dose accumulation after left-sided breast cancer adjuvant radiotherapy"

**Publications and Presentations**

**PEER-REVIEWED MANUSCRIPTS: PUBLISHED**


**PEER-REVIEWED MANUSCRIPTS: SUBMITTED**


**PEER-REVIEWED MANUSCRIPTS: IN PREPARATION**


**CONFERENCE PROCEEDINGS**


**ORAL PRESENTATIONS**

07/15 American Association of Physicists in Medicine Annual Meeting, Anaheim CA, “Hybrid PET-MRI Imaging of Acute Radiation Induced Cardiac Toxicity”

04/15 Cancer Imaging Network of Ontario Symposium, London ON, “Dosimetric Analysis of Respiratory-Induced Cardiac Intrafraction Motion in Left-sided Breast Cancer Radiotherapy”

07/14 Canadian Organization of Medical Physicists, J.R. Cunningham young investigator symposium, Banff AB, "Dosimetric Analysis of Respiratory Induced Cardiac Intrafraction Motion in Left-sided Breast Cancer Radiotherapy"

06/14 Oncology Research and Education Symposium, London ON, “Protecting the heart during left-sided breast cancer radiotherapy”

01/14 Translational Breast Cancer Research Retreat, London ON, “Respiratory cardiac motion and dosimetric impact on left-sided breast irradiation”

03/13 Western University, Department of Medical Biophysics Seminar Series, "4D-CT for left-sided breast cancer patients: Towards patient-specific radiation treatment planning”

09/12 Western University, Department of Medical Biophysics Seminar Series, “3D vs 4D Dose Calculations for Lung Radiotherapy”

03/11 Western University, Department of Medical Biophysics Seminar Series, “Minimally Constrained Deconvolution: A Novel Approach for Quantifying Myocardial Blood Flow”

04/10 Western University, Department of Medical Biophysics Seminar Series, “Cardiac Magnetic Resonance Imaging: Quantifying Myocardial Blood Flow”

06/09 Western University, Department of Medical Biophysics Seminar Series, ”Cardiac Magnetic Resonance Imaging: Quantifying Myocardial Blood Flow”

**POSTER PRESENTATIONS**


RESEARCH RELATED PRESS


04/14 The Breast Cancer Society of Canada, "Protecting the heart during breast cancer radiotherapy" (http://bit.ly/1Rit1Qp)

Affiliations

- International Society of Magnetic Resonance in Medicine (ISMRM) – Student Member
- Canadian Organization of Medical Physicists (COMP) – Student Member
- American Association of Physicist in Medicine (AAPM) – Student Member