Early cardiac remodeling in women with breast cancer, receiving sequential therapy with anthracycline and trastuzumab – Cardiac MRI study

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science

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Cardiac Remodeling during Cancer Therapy in Women with Breast Cancer

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2019

Abstract

Background: Cardiotoxicity is an adverse prognostic marker in women with early-stage breast cancer. Understanding cardiac changes using cardiac-MRI will provide the opportunity to establish predictive models for early detection of cardiotoxicity.

Methods: Eighty-three patients had cardiac MRI pre-anthracycline, within three weeks post-anthracycline, and at five months (~three months into trastuzumab therapy) on a 1.5T scanner; along with thirty volunteers scanned at matched time points.

Results: Temporal and inter-observer test-retest variability, repeatability, and reproducibly of left and right ventricular volumetric parameters in healthy volunteers were statistically small. Ten patients (12.1%) developed LV-cardiotoxicity, 1 (10%) at 2 months and 9 (90%) at 5 months. LV-cardiotoxicity was associated with a significant increase in LVESV in 91% of the patients.

Conclusions: Ventricular remodeling occurs during cancer therapy. The primary mechanism of cardiotoxicity is likely a reduction in contractility. Change in LVESV at 2 months is an early predictor of cardiotoxicity by 5 months.

Acknowledgments

To my lord, the most gracious and merciful.

To my primary supervisors, Dr. Paaladinesh Thavendiranathan and Dr. Kim Connelly. Thank you for being there for me at all times and for all your excellent teaching and support. I have been most fortunate to have such great mentors and teachers as my supervisors. I remember when my leg was broken in my second year, how you supported me unconditionally during that difficult time.

To my Mom and Dad, Dr. Amira Ibrahim Al-Bayati and Dr. Abdul-Halim Abdul-Selam Altaha. I can't thank you enough for your unconditional love and support. Your inspiration has been my biggest motivation to continue walking the path of success despite the many challenges, especially that I have been living far from you. I have been through many tough times, but you constantly checking on me has kept me determined to achieve my goals.

To my beautiful wife Maryam Altaha and my two angels Taha and Teba. The long hours away from you do not go in vain. I love you and I want you to know that none of my success would have been possible without your presence in my life.

To my dearest brother Ahmed Altaha - the wise young man, I won't forget your frequent support, including and not limited to proof-reading my English writings early during my MSc. To my tender loving sister Dr. Zainab Abdul Selam, I still remember when you helped me prepare for my grade 12 exams by reading to me some of my text books when I was sick and couldn't open my eyes for many days.

To my PAC member and my previous supervisor, Dr. Narinder Paul. Thank you for believing in me and for all your support and advice. I remember when I started my very first research project under your supervision. With your excellent mentorship, I got into MSc and later into my dream residency training in Diagnostic Radiology. Also, to my PAC member, Dr. Eitan Amir. Thank you for being kind and flexible to meeting with me and for your advice.

To my best friend and big brother, Dr. Hatem Mehrez. Thank you for your noble altruistic character, as you always made time for me even when you have been busy. Your mentorship of me in the many aspects of life was one of my primary tools for success.

To all my colleagues and friends who contributed to this work or to my knowledge, including Dr. Mark Nolan and Roula Raptis. And to all doctors and scientists who taught me something within or outside the University of Toronto, including: Dr. Bernd Wintershperger, Prof. Jack Goodman, Dr. Andrew Yan, and Dr. Rachel Wald.

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Chapter 1

1 INTRODUCTION

1.1 Background and Rationale

Every year, about 26,300 new cases of breast cancer are identified in Canadian women(1). Up to one fourth of these new cases have an aggressive subtype due to over-expression of the human epidermal growth factor receptor 2 gene (HER2+) (1,2). For this aggressive subtype, chemotherapy with anthracyclines is routinely supplemented with trastuzumab - a revolutionary, monoclonal antibody that can reduce breast cancer recurrence by 50% and mortality by 30% (3-5). Unfortunately, however, this combined therapy is also associated with a 10-30% risk of cardiac-toxicity, defined as left ventricular dysfunction or heart failure (HF) (6,7). Cardiac-toxicity is a serious, life threatening complication; once HF ensues, about 60% of patients die within 2 years (8); furthermore, HF associated with cancer therapy was linked to 3.5-fold increase in mortality risk compared to idiopathic dilated cardiomyopathy(8,9). It is therefore important to be able use this combined cancer therapy to treat this common and aggressive form of breast cancer, yet without allowing for cardiac-toxicity to compromise patients' overall outcome.

The point at which cardiac-toxicity with this combined cancer therapy occurs is still not known. Many HER2+ breast cancer survivors don't experience any cardiac-toxicity while others develop heart failure (1 to 5%) and/or cardiac dysfunction (10 to 20%) during or even many years after treatment completion(6,7,10). There are many known risk factors for the development of cardiac-toxicity in these patients; however, cardiac-toxicity still occurs even in the absence of these risk factors. It is therefore important to use more sensitive methods to identify patients at high risk for cardiac-toxicity, so that ultimately heart failure and cardiac mortality can be prevented.

Traditionally, when cardiac-toxicity was first identified as a serious side effect of anthracycline therapy in the 70s, patients were frequently tested with myocardial biopsy during chemotherapy to identify those that develop myocardial pathological changes suggestive of cardiac-toxicity, so chemotherapy can be stopped to avoid heart failure. Soon this method was deemed ineffective

due to inconsistent results, sampling challenges, and aggressiveness of the procedure reducing the enthusiasm for it routine use. Since then cardiac-toxicity identification has been based on non-invasive methods to identify myocardial functional changes suggestive of myocardial injury. For this purpose, MUGA scans became the standard of practice with more recent move towards 2D/3D echocardiography due to the risk of exposure to radiation and limited information of myocardial structure obtained from MUGA scans. One of the popular definitions of cardiac-toxicity is proposed by the Cardiac Review and Evaluation Committee (CREC) as: "(1) cardiomyopathy characterized by a decrease in cardiac LVEF that was either global or more severe in the septum; (2) symptoms of congestive heart failure (CHF); (3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and (4) decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of CHF, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms" (11). Since most of current objective definitions of cardiac-toxicity require a significant drop in LVEF of at least 10% in the absence of signs and symptoms of heart failure, the inherent variability in echocardiography based LVEF measurements has raised concerns about its value in identifying early myocardial dysfunction. In fact, several studies have demonstrated that once there is a significant reduction in LVEF identified by echocardiography or MUGA, many patients will not have complete recovery despite current heart failure medications.

In the recent years there has also been a growing interest in using serum biomarkers (12); (13), and novel echocardiographic and other advanced cardiac imaging techniques to detect early cardiotoxicity (14). Most commonly studied serum biomarkers include high sensitivity troponin-I (hs-TnI) and Brain Natriuretic Peptide (BNP). Although not consistent amongst studies, hs-TnI has been shown to increase immediately post anthracycline administration and predicted future development of cardiac-toxicity (15-17). Increase in BNP levels appear to occur after more severe cardiac injury and have not been shown to be a good predictor of future cardiotoxicity. Measurement of myocardial deformation using novel echocardiographic strain techniques has been shown to better predict future reductions in ventricular function than conventional LVEF measurements. Echo-measured global longitudinal strain (GLS) is currently suggested as a useful tool to follow cancer patients during cancer therapy to identify early myocardial injury. However, echo-measured GLS has limited positive predictive value (50%) for subsequent fall in LVEF.

Cardiac MRI (CMR), is a safe non-invasive imaging technique that has the potential to identify subtle myocardial morphological and functional changes (18). This is due to the superior accuracy and reproducibility of CMR techniques to measure left ventricular volumes and function. Our group hypothesizes that in patients receiving cancer therapy early ventricular remodeling is more likely to happen before a change in myocardial function (i.e cardiotoxicity) can occur. This ventricular remodeling is best assessed using CMR over other cardiac imaging techniques. However, normal physiological changes over time in healthy volunteers and those receiving cancer therapies as measured by CMR are still unknown. This knowledge would be necessary to then define physiological versus pathological remodelling. A large study in homogenous patient group and with similar cancer therapy regimens, who are imaged frequently at constant intervals using CMR and biomarkers are ideal to study the process of ventricular remodeling. Understanding cardiac remodeling longitudinally can provide the opportunity to establish predictive models for early detection of cardiac-toxicity.

1.2 Hypothesis

1. Cardiac MRI measured ventricular function and volumes will have excellent temporal and observer variability and will be lower than that described for 2D and 3D echocardiography in the literature.

2. Significant increase in left ventricular end-systolic volume (i.e. a surrogate measure of reduced myocardial contractility) measured using cardiac MRI will precede the reduction in LVEF in women with HER2+ breast cancer who develop cardiotoxicity

1.3 Objectives

1. To define the temporal, inter-observer, intra-observer, and inter-observer test-retest variability for left ventricular function, mass, and volumes measurements in healthy volunteers.

2. To define the temporal and intra-observer variability for right ventricular function and volumes measurements in healthy volunteers.

3. To describe the cardiac remodeling of LV/RV during anthracycline and trastuzumab therapy in women with HER2+ breast cancer

4. To determine if an increase in LV-ESV can predict the development of cardiotoxicity during treatment

5. To determine the association between ventricular remodeling and blood markers (Troponin I, BNP)

Chapter 2

2 REVIEW OF LITRATURE

2.1 Cancer and Cardiovascular Disease

Cancer and cardiovascular disease (CVD) are the two most common causes of death globally. In Canada, cancer and CVD account for 30.2% and 19.7% of all deaths, respectively (Figure 2.1) (19). It is estimated that half of Canadians will develop cancer in their lifetime and that 50% of these patients will die from it (19).

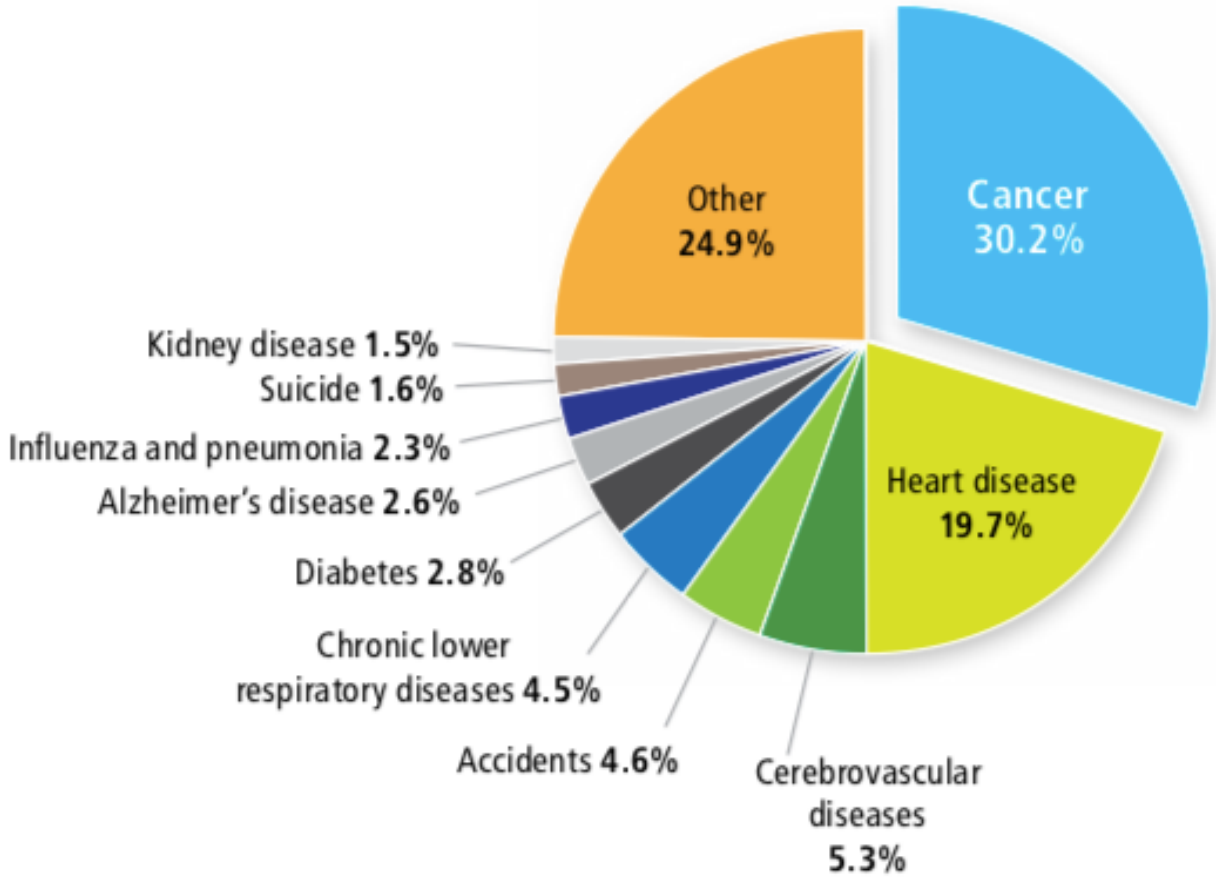


Figure 2.1. Statistics of Breast Cancer in Canada (2012)
Data source: Canadian Vital Statistics Death database at Statistics Canada

The second most common cancer in Canada is breast cancer with a 5-year net survival estimated at 89%. Cancer survivors have multiple causes for premature mortality including infections, heart disease, stroke, hypertension, lung disease, increased risk of other cancers, osteoporosis, and depression (20).

It is estimated that 2.4 million Canadian adults live with heart disease of whom 12 die every hour. Ischemic heart disease is the leading cause of premature mortality. A diagnosis of heart failure in adults (40 years or older) portends 6 times higher rate of death compared to non-heart failure patients. Heart failure, not only is a prevalent disease with significant morbidity and poor prognosis, but also has the highest hospital readmission rate (21). Majority of patients with heart failure get admitted at least once in their life time and about 22% of heart failure patients get readmitted again within the first month. These hospitalizations contribute to a large health care burden and cost. In the US, it has been estimated that heart failure hospitalizations per patient costs 83, 980USD over each patient's lifetime (22). In cancer patients, it is crucial, therefore, to prevent iatrogenic heart failure from developing during or after cancer therapy.

2.2 Cancer Survival

Cancer survival has steadily improved for the majority of cancer types over the last 30 – 40 years in Canada and in most developed countries across both adult and pediatric populations (17-21). This improvement can be attributed to many factors, but is mainly due to better cancer detection and treatment (23-25). The improvement in imaging techniques including CT scans and MRIs has made early detection feasible for many cancers. Chemotherapy and targeted therapy have significantly improved survival of most cancers. Therefore, with the advancement and availability of cancer therapies, millions of treated patients are now cancer survivors(19).

2.3 Cardiovascular disease in patients with cancer

Unfortunately with improved cancer survival, cardiovascular (CV) complications have become an important cause of morbidity and mortality in survivors (26). This is attributable to various

different reasons including shared risk factors and biology and the direct impact of cancer therapy on the cardiovascular system.

2.3.1 *Shared Risk Factors of Cancer and CVD*

We are increasingly learning that cancer and CVD are highly interconnected (27). Risk factors of CVD including cigarette smoking, obesity, unhealthy diet, and physical inactivity are also associated with increased incidence of cancer and worse outcomes (28) (Figure 2.2). Although cigarette smoking has been decreasing; according to Canadian statistics, as much as 17% of Canadians aged ≥12 years smoked either daily or occasionally in 2016. Smokers have 2 to 3 times higher risk of death mostly related to development of cancer and CVD, with recent data showing a direct link between smoking and breast and prostate cancers, as an example (29). Incidence of obesity over the last decade in Canada, has been relatively stable in children and adolescents compared to adults. However, 1 in 7 Canadian children and adolescents and 1 in 4 Canadian adults are obese (30). Obesity increases cancer incidence by at least 8%. Metabolic syndrome and its risk factors that are associated with obesity such as hyperglycemia, dyslipidemia, and elevated body mass index, all increase the incidence and mortality from cancer and CVD (31,32). It is intriguing to realize that obesity may increase CVD-related mortality indirectly through increased myocardial susceptibility to injury from cancer therapy; and that obesity may increase cancer-related mortality indirectly through decreased cardiovascular reserve due to pre-existing CVD (28). This double insult phenomena of obesity on patients with cancer and CVD may also be true for other shared risk factors of cancer and CVD, including smoking, poor diet, and sedentary lifestyle.

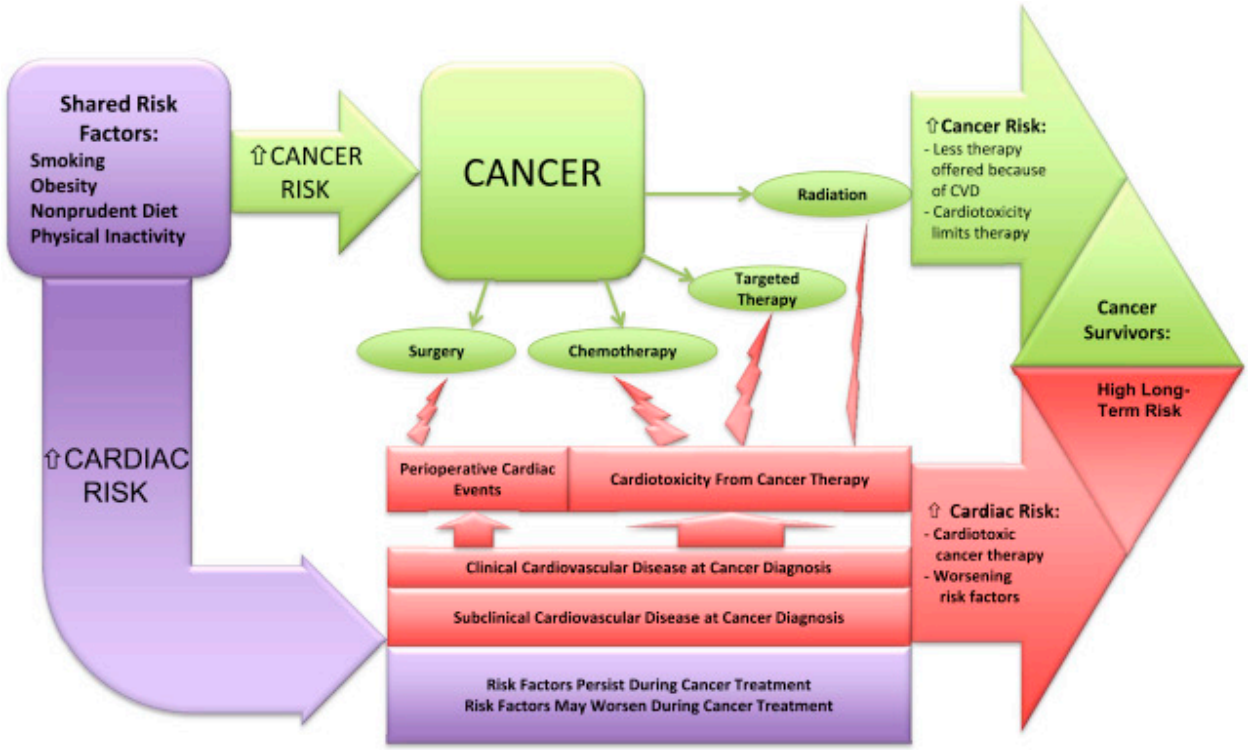


Figure 2.2. Shared risk factors of cancer and cardiovascular disease

2.3.2 *Shared Biology for Cancer and Cardiovascular Disease*

CVD risk is further heightened in cancer patients because of shared biology of the two diseases. Inflammation is a common factor in the etiology and progression of both cancer and CVD (33,34). Not only chronic inflammation can promote tumor formation, but also tumor formation can blunt the systemic inflammation response mediated by the host-immune system (33). High concentrations of cytokines and chemokines released by cancer inflammatory component attract leukocytes including: neutrophils, macrophages, lymphocytes, dendritic cells, eosinophils, and mast cells. These leukocytes then differentiate and can potentiate neoplastic process by producing wide range of mediators including chemokines, extracellular proteases, pro-angiogenic factors (such as vascular endothelial growth factor), and cytotoxic mediators (such as reactive oxygen species) (33,35). Likewise, Inflammatory mediators are long recognized as being central in the initiation and development of atherosclerosis leading to myocardial infraction (MI) as well as in complicating MI with fibrosis. Atherosclerosis formation starts

when inflammation causes changes in the endothelium leading to monocytes migration to the intima, followed by monocyte transformation to macrophages to engulf lipids to become foam cells and to initiate fatty streaks, and finally complex atherosclerotic plaques ensues as inflammation persists (34). Traditional CVD risk factors including obesity, diabetes, hypertension, and cigarette smoking are associated with inflammation, which also promotes carcinogenesis and tumor growth (36-41). Oxidative stress due to endogenous metabolic reactions or exogenous sources (e.g. smoking) is associated with cancer and CVD (41-43). An increasing number of hormones (e.g. leptin), cytokines, and growth factors are linked to both cancer and CVD (44,45). Consequently, patients who develop cancer are inherently at higher risk for CVD or may already have subclinical CVD.

2.3.3 *Cancer therapy as a cause for CVD*

In addition to shared risk factors and shared biology between cancer and CVD there is also a growing interest in the direct cardiovascular impact of cancer therapy (26). Cancer therapy can cause injury to the different structures of the heart and/or the peripheral vascular system depending mainly on the type of drug used (46). CV injuries differ in incidence, severity, and impact on clinical outcome mainly based on patient's characteristics (e.g. age, sex, comorbidities, risk factors, receptors status, genetic predisposition, etc.). Congestive heart failure with anthracycline therapy, for instance, has an incidence of ~1-5% but is associated with poor prognosis with mortality rates of 60% within 2 years (8). Examples of other common complications include coronary artery disease/acute coronary syndrome, LV systolic and/or diastolic dysfunction, acute myocarditis, pericarditis, arrhythmia, bradycardia, cardiogenic shock, hypotension, valvular heart disease, thromboembolism, angioedema, and myocardial fibrosis (47). CV complications can occur not only with the first line chemotherapeutic agents such as: anthracyclines (e.g. doxorubicin), alkylating agents (e.g. cyclophosphamide), and humanized monoclonal antibody (e.g. trastuzumab), but also with other currently used chemotherapeutic agents such as: antimetabolites (e.g. 5-fluorouracil), hormonal therapies (e.g. tamoxifen), anti-microtubule agents (e.g. paclitaxel), VSP inhibitors (e.g. bevacizumab), and proteasome inhibitors (e.g. proteasomib) (27,48). CV complications can also be caused by other forms of cancer treatment such as involved field radiation (26,27) and stem cell transplant. Table 2.1 shows some common chemotherapeutic drugs and their common CV complications.

Cardiovascular complications of cancer therapy have therefore become an important focus of research, with special interest in CHF due to its associated poor prognosis. And this focus is specifically relevant in Breast Cancer due to the fact it is the most common cancer amongst women and it has good survivorship.

Table 2.1. Summary of potential mechanisms of cardiovascular damage induced by common anticancer treatments

Examples of chemotherapeutics	Possible cardiovascular complications	DNA damage	ATP block	Apoptotic protein release	ROS generation	Endothelial cell damage/spasms
Anthracyclines	CHF, LVD, acute myocarditis, arrhythmia	+	+	+	+	–
Trastuzumab	Arrhythmias, CHF, angioedema, LVD	–	–	–	–	<>
Cyclophosphamide	Neurohumoral activation, mitral regurgitation	+	?	?	?	+
5-fluorouracil, Cytarabine	Ischemia, pericarditis, CHF, cardiogenic shock	+	+	+	+	+
Paclitaxel, Vinca Alkaloids	Sinus bradycardia, ventricular tachycardia, atrioventricular block, hypotension, CHF, ischemia	+	?	?	?	?
SERMs	LDL/HDL modulation, thromboembolism	–	–	–	–	–
Imatinib	Arrhythmias, CHF, angioedema, LVD	–	+	+	–	<>
Bevacizumab	Hypertension, thromboembolism, GI tract bleeding	–	–	–	–	<>
Thorax irradiation	Myocardial fibrosis, valvular heart disease, LVD	+	–	<>	+	+

(+) = likely; (−) = unlikely; (?) = unknown; (< >) = probable.
ROS = reactive oxygen species; CHF = congestive heart failure; LVD = left ventricular dysfunction; HDL = high-density lipoprotein; LDL = low-density lipoprotein; GI = gastrointestinal tract; SERMs = selective estrogen receptor modulators

2.4 Breast Cancer

Breast cancer is the most common cancer diagnosed among women in Canada, accounting for nearly 1 in 4 cancers (26%). It is also the second leading cause of cancer death among Canadian

women after lung cancer, accounting for 13% of all cancer-related deaths. One in 9 women in Canada is expected to develop breast cancer in her lifetime(1).

Breast Cancer mortality has improved by 44% since 1986 due to advancement in screening and treatment. The 5-year relative survival ratio has improved from 82% in 1992 to 87% in 2018. It is expected that breast cancer will continue to be a leading contributor to cancer burden moving forward. This is because, breast cancer incidence and mortality are many fold higher as women age, with the majority of breast cancer deaths (52%) occurring in women over 70 years of age(1).

2.4.1 *Breast Cancer types and taxonomy*

Breast cancer is a complex and heterogenous disease, which can be categorized based on histologic type, receptor status, clinical stage, and molecular taxonomy. The major breast cancer types are: invasive, non-invasive, inflammatory, and Paget's disease of the nipple(1). Based on the histological type, invasive breast carcinoma (IBC) is divided into 18 types; the majority of IBC (50-80%) fails to identify clear morphological features and thus grouped into IBC-not otherwise specified. The remaining 17 IBC types exhibit specified features and termed histological-special types (Table 2.2) (49) (50-52). Non-invasive breast cancer - AKA carcinoma in situ - includes mainly either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ, with DCIS being the most common of the non-invasive types. Breast cancer is further characterized by its receptor status. There are three main receptors clinically relevant to breast cancer: estrogen, progesterone, and HER2, each of which can be either status positive or negative. Breast cancer receptor status is an important factor in determining appropriate therapy. Breast cancer is divided into four stages (I-IV) depending on extent of the disease, with I-III considered early stage and IV as advanced or metastatic. Breast cancer can also be classified based on the molecular genotype. Molecular taxonomy is a new approach that has the potential to become the gold standard for breast cancer classification as it categorizes breast cancers based on transcriptomic features and patient outcome (53). Based on this method, three main types of breast cancer were identified: HER2+, luminal, and basal-like. As more molecular genotypes get identified, this model could be used to individualize therapy for breast cancer patients based on gene expression patterns (51).

Table 2.2. Main histological types, frequency, and outcome of invasive breast carcinoma

Histopathological type of invasive breast carcinoma	Frequency	10-year overall survival rate
Invasive ductal carcinoma not otherwise specified (IDC NOS)	50–80%	35–50%
Invasive lobular carcinoma (ILC)	5–15%	35–50%
Adenoid cystic carcinoma	0.1%	90–100%
Apocrine carcinoma	0.3–4%	Like IDC NOS
IDC with osteoclastic giant cells	Unknown	Like IDC NOS
Medullary carcinoma	1–7%	50–90%
Metaplastic carcinoma	< 5%	Unknown
Micropapillary carcinoma	< 3%	Unknown
Mucinous carcinoma	< 5%	80–100%
Neuroendocrine carcinoma	2–5%	Unknown
Tubular carcinoma	1–6%	90–100%

2.4.2 *Human Epidermal Receptor-2 Positive (HER2+) Breast Cancer*

The receptor of the endothelial growth factor (EGFR) in humans and experimental animals was discovered in 1975 (54-56). Around 1985, another member of the RTK family was identified that looked quite similar to the human EGFR, and thus was named human EGFR-related 2 (HER2) (57). As a member of the RTK, HER2 is a fundamental signaling-pathway receptor that regulates key cellular processes including cell division, migration, proliferation, metabolism, differentiation, and death, as well as intracellular communication during development (58,59). Other names of HER2 are HER2/neu and erbB2; with the neu being a name that was given to a rat oncogene that shares the same chromosomal location as that of HER2, and erbB2 is the same receptor but was named by a different research group independently (60). In 1987, Slamon et al showed for the first time that HER2 gene amplification is a significant predictor of both overall survival and time to relapse in patients with primary breast cancers. This discovery allowed

genomic researchers to develop a specific cancer therapy targeted against the HER2 receptor – the antibody trastuzumab.

Now, we know that about 20 to 25% of all primary breast cancers are HER2 positive (2). HER2 positive breast cancers amplify the HER2 gene leading to overexpression of HER2 receptors (up to 100 times the ordinary) making this subtype clinically more aggressive than the HER2 negative breast cancer (59). Recent studies have demonstrated that overexpression of HER2 receptor portends poor outcomes because this subtype is more likely to be associated with poorly differentiated high-grade tumors, increased rates of cell proliferation, high risk of lymph node involvement, resistance to certain types of chemotherapy, and greater rate of recurrence.

2.5 Breast Cancer Therapy

With improved screening, the majority of patients with newly diagnosed breast cancer today have curable early stage disease. The treatment of breast cancer depends on the stage of disease and tumor characteristics (61). Along with surgery, nearly all early stage patients receive either adjuvant or neoadjuvant systemic therapy to eradicate micro-metastases and a subgroup receives radiotherapy (61,62). The decision for systemic therapy is based on tumor characteristics, estrogen (ER) and progesterone (PR) receptor status, and amplification of the HER2 gene (61). Systemic therapy options for breast cancer are chemotherapy and endocrine therapy. Chemotherapy includes anthracycline or taxane-based treatment, and in HER2+ disease, the addition of trastuzumab. Adjuvant chemotherapy improves survival and reduces the recurrence of cancer by approximately 20-38% in early-stage breast cancer (63,64). The addition of ER blockade provides a 26% reduction in mortality and up to a 47% reduction in local recurrence at 10-year follow-up in ER+ disease (64,65). The use of radiotherapy reduces the absolute risk of cancer recurrence by 15.4-21.2% and mortality by 3.3-8.5%. Finally, the use of trastuzumab with chemotherapy is associated with an approximate 30% reduction in mortality and a 50% reduction in the recurrence of cancer in HER2+ patients (3-5,66,67). Therefore, with the available therapy for early stage disease, millions of treated patients are now cancer survivors.

2.5.1 *HER2+ Breast Cancer Therapy*

Many randomized controlled trials have shown that HER2 positive cancer is particularly sensitive to anti-HER2 agents, including trastuzumab, as well as to anthracycline-based chemotherapy (68-78).

2.5.2 *Anthracyclines*

Anthracyclines are an old class of chemotherapy, with daunomycin/daunorubicin being the first approved for clinical use more than 50 years ago. Anthracyclines have always been the corner stone of chemotherapy in many cancers and remain widely used in both early stage and metastatic breast cancers. Unfortunately, anthracyclines can cause injury to myocardial cells. As early as 1967, the first anthracycline used clinically (daunomycin/daunorubicin) was recognized to have serious cardiotoxicity including heart failure (79). Similarly in 1984, Adriamycin, which is a very potent anthracycline, was noticed to cause significant cardiotoxicity (80). Many studies have confirmed anthracyclines side effects on the heart and its mechanism of cardiotoxicity. Anthracyclines cause type-1 myocardial injury (Table 2.3) (81).

2.5.2.1 Mechanism of cardiotoxicity with Anthracycline

Proposed mechanisms of anthracycline cardiotoxicity include interference with cardiac topoisomerase Iβ and ROS mediated injury leading to cardiomyocyte apoptosis initially and necrosis with higher doses, as well as fibrosis of the extracellular space (82) (83).

Table 2.3. Main types of cancer therapy-related cardiac dysfunction

Type 1 **e.g. Doxorubicin**	**Type 2** **e.g. Trastuzumab**
Cellular death	Cellular dysfunction
Damage starts with first administration	
Biopsy changes	No typical biopsy changes
Cumulative dose related	Not cumulative dose related
Permanent damage (myocyte death)	Predominantly reversible (myocyte dysfunction)
Risk factors	
Prior/concurrent radiotherapy	Prior/concurrent anthracycline
Combination chemotherapy	Paclitaxel
Age	Age
Previous cardiac disease	Previous cardiac disease
Hypertension	Obesity (BMI > 25)

Typical anthracyclines that are in use today for HER2+ early breast cancer is doxorubicin and epirubicin. Myocardial injury form anthracyclines is dose dependent (84). Heart failure during Anthracycline therapy is reported in up to 18% of patients at cumulative doses of 700mg/m^2 of doxorubicin (85); however, even with lower cumulative doses and less toxic anthracyclines, cardiotoxicity is still reported in 3.3-18% of patients (83). Current suggested threshold of cumulative anthracycline doses to minimize the risk of cardiac dysfunction has been reduced to 240–360 mg/m2 doxorubicin and 450–600 mg/m2 epirubicin. At low cumulative dose of anthracycline, mild cardiac damage can be masked by cardiac compensatory mechanisms keeping LVEF in the normal range during this compensated stage (86). At moderate-high cumulative dose of anthracycline, the heart's compensatory reserve can get exhausted, leading to

development of overt cardiac-toxicity. Risk factors that increase cardiac-toxicity include: pre-existing co-morbidities, CVD or their risk factors, old age, genetics (87), previous exposure to chemotherapy or radiation, and concurrent exposure to chemotherapeutic drugs, especially to trastuzumab (Figure 2.3) (88). Even if the heart's function remains compensated during anthracycline therapy, the irreversible anthracycline-induced cardiac damage lingers in the heart forever as the cardiomyocytes do not have the capacity to regenerate (89). The heart function may then worsen overtime due to physiological and/or pathological reasons such as new onset hypertension or diabetes. This possibly explains the occurrence of late cardiac dysfunction after anthracycline exposure (90).

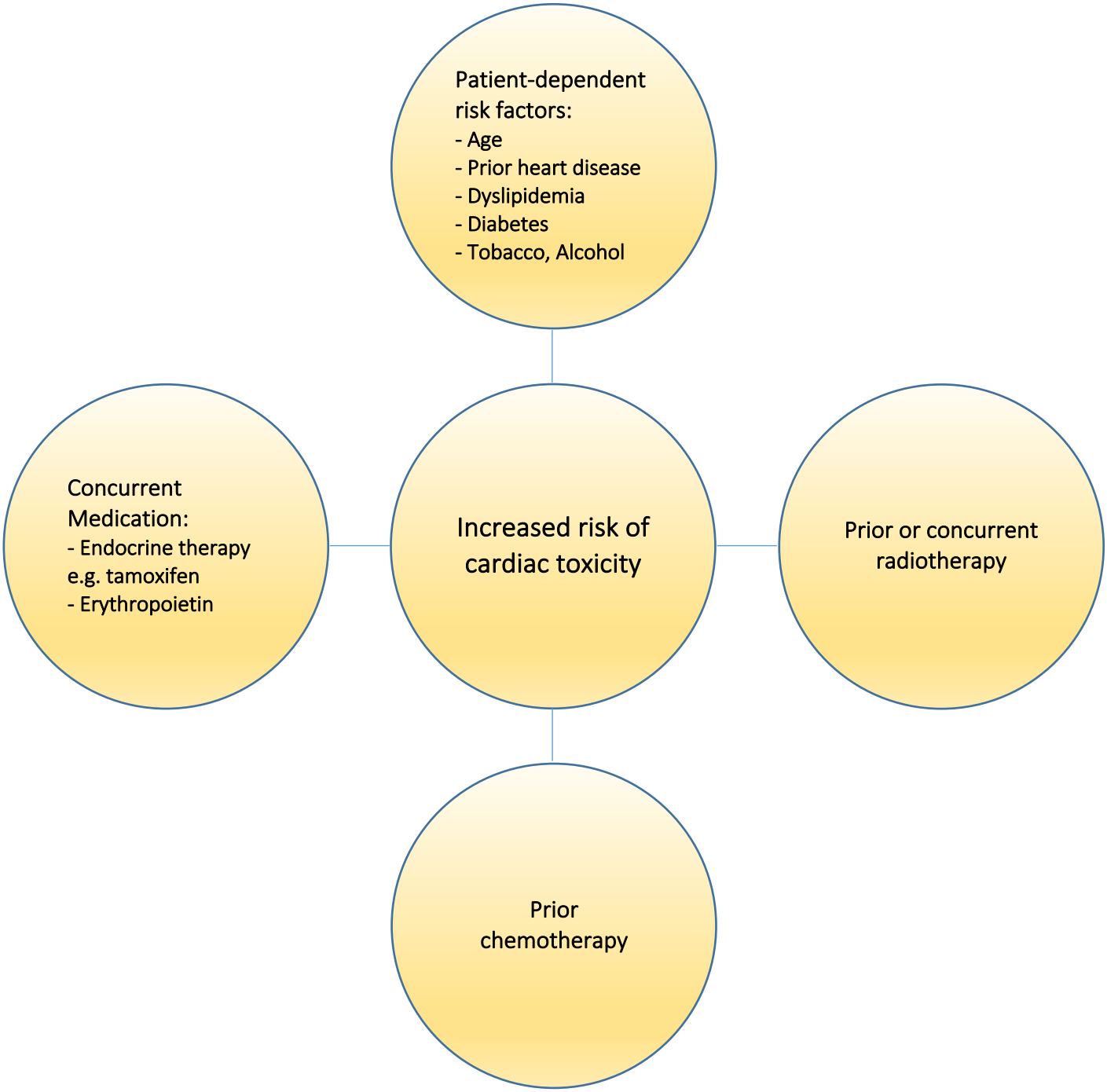


Figure 2.3. Risk factors of cardiotoxicity

2.5.3 *Trastuzumab*

Trastuzumab, a monoclonal antibody against HER2 receptor, was initially approved as a first-line treatment for metastatic HER2-positive breast cancer, in 1998 (5,72). In 2005, results from five important randomized controlled trials (RCTs) showed a significant survival advantage with use of adjuvant trastuzumab, in addition to the conventional therapy, in patients with early stage HER2+ breast cancers leading to the approval of trastuzumab as a first line therapy in this patient population (72,76). Based on short- and long-term results from these five RCTs and other RCTs subsequently: 12 months of treatment with trastuzumab became the standard of care for early stage HER2+ breast cancer (76). Some of the clinically important conclusions from these RTCs are the following:

1- The addition of trastuzumab to chemotherapy improved disease-free survival and overall survival significantly in comparison to chemotherapy alone (70-72).

2- Trastuzumab administered sequentially to chemotherapy that included anthracycline showed numerically better disease-free survival and overall survival than when administered with chemotherapy that didn't include anthracycline (69).

3- Trastuzumab initiated sequentially to anthracycline treatment showed less cardiotoxicity and better outcome than given concurrently (91,92).

4- Trastuzumab given for 52 weeks (12 month) with paclitaxel (subsequent to completion of anthracycline) had superior disease-free survival and overall survival when compared to no-trastuzumab administration; furthermore, trastuzumab administered concurrently with paclitaxel for 52 weeks showed numerically better (not statistically significant) disease-free survival and overall survival in comparison to trastuzumab administered sequentially (75,76).

5- Two years of trastuzumab offers no additional benefits to patients compared to 12 months of trastuzumab (73); of note, outcome of 6 months of trastuzumab treatment was recently tested versus 12 months and showed a non-inferior results, yet was still considered not significant enough to alter the current standard of 12 months treatment course of trastuzumab (74).

2.5.3.1 Mechanism of action of trastuzumab

Trastuzumab is a monoclonal antibody that binds to domain IV of HER2 receptor on the surface of breast cancer cells as well as cardiomyocytes interrupting HER2 signaling (59). Disrupting HER2 signaling of the overexpressed HER2 receptors interferes with tumor progression leading to reduced aggressiveness and improved outcome in HER2+ breast cancer patients making trastuzumab an essential treatment in this patient population.

2.5.3.2 Mechanism of cardiotoxicity with trastuzumab

Unfortunately, HER2 signaling is indispensable for normal cardiac function. Furthermore, HER2 signaling has been shown to have a cardioprotective effects in both human and animal hearts (92). Trastuzumab binding to HER2 receptors can halt important cellular mechanisms that are normally cardio-protective, including during stress. Some cardio-protective mechanisms of normal HER2 signaling include promotion of: "antiapoptotic pathways (93-95), hypertrophic and even mitotic growth (87,88,90), cell elongation with improved cell-cell adhesion (96), angiogenesis (97), and reduced sensitivity to adrenergic stimulation (98)" (99). These important cardio-protective mechanisms of HER2 signaling are referred to as survival pathways. Interrupting these survival pathways in the heart can cause cardiac dysfunction. Cardiac-dysfunction can occur even when trastuzumab is used as monotherapy with incidence rate reported at 2-7% (11). Trastuzumab's mechanism of myocardial dysfunction is likely through impairing contractility and not through death of myocytes and permanent myocardial damage as in the case of anthracyclines. Trastuzumab causes type-II cardiotoxicity, as opposed to the classic type-I cardiotoxicity caused by the prototype anthracyclines (100), (Table 2.3). The main feature of trastuzumab's type-II cardiotoxicity is its high likelihood for recovery to baseline or to near baseline cardiac status within 2-4 months of trastuzumab discontinuation reflecting its half-life of 20-25 days. Heart function can return to normal post discontinuation of trastuzumab allowing for future re-administration of trastuzumab when required. Other proposed mechanisms of trastuzumab-induced myocardial dysfunction, however, include mitochondrial dysfunction with ATP depletion and immune-mediated destruction of cardiomyocytes (101).

2.6 Cardiotoxicity

With improved breast cancer survival, the cardiac-toxicity due to cancer therapy has become an important cause of morbidity and mortality in survivors (6,83,102,103). The main drugs implicated with cardiotoxicity in patients with HER2+ breast cancer are anthracyclines and trastuzumab (83). The five-year cumulative incidence of major cardiac events due to cancer therapy in HER2+ breast cancer is 4.13%, whereas it is 1.68% in the general population with no cancer (104). Although disease-free survival and overall survival is significantly better when anthracyclines are used and sequentially followed by trastuzumab, the incidence of cardiotoxicity is higher with this sequential regimen than with either drug alone or anthracycline- and trastuzumab-free regimens. Incidence of major cardiac events during sequential therapy with anthracycline and trastuzumab in clinical trials ranges from 3.9% in NSABP-B31 trial (105), to 2.0% in BCIRG 006 trial (106), and to 1.7% in a meta-analysis of clinical trials (107). Specifically in the NSABP-31 trial of sequential anthracyclines and trastuzumab therapy, the incidence of HF and subclinical LV dysfunction was 3.6% and 14.0% respectively (108). Even the development of subclinical LV dysfunction is not benign, as data from the Framingham study suggests a 4.8 fold higher risk of subsequent symptomatic HF and 1.6 fold higher risk of mortality (10). Outside of the clinical trial setting, cardiomyopathy and HF among anthracycline and trastuzumab-treated patients have ranged between 15.5-41.9% over long-term follow-up (6,7). The higher toxicity with the combined therapy can result from a “two-hit” hypothesis which proposes that the myocardial injury and activation of cardiomyocyte death pathways by anthracyclines is not naturally opposed by the survival pathways mediated by the HER2 receptor activation (109). Inhibition of the survival pathways that can be mediated by trastuzumab, can aggravate cardiomyocyte death, LV dysfunction, and subsequent HF (110).

2.6.1 *Definition of Cardiotoxicity*

There are several definitions for cardiotoxicity. They typically vary based on the modality used, inclusion of symptoms, and lower cut-off of normal depending on the experience of the different cardiology and oncology organizations (111). The National Cancer Institute, for example,

defines cardiotoxicity as “toxicity that affects the heart”; although this definition is quite simple, it includes direct and indirect effects of the cancer therapy on the heart (46,112). The Cardiac Review and Evaluation Committee (CREC), as mentioned earlier is more comprehensive as it defines cardiotoxicity as “(1) cardiomyopathy characterized by a decrease in cardiac LVEF that was either global or more severe in the septum; (2) symptoms of congestive heart failure (CHF); (3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and (4) decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of CHF, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms” (11). Although, this definition is clinically used, it is not sensitive to the subclinical injury that can happen with cancer therapy (46). In our study, we used the fourth criterion of the CREC definition since this definition was also used in the Cardiac Dysfunction in the Trastuzumab Clinical Trials Experience (11).

2.6.2 *Cardiotoxicity due to Radiotherapy*

Radiotherapy can also be associated with cardiotoxicity due to micro or macro-vascular coronary disease or acute myocarditis; but, the incidence is not well established (113,114). Most clinically-important cardiotoxicity due to radiotherapy is in patients with early breast cancer and Hodgkin’s lymphoma, as these patients are typically young and survive longer to experience the late effects of cardiotoxicity, including fatal cardiovascular events in up to 2.2% and 7% of the patients respectively (115).

2.6.3 *Cardiotoxicity due to Endocrine Agents*

Tamoxifen and/or aromatase inhibitors (AIs) are established agents for the treatment of post-menopausal hormone receptor-positive early breast cancer. Third generation AIs, however, have an advantage over tamoxifen for treatment of estrogen-receptor positive breast cancer by significantly reducing recurrence and mortality and improving overall survival. AIs, nevertheless, increase the risk of cardiovascular disease including ischemic heart disease in comparison to tamoxifen as the latter has a cardioprotective effect; the absolute risk of

developing cardiac-toxicity has been shown to be 4.2% with aromatase inhibitors and 3.4% with tamoxifen (116-118).

2.6.4 *Outcomes of Cardiotoxicity*

The development of symptomatic HF with anthracyclines is associated with poor prognosis (8), therefore, attention has been directed towards recognition of patients during stage B HF. Stages of heart failure defined by ACCF/AHA are: stage A, at high risk of HF but without structural heart disease or symptoms; stage B, structural heart disease but without signs or symptoms of HF, stage C, structural heart disease with prior or current symptoms of HF; stage D, refractory HF requiring specialized interventions(119). Based on HF guidelines, patients in stage B HF generally benefit from treatment with beta-blockers and ACE inhibitors (120). Currently, in HER2+ breast cancer patients receiving cancer therapy, stage B HF is identified by repeated LVEF measurements pre-therapy, prior to initiation of trastuzumab, and every 3 months thereafter. First, modification to cancer therapy or addition of cardiac medication is dependent on a reduction in LVEF. This approach has limitations since measurement variability for LVEF using 2D or 3D echocardiography (echo) in breast cancer patients is ~6-10% (14). Even with multi-gated acquisition scans (MUGA), LVEF measures are affected by physiological changes (86). However, cardiac MRI-measured LVEF can provide more accurate diagnosis of cardiotoxicity given its lower measurement variability. Secondly, once LVEF falls, it frequently necessitates interruptions to cancer treatment possibly affecting cancer outcomes. Thirdly, although traditionally, stage B HF is defined by an asymptomatic drop in LVEF, for chemotherapy-induced cardiomyopathy, even this definition appears to be too late. In studies of anthracycline- (121) or trastuzumab- (121-123) treated breast cancer patients, once LV dysfunction occurred, 40-58% of the patients had no subsequent recovery. These patients then had more adverse cardiovascular events in follow-up. Therefore, for chemotherapy-induced cardiotoxicity it is important to move beyond the traditional definition of stage B HF and identify other surrogate markers that indicate ventricular injury before LVEF falls, including LV end-systolic volume (ESV), LV end-diastolic volume (EDV), myocardial strain, and tissue characterization techniques. These early changes may trigger the initiation of therapies to prevent subsequent drop in LVEF and HF.

2.6.5 *Why not treat everyone at risk for cardiotoxicity prophylactically?*

One approach to preventing cardiotoxicity is to treat everyone receiving cancer therapy prophylactically with cardio-protective therapy including angiotensin converting enzyme inhibitors (ACEi) and beta-blockers (BB) (124). Although this approach is attractive, the literature on the benefit of this approach is controversial. (125,126). Two recent randomized controlled trials (PRADA and MANTICORE) have tested the effect of using cardioprotective medications during treatment of early breast cancer therapy (127,128). In PRADA trial, candesartan, but not metoprolol mitigated the decline in LVEF (125). In the MANTICORE trial, where the effects of perindopril and bisoprolol were studied for the prevention of trastuzumab-mediated cardiotoxicity; neither was able to prevent ventricular remodeling, despite having attenuated trastuzumab-mediated declines in LVEF (126). Another limitation of the "treat-all approach" include the that patients receiving chemotherapy commonly experience vomiting, dehydration, pre-syncope, renal impairment, and fatigue; therefore, the non-selective addition of medications such as beta-blockers and ACE inhibitors may be poorly tolerated as recently demonstrated (124). Also, since in clinical practice ~75% of the patients will not experience cardiotoxicity, the need for close monitoring with the cardiac drugs in all patients will make this approach costly. At present, an approach focused on identification of patients at the highest risk for developing cardiac dysfunction and initiating targeted therapy appears more appealing.

2.6.6 *Current methods to identify cardiotoxicity*

2.6.6.1 Biopsy

Endomyocardial biopsy was first successfully performed in 1956 (129) and it underwent many refinements to become more safe over the years (130). Endomyocardial biopsy has some advantages for cardiotoxicity identification over the conventional non-invasive methods: (1) it can identify early changes of cardiotoxicity at the cellular level before the functional and clinical changes occur; (2) it can differentiate, to a certain degree between cardiac pathological changes

specific to cancer therapy versus other types of heart disease; and (3) it can determine the grade of overall pathology, and whether further cancer therapy can be safely given (131). The challenges with biopsy, however, include: (1) patient safety, (2) limited accessibility and (3) its limitation to subendocardial myocardium. Perhaps the main limitation of biopsy in the context of cardiotoxicity is secondary to its false positive and false negative results where pathology in cardiotoxicity is typically diffuse, and biopsy is usually limited to a number of small-sized tissue specimens usually accessed from the right ventricle only. Biopsy thus can frequently miss significant lesions, or sometimes lands on a pathological lesion that is otherwise not clinically representative of the entire heart. As a consequence correlation between histologic and functional changes has been deemed imperfect (132). Endomyocardial biopsy therefore has limited usefulness for serial assessments in the context of early detection of cardiotoxicity during cancer therapy.

2.6.6.2 MUGA

Multi-gated radionuclide angiography (MUGA) is a conventional, non-invasive technique that uses 99m Technetium (Tc)-erythrocyte labeling to visualize the cardiac blood pool by gama-camera (133). MUGA provides a precise and reproducible measurement of LV volumes and function independent of geometric assumptions, yet still slightly less accurate than CMR (134). For many decades, MUGA was the standard technique used clinically to measure left ventricular volumes and function including adult patients treated with cardiotoxic agents (135). However, due to its limitations, MUGA is now reserved as a third modality in cardio-oncology after echo and CMR (136). These limitations include: exposure to ionizing radiation, susceptibility to soft tissue attenuation artifacts affecting image quality, and susceptibility to patients' physiological changes resulting in inaccurate LVEF measurement affecting its usefulness in the serial assessment of cardiotoxicity (83,130). Recent studies using more contemporary equipment have demonstrated better agreement between MUGA measured LVEF and cardiac MRI specifically in patients receiving cancer therapy, but radiation continues to be a big limiting factor (137).

2.6.6.3 Blood biomarkers to predict cardiotoxicity

Amongst blood biomarkers, the one that is best associated with early cardiac injury is cardiac troponin. Cardinale et al demonstrated that patients who had troponin increase early post high-dose anthracycline, developed cardiac dysfunction many years later (138). They also showed that post trastuzumab-based therapy, an early rise in troponin was an independent predictor of future development of cardiac dysfunction (12). However, data for troponin levels to predict LV dysfunction or HF are conflicting (15-17). The type of troponin assay to use, the optimal time to measure during treatment, and the threshold to define abnormality are unknown. The largest studies that have illustrated the utility of troponin as a marker of early injury performed blood sampling at 5 time points around each chemotherapy dose (15-17,138). Clinical application of such a strategy is challenging. The two most recent studies of breast cancer patients receiving adjuvant trastuzumab therapy have shown that even high sensitivity troponin I (hs-TnI) only had a positive predictive value between 44-50% (139) (140) for subsequent cardiotoxicity.

Other novel biomarkers that have been recently shown to have potential value in predicting cardiotoxicity are myeloperoxidase (MPO) and growth differentiation factor (GDF)-15 (13). MPO is believed to be a marker of oxidative stress, which may represent the central mechanism of anthracycline cardiotoxicity (141,142), as well as ErbB2 inhibition, by trastuzumab (143). Similarly GDF-15 is a member of the TGF-cytokine family with increased expression with oxidative stress and inflammation (144,145). There is currently limited literature on the use of these latter biomarkers and the timing of measurements or the thresholds to detect early cardiac injury have not been defined.

2.6.6.4 Echocardiography

Two-dimensional echocardiography (2D echo) is currently the most widely used method to assess cardiac function in cancer population. It remains a preferred method for LVEF assessment over MUGA since the latter exposes cancer patients to radiation. 2D echo determines cardiac function by measuring LVEF indirectly by utilizing geometric assumptions. 2D echo reproducibility is however limited by geometric assumptions and by being operator dependent. 3D echo is a newer, less operator dependent technique when compared to 2D echo as it uses semi-automated algorithms to assess LVEF, which rely less on geometric assumptions but is

dependent upon good acoustic windows. As a result of the above limitations,2D and 3D echo inter-observer variability has been shown to be high (when compared to MUGA or CMR), limiting their utility in sequential screening and early detection of cardiotoxicity. Thavendiranathan et al showed that 2D and 3D echo inter observer variability to be 11% and 8%, respectively (14). 2D echo represents a significant advance in those with poor acoustic windows enabling superior assessment of regional function, but also suffers from high inter-observer variability because of limited visualization of the base of the heart and also suffers from operator dependence. Regardless of these limitations, the ability of echocardiography to assess both LV and RV systolic and diastolic function, volumes, pericardial disease and valvular dysfunction in a portable, cost effective manner has maintained echocardiography place as the preferred modality used to assess cardiotoxicity.

2.6.6.5 Peak systolic global longitudinal strain (GLS) to predict cardiotoxicity

Echo measured GLS is deemed sensitive method for screening and for measuring subclinical LV dysfunction (17,146,147). An early reduction in GLS during breast cancer therapy appears to predict subsequent LV systolic dysfunction (139,140). However, obtaining diagnostic quality images with echo is often difficult due to post-operative chest-wall pain and acoustic window limitations, especially in patients with breast cancer. Even in healthy volunteers, strain measurements may not be obtained in 1 in 5 subjects (148). Secondly, the relative change in myocardial strain values that have been shown to predict subsequent cardiotoxicity for the population is between 10-15% (139,140,149,150). However, the reported 95% confidence interval for the inter-observer variability for GLS even in healthy volunteers has been between -11.4% and +11.8%, while the test-re-test variability was -9.6% to +9.7% (148). Therefore, using such a threshold in an individual patient can be challenging especially in patients with significant hemodynamic variations due to chemotherapy. As a consequence, early GLS changes have only shown modest sensitivity and specificity and poor positive predictive value (50%) for subsequent cardiotoxicity (139,140,151).

Therefore, given the limitation of existing methods to predict cardiotoxicity, a large study that examines all these potential predictors along with novel markers of cardiotoxicity is needed to identify the most robust predictor of subsequent cardiotoxicity. This will allow investigation of

patient-specific cardio-protective strategies to prevent cardiotoxicity and its associated morbidity/mortality and allow uninterrupted completion of cancer therapy.

2.6.6.6 Cardiac MRI to predict cardiotoxicity

Cardiac MRI, a non-invasive non-radiation based imaging technique is currently the most precise and accurate technique to identify a drop in LVEF (152). Furthermore, CMR's precision allows for accurate quantification of two important determinants of LVEF, namely end-diastolic (EDV) and end-systolic volumes (ESV). Physiologically, LV-EDV and/or LV-ESV must change before a drop in LVEF can occur. Changes in LV-ESV and/or LV-EDV can represent changes at the morphological level, whereas a decrease in LVEF reflects a change at the functional level. The changes at the morphological and functional cardiac levels are collectively known as cardiac remodeling. Studying cardiac remodeling longitudinally may provide novel knowledge as to whether a change in LV-EDV and/or LV-ESV beyond a certain threshold can predict a subsequent drop in LVEF.

Cardiac MRI also have the advantage of providing information beyond quantification of LV and RV EF, volumes, and mass. Cardiac MRI can assess myocardial tissue characterization, myocardial deformation as well as atrial and vesicular flow assessments, making cardiac MRI the obvious go to technique for accurate and comprehensive assessment of heart function. Table 2.4 compares the main imaging modalities used for assessment in cardiotoxicity.

Table 2.4. Cardiotoxicity imaging modalities' characteristics

Imaging modality	MUGA	Echocardiography			Cardiac MRI
		2D	3D	Strain	
Availability	Widely available	More widely available	Less widely available	Less widely available	Least widely available
Cost	Expensive	least expensive	Less expensive	Less expensive	Expensive
Reproducibility	Reproducible	Least reproducible	less reproducible	less reproducible	More reproducible
Risk of radiation	Yes	No	No	No	No
Geometric assumptions	No	Yes	No	No	No
Operator dependency	Minimal	Yes	Minimal	Yes	Minimal
Feature tracking	No	No	No	Yes	Yes
Tissue characterization	No	No	No	No	Yes

2.6.7 *Current knowledge gap in identifying cardiotoxicity*

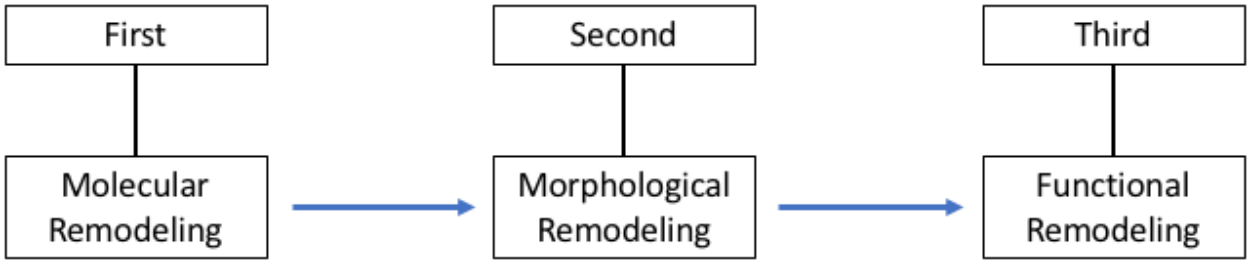
Early detection of cardiac-injury to prevent cardiac-toxicity is a research priority. Currently there are no risk prediction models for early detection of cardiac-toxicity. Although there has been a lot of enthusiasm about new techniques such as 2D and 3D echo, echo-measured GLS, and hs-TnI, they all have intrinsic limitations. For example, the measurement variability for LVEF using 2D or 3D echo in breast cancer patients is >6-10% (14); also the positive predictive value of echo-measured GLS as well as hs-TnI for detecting subsequent cardiac-toxicity is only around

50% (139,140,151). CMR has not been studied formally for early detection yet. CMR has a better precision and accuracy to detect small changes in LVEF (<5%); CMR can also detect changes in myocardial volumes (morphological changes) that precede the small changes in LVEF (functional changes). Furthermore, there is a limited knowledge of the association of these cardiac morphological and functional changes in cardiac-toxicity. Understanding these associations can potentially help in early prediction of future cardiac-toxicity. Understanding these associations requires examining these changes longitudinally in a large, homogenous cohort of patients. In addition, it is also important to understand normal physiological variability in healthy individuals over time in order to define thresholds above which a change can be considered to be pathological. Therefore, understanding remodeling in both healthy people and breast cancer patients will allow us to define the volumetric and functional changes associated with development of cardiac-toxicity. Our work will help establish this new knowledge.

2.7 Cardiac Remodeling

2.7.1 *Definition of Cardiac Remodeling*

The term “cardiac remodeling” was first referred to by Pfeffer et al in 1985 to describe the ventricular dilatation post-MI in a rat model (153). The term has since been used to describe various ventricular changes occurring in response to chronic stresses on the heart that are typically mechanical in nature (154). However, remodeling can essentially occur in response to any stressors, even due to cardiotoxic drugs such as anthracyclines and trastuzumab (155). Ventricular changes of remodeling can be simply classified as changes that occur at three main levels: molecular level, morphological level, and the functional level. Naturally, remodeling at the molecular level precedes that of the morphological level, and the latter precedes that of the functional level (Diagram below).



Cardiac remodeling not only affects myocytes, but also affects the interstitium, fibroblasts, and surrounding coronary vasculature, leading to gradual and progressive changes in chamber architecture. Examples of changes at the morphological level are: ventricular dilatation, concentric hypertrophy, and eccentric hypertrophy; and at the functional level are: reduction in LVEF myocardial deformation indices and heart failure. In the case of changes in ventricular loading conditions such as in mitral regurgitation, aortic stenosis, myocardial infarction, or even during chronic endurance exercise, the heart adapts by trying to increase its function to be able to maintain systemic perfusion. Cardiomyocytes have a high capacity to differentiate by changing its structure to adapt during these various stressors (156). Studies since the 1940s has shown that the cardiomyocyte does adapt by undergoing hypertrophy but cannot undergo hyperplasia. Cardiomyocyte hypertrophies by increasing its size and/or length leading to ventricular remodeling in its entirety. Cardiomyocyte can only hypertrophy can only occur up to a certain limit, after which it undergoes apoptosis (programed cell death). Cardiomyocyte decision to hypertrophy or die is also affected by the type of signaling pathways active. For example, in the context of un inhibited HER2-mediated survival pathways, growth and hypertrophy predominates. The ventricular capacity to adapt to stressors is therefore limited by number of healthy cardiomyocyte available, their capacity to hypertrophy, and by the type of activated signaling pathways, among other factors. Based on studies of cardiac remodeling in loading conditions, the heart adaptation can be physiological, pathological, or a mix of both depending on type and severity of cardiac injury. During physiological remodeling the cardiomyocyte and therefore the entire ventricle adapts to improve function (physiological functional adaptation). When the heart exhausts its capacity to adapt, it starts decompensating leading to pathological remodeling. Pathological remodeling happens when the adaptation no longer improves function, but rather only cause change in the structure of the cardiomyocytes and over time leads to

structural change of the entire ventricle (pathological structural adaptation). Pathological structural adaptation eventually leads to ventricular dysfunction and heart failure (157).

Cardiac physiological and pathological remodeling in response to alterations in loading conditions has been studied extensively with the intention to develop treatments to prevent or reverse heart failure by preventing or reversing pathological remodeling. Cardiac physiological and pathological remodeling during cardiotoxic medications, has not been adequately investigated, especially with the use of contemporary imaging techniques.

2.7.2 *Cardiac Remodeling due to loading conditions*

Three main loading conditions have been described: pressure overload, volume overload, and a mix of pressure and volume overload. In each one of these loading conditions, myriad of cardiac compensatory mechanisms are triggered to maintain adequate cardiac output to ensure sufficient systemic perfusion. These compensatory mechanisms initially increase cardiac function by inducing changes at the cardiomyocyte level (cardiac functional unit) to increase contractility and changes at the surrounding coronary vasculature to enhance fueling of the heart (adaptive remodeling). Cardiac compensatory mechanisms can also stimulate proliferation of fibroblasts leading to fibrosis and thickening (maladaptive remodeling) leading eventually to myocardial dysfunction (158). The adaptive remodeling occurs by means of physiologic hypertrophy (159).

2.7.2.1 Physiological and Pathological Remodeling

Physiological remodeling is best exemplified in athletes. Hearts of athletes who do regular heavy physical work increase in weight from 300gm to 500gm (LV from 100gm to 300gm) by increasing length and thickness of cardiomyocytes and by slight increase in the width of the capillary network making their hearts hypertrophy and more able to produce higher cardiac output (157). The hypertrophy in athletes is mostly physiological as the loading stressor is gradual in nature and voluntary.

Pathological remolding can happen when the loading stressor is sudden and significant (aortic stenosis, mitral regurgitation), or when there is direct injury to the myocardium (MI,

anthracyclines). As the systemic perfusion demands exceeds the limits of heart's-functional adaptation, the myocardium undergoes structural adaptation leading to decompensated heart - signified by failure and dilatation (157).

The extent of physiological/pathological cardiac remodeling is influenced by the severity of the loading stressor and by the cross-sectional area of normal cardiac tissue capable for adaptation.

2.7.2.1.1 Physiological Remodeling – at the molecular level

Biomechanical stress due to increased LV stretch during diastole and/or due to increased LV load during systole induces both local and systemic neurohormonal stimuli. Both stimuli are sensed by specialized myocardial receptors leading to activation of intracellular signaling pathways activating nuclear responses and gene expression. This lead to changes in cardiomyocyte phenotype and new cellular and extracellular growth (160). Multiple molecular signaling pathways have been identified, some leads to physiological remodeling, and others lead to pathological remodeling, but each one is associated with distinctive biochemical and molecular signatures (161) (158). Unfortunately, patients frequently have a mix of physiological and pathological remodeling taking place on different parts of the heart at the same time.

2.7.2.1.2 Physiological Remodeling – at the morphological level

In the case of a relatively mild loading stressor, cardiac remodeling at the cardiomyocyte and heart's morphological levels has been divided into three main types: (1) in case of pressure overload such as due to mild aortic stenosis: myocytes thicken leading to concentric LV hypertrophy to normalize wall stress; (2) in case of volume overload such as due to mitral regurgitation: myocytes elongate leading to eccentric LV hypertrophy to accommodate the increased volume; (3) in the case of mild to moderate post-myocardial infarction: the LV can be subjected to both concentric and eccentric remodeling (154). In all of these scenarios, the myocardium hypertrophies typically to increase its energy-producing mass and its contractile structure so it can meet the body's demand by maintaining adequate perfusion. In any of these

three types of loading stressors, if functional hypertrophy predominates then cardiac remodeling can be considered physiologic (162).

2.7.2.1.3 Pathological Remodeling – at the morphological level

In the case of moderate or severe hemodynamic load, a cardiomyocyte can either survive the loading stress and augment heart's function through further hypertrophy, or undergo apoptosis (programed cell death) (154). One of the known characteristics of cardiomyocyte's pathological hypertrophy, is that there is gene alteration in the type of the cardiac myosin heavy-chain due to decreased ATPase activity. This alteration causes re-induction of the fetal isoform of myosin heavy-chain rather than the adult's isoform. The problem with the fetal isoform is its slower rate of contractility (163). Since this alteration reduces contractility it potentially contributes to the development of ventricular dysfunction as a consequence of pathologic remodeling (164). Pathological remodeling also at the extracellular level is characterized by proliferation of cardiac fibroblast and enhanced collagen synthesis leading to progressive interstitial and perivascular fibrosis and eventually myocardial stiffening. This myocardial stiffening, although not know why it happens, plays an important role in the development of myocardial dysfunction (165).

2.7.2.1.4 Pathological Remodeling – at the molecular level

Increased oxidative stress has been shown, both in animal and human studies, to play an important role in the development of pathological hypertrophy and heart failure (166) (165). Oxidative stress happens when production of reactive oxygen species (ROS) occurs in excess of cell's natural antioxidant capacity (158) (165). ROS are a natural by-product of mitochondrial energy generation that exist in all aerobic cells including cardiomyocyte. Also, in cardiomyocytes, ROS can be produced by xanthine oxidase, NADH/NADPH oxidase, and endothelial nitric oxide synthase (167,168). ROS are a group of highly toxic oxygen containing reactive free radicals that have important roles in cell signaling and homeostasis (169). Under normal conditions, body's natural anti-oxidants degrade ROS to nontoxic molecules. Excessive production of ROS during pathological conditions cause cell dysfunction, apoptosis, DNA damage, protein and lipid peroxidation, cardiac fibroblast proliferation, and activation of matrix

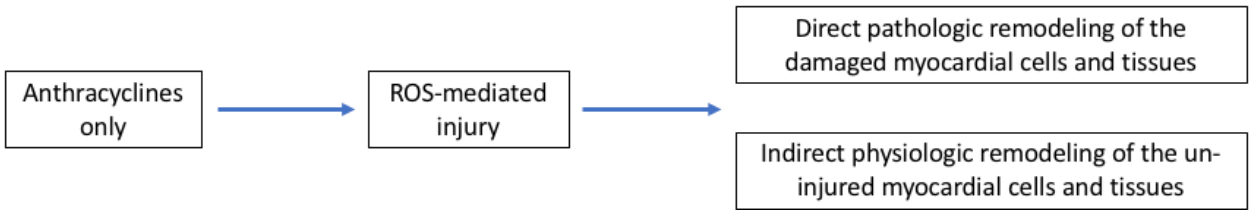
metalloproteinases leading to cellular and extracellular myocardial pathological remodeling and heart failure (168) (170-173).

2.7.3 *Cardiac Remodeling due to cardiotoxic cancer therapy*

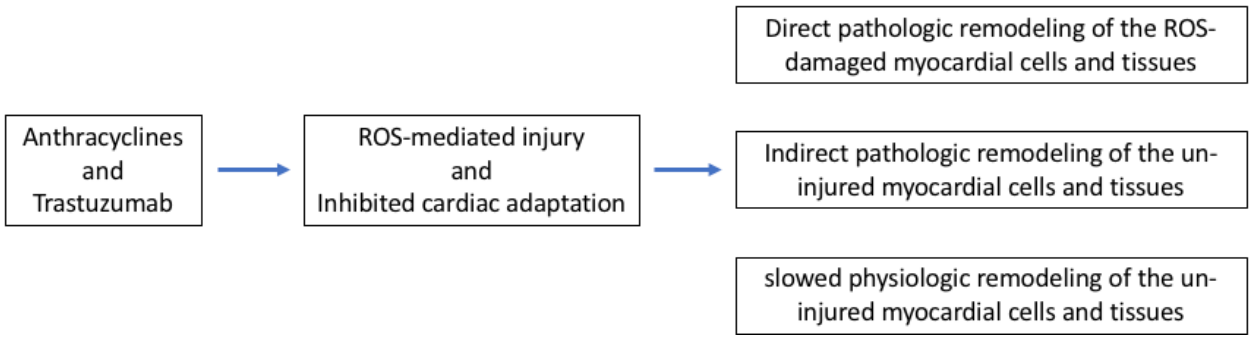
Many chemotherapeutic agents, including anthracyclines and trastuzumab are known to cause cardiotoxicity, but their mechanism for cardiac remodeling is not fully understood. The morphological remodeling is a reflection of the molecular remodeling. As these molecular changes increase in magnitude and reach a certain threshold, changes at the morphological level become evident. By the same token, structural changes need to reach a threshold point before changes in cardiac function becomes apparent, and eventually signs and symptoms of heart dysfunction manifest, if this cycle is not interrupted.

2.7.3.1 Molecular remodeling due to cancer therapy

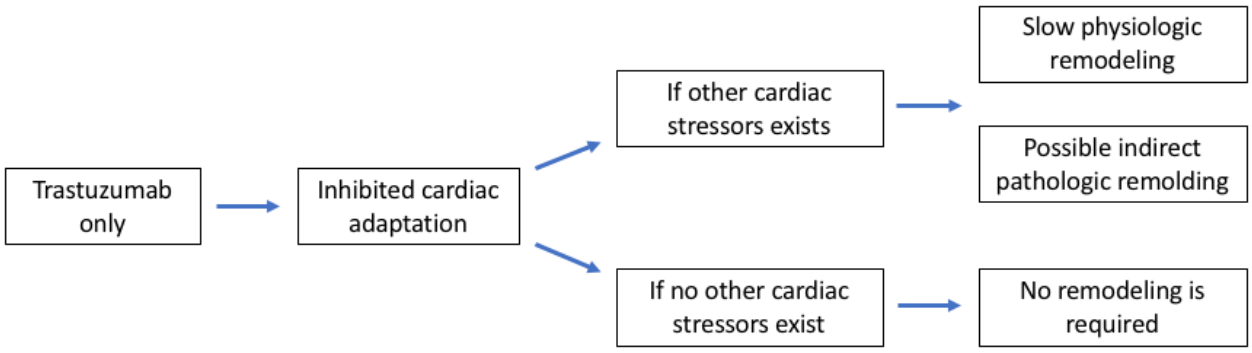
There is limited data regarding molecular remodeling during breast cancer treatment. As described earlier, anthracyclines induce ROS production leading to direct myocardial cell damage, whereas trastuzumab seems to inhibit important cellular functions leading to inhibition of survival pathways. In light of our understanding of physiological and pathological cardiac remodeling due to loading conditions, anthracyclines can be considered to essentially cause pathological remodeling by directly injuring myocytes through the generation of ROS leading to irreversible cellular (apoptosis and necrosis) and extracellular damage (fibrosis, and thickening). Biopsy and necropsy studies have confirmed these cellular and extracellular injuries post anthracyclines treatment. Small studies using cardiac MRI tissue characterization techniques such as T1 and T2 mapping and ECV has demonstrated edema, inflammation, and fibrosis post anthracycline use. The heart adapts to the insult of anthracycline injury by augmenting physiological remolding of the uninjured cardiomyocytes to preserve heart structure and function. Therefore, during anthracycline treatment the heart undergoes both direct pathologic remolding and indirect physiological remolding (Diagram below).



Molecular Remodeling due to trastuzumab therapy is variable depending on the level of concomitant stress exerted on the myocardium during the presence of trastuzumab in the circulation. If the trastuzumab-inhibited heart gets exposed to high loading stressor requiring compensation and hypertrophy, then this hypertrophy becomes maladaptive (due to inhibited cardiac survival pathways) leading to pathological remodeling. Examples of high stresses include anthracycline-induced ROS cell damage, hypertension, MI, or any other loading conditions. Even in patients with unrecognized subclinical heart dysfunction trastuzumab therapy might tip these patients over into developing heart failure. This is especially important in older patients with little reserve as trastuzumab inhibits heart capacity to adapt and hypertrophy (Diagram below).



Without these stressors trastuzumab is generally believed to have no cardiotoxic effects or only cause subclinical transient reversible ventricular dysfunction (Diagram below).



2.7.3.2 Morphological remodeling due to cancer therapy

The current understanding of the morphological remodeling during treatment with anthracyclines and/or trastuzumab is conflicting since only a few studies with small sample size have studied remodeling using the non-invasive gold standard cardiac MRI. Other limitations of existing data include: studying remodeling in a mix of tumors, with mixed treatment regimens, and variable imaging techniques. Over the recent years, controversy exist regarding the mechanism of pathological morphological remodeling that leads to drop in LV ejection fraction and development of cardiac dysfunction. Some groups believe that LV diastolic remodeling (measured by increase in LVEDV) is the primary insult leading to LV dysfunction (126,174); Haykowsky et al. reported that LV EDV and ESV at rest and peak (dobutamine stress) were significantly increased after the 4-month intervention period (174); Bergamini et al, further showed that baseline LVEDV is an independent predictor of LV dysfunction (175). Other groups including ours, believe that reduction in LV contractility is the first insult leading to LV dysfunction. More recently, Hundley's group showed that isolated reduction in preload (measured by reduction in LVEDV) can lead to LV dysfunction in about 20% of patients during chemotherapy (176,177).

Uncertainties exist regarding the remolding of LVmass during or post cancer therapy. Lipshultz and Neilan showed that myocardial mass reduces significantly many years post cancer therapy in pediatric an adults cancer patients, respectively (178,179). Haykowsky et al showed that myocardial mass increases significantly during trastuzumab therapy in HER2 positive breast cancer (174). Yet, Avelar et al did not demonstrate significant change of myocardial mass during cancer therapy, but did show that LVmass/LVEDV decreased significantly during breast cancer therapy with anthracycline or trastuzumab for early stage breast cancer (180).

The aforementioned controversies and uncertainties clearly demonstrates the need for a carefully performed longitudinal study to delineate the process of ventricular remodeling using cardiac MRI-derived volumes, function, and mass in a uniform population of cancer during standardized treatment. Such longitudinal cardiac MRI study will also provide an understanding of cardiac remodeling which can then be used to study methods to prevent progression to ventricular dysfunction and heart failure.

Chapter 3

3 MATERIALS AND METHODS

3.1 Patient population

Eighty-seven consecutive women with early stage HER2+ breast cancer who were scheduled to receive cancer therapy were recruited prospectively from outpatient clinics between January 2014 and April 2017 from Princess Margaret Cancer Centre/University Health Network (UHN), St. Michael's Hospital, and the Odette Cancer Centre/Sunnybrook Health Sciences Center. Thirty-two healthy volunteers were also recruited between July 2014 and November 2016 through advertisements at the Toronto General Hospital. The study was approved by the research ethics board at the respective sites and all patients and volunteers signed informed consent. Funding of this study was made possible by the Canadian Institutes of Health Research (CIHR).

3.1.1 *Patients' Inclusion criteria*

(1) Women ≥18 years with stage I-III, HER2+ breast cancer scheduled to undergo treatment with one of: (a) 5-fluorouracil, epirubicin, cyclophosphamide, followed by docetaxel and trastuzumab, (b) adriamycin, cyclophosphamide, followed by docetaxel and trastuzumab, (c) adriamycin-cyclophosphamide with weekly paclitaxel and trastuzumab, or (d) dose-dense adriamycin and cyclophosphamide followed by dose-dense paclitaxel and trastuzumab; (2) able to tolerate five ~60 minute CMR examinations over 15 months; (3) able to give informed consent; and (4) able to travel to the University Health Network (UHN) hospitals for cardiac imaging.

3.1.2 *Patients' Exclusion criteria*

(1) Life expectancy <12 months; (2) participating in a clinical trial of a new cancer drug; (3) having received previous anthracycline or radiotherapy to the thoracic region; (4) history of myocardial infarction or HF; (5) current unstable angina, persistent atrial fibrillation or other irregular rhythm, or a history of more than mild regurgitant or stenotic valvular heart disease; (6) severely reduced renal function (GFR ≤ 30ml/min); (7) general contraindications to MRI (e.g. pacemaker, breast expanders); (8) echo image quality inadequate for strain analysis (unable to adequately visualize 2 contiguous segments in 2 out of the 3 long axis views).

3.1.3 *Healthy Volunteers' Inclusion and Exclusion Criteria*

Inclusion criteria for healthy volunteers were: (1) men and women ≥18 years of age willing to undergo three CMR scans with contrast over a 6 months period, (2) no prior cardiovascular disease history, (3) no history of hypertension, diabetes, or hyperlipidemia, (4) not on any cardiac medications, and (5) no other known systemic diseases. We excluded volunteers with general contraindications to CMR (e.g. ferromagnetic implants, renal dysfunction).

3.2 Data collection

A research coordinator at each site screened patients at the breast cancer clinic using standard screening forms. Patients meeting enrollment criteria were approached by the primary oncologist for participation. The research coordinator obtained written informed consent from all patients and healthy volunteers. Unique identification numbers were assigned to all consented patients and healthy volunteers and were linked to their demographic and clinical data in a separate secure database.

3.3 Cancer therapy

All included patients received either adjuvant (i.e. after surgery) or neoadjuvant (i.e. prior surgery) cancer therapy that consisted of two months of concurrent administration of anthracycline, and cyclophosphamide +/- 5-flurouracil, followed subsequently by 52 weeks of concurrent taxane and trastuzumab administration every 3 weeks (71,72). Taxanes were only administered with trastuzumab during the first 3-4 cycles. The total duration of cancer therapy was approximately 15 months.

3.4 Timing of imaging in relation to cancer therapy

Cardiac MRI imaging was scheduled, during the 15 months of cancer therapy administration, at 5-time points (Figure 3.A): (1) at baseline (i.e. prior to any cancer therapy), (2) at ~2-3 months (immediately post completion of anthracyclines but prior to first dose of trastuzumab), (3) at ~5-6 months (3 months into trastuzumab therapy), (4) at ~9 months (6 months into trastuzumab therapy), and (5) at ~15months (completion of cancer therapy). During base line visit, cardiac MRI scan, Echocardiography, and blood samples were performed, as well as the following clinical data were extracted from hospital electronic patient records or direct patient history using standardized forms: age, coronary risk factors (hypertension, diabetes, hypercholesterolemia, smoking, family history of coronary artery disease, and obesity), current medications and doses, date of cancer diagnosis, tumor characteristics (size, grade, ER, PR status, laterality), vital signs, height, and weight. All data were entered prospectively into a Medidata RAVE online database system.

Patients' first follow up visit (time point 2) was scheduled within 2-3 week of completion of chemotherapy administration (~2-3 months from baseline). Subsequent Patients' follow up visits were scheduled at 3-months, 6-months, and 12-months through trastuzumab therapy (~5-6 months, 9 months, and 15 months from baseline). Cardiac MRI, Echocardiography, and blood samples were performed as well as patients' clinical data were extracted for all patients at all these four follow-up time points. Further, a research coordinator recorded medication use and cumulative chemotherapy doses for patients during these follow up visits. In patients receiving

radiotherapy, total cardiac radiation dose volume histogram data were collected. A standardized questionnaire was used to collect cardiac symptoms and any cardiac related hospitalizations.

Healthy volunteers' baseline and follow up visits were scheduled to correspond to the first 3-time points only (at baseline and at ~2-3 months and ~5-6 months from baseline). Similarly, all volunteers had cardiac MRI, echocardiography, and blood samples performed as well as clinical data recorded at all 3-time points. All data were entered prospectively into a Medidata RAVE database system.

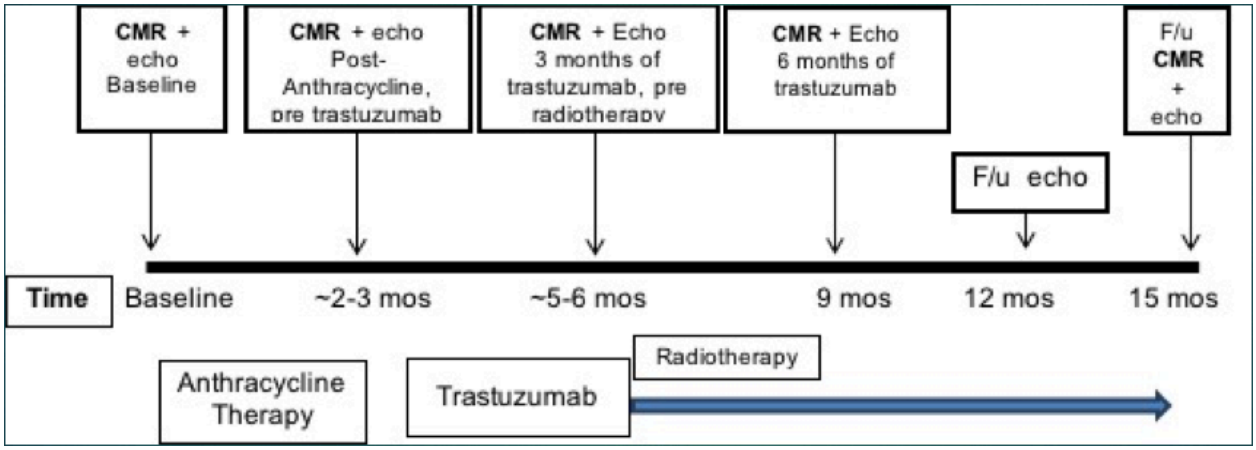


Figure 3.1. Timing of cardiac MRI imaging in relation to cancer therapy

3.5 Primary outcome – (and definition of LV-cardiotoxicity)

For the current study only, cardiac MRI imaging performed at the first 3 time points were included in the analysis. These time points were chosen to gain an understanding of the early remodeling during cancer therapy in our patient cohort. The primary outcome for the current study was the development of cardiotoxicity at any of the first 2 follow up visits. Our primary definition for cardiotoxicity was based on the CREC criteria as follows: **(1)** using cardiac MRI, a ≥5% reduction in LVEF from baseline and to <55% with signs or symptoms of HF, **OR (2)** using cardiac MRI, a ≥10% reduction in LVEF from baseline to <55% without accompanying signs or symptoms of HF (11). Presence or absence of heart failure symptoms was determined at time of each CMR examination by clinical review by a cardiologist with > 5-year experience in cardio-

oncology using a synthesis of clinical history, physical examination and results of cardiac investigations.

3.6 Secondary outcome – (and LVEF sensitivity analysis)

Our study secondary outcome was based on a pre-defined sensitivity analyses including a mild reduction in LVEF of >5% at any of the follow-up time-points compared to baseline, using cardiac MRI. We used this cut-off based on published recent data showing that: (1) in breast cancer patients treated with anthracyclines and/or trastuzumab the mean drop in LVEF using cardiac MRI was 5.0-5.2% (181,182); (2) cardiac MRI's excellent observer and test-re-test reproducibility makes detection of a 5% change over time reliable (177,183,184); (3). This precision is also confirmed with our own data (results section) where intra-observer, inter-observer, and temporal variabilities for LVEF and ventricular volumes using cardiac MRI are well below 5% for our cohort of healthy volunteers.

3.7 Definition of RV-cardiotoxicity

RV cardiotoxicity was defined as a >10% drop in RVEF to <51% based on recently published normal values by Kawel-Boehm et al (185).

3.8 Definition of significant change in volumes

Significant changes in cardiac MRI measured LV volumes were defined to be consistent with published literature as LVEDV reduction >10 mL and LVESV increase >5 mL (177). These thresholds are also higher than the inter-study variability for these measurements reported in the literature (186). Arterial elastance (EaI) was calculated as end-systolic pressure (0.9 x brachial systolic blood pressure)/LV stroke volume indexed to BSA (176)

3.9 Bio-specimen collection and bio-banking

Blood sample was collected by venipuncture from every volunteer at every time point within 1 hour of the CMR study. hs-TnI, and BNP measurements were obtained using the central hospital laboratory. High sensitivity Troponin-I and BNP assays were performed on the ARCHITECT i2000 immunoassay analyzer (Abbott Diagnostics, Abbott Park, IL, USA) using the manufacturer's reagents. The limit of detection for the troponin assay is 2 pg/mL with routine CV of 5.6% at 46 pg/mL and 4.3% at 1400 pg/mL. The limit of detection for the BNP assay is 10 pg/mL with routine CV of 8.8% at 67 pg/mL and 8.2% at 300 pg/mL.

3.10 Cardiac MRI acquisition

Cardiac MRI studies were performed on a 1.5T scanner (Siemens) using a multi-element receiver-coil array. The study consisted of: (1) long axis (3, 2, 4 chamber) and a stack of short axis (SAX) cine steady state-free precession (SSFP) slices for LV function assessment. Acquisition parameters for the CMR techniques are summarized in Table 3.1. Cardiac MRI studies were performed pre-therapy, at ~2-3 months (post-anthracycline completion but pre trastuzumab initiation), at ~5-6 months (3 months into trastuzumab), at ~9 months (6 months into trastuzumab), and at completion of trastuzumab (~15 months from baseline - Figure A).

Table 3.1. Cardiac MRI Acquisition parameters

Technique	**CINE**
Sequence	SSFP
Receiver Coil	24-Element Body Matrix
Breath-hold	Yes
Parallel Imaging	*GRAPPA; R*=2
In-plane resolution (mm)	1.6 x 1.6
Slice thickness (mm)	8
TR / TE / (ms)	2.8/1.2
Flip angle (˚)	65
Miscellaneous	Temporal resolution: 36ms (13 lines/segment)

3.11 Cardiac MRI post-processing

3.11.1 *Image setup*

a) Before performing LV and RV volumetric analysis on the short axis cine images, the workstation windows are organized to have, in addition to the main SAX cine sequence quantification window, a cross reference window (4-ch, 2-ch, or 3ch) and the same SAX cine sequence playing in a separate window (figure 3.2).

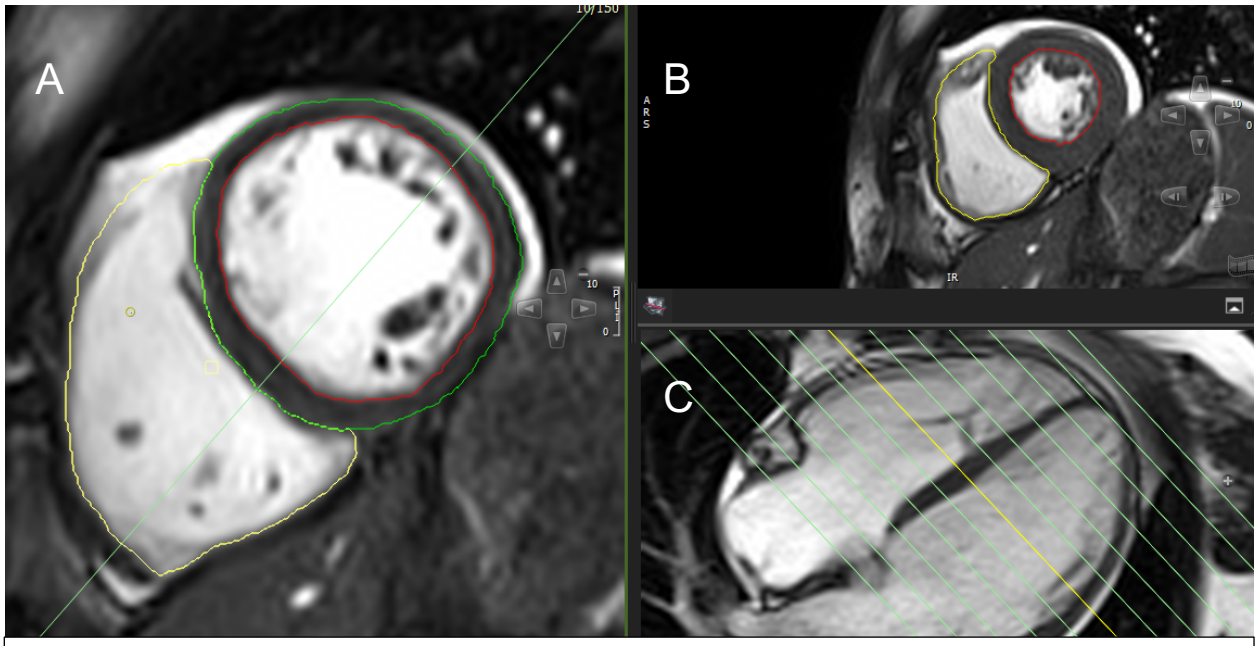


Figure 3.2. CMR post-processing - window layout:

A. SAX cine primary quantification window showing tracing at end-diastolic phase: LV endo-cardial contour (red), LV epi-cardial contour (green), RV endo-caridial contour (yellow), and cross-reference line (white);
B. A movie window playing the same SAX cine sequence at the same slice level. The current phase is at end-systole showing similarly, yet only, LV endo-cardial contour (red) and RV endo-cardial contour (yellow);
C. A 4-chamber cross-reference window showing all cross-referencing lines spanning both LV and RV from base to apex. Current slice position is at mid-ventricle (yellow line) and at end-diastole (synchronized with the primary quantification window).

b) Windows' size and contrast are optimized, quality of image planning is checked, and a 4 chamber cines sequence is checked in movie mode to note the presence/absence of any valvular regurgitation (Figure 3.2).

c) A single LV end-diastolic phase is defined by identifying visually the phase with the largest LV blood volume. Also, a single LV end-systolic phase is defined by identifying visually the phase with the smallest LV blood volume.

d) Similarly, a single RV end-diastolic phase is defined by identifying visually the phase with the largest RV blood volume and a single RV end-systolic phase defined as the phase with the smallest RV blood volume.

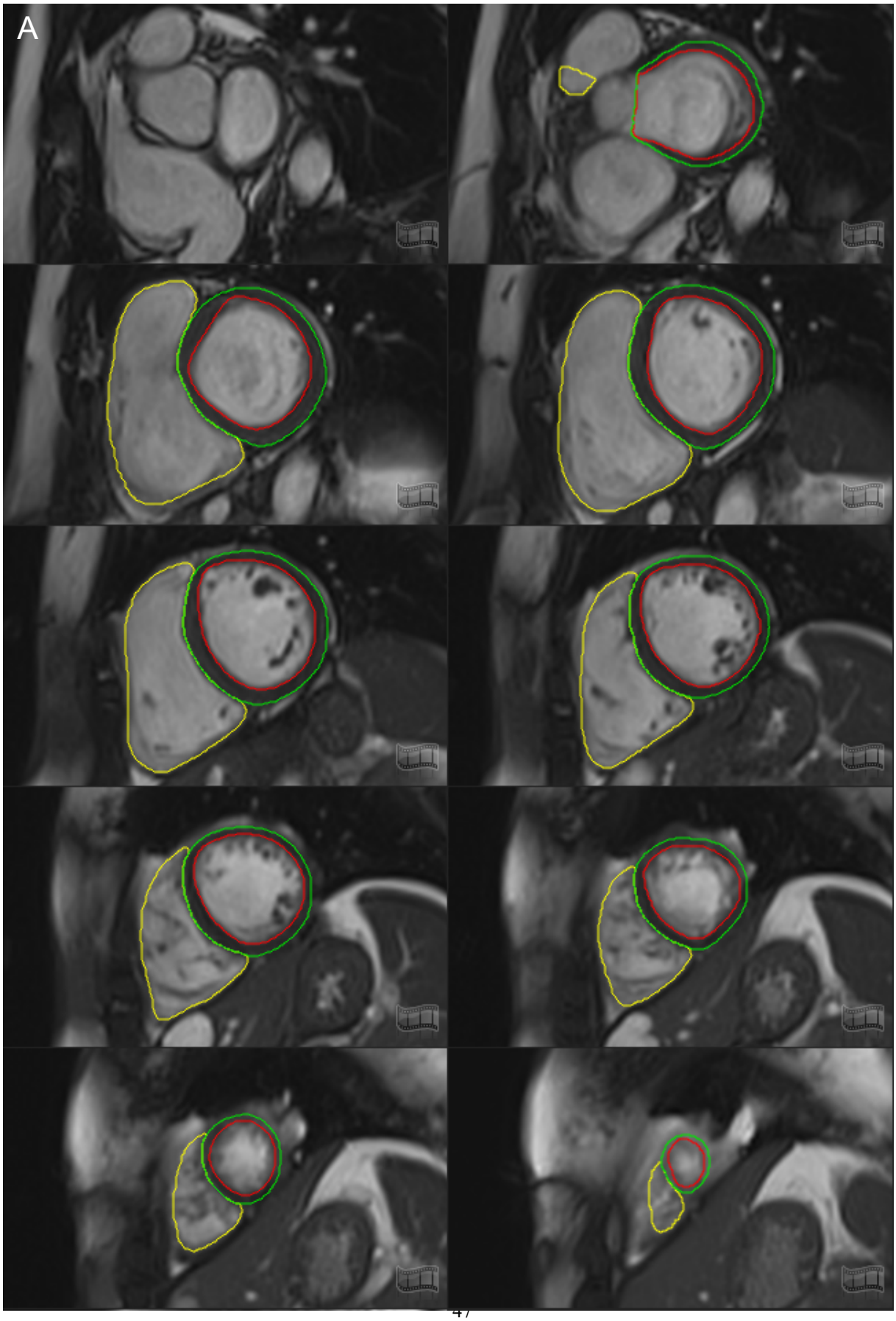
e) LV and RV end-diastole are usually on the same phase; however, LV and RV end-systole may not necessarily be on the same phase.

f) First basal end-systolic slice position is usually lower than that of the first basal end-diastolic slice position for both LV and RV due to the systolic motion of the annular ring toward the apex (basal descent).

g) In the case of difficult LV end-systolic phase determination, phase that precedes closure of the aortic valve is selected.

h) LV and RV parameters recorded were: end-diastolic volume, end-systolic volume, ejection fraction, stroke volume, mass (latter only for LV), and body-surface area-indexed values of all except ejection fraction.

i) Endocardial borders are contoured at end-diastole and end-systole for both LV and RV; epicardial borders are only contoured at end-diastole for LV only (Figure 3.3).


A

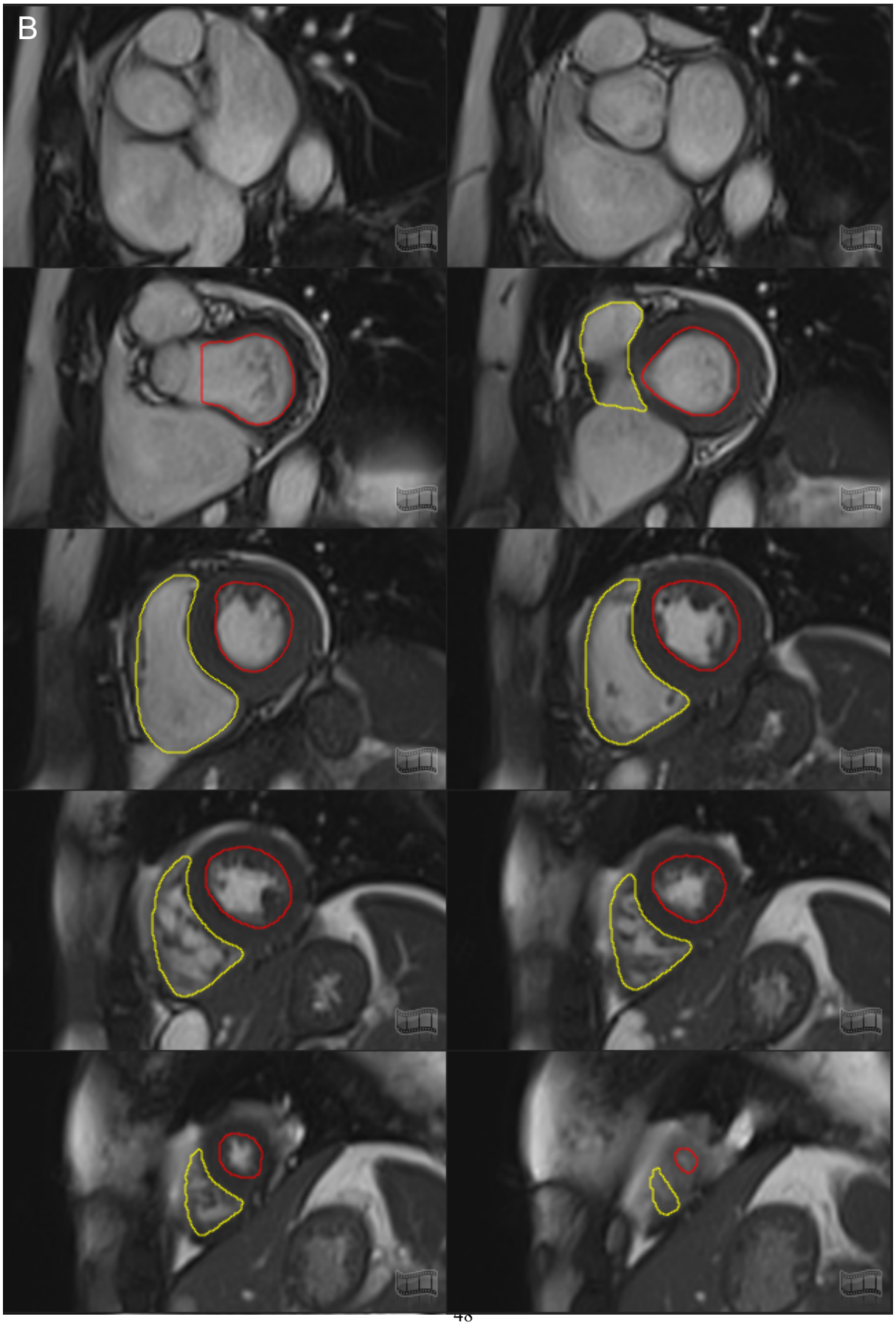

B

Figure 3.3. CMR post-processing – complete tracing of a single case
A. All slices at end-diastolic phase
B. All slices at end-systolic phase

3.11.2 *Volume, function, mass quantification*

3.11.2.1 General

a) Smooth endocardial borders are drawn right where the compact and trabecular myocardium meet, making sure no white areas (blood) are seen outside the traced line (Figure 3.3).

b) Smooth epicardial borders are drawn so no white areas (fat) are seen inside the traced line. In case of chemical shift (thick black) artifact line, epicardial contours should be drawn right on the middle of the chemical shift artifact line (Figure 3.4).

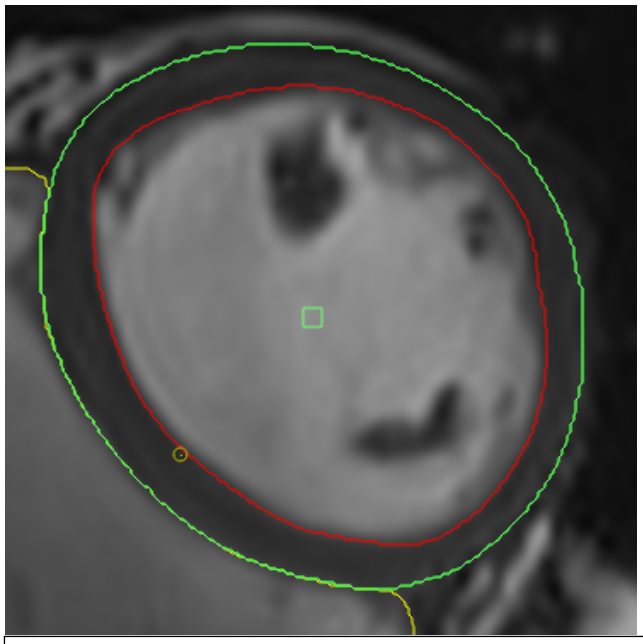


Figure 3.4. CMR post-processing – LV-epicardial contours, chemical shift tracings

c) LV Papillary muscles are included in the LV blood pool volume (excluded from LV mass); similarly, RV trabecular tissue and papillary muscles are included in the RV blood pool volume (Figure 3.3).

d) All automatic contour tracings, if any, are checked for appropriateness by the reader.

e) All contours are confirmed carefully for appropriateness with the help of the SAX cine window.

f) LV and RV outflow tracts are included in blood volume quantification.

g) Basal slices that contain partial segments of the ventricle are traced to be included in the blood volume, and in LV mass quantification (Figure 3.2).

h) All LV end-diastolic phase contours should typically have both endocardial and epicardial contours even for LV chambers with small or partial volumes.

i) Straight lines are drawn to separate ventricles from atria and to separate outflow tracts from arteries (Figure 3.2).

j) For RV basal slice tracing, if RVOT is separated completely from the rest of RV chamber in the same slice, by the aortic root for example, the larger of the two RV parts is traced.

3.11.2.2 Basal slice

Consistency in the method for cardiac MRI basal slice quantification greatly improves precision of CMR assessment. Depending on patient's heart size, shape, clinical condition, and technical image planning, basal slices normally vary in the degree of partiality of ventricular chambers wall size, therefore requiring a robust method that is both repeatable and reproducible. I have illustrated here basal slice quantification for both the left and right ventricles based on my 5-year experience working with experts in the field at the University Health Network and St. Michaels Hospital and supported by published literature.

3.11.2.2.1 Left ventricle – End-diastole

1) LV wall in end-diastole can be identified through the following:
    a) Thick myocardial wall – trabeculated (Figure 3.5A)
    b) Dilates during diastole (Figure 3.5)

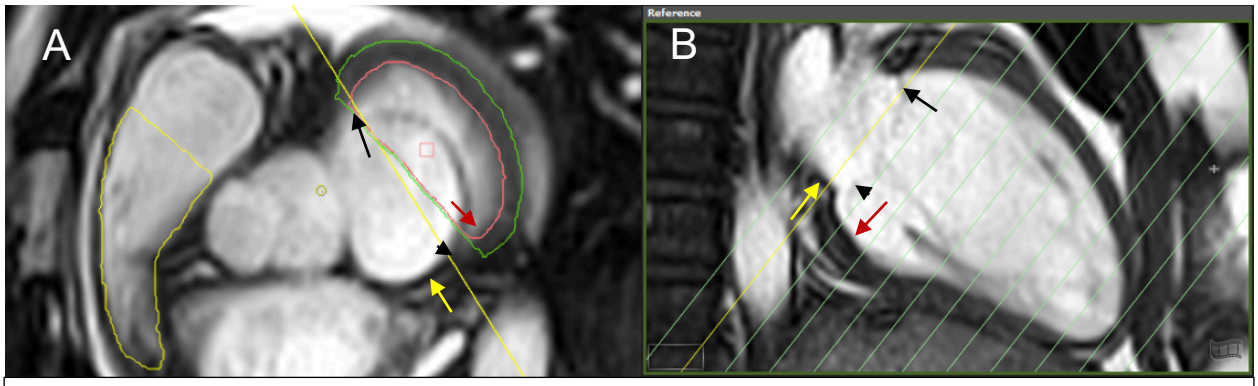


Figure 3.5. CMR post-processing – LV basal slice quantification in end-diastole:
A. SAX image - Thick trabeculated myocardial wall, dilates during diastole (Red arrow); Thin non-trabeculated atrial wall, constricts during diastole (yellow arrow); Mitral valve leaflets (black arrows).
B. 2-chamber image is ideal to cross-reference mitral ring. Black arrow head shows that the mitral valve insertion is below the yellow cross-referencing line (left atrium), explaining why it was excluded from LV wall quantification in A.

2) LV outflow tract wall in end-diastole can be identified through the following:
   a) Relatively thin straight wall up to, but not including any bulge that represent aortic cusps (Figure 3.6A)

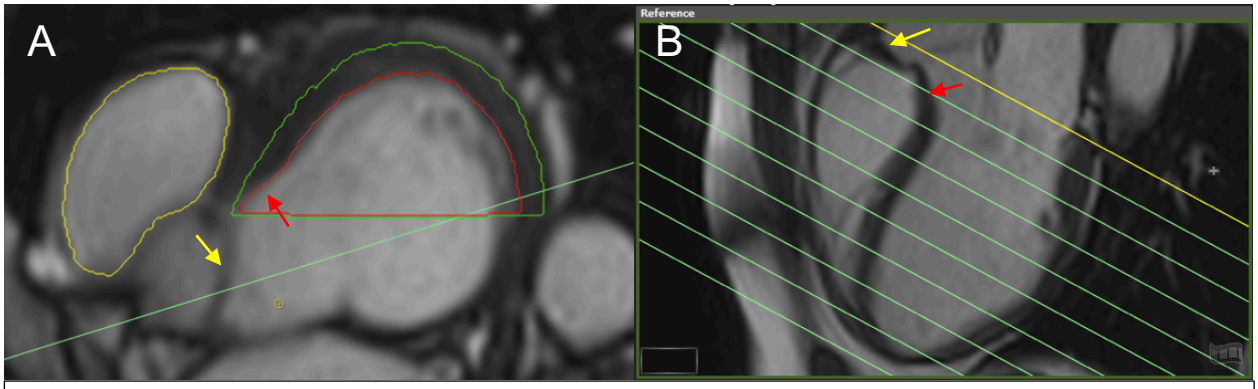


Figure 3.6. CMR post-processing – LVOT in end-diastole:
A. Straight thin walled LVOT (red arrow); Aorta (yellow arrow).
B. 3-chamber image is ideal to cross-reference the LVOT.

   b) Does not constrict during diastole

3.11.2.2.2 Left ventricle – End-systole

3) LV wall in end-systole can be identified through the following:
   a) Thick myocardial wall – trabeculated (Figure 3.7A)

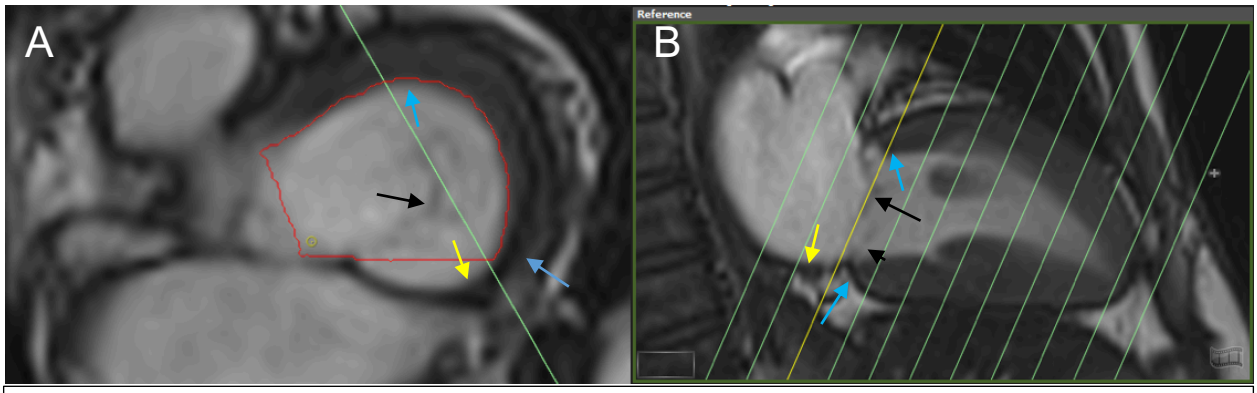


Figure 3.7. CMR post-processing – LV basal slice quantification in end-systole:
A. SAX image - Thick trabeculated myocardial wall constricts during systole (Blue arrow); Thin non-trabeculated atrial wall, dilates during systole (yellow arrow); Mitral valve closed (black arrows).
B. 2-chamber image is ideal to cross-reference mitral ring attachments – Arrows correspond to A; Black arrow head shows that the mitral valve insertion is below the yellow cross-referencing line (left atrium), explaining why it was excluded from LV wall quantification in A.

   b) constricts during systole (Figure 3.7)
   c) Sometimes a thin annular ring can be confused for atrial tissue. But it can be clearly visualized by the corresponding cross-referencing long axis images as being part of the LV chamber (below the plain of mitral valve) (Figure 3.8 and 3.9).

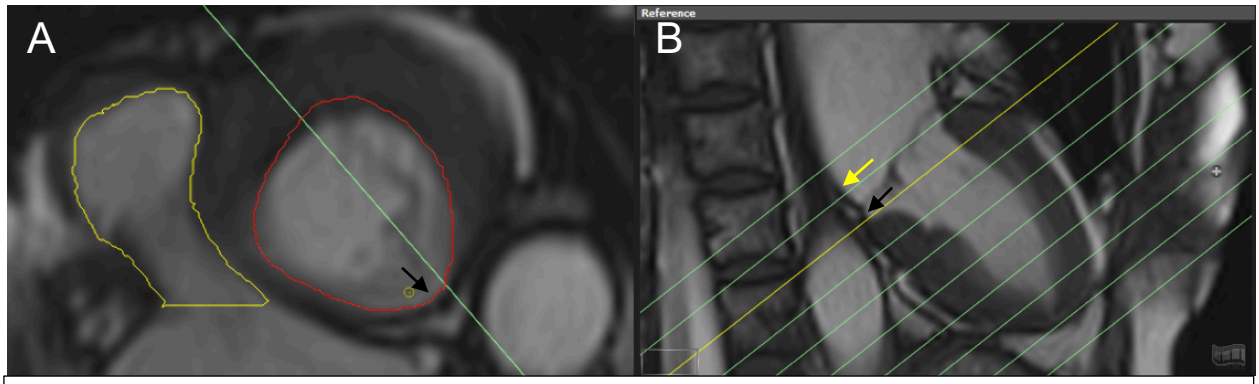


Figure 3.8. CMR post-processing – LV basal slice quantification in end-systole:
A. Thin walled LV annular ring (black arrow).
B. 2-chamber image is ideal to cross-reference the LV annular ring when it exists – The thin walled LV annular ring (black arrow), although out-pouching, but can be easily distinguished from the left atrium (yellow arrow) by being below the mitral valve attachment.

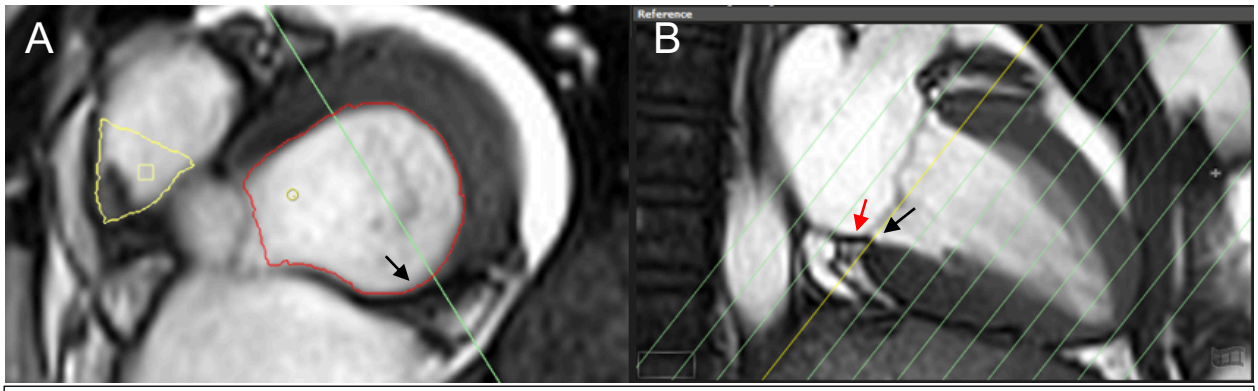


Figure 3.9. CMR post-processing – LV basal slice quantification in end-systole:
A. Thin walled LV annular ring (black arrow).
B. 2-chamber image is ideal to cross-reference the LV annular ring when it exists – The thin walled LV annular ring (black arrow), can be easily distinguished from the left atrium (red arrow) by being below the mitral valve attachment.

4) LV outflow tract wall in end-systole can be identified through the following:
   a) Relatively thin straight wall up to, but not including any bulge that represent aortic sinuses (Figure 3.10)

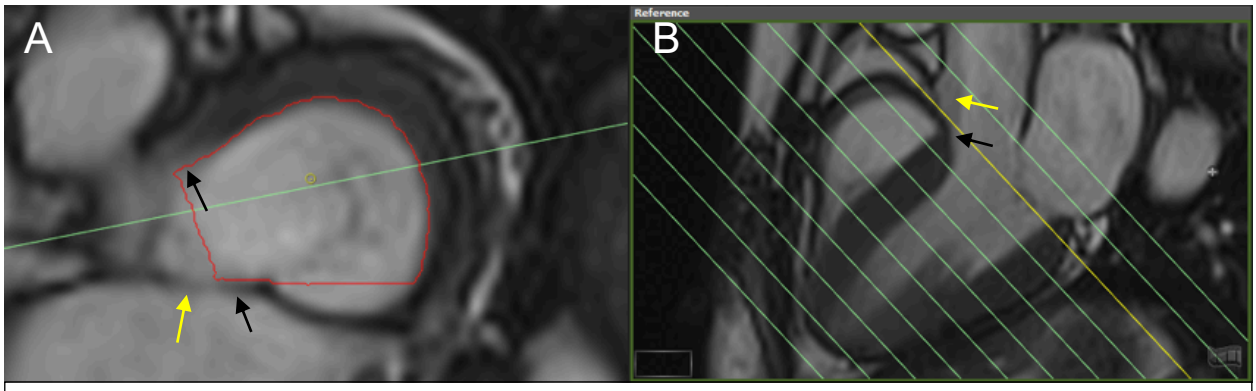


Figure 3.10. CMR post-processing – LVOT in end-systole:
A. Straight thin walled LVOT (black arrows); Aortic cusp (yellow arrow).
B. 3-chamber image is ideal to cross-reference the LVOT.

b) Does not dilate during systole

3.11.2.2.3 Right ventricle – End-diastole

5) RV wall can be identified through the following:
   a) Thin to mildly thick and trabeculated wall (Figure 3.1 and 3.10)
   b) Dilates during diastole (Figure 3.11)

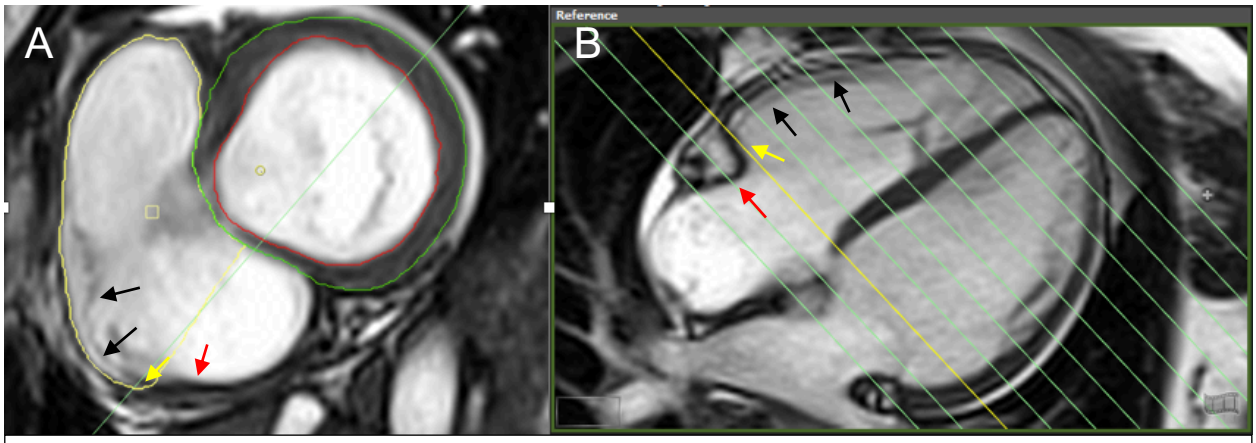


Figure 3.11. CMR post-processing – RV in end-diastole:
A. SAX image: trabeculated myocardial wall (black arrows), dilates during diastole (yellow arrow); Thin non-trabeculated right atrial wall that constricts during diastole (red arrow).
B. 4-chamber image is ideal to cross-reference RV wall and tricuspid valve.

6) RV outflow tract wall in end-diastole can be identified through the following:
   a) Long and thin wall up to, but not including any bulge that represent pulmonary valve cusps (Figure 3.12)

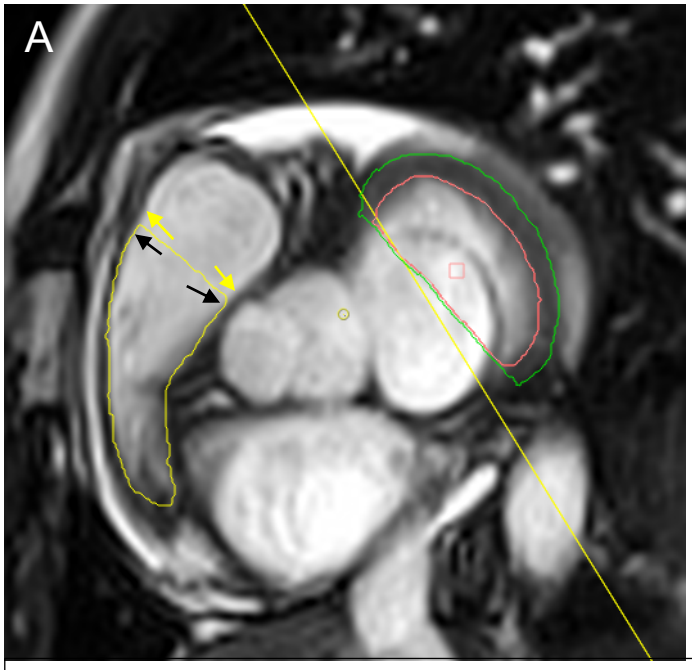


Figure 3.12. CMR post-processing – RVOT in end-diastole:
A. Straight thin walled LVOT (black arrows); Neck of the pulmonary valve (yellow arrows).

   b) Expands during diastole

3.11.2.2.4 Right ventricle – End-systole

7) RV wall in end-systole can be identified through the following:
   a) Relatively thick trabecular wall (Figure 3.13)
   b) constricts during systole (Figure 3.13)

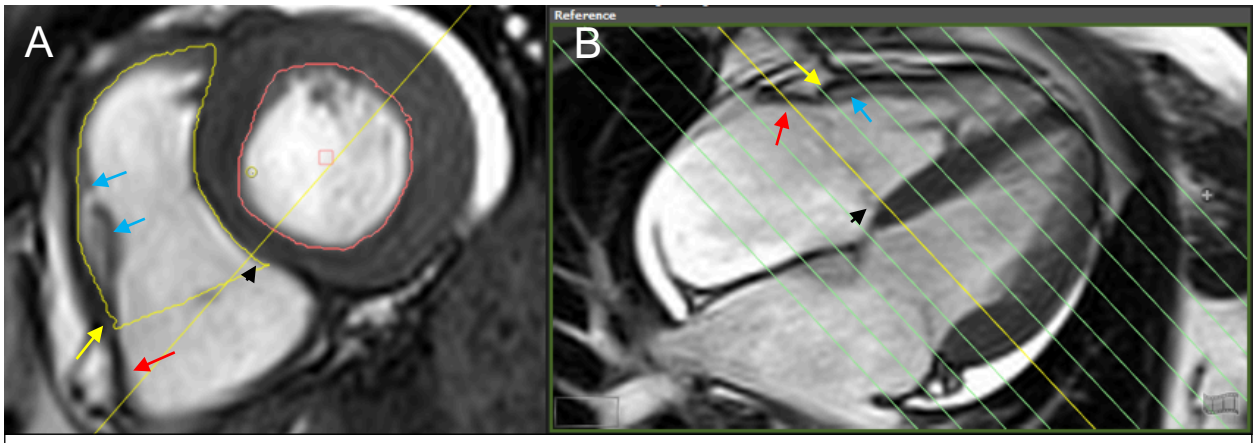


Figure 3.13. CMR post-processing – RV in end-systole:
A. Trabeculated myocardial wall (blue arrows), constricts during systole (yellow arrow); Thin non-trabeculated right atrial wall that dilates during systole (red arrow); cross reference line identifies attachment point of tricuspid valve on the interventricular septum (black arrow head).
B. 4-chamber image is ideal to cross-reference RV wall and tricuspid valve.

8) RV outflow tract wall in end-systole can be identified through the following:
   a) Long and thin wall (constricts during systole) up to but not including any bulge that represent pulmonary artery sinuses (Figure 3.14 and 3.15)

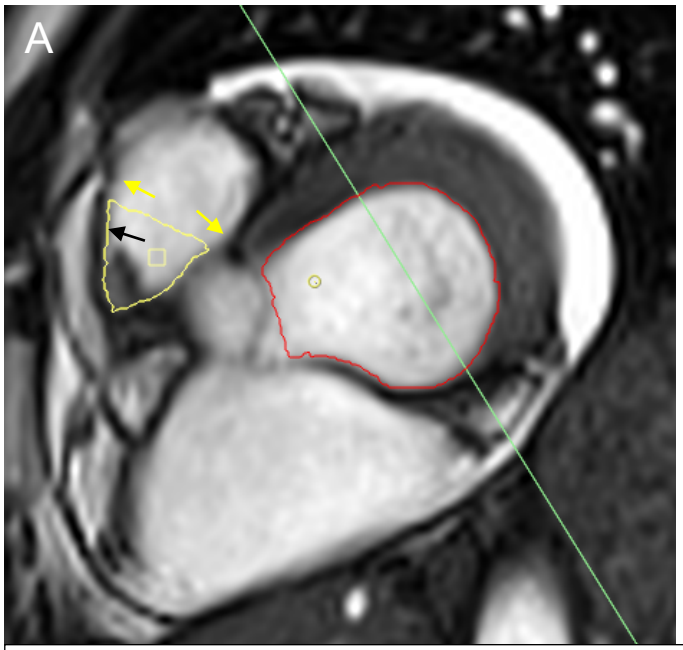


Figure 3.14. CMR post-processing – RVOT in end-systole:
A. Straight and thin walled LVOT (black arrows); Neck of the pulmonary valve (yellow arrows).

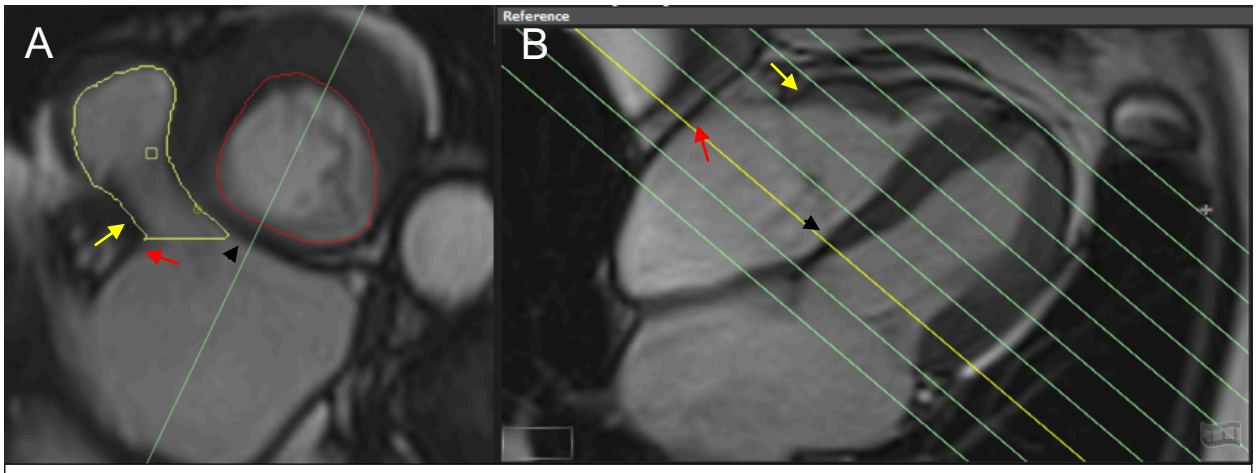


Figure 3.15. CMR post-processing – RVOT in end-systole:
A. RVOT constricts during systole (yellow arrow); Left atrium dilates during systole (red arrow); cross reference line identifies the approximate attachment point of tricuspid valve on the interventricular septum (black arrow head).
B. 4-chamber image.

3.11.3 *Confirmation of results*

"If no intracardiac shunts or valvular regurgitation is present, the RV and LV stroke volumes should be nearly equal (small differences are seen as a result of bronchial artery supply). Since the LV stroke volume is more reliably determined than the RV stroke volume, the LV data can be used to validate RV data" (187).

3.12 Intra-observer and inter-observer, test-retest and temporal variability quantification – Healthy Volunteers

For the healthy volunteers, temporal variability for LV and RV parameters (baseline, 3-month, and 6-month; 90 CMR studies), were analyzed by the same reader (MA). For intra- and inter-observer variability the primary reader (MA) repeated 45 CMR studies (15 random patients x 3 time points) 4 weeks later blinded to prior measurements and in a random fashion to eliminate any memory of the first measurements. A second reader (MN) independently performed the analysis on the same 45 scans and repeated it 4 weeks later blinded to prior measurements and in

a random fashion. The inter-observer variability is the average of 2 readers, while the inter-observer variability accounted for both within reader and between reader variability (14,188). We also calculated the 6-month inter-observer test-retest variability by randomly using baseline first measurement by reader one compared to 6-month first measurement by reader two (15 volunteers x 1 time-point x 2 readers x 2 measurements each reader = 60 scans). This represents variability in measurements when measurements are performed by two different readers at two separate time points as is the case in routine clinical practice.

For the patients, Intra- and inter-observer variability was assessed in 20 randomly chosen single time point CMR data sets. Intra-observer variability was assessed by a single observer repeating the analysis (about 3 months apart) blinded to previous results and clinical data. Inter-observer variability was assessed by two observers blinded to clinical and each other data.

3.13 Statistical analysis

Data are summarized as mean (SD) or median (IQR) as appropriate. All data were first assessed for normality based on skewness, kurtosis, and the Kolmogorov-Smirnov test. No transformations were necessary.

3.13.1 *Volunteers*

To assess if there was significant temporal variability in LVEF, LVEDV and LVESV we performed linear mixed model analysis with the variable of interest as the dependent, volunteers as random effect, and time (in days) as a covariate. We used compound symmetry as our model for covariance structure since our healthy volunteers were expected to have constant variances over time and constant correlation between measurement times. Repeated measured analysis of variance was used to calculate the mean square error using the parameter of interest (LVEF, LVEDV, LVESV, LVSV, LVmass, RVEF, RVEDV, RVESV, RVSV, hs-TnI, or BNP) as the dependent variable, subjects as fixed factors, and time as the observer. The square root of the error term provided the standard error of the measurement (SEM) for each parameter over the 3

time points and was the primary measure of temporal variability. The 95% confidence interval (CI) for the SEM was calculated as:

$$\frac{(n-1)S^2}{Xu^2} < \sigma^2 < \frac{(n-1)S^2}{Xl^2}$$

Where XU2 is the upper-tail of the chi-square distribution for df=n-1 with area α/2 to its right and XL2 is the lower-tail value with α/2 to its left. The minimal temporal change in the measured parameters above which measurements over time in the same patient could be considered to be truly different was taken as twice the SEM for each technique. In addition, the coefficient of variation (COV) and 95% CI (as described above) was also calculated as a second measure of temporal variability for each method in order to allow inter-method comparisons. The absence of overlap in 95% CI for various SEM and COV measurements were used as a measure of significant differences in measurements.

Intra- and inter-observer variability were determined using the 2-way ANOVA approach as described by Eliasziw et al (188) with observers used as random factors. This technique was recently described specifically for use with cardiac imaging studies (189). In this analysis the inter-observer variability consists of the variability between the measurements of the two readers as well as each reader's intrinsic variability. The inter-observer test-retest variability was also calculated using the 2-way ANOVA and consists of variability within observers, between observers, and over time. For all 3 measures, the 95% CI was calculated as a measure of the smallest difference between 2 measurements above which the measurements can be considered to be truly different (189). The method used to calculate the 95% CI has been previously described (189). All statistical analysis was performed with SPSS (version 24.0.0.0, SPSS, Chicago, Illinois).

3.13.2 *Patients*

Comparison of means was performed using paired or unpaired t-test as appropriate. Longitudinal data analysis was performed using linear mixed models to determine changes in vitals and imaging parameters over study period. The frequency of LVEDV declines or LVESV increases were calculated based on above criteria in those with cardiotoxicity. Association between

cardiotoxicity and various parameters at baseline or their early change were assessed using univariable logistic regression models. To assess whether early changes predict cardiotoxicity, the changes in the following parameters were considered: LV and RV volumes and EF, serum biomarkers, BSA, EaI, and NYHA. Multivariable adjustments could not be performed due to the small number of cardiotoxicity events. Intra-observer variability were calculated using co-efficient of variation and Bland-Altman analysis. A two-sided p-value <0.05 was considered significant. Statistical analysis was performed using SPSS version 24 (IBM Corp., Armonk, NY) and R statistical software (Vienna, Austria).

Chapter 4

4 RESULTS – 1 (Validation, Healthy Volunteers)

4.1 Healthy Volunteers

Amongst the 32-healthy volunteers recruited, 2 dropped out after their first CMR study. Thirty healthy volunteers, 60% female, mean age ± SD = 50.0 ± 13.5 years (range 23.3–80.4) completed all three time points and were included in the analysis (30 volunteers x 3 time points = 90 studies). Volunteers' vitals at all 3 time points are summarized in Table 4.1. The systolic blood pressure was higher on the first exam compared to the subsequent studies. There was no difference in diastolic blood pressure, or heart rate over the study period.

Table 4.1. Clinical Characteristics and Demographic Data for Healthy Volunteers

Variable	All Time-Points	Baseline	3-Months	6-Months	p-Value*
Age		**50.0 ± 13.5**			
Females		**18 (60%)**			
Heart Rate (beat/min)	**64.6 ± 8.8**	**65.6 ± 8.6**	**63 ± 8.9**	**65.1 ± 9**	**0.249**
Systolic BP (mmHg)	**129.2 ± 16.6**	**134.7 ± 19.6**	**126.6 ± 14.8**	**126.3 ± 14.1**	**0.011**
Diastolic BP (mmHg)	**77.4 ± 11**	**77.9 ± 12.4**	**76.8 ± 9.8**	**77.4 ± 10.9**	**0.986**
Body Mass Index (kg/m^2)	**25.2 ± 4.5**	**24.9 ± 4.9**	**25.5 ± 4.6**	**25.2 ± 4.2**	**0.742**
eGFR (ml/min/1.73 m^2)	**73.1 ± 12**	**73.3 ± 12.6**	**73.4 ± 12.1**	**72.6 ± 11.8**	**0.796**
Hematocrit (%)	**40.8 ± 3.2**	**40.1 ± 3**	**40.9 ± 3.2**	**40.7 ± 3.4**	**0.818**
Troponin-I (ng/ml)	**2.2 ± 0.9**	**2.1 ± 0.6**	**2.3 ± 1**	**2.3 ± 1.1**	**0.203**
BNP (pg/ml)	**23.0 ± 21.3**	**22.5 ± 22**	**22.1 ± 15.7**	**24.3 ± 25.6**	**0.620**
LVEF (%)	**59.5 ± 4.4**	**59.9 ± 3.8**	**59.0 ± 4.7**	**59.5 ± 4.5**	**0.372**

Values provided are mean ± SD, * Linear mixed model - correlation compound symmetry

4.2 Ventricular Volumes, Mass, and Function

4.2.1 *Normal Values – Left Ventricle*

All left ventricular (LV) data were analyzable in all 3 time points from all 30 volunteers. Considering all 90 studies, the mean ± SD value (range) for LV-EF was 59.5 ± 4.4%, (50.0-68.6%); for LV-EDV was 153.4 ± 36.3ml, (103.5-279.7ml); for LV-ESV was 62.8 ± 18.8ml, (35.0-128.3ml); LV-stroke volume (SV) was 90.7 ± 19.5ml, (64.3-151.9ml); and LV-mass was 74.4 ± 18.9g, (39.6-126.9g). LV parameters at baseline and follow-up time points are summarized in Table 2; there were no significant differences over the 3 time points in LV-EF, EDV, ESV, SV, or Mass values (p-value >0.05 for all) (Table 4.2).

Mean LVEF was normal at baseline (59.9 ± 4.0%); however, 3 healthy volunteers had LVEF between 50-55%, and no-one had an LVEF below 50% at baseline. Similarly, mean LVEF was normal at the follow-up time points (58.9 ± 4.7% at 3-month and 59.7 ± 4.4% at 6-month). No healthy volunteer had an LVEF below 50% at the follow-up time points.

Table 4.2. Normal Values for Left and Right Ventricular Volumetric Parameters in healthy volunteers

Variable	All Time points	SD	Range	Baseline	SD	3-Months	SD	6-Months	SD	*P-Value
Left Ventricular Parameters:										
EF (%)	**59.5**	4.4	50.0-68.6	**59.9**	4.0	**58.9**	4.7	**59.7**	4.4	0.350
EDV (ml)	**153.4**	36.3	103.5-279.7	**153.3**	36.0	**153.0**	38.4	**154.0**	35.5	0.889
ESV (ml)	**62.8**	18.8	35.0-128.3	**61.9**	17.2	**63.6**	20.6	**62.8**	19.0	0.445
SV (ml)	**90.7**	19.5	64.3-151.9	**91.4**	20.7	**89.4**	19.7	**91.2**	18.8	0.443
Mass (gm)	**74.4**	18.9	39.6-126.9	**74.1**	18.5	**76.1**	20.2	**73.1**	18.3	0.059
Right Ventricular Parameters:										
EF (%)	**54.7**	4.9	44.8-66.1	**55.2**	5.0	**54.2**	5.1	**54.8**	4.7	0.230
EDV (ml)	**167.1**	38.8	111.2-285.5	**166.6**	40.5	**167.2**	39.3	**167.5**	37.9	0.931
ESV (ml)	**76.2**	21.7	41.3-133.3	**75.2**	22.3	**77.1**	21.9	**76.4**	21.5	0.373
SV (ml)	**90.8**	19.9	62.5-153.2	**91.4**	21.3	**90.1**	20.1	**91.1**	18.8	0.731

* Linear mixed model - correlation compound symmetry

4.2.2 *Normal Values – Right Ventricle*

All right ventricular (RV) data were analyzable in all 3 time points from all 30 volunteers. Considering all 90 studies, the mean ± SD value (range) for RV-EF was 54.7 ± 4.9%, (44.8-66.1%); for RV-EDV was 167.1 ± 38.8ml, (111.2-285.5ml); for RV-ESV was 76.2 ± 21.7ml, (41.3-133.3ml); and for RV-SV was 90.8 ± 19.9ml, (62.5-153.2ml). RV parameters at baseline and follow-up time points are summarized in Table 2; there were no significant differences over the 3 time points in RV-EF, EDV, ESV, or SV values (p-value >0.05 for all) (Table 4.2).

Mean RVEF was normal at baseline (55.2 ± 5.0%); however, 3 healthy volunteers had RVEF between 45-50%, and only one had an RVEF below that range at 44.8% at baseline. Similarly, mean RVEF was normal at the follow-up time points (54.2 ± 5.1% at 3-month and 54.8 ± 4.7% at 6-month). No healthy volunteer had an RVEF below 45% at the follow-up time points.

4.2.3 *Temporal Variability (table 4.3)*

The temporal variability as measured by SEM (95%CI), was small for all LV and RV volumetric parameters, (table 4.3, figure 4.1). Based on our data the temporal variability for EF for both ventricles was approximately 2.5% with upper 95%CI below 3.0%, while the temporal variability for LV-EDV and LV-ESV, as measured by SEM (upper 95%CI), was 8.3ml (9.7ml) and 5.2ml (6.1ml), respectively.

The temporal variability as measured by COV (95%CI), were within a close range of each other for all LV and RV corresponding volumetric parameters, (table 4.3, figure 4.2). The temporal variability as measured by COV (95%CI), comparing LV volumetric parameters, was smallest for LV-EF at 3.8% (3.1–4.6%), followed by LV-EDV at 4.3% (3.0%–5.7%), and then LV-ESV at 7.1% (5.6– 8.6%). Similarly, the temporal variability measured by COV (95%CI) comparing RV volumetric parameters, was smallest for RV-EF at 3.9% (3.4–4.5%), followed by RV-EDV at 4.9% (3.8%–6.0%), and then RV-ESV at 6.2% (4.8– 7.6%).

Table 4.3. Temporal Variability of **Left and Right Ventricular** Volumetric Parameters for all Time Points in Healthy Volunteers, Represented as Standard Error of Measurement (SEM) 95% CI, and as Coefficient of Variation (COV) 95% CI.

Variable	Temporal Variability by SEM	Lower 95%CI	Upper 95%CI	Temporal Variability by COV	Lower 95%CI	Upper 95%CI
Left Ventricular Parameters:						
EF	**2.55%**	2.23	2.99	**3.82%**	3.05	4.59
EDV	**8.26 ml**	7.20	9.68	**4.33%**	2.99	5.67
ESV	**5.22 ml**	4.55	6.12	**7.08%**	5.55	8.61
SV	**6.83 ml**	5.96	8.00	**6.26%**	4.81	7.72
Mass	**4.86 gm**	4.24	5.69	**5.48%**	4.26	6.7
Right Ventricular Parameters:						
EF	**2.28%**	1.99	2.67	**3.93%**	3.36	4.5
EDV	**8.96 ml**	7.82	10.50	**4.88%**	3.81	5.95
ESV	**5.32 ml**	4.64	6.24	**6.19%**	4.83	7.55
SV	**6.81 ml**	5.94	7.98	**6.33%**	4.85	7.8

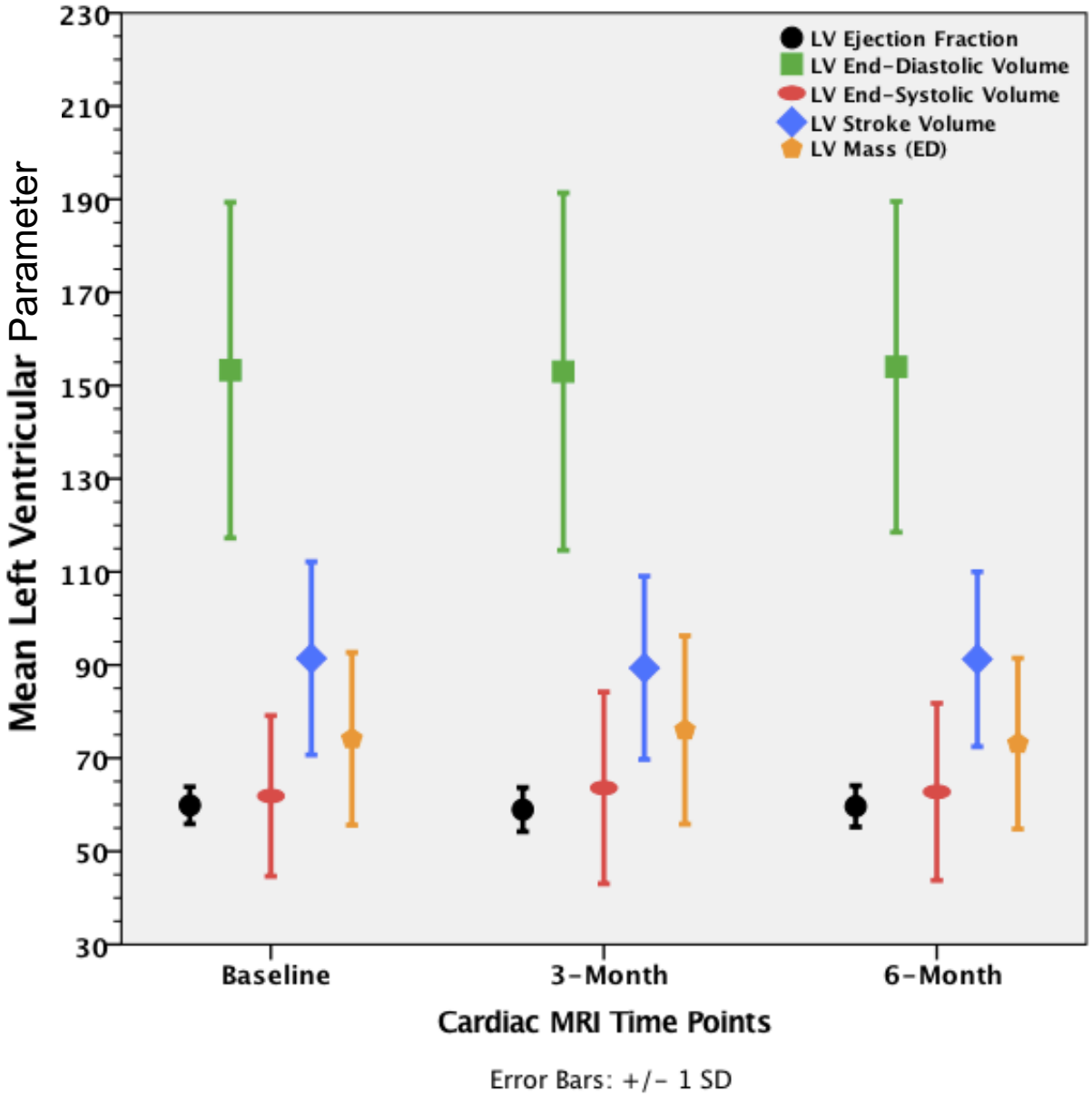


Figure 4.1. Mean +/- standard deviation of Normal values in health volunteers for LV ejection fraction, LV end-diastolic volume, LV end-systolic volume, LV stroke volume, and LV mass over three time points (baseline, 3 months, and 6 months).

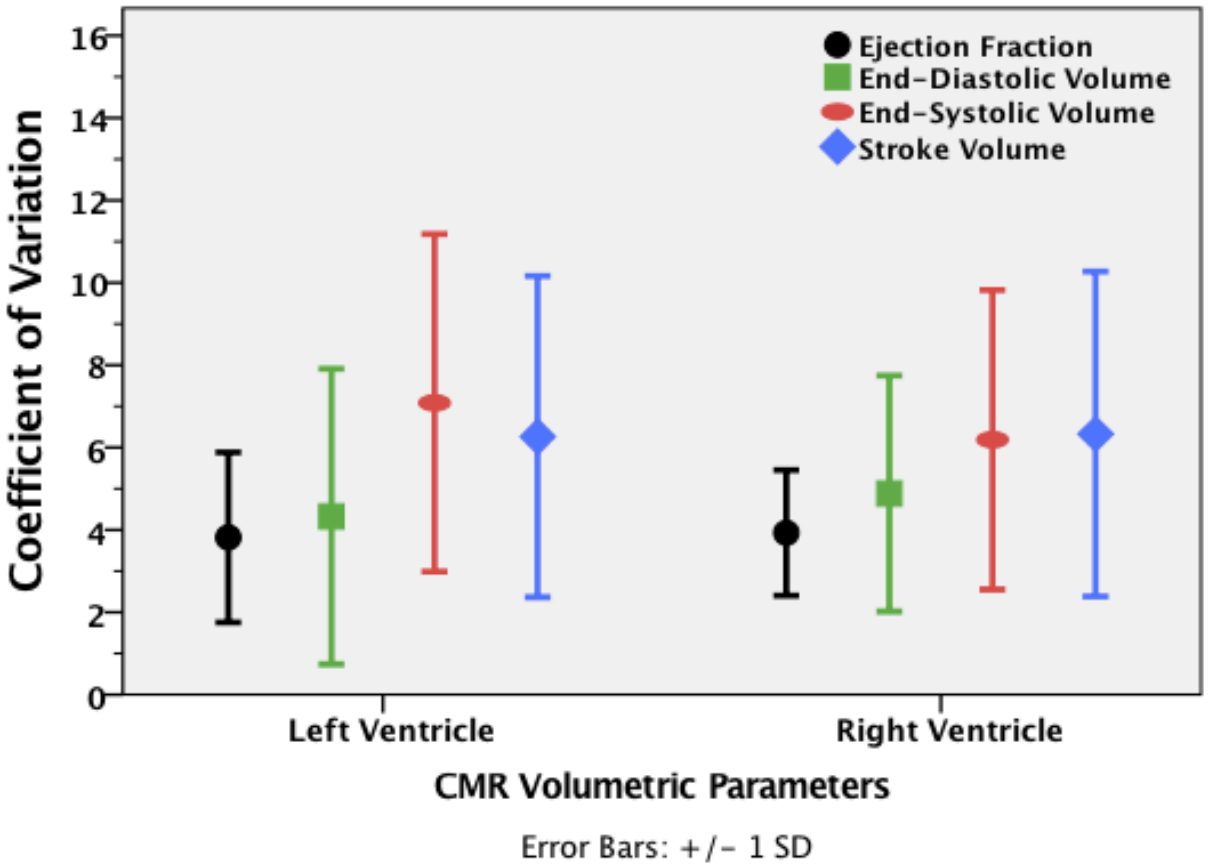


Figure 4.2. Temporal variability demonstrated as coefficient of variation (CoV) in healthy volunteers comparing side by side left and right ventricular ejection fraction, end-diastolic volume, end-systolic volume, and stroke volume over the 6-month follow-up period.

4.2.4 *Intra-Observer Variability (table 4.4)*

Intra-observer variability of LV volumetric parameters was calculated as an average of two readers using SEM and COV. The two-reader intra-observer variability for LV parameters using SEM (minimum detectable change) and COV (upper 95%CI) were as follows: for LV-EF = 1.3 (3.5)% and 1.1 (1.3)%; for LV-EDV = 3.3 (9.1)ml and 1.0 (1.3)%; for LV-ESV = 2.3 (6.5)ml and 1.2 (1.5)%; for LV-SV = 3.2 (8.8)ml and 1.9 (2.4)%; and for LV-mass = 4.2 (11.6)gm and 3.5 (4.5)%, Table 4.4a.

Table 4.4a. Intra-Observer Variability of **Left Ventricular** Volumetric Parameters for all Time Points in Healthy Volunteers, Represented as Standard Error of Measurement (SEM) 95% CI, and as Coefficient of Variation (COV) 95% CI.

Variable	Intra-Observer Variability by SEM	Min Detectable Change	Intra-Observer Variability by COV	Lower 95%CI	Upper 95%CI
Left Ventricular Parameters:					
EF	**1.3%**	3.5%	**1.1%**	0.8	1.3
EDV	**3.3 ml**	9.1 ml	**1.0%**	0.7	1.3
ESV	**2.3 ml**	6.5 ml	**1.2%**	0.9	1.5
SV	**3.2 ml**	8.8 ml	**1.9%**	1.4	2.4
Mass	**4.2 gm**	11.6 gm	**3.5%**	2.5	4.5

Intra-observer variability of RV volumetric parameters was calculated for a single reader using SEM and COV. The single-reader intra-observer variability scores for RV parameters using SEM (minimum detectable change) and COV (upper 95%CI) are as follows: for RV-EF = 1.0 (2.9)% and 1.5 (1.9)%; for RV-EDV = 3.8 (10.4)ml and 1.6 (2.0)%; for RV-ESV = 2.8 (7.8)ml and 2.5 (3.1)%; and for RV-SV = 2.4 (6.8)ml and 2.1 (2.7), Table 4.4b.

Table 4.4b. Single-Reader Intra-Observer Variability of **Right Ventricular** Volumetric Parameters for all Time Points in Healthy Volunteers, Represented as Standard Error of Measurement (SEM) 95% CI, and as Coefficient of Variation (COV) 95% CI.

Variable	Intra-Observer Variability by SEM	Min Detectable Change	Intra-Observer Variability by COV	Lower 95%CI	Upper 95%CI
Right Ventricular Parameters:					
EF	**1.0%**	2.9%	**1.5%**	1.2	1.9
EDV	**3.8 ml**	10.4 ml	**1.6%**	1.1	2.0
ESV	**2.8 ml**	7.8 ml	**2.5%**	1.9	3.1
SV	**2.4 ml**	6.8 ml	**2.1%**	1.6	2.7

4.2.5 *Inter-Observer Variability (table 4.5)*

Inter-observer variability of LV volumetric parameters was calculated between the two readers using SEM and COV. The LV inter-observer variability using SEM (minimum detectable change) and COV (upper 95%CI) were as follows: for LV-EF = 1.7 (4.8)% and 2.5 (2.8)%; for LV-EDV = 5.9 (16.5)ml and 2.6 (3.2)%; for LV-ESV = 3.5 (9.7)ml and 4.1 (4.5)%; for LV-SV = 5.0 (13.8)ml and 3.8 (4.7)%; and for LV-mass = 5.6 (15.4)g and 5.7 (6.5)%, Table 4.5.

Table 4.5. Inter-Observer Variability of **Left Ventricular** Volumetric Parameters for all Time Points in Healthy Volunteers, Represented as Standard Error of Measurement (SEM) 95% CI, and as Coefficient of Variation (COV) 95% CI.

Variable	Inter-Observer Variability by SEM	Min Detectable Change	Inter-Observer Variability by COV	Lower 95%CI	Upper 95%CI
Left Ventricular Parameters:					
EF	**1.7%**	4.8%	**2.5%**	2.1	2.8
EDV	**5.9 ml**	16.5 ml	**2.6%**	2.0	3.2
ESV	**3.5 ml**	9.7 ml	**4.1%**	3.6	4.5
SV	**5.0 ml**	13.8 ml	**3.8%**	2.9	4.7
Mass	**5.6 g**	15.4 g	**5.7%**	4.9	6.5

4.2.6 *Inter-Observer Test-Retest Variability (table 4.6)*

The inter-observer test-retest variability of LV volumetric parameters was calculated between the two readers (variability within readers, between readers, and over time) using SEM and COV. The LV inter-observer test-retest variability using SEM (minimum detectable change) and COV (upper 95%CI) were as follows: for LV-EF = 2.1 (5.7)% and 2.6 (3.6)%; for LV-EDV = 12.6 (34.9)ml and 4.7 (8.0)%; for LV-ESV = 7.4 (20.5)ml and 6.9 (10.3)%; for LV-SV = 7.4 (20.6)ml and 4.8 (8.2)%; and for LV-mass = 5.7 (15.7)g and 6.0 (7.3)%, Table 4.6.

Table 4.6. Inter-Observer Test-Retest (6-month) Variability of **Left Ventricular** Volumetric Parameters for all Time Points in Healthy Volunteers, Represented as Standard Error of Measurement (SEM) 95% CI, and as Coefficient of Variation (COV) 95% CI.

Variable	Inter-Observer Variability by SEM	Min Detectable Change	Inter-Observer Variability by COV	Lower 95%CI	Upper 95%CI
Left Ventricular Parameters:					
EF	**2.1%**	5.7%	**2.6%**	1.6	3.6
EDV	**12.6 ml**	34.9 ml	**4.7%**	1.4	8.0
ESV	**7.4 ml**	20.5 ml	**6.9%**	3.5	10.3
SV	**7.4 ml**	20.6 ml	**4.8%**	1.5	8.2
Mass	**5.7 g**	15.7 g	**6.0%**	4.7	7.3

4.3 High-Sensitivity Troponin-I and BNP

4.3.1 *Normal Values (table 4.7)*

Hs-TnI blood values were available in all 3 time points from all 30 volunteers; BNP blood values were available in all except one time point. Considering all 90 studies, the median and IQR (range) for hs-TnI was 2.0 and 0.0 (2.0-8.0) ng/ml; for BNP was 15.3 and 16.1 (10.0-127.7) pg/ml. Hs-TnI and BNP values at baseline and follow-up time points are summarized in Table 4.8; there were no significant differences over the 3 time points in hs-TnI or BNP (p-value >0.05 for all) (Table 4.7).

Mean hs-TnI was normal at baseline (2.1 ± 0.6ng/ml), as well as at the follow-up time points (2.3 ± 0.9 at 3-month and 2.3 ± 1.1% at 6-month). No healthy volunteer had hs-TnI above 27 at baseline or at the follow-up time points.

Mean BNP was normal at baseline (22.5 ± 21.6pg/ml); as well as at the follow-up time points (22.1 ± 15.5pg/ml at 3-month and 24.3 ± 25.2pg/ml at 6-month) with only one healthy volunteer had BNP above 100 at 6 months.

Table 4.7. Normal Values for high-sensitivity troponin-I (hs-TnI) and BNP in Healthy Volunteers

Variable		All Time-Points	Baseline	3-Months	6-Months	P-Value*
hs-TnI	**Mean**	**2.2**	**2.1**	**2.3**	**2.3**	**0.203**
(ng/ml)	95% CI	(2.0–2.4)	(1.9– 2.3)	(1.9–2.6)	(1.9–2.7)	
	Median	**2.0**	**2.0**	**2.0**	**2.0**	
	IQR	0.0	0.0	0.0	0.0	
BNP	**Mean**	**23.0**	**22.5**	**22.1**	**24.3**	**0.620**
(pg/ml)	95% CI	(18.5–27.5)	(14.3–30.7)	(16.1–28.1)	(14.7–33.8)	
	Median	**15.3**	**14.1**	**14.7**	**16.3**	
	IQR	16.1	14.6	54.3	15.6	

* Linear mixed model - correlation compound symmetry

4.3.2 *Temporal Variability (table 4.8)*

The temporal variability for hsTn-I and BNP measured by SEM (95% CI) were 0.4ng/ml (0.4–0.5) and 9.8pg/ml (8.6–11.5), respectively (Table 4.8). The respective COV (95%CI) values were 5.7% (1.6-9.8) and 24.3% (18.5-30.2) (Table 4.8).

Table 4.8. Temporal Variability for high-sensitivity troponin-I (hs-TnI) and BNP in Healthy Volunteers

Variable		Temporal Variability	
		SEM	**CoV**
hs-TnI (ng/ml)	**Mean**	**0.40**	**5.68**
	95% CI	(0.35–0.46)	(1.57–9.79)
BNP (pg/ml)	**Mean**	**9.81**	**24.32**
	95% CI	(8.55–11.51)	(18.5–30.2)

Chapter 5

5 RESULTS – 2 (Patients with Breast Cancer)

5.1 Patient Population

Out of the 87-breast cancer patients recruited, 4 dropped out after their first or second CMR studies. Eighty-three women mean age ± SD = 51.0 ± 9.5 years (range 27–70 years) completed the first three time points and were included in the analysis. Patient clinical characteristics are summarized in Table 5.1. At least one cardiovascular risk factor was present in 34 patients (41.0%). Over the six-month follow-up period there was no statistically significant change in systolic blood pressure, or arterial elastance. There was, however, a small but statistically significant increase in mean weight and HR over the follow-up period ($p < 0.001$) (Figure 5.1). There was no change in the mean weight between baseline and 3 months (66.7 ± 13.5kg to 66.7 ± 13.6kg, $p = 0.99$), but the weight increased significantly at 6 months (68.1 ± 13.9kg, $p = 0.006$). All HER2+ patients received either adjuvant or neoadjuvant cancer therapy that consisted of two months of concurrent anthracycline, and cyclophosphamide +/- 5-flurouracil, followed subsequently by 52 weeks of trastuzumab administration every 3 weeks (71,72). All patients also received taxanes along with trastuzumab every 3 weeks during the first 3-4 cycles. Taxanes have the potential to increase fluid retention and therefore can affect measurement of cardiac function artifactually.

Table 5.1. Clinical and demographic data for the included patient's pre-cancer treatment

Demographics	(N=83)
Age (years)	51.0 ± 9.5
Body weight (kg)	66.6 ± 13.5
Body surface area, (m^2)	1.7 ± 0.2
Body Mass Index, (kg/m^2)	25.8 ± 5.1
Heart rate (bpm)	74.5 ± 10.6
Systolic blood pressure (mmHg)	128.5 ± 17.6
Diastolic blood pressure (mmHg)	78.9 ± 12.2
Cardiovascular Risk Factors, n (%):	
Hypertension	12 (14.5%)
Diabetes	3 (3.6%)
Hypercholesterolemia	9 (10.8%)
Smoking History, Any	23 (27.7%)
Coronary artery disease	0 (0%)
Chemotherapeutic Regimen:	
Epirubicin dose (mg/m^2), n=82	301.8 ± 12.3
Doxorubicin dose (mg/m^2), n=1	253.1
Cardiac Medications:	
Beta-blocker	4 (4.8%)
ACE inhibitor / ARB	10 (12.0%)
Statins	6 (7.2%)

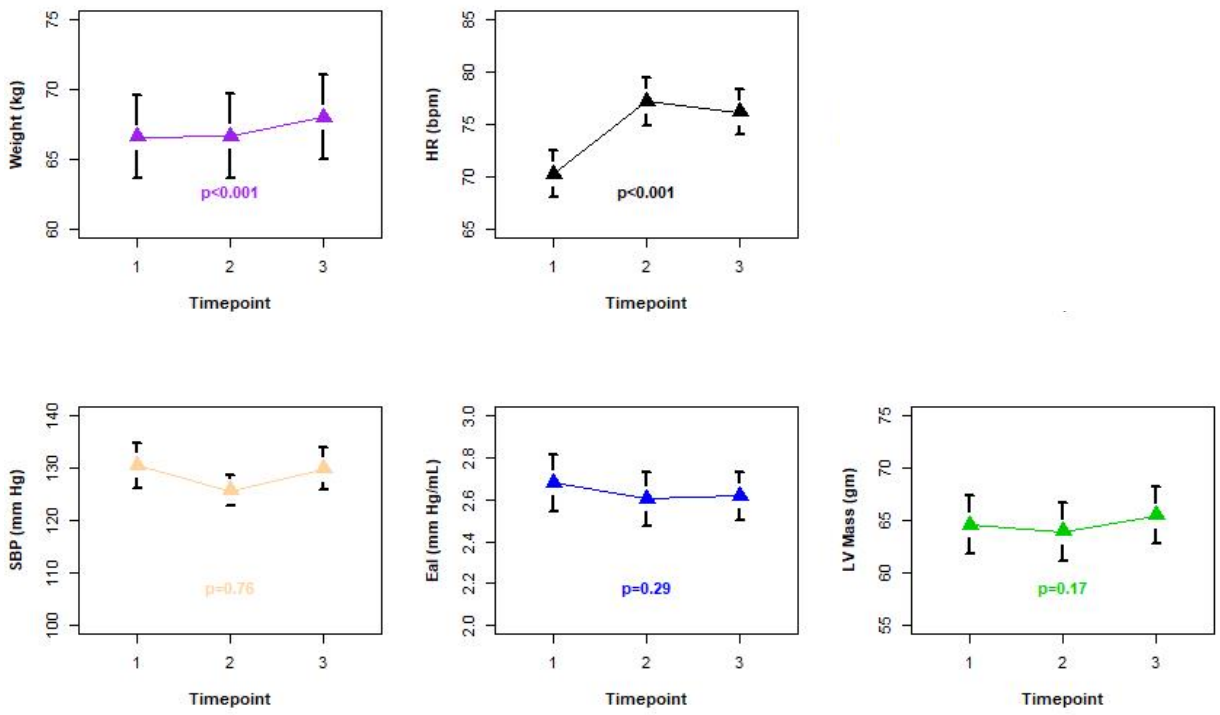


Figure 5.1. Changes in hemodynamic variables and LV mass over time with p-values calculated using linear mixed models. A) weight, B) heart rate, C) arterial elastance, D) systolic blood pressure, E) diastolic blood pressure and e) LV mass.

5.2 Overall Changes in Left Ventricular Volumes, Mass, and Function

LV CMR parameters at baseline and follow-up are summarized in Table 5.2. Mean (± SD) LVEF was normal with 60.1% ± 4.4% at baseline, however, 12 patients had LVEF between 50 – 55% and one patient had an LVEF of 47% at baseline. These patients with a mild reduction in LVEF still received treatment since cardiac screening prior to cancer therapy was based on 2D echocardiography (which did not identify the low normal/mild LV dysfunction) as per standard of care. Over the three-month period, there was an overall significant increase only in LVESV (including after being normalized to BSA) and an overall significant reduction in LVEF ($p<0.01$ for all). Over the six-month period, there was an overall significant increase in LVEDV, LVESV, and LV-mass (including after being normalized to BSA), and a significant reduction in LVEF

($p<0.05$ for LV-mass and $p<0.001$ for the rest). Changes are summarized in Table 5.2 and Figures 5.2A and 5.3.

Table 5.2. Sequential change in patients' LV cardiac MRI parameters

Characteristic	Pre-Cancer therapy		~2 Months*		P-value	~5 Months[&]		P-value
	Mean	SD	Mean	SD	(0-2)	Mean	SD	(0-5)
Cardiac MRI Left Ventricular Parameters:								
LVEF (%)	**60.1**	4.4	**58.8**	4.3	**0.004**	**55.6**	4.1	**<0.001**
LVEDV (ml)	**130.1**	23.0	**131.6**	25.2	0.334	**141.9**	23.0	**<0.001**
LVEDV indexed (ml/m^2)	**76.5**	10.3	**77.8**	12.4	0.199	**83.1**	11.6	**<0.001**
LVESV (ml)	**52.2**	12.0	**54.4**	12.5	**0.006**	**63.4**	13.8	**<0.001**
LVESV indexed (ml/m^2)	**30.7**	6.0	**32.1**	6.6	**0.003**	**37.0**	7.4	**<0.001**
LVSV (ml)	**77.9**	13.5	**77.3**	15.2	0.474	**78.6**	11.7	0.572
LVSV indexed (ml/m^2)	**45.8**	6.1	**45.6**	7.5	0.584	**46.0**	6.0	0.772
LV mass, (gm)	**61.0**	12.4	**61.5**	12.8	0.501	**62.8**	12.2	**0.007**
LV mass indexed, (gm/m^2)	**35.8**	5.5	**36.1**	5.6	0.387	**36.6**	5.5	**0.022**

Data presented as frequency mean ± standard deviation,
*Within 1 month of completion of anthracycline,
[&]Within 2-3 months of initiation of trastuzumab

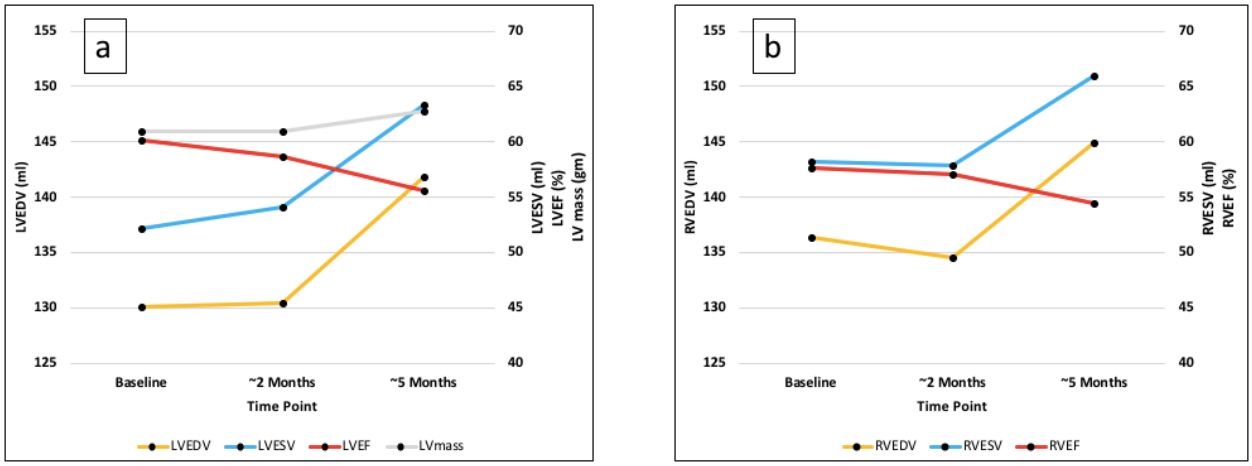


Figure 5.2. Changes in LV and RV volumes and ejection fraction and LVmass. (A) Changes over time in LV end-diastolic and end-systolic volumes, LVEF, and LV-mass in 83 patients. (B) Changes over time in RV end-diastolic and end-systolic volumes and RVEF in 83 patients.

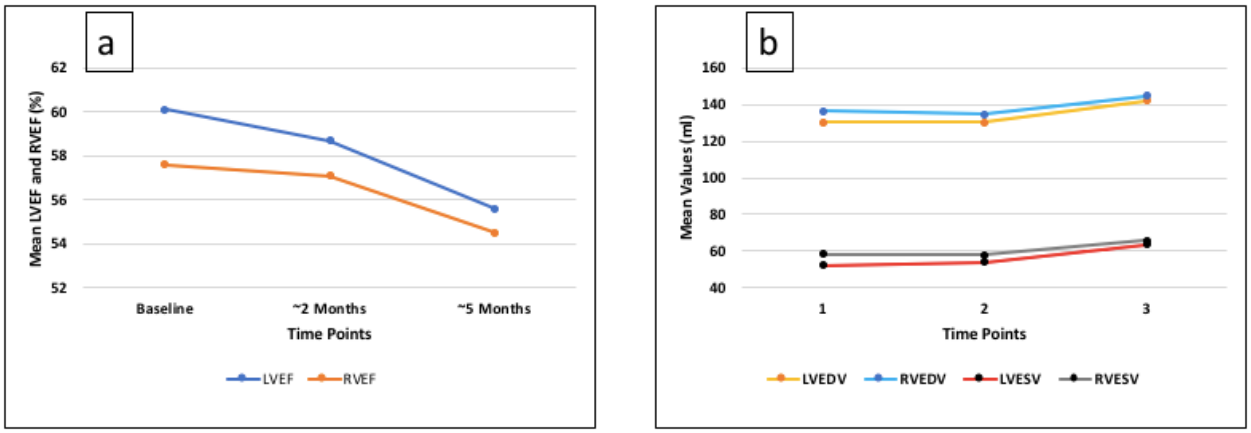


Figure 5.3. Side by side comparison of changes in LV and RV volumes and ejection fraction. (A) Changes over time in LV and RV ejection fraction in 83 patients. (B) Changes over time in LV and RV end-diastolic and end-systolic volumes in 83 patients.

5.3 Overall Changes in Right Ventricular Volumes and Function

RV CMR parameters at baseline and follow-up are summarized in Table 5.3. All patients had normal RVEF at baseline with Mean (± SD) RVEF = 57.6 ± 4.5%. Over the three-month period, there was no significant change overall in any of the RV volumes nor in RVEF ($p>0.05$ for all). Over the six-month period, however, there was an overall significant increase in both RVEDV

and RVESV (including after being normalized to BSA), and a significant reduction in RVEF ($p<0.001$ for all). Changes are summarized in Table 5.3 and Figures 5.2B and 5.3.

Table 5.3. Sequential change in patients' RV cardiac MRI parameters

Characteristic	Pre-Cancer therapy		~2 Months*		P-value	~5 Months&		P-value
	Mean	SD	Mean	SD	(0-2)	Mean	SD	(0-5)
Cardiac MRI Right Ventricular Parameters:								
RVEF (%)	57.6	4.5	57.2	4.4	0.320	54.5	3.9	**<0.001**
RVEDV (ml)	136.4	25.6	135.8	26.2	0.637	144.9	24.4	**<0.001**
RVEDV indexed ml/m^2)	80.2	11.8	80.3	13.1	0.853	84.8	12.4	**<0.001**
RVESV (ml)	58.2	14.1	58.3	13.3	0.997	66.0	14.4	**<0.001**
RVESV indexed (ml/m^2)	34.2	7.1	34.5	7.2	0.754	38.6	7.5	**<0.001**
RVSV (ml)	78.2	13.8	77.5	15.3	0.463	78.6	12.1	0.773
RVSV indexed (ml/m^2)	46.0	6.3	45.8	7.7	0.582	46.0	6.2	0.965

Data presented as frequency mean ± standard deviation,
*Within 1 month of completion of anthracycline,
&Within 2-3 months of initiation of trastuzumab

5.4 Overall Changes in High Sensitivity Troponin-I (Hs-TnI) and BNP

Hs-TnI and BNP values at baseline and follow-up are summarized in Table 5.4. All patients had normal Hs-TnI and BNP at baseline with mean (± SD) Hs-TnI and BNP were 2.4 (± 1.5) ng/ml and 21.9 (± 14.5) pg/ml, respectively. No patient had an above normal hs-TnI (>27ng/ml) or BNP (>100pg/ml) values during the follow-up time points. At the three-month time point, there was an overall significant increase only in hs-TnI ($p<0.001$). At the six-month time point, hsTnI values decreased compared to 3 months, yet the values still remained elevated compared to

baseline. There was a mild but non-significant increase in BNP levels between baseline and 3 months, which subsequently decreased to below baseline by 6 months ($p<0.01$). Changes are summarized in Table 5.4.

Table 5.4. Sequential changes in patients' biomarkers

Blood Marker	Pre-Cancer therapy		~2 Months*		P-value (0-2 months)	~5 Months&		P-value (0-5 months)
	Mean/ Median	SD/ IQR	Mean/ Median	SD/ IQR		Mean/ Median	SD/ IQR	
High Sensitivity Troponin-I	2.4	1.5	8.2	7.2		5.9	8.4	
(**Hs-TnI**) ng/ml	2.0	0.0	6.0	7.2	**<0.001**	4.0	3.8	**<0.001**
Brain Natriuretic Peptide	21.9	14.5	24.7	19.5		17.5	13.1	
(**BNP**) pg/ml	18.0	18.0	18.1	20.1	0.099	11.1	11.1	**0.006**

Data presented as frequency mean ± standard deviation (SD), and as median ± Interquartile range (IQR)
*Within 1 month of completion of anthracycline,
&Within 2-3 months of initiation of trastuzumab

5.5 Comparison of Patients with and without Cardiac-Toxicity *LV Cardiotoxicity – Primary Definition*

Using our primary definition, 10 (12.1%) patients developed LV-cardiotoxicity. For this cardiotoxicity subgroup by 5 months, there was significant reduction in LVEF (62.1±5.0% to 51.8±3.9%, $p<0.001$), significant increase in LVESV (49.2±11.4 mL to 69.4±15.7 mL, $p<0.001$), and a nonsignificant increase in LVEDV (129.9±27.7 mL to 143.4±27.6 mL, $p=0.08$).

Except for LVEF, there was no difference amongst the baseline characteristics between the patients who developed LV cardiotoxicity (n=10) and the ones who didn't' develop LV cardiotoxicity (n=73) by 5 months. The baseline mean LVEF was statistically higher in the 10 patients who subsequently developed LV cardiotoxicity (Table 5.5).

Table 5.5. Baseline characteristics of two subsets of patients: who did subsequently developed cardiotoxicity and who did not develop cardiotoxicity by 5 months.

Baseline Characteristics	No cardiotoxicity (n=73)		Cardiotoxicity (n=10)		P-value
	Mean	SD	Mean	SD	*†
Age	50.1	8.9	57.8	11.2	0.060
Weight (kg)	66.4	13.5	68.1	14.1	0.723
BSA (kg/m^2)	1.7	0.2	1.7	0.2	0.790
HR (b/min)	70	10.2	72.5	10	0.470
DBP (mmHg)	79.6	13.9	75.1	13.3	0.337
SBP (mmHg)	130.1	20.3	133	19.7	0.674
Troponin I (ng/ml)	2.4	1.5	2.4	1.3	0.923
BNP (pg/ml	20.4	12.6	32.5	22.6	0.129
LVEF (%)	**59.7**	4.3	**62.7**	3.7	**0.034**
LVEDVi (ml)	76.4	9.9	77.3	13	0.830
LVESVi (ml)	31	6	28.8	5.4	0.269
LVSVi (ml)	45.4	5.7	48.7	8.7	0.274
LVmass i (gm)	36.1	5.6	34	4.8	0.239
RVEF (%)	57.3	4.4	59.6	4.6	0.159
RVEDVi (ml)	79.9	11.5	82.2	14.3	0.635
RVESVi (ml)	34.3	7.1	33.3	7.1	0.663
RVSVi (ml)	45.6	5.9	49	8.6	0.260
Total Epirubicin (mg)‡	512.8	54	511	61.1	0.932
Epirubicin Dose i (mg/m^2)‡	302.3	11.6	298.1	16.7	0.455

*Independent T-test, †Equal variances not assumed, ‡Not at baseline

LV- cardiotoxicity was identified in only 1 patient by two months (who continued to have cardiotoxicity by 5 months) and the remaining 9 patients developed cardiotoxicity by five months. Among cardiotoxicity patients, eight patients had LVEF declines to between 50% and 55%, while 2 patients had a decline to <50%. Also, from the cardiotoxicity group, seven patients had reduction in LVEF of >10% while three had symptomatic declines in LVEF between 8.1% and 9.7% with HF symptoms progressing from NYHA class 0-I to II. Of the 12 patients with baseline LVEF <55% by CMR, only one developed LV- cardiotoxicity, as diagnosed by a symptomatic 9.4% absolute decline in LVEF from baseline value of 54.3%.

Of the 10 patients with LV-cardiotoxicity, 8 (80.0%) had isolated increase in LVESV, and 2 (20.0%) had combined significant increase in LVESV and decrease in LVEDV; none developed LV-cardiotoxicity due to isolated reduction in LVEDV (Table 5.6, Figure 5.4a).

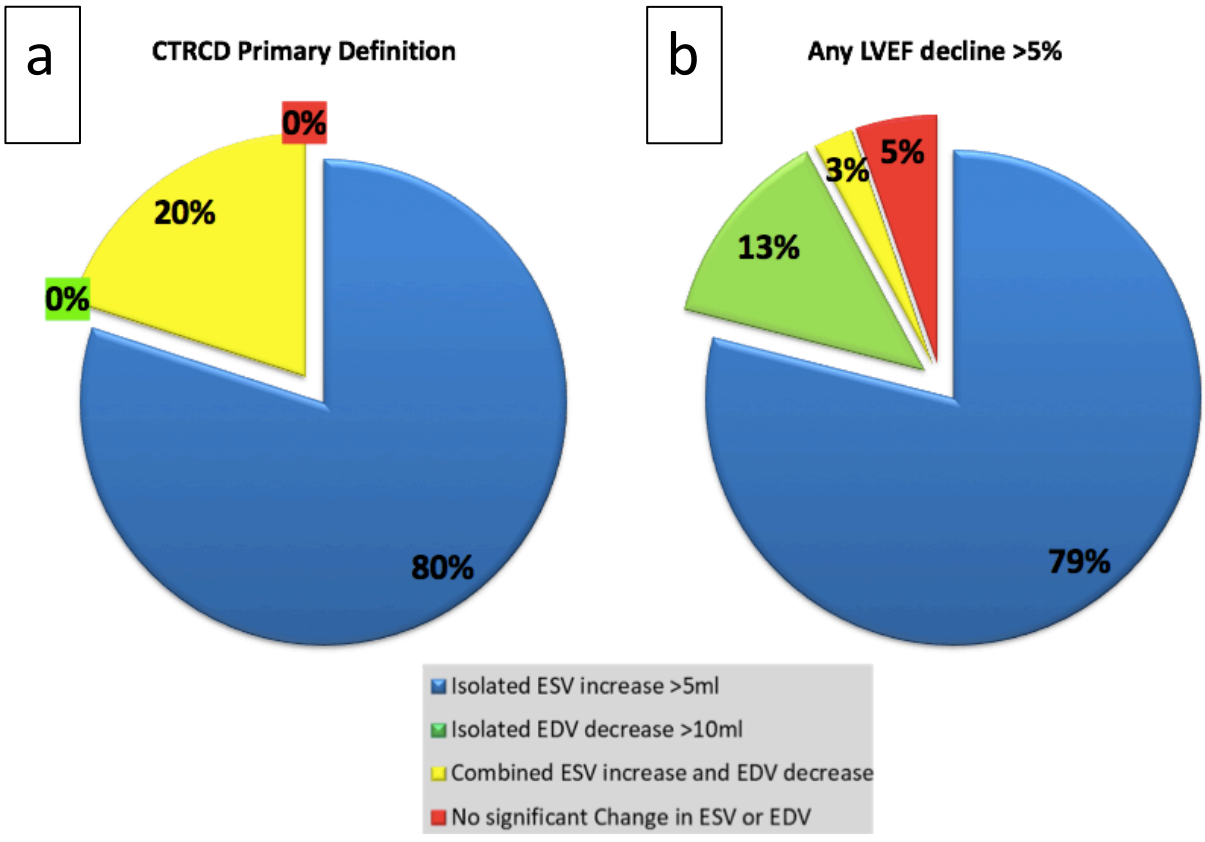


Figure 5.4. Causes for reduction in LVEF. (A) cardiotoxicity was defined as per CREC recommendations. (B) Any >5% reduction in LVEF at 3 or 5 months compared to baseline.

Table 5.6. CMR Volumetric determinants of left ventricular ejection fraction decline categorized by different cardiotoxicity definitions and the time of occurrence.

LV-Cardiotoxicity*†	**~2 Months** **n**	**~5 Months** **n**	**Entire Follow Up** **n (%)**
Isolated ESV increase	1	7	8 (9.6%)
Isolated EDV decrease	0	0	0
Combined ESV increase and EDV decrease	0	2	2 (2.4%)
No significant Change in ESV or EDV	0	0	0
Total	1	9	10 (12.1%)
LVEF decline >5% (sensitivity definition)†	**~2 Months** **n**	**~5 Months** **n**	**Entire Follow Up** **n (%)**
Isolated ESV increase	9	21	30 (79.0%)
Isolated EDV decrease	3	2	5 (13.2%)
Combined ESV increase and EDV decrease	0	1	1 (2.6%)
No significant Change in ESV or EDV	2	0	2 (5.3%)
Total	14	24	38 (42.2%)
RV-Cardiotoxicity‡#	**~2 Months** **n**	**~5 Months** **n**	**Entire Follow Up** **n (%)**
Isolated ESV increase	1	0	1 (1.2%)
Isolated EDV decrease	0	0	0
Combined ESV increase and EDV decrease	0	1	1 (1.2%)
No significant Change in ESV or EDV	0	0	0
Total	1	1	2 (2.4%)

EDV, end-diastolic volume; ESV, end-systolic volume

*LV-Cardiotoxicity is defined by >5% Symptomatic drop in LVEF to <55%, or asymptomatic >10% drop in LVEF to <55%
‡ RV-Cardiotoxicity is defined by a >10% drop in RVEF to <51%
†Significant LVEDV decrease is >10ml, Significant LVESV increase is >5ml
Significant RVEDV decrease is >10ml, Significant RVESV increase is >5ml

5.5.1.1 LVEF Changes in patients' with and without LV-cardiotoxicity

In patients who developed cardiotoxicity, the mean LVEF declined significantly at two and five months. In patients not meeting our binary cardiotoxicity criteria, there was still a statistically significant reduction in LVEF by five months (table 5.7).

5.5.1.2 RVEF Changes in patients' with and without LV-cardiotoxicity

In both patients with or without LV-cardiotoxicity, mean RVEF declined significantly only at 5 months (table 5.7). Mean (± SD) LVEF and RVEF at baseline, ~2-month, and ~5-month for all patients and those with and without cardiotoxicity are summarized in table 5.7.

5.5.1.3 Biomarker changes in patients with and without LV-cardiotoxicity

In both patients with and without cardiotoxicity, mean hs-TnI increased significantly at 2 months. However, in both groups there was reduction in LVEF at 5 months although the values remain significantly higher than baseline only in the cohort that did not develop LV cardiotoxicity (table 5.7). This possibly reflects the sample size differences between the two groups.

In patients with cardiotoxicity, mean BNP did not change significantly at the follow up time points. However, in patients without cardiotoxicity, the 5-month's mean BNP was significantly lower than that of the baseline. (table 5.7).

Table 5.7. Summary of changes in left and right ventricular ejection fraction, high-sensitivity troponin-I, and BNP between pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

Characteristics	Pre-Cancer therapy	~2 Months*	P-value‡ (0-2)	~5 Months†	P-value‡ (0-5)
Left Ventricular Ejection fraction, (%)					
All patients (n=83)	60.1 ± 4.4	58.8 ± 4.3	**.003**	55.6 ± 4.1	**<.001**
In pts with LV dysfunction	62.7 ± 3.7	57.7 ± 4.1	**.001**	51.3 ± 3.5	**<.001**
In pts with No LV dysfunction	59.7 ± 4.3	58.9 ± 4.3	.076	56.2 ± 3.9	**<.001**
Right Ventricular Ejection fraction, (%)					
All patients (n=83)	57.6 ± 4.5	57.2 ± 4.4	.299	54.5 ± 4.0	**<.001**
In pts with LV dysfunction	59.6 ± 4.6	58.7 ± 5.1	.339	53.5 ± 3.4	**.002**
In pts with No LV dysfunction	57.3 ± 4.5	57.0 ± 4.3	.429	54.6 ± 4.0	**<.001**
High-Sensitivity Troponin-I (ng/ml)					
All patients (n=83)	2.4 ± 1.5	8.2 ± 7.2	**<.001**	5.9 ± 8.4	**<.001**
In pts with LV dysfunction	2.5 ± 1.6	8.0 ± 7.2	**.005**	6.0 ± 8.9	.077
In pts with No LV dysfunction	2.4 ± 1.3	11.0 ± 8.5	**<.001**	6.1 ± 7.0	**.001**
Brain Natriuretic Peptide (pg/ml)					
All patients (n=83)	21.9 ± 14.5	24.7 ± 19.5	.099	17.5 ± 13.1	**.006**
In pts with LV dysfunction	32.5 ± 22.6	43.3 ± 30.0	.207	29.6 ± 23.6	.666
In pts with No LV dysfunction	20.4 ± 12.8	22.5 ± 16.5	.263	16.0 ± 12.2	**.004**

Data presented as frequency mean ± standard deviation,
*Within 3 weeks of completion of anthracycline,
†Within 3 months of initiation of trastuzumab
‡Paired t-test.

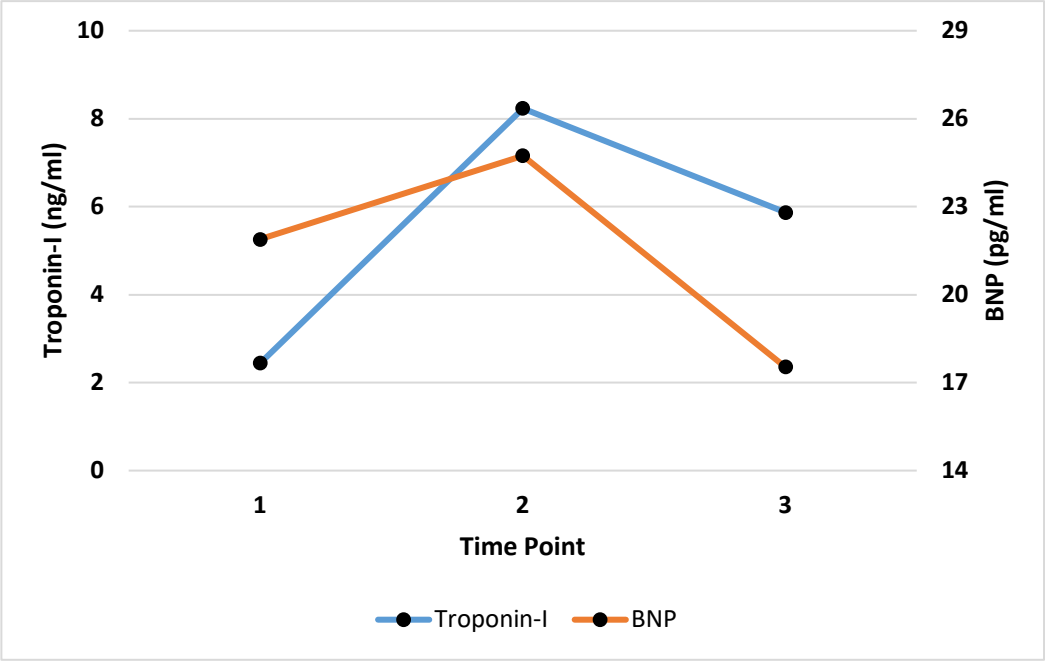


Figure 5.5. Changes over time in high sensitivity Troponin-I and BNP in 83 patients.

5.5.2 *LV-Cardiotoxicity – Sensitivity Definition*

Using the sensitivity definition for LV-cardiotoxicity, a >5% reduction in LVEF occurred in 38 (45.8%) patients (Table 5.6). Amongst these patients 30 (79.0%) had an isolated increase in LVESV, five (13.2%) had isolated decrease in LVEDV, one had both, and two had neither (Table 5.6, Figure 5.4B).

5.5.3 *RV Cardiotoxicity*

Using our definition for RV-cardiotoxicity, two patients (2.4%) developed RV-cardiotoxicity, one by 2 months and the second by 5 months. Of these two patients with RV-cardiotoxicity, none developed RV-cardiotoxicity due to isolated reduction in RVEDV, one patient had isolated significant increase in RVESV, and one had combined significant increase in RVESV and decrease in RVEDV, of whom one patient also had LV-cardiotoxicity (Table 5.6).

Unlike in patients with LV dysfunction, where mean LVEF significantly dropped at both 2 and 5 months; significant reduction in mean RVEF only occurred at 5 months (Figure 5.6a, Table 5.7).

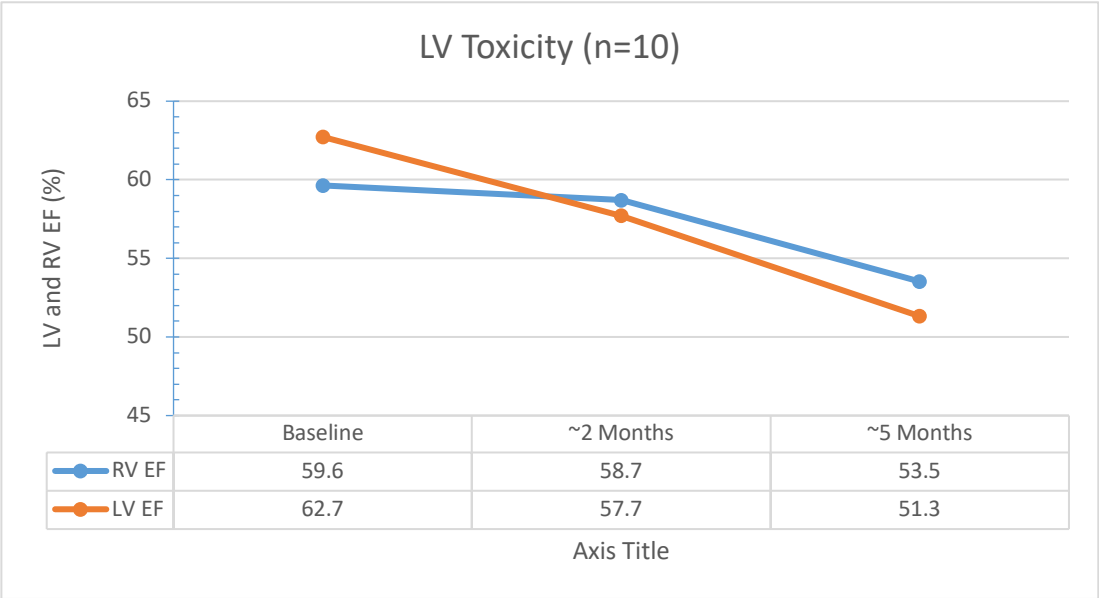


Figure 5.6a. Comparison between mean changes in LVEF and RVEF in patients with LV dysfunction at pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

Changes in RVEF mirrored that of LVEF in the patients who did not develop LV cardiotoxicity figure 5.6b.

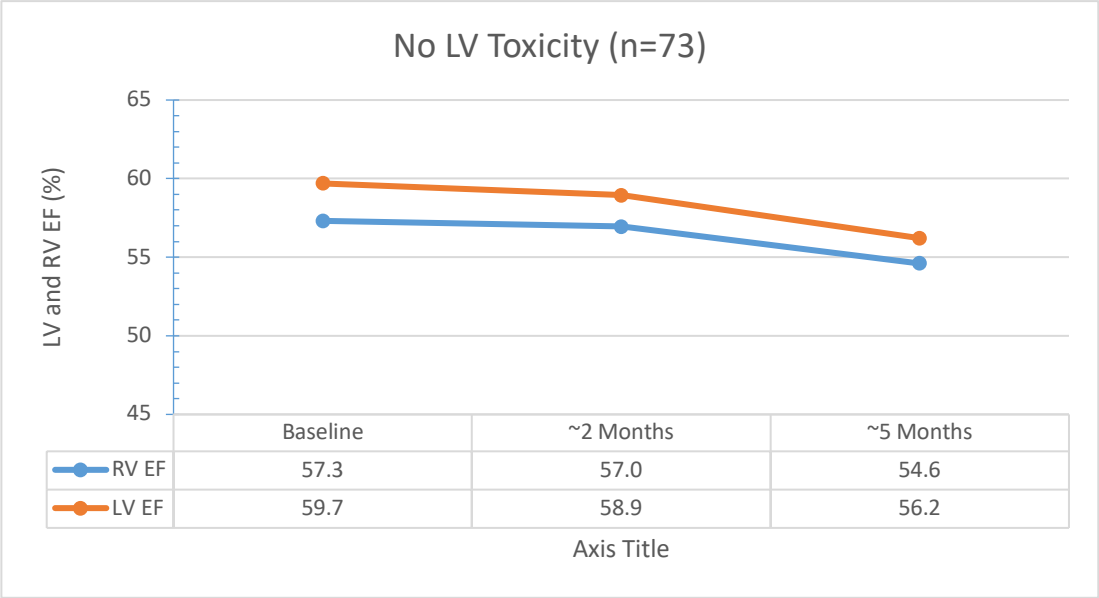


Figure 5.6b. Comparison between mean changes in LVEF and RVEF in patients with no LV cardiotoxicity at pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

5.6 Association Between Changes in LV Volumes and Cardiotoxicity

On univariable logistic analysis, only age, BNP, and LVEF values at baseline were significantly associated with development of LV-cardiotoxicity (Table 5.8). So, an increase in age by one year, BNP by one pg/ml, or LVEF by one percent at base line increase the probability of developing cardiotoxicity at 5 months by 11%, 4%, and 21% respectively.

Table 5.8. Univariable logistic regression analysis of association between baseline variables and development of cardiotoxicity by 5 months (~3 months into trastuzumab).

Baseline variables*†	**OR**	**LV-cardiotoxicity**		**p-value‡**
		95%CI		
Age	**1.11**	1.02	1.20	**0.020**
Weight (kg)	1.01	0.96	1.06	0.705
BSA (kg/m^2)	1.68	0.04	77.15	0.791
HR (b/min)	1.02	0.96	1.09	0.460
DBP (mmHg)	0.98	0.93	1.03	0.333
SBP (mmHg)	1.01	0.98	1.04	0.670
Troponin I (ng/ml)	0.98	0.61	1.56	0.932
BNP (pg/ml)	**1.04**	1.01	1.09	**0.027**
LVEF (%)	**1.21**	1.00	1.45	**0.045**
LVEDVi (ml/m2)	1.01	0.95	1.08	0.785
LVESVi (ml/m2)	0.94	0.84	1.06	0.286
LVSVi (ml/m2)	1.09	0.98	1.23	0.120
LVmass I (g/m2)	0.93	0.81	1.06	0.268
RVEF (%)	1.11	0.97	1.28	0.132
RVEDVi (ml/m2)	1.02	0.96	1.08	0.562
RVESVi (ml/m2)	0.98	0.89	1.08	0.651
RVSVi (ml/m2)	1.09	0.98	1.22	0.120
EaI (mmHg/ml/m2)	0.85	0.27	2.70	0.778

*Variables at baseline,
†i = indexed for BSA,
‡Univariable logistic regression

On univariable logistic regression analysis for changes at 2 months, only an increase in LVESV-normalized (OR 1.43, 95%CI 1.14–1.80, p=0.002), and an increase in LVEF (OR 0.75, 95%CI 0.62–0.91, p=0.004) were significantly associated with development of LV-cardiotoxicity by 5 months (Table 5.9). So, an increase in LVESV-normalized by one ml, or a drop in LVEF by one percent by 2 months increases the probability of developing cardiotoxicity at 5 months by 43% and 33%, respectively. Changes at two months in LVEDV, LVmass, RV volumes, RVEF, h-TnI, and BNP were not significantly associated with development of LV-cardiotoxicity by 5 months (Table 5.9). Furthermore, epirubicin cumulative dose by 2 months normalized to BSA was not associated with cardiotoxicity by 5 months (OR 0.98, 95%CI 0.94–01.02, p=0.319).

Table 5.9. Univariable logistic regression analysis of association between changes at 2 months (post anthracycline) and development of cardiotoxicity by 5 months (~3 months into trastuzumab).

Change in variables at 2 months *†	**OR**	**LV-cardiotoxicity**		**p-value** ‡
		95%CI		
BSA (kg/m^2)	0.00	0.00	4.38	0.066
LVEF (%)	**0.75**	0.62	0.91	**0.004**
LVEDVi (ml)	1.04	0.96	1.14	0.35
LVESVi (ml)	**1.43**	1.14	1.80	**0.002**
LVSVi (ml)	0.91	0.80	1.04	0.173
LVmass-i (gm)	1.08	0.90	1.31	0.411
RVEF (%)	0.96	0.80	1.14	0.64
RVEDVi (ml)	0.99	0.93	1.04	0.593
RVESVi (ml)	1.00	0.89	1.14	0.948
RVSVi (ml)	0.96	0.88	1.05	0.379
Trop (ng/ml)	1.05	0.97	1.13	0.213
BNP (pg/ml)	1.03	0.99	1.07	0.142
Eal (mmHg/ml)	1.40	0.36	5.53	0.627
NYHA	2.48	0.65	9.45	0.184

*Change between baseline and post completion of anthracycline (0 and 3 months).
†i = indexed for BSA
‡Binary univariable logistic regression

For univariable analysis using the LVEF sensitivity definition, similarly, change in LVESV by two months was significantly associated with a decrease in LVEF of >5% by 5 months, but change in LVEDV was not associated with a decrease in LVEF of >5% (Table 5.10).

Table 5.10. Univariable logistic regression analysis of association between changes in ventricular volume and function measurements and development of cardiotoxicity

Changes in Left Ventricular Parameters	LVEF decrement >5%		
	OR	95%CI	p-value
ΔEDV (mL)	1.00	0.97 – 1.02	0.75
ΔESV (mL)	1.06	1.01 – 1.14	**0.04**

EDV – End-Diastolic volume;
ESV- End-Systolic Volume;
LVEF – Left Ventricular Ejection fraction

5.7 Intra-observer Variability in Patients

Intra-observer variability of the primary reader was tested in 20 randomly selected patients with a 4-week interval between the two reads. Bland-Altman plots show small intra-observer variability in LV parameter (LVEF, LVEDV, LVESV, and LV mass), figure 5.10.

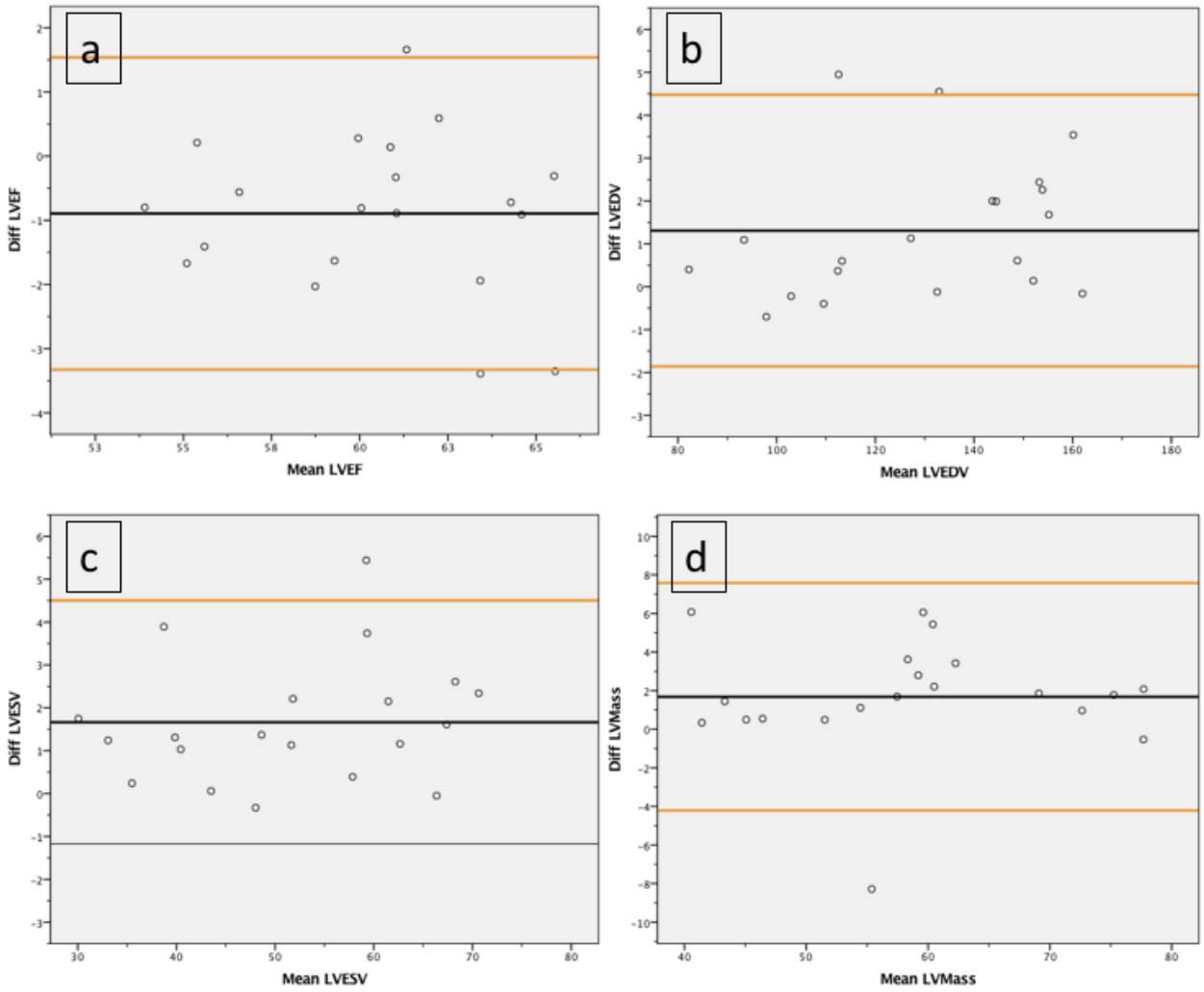


Figure 5.7. Intra-observer variability in 20 patients using Bland Altman Plots. (a) LVEF (%), (b) LVEDV (ml), (c) LVESV (ml), and (d) LVmass (g).

Chapter 6

6 DISCUSSION

6.1 Healthy volunteers' variability

6.1.1 *Normal values*

Our randomly selected sample of volunteers included a balanced mix of males and females (60% females) and a wide age range (23 – 80 years). Volunteer's clinical characteristics were normal at baseline and remained stable over the 6-month follow up period. Similarly, mean values for all LV and RV volumetric parameters and for blood markers were all within normal range at baseline and remained so over the follow up time points.

6.1.2 *Temporal Variability*

In order to identify cardiac toxicity using a threshold change in left ventricular ejection fraction during cancer therapy, a robust imaging method with excellent accuracy and precision for LVEF measurements is needed. Traditionally oncologists have used echocardiography or MUGA scans for this purpose. Although readily available clinically, both techniques have intrinsic limitations as previously discussed. Although these methods will perhaps remain the method of choice to monitor for cardiotoxicity, CMR is likely the ideal method to understand ventricular remodeling and early changes to myocardial function. Our work in healthy volunteers was performed to establish the expected temporal, inter-observer, and intra-observer variability for ventricular volume and ejection fraction measurements when repeated at intervals similar to that performed in patients undergoing cancer therapy. This allowed us to establish threshold values above which a change could be considered to have occurred beyond physiological, imaging, and analysis related variability.

The majority of analysis for temporal variability use measures of co-variance or correlation to quantify stability. Although these methods are well established, for practical purposes it would be important to know the variability measured in the same units as the primary measure. Therefore, we used a recently described method to quantify temporal and observer variability for cardiac imaging studies (189). Using this method, we demonstrate that the temporal variability of LVEF and RVEF measurements over a 6-month period is < 3.0% (absolute); and using CoV method, temporal variability for LVEF was 3.8% and for RVEF was 3.9%. In addition, the temporal variability for left ventricular volumes using SEM was <9ml for LV-EDV and <5.5ml for LV-ESV; and using CoV, for LVEDV was 4.3% and for LVESV was 7.1%. Similarly, for right ventricular volumes the temporal variability using SEM was <9ml for RV-EDV and <5.5ml for RV-ESV; and using CoV, for RVEDV was 4.9% and for RVESV was 6.25. This low absolute temporal variability in LVED and LESV is important as it confirms that the changes in left ventricular end-diastolic volumes of 10ml and end-systolic volumes of 5 ml used to define significant changes in our cardiotoxicity population are likely to represent changes beyond expected variability. Furthermore, the temporal variability for available biomarkers over 6-month period using SEM method was 0.4ng/ml for hs-TnI and 9.8pg/ml for BNP; and using CoV method, temporal variability for hs-TnI was 5.7% and for BNP was 24.3%. longitudinal variability in routinely used biomarkers (BNP and hs-TnI) were twice higher for hs-TnI and 6-times higher for BNP than that seen with LVEF and RVEF, demonstrating the robustness of LVEF and RVEF for following myocardium temporal changes in the various disease.

6.1.3 *Observer Variability*

The data in the healthy volunteers also demonstrates the excellent inter- and intra-observer variability for the left and right ventricular volumes and ejection fraction again confirming the strength of cardiac MRI as a robust technique for quantification of cardiac volumes and function. These variabilities are significantly smaller than what has been reported, using the same statistical methodology, using 2D and 3D echocardiography recently (150). The larger inter-observer test re-test variability than the temporal variability (measured by a single reader) suggests that in order to identify small changes in ventricular volumes and function it is more ideal for the same user to analyze all data over time for the same patient. The use of COV data presented allows us to compare between various measures. For temporal and observer variability

we demonstrated that measurements of end-systolic volume are more variable than end-diastolic volume. This emphasizes the importance of careful attention to end-systolic endocardial contours during cardiac MRI analysis.

6.2 LV – Cardiotoxicity

In our cohort of consecutively-recruited HER2-positive breast cancer patients receiving uniform cancer treatment, LV-cardiotoxicity by five months was seen in 12.1% of the patients. The reduction in LVEF was associated with a significant increase in LVESV in 100% of the patients. None of the patients had an isolated reduction in LVEDV, as would be expected with reduced preload. These findings suggest that the primary mechanism of reduction in LVEF from cancer therapy is due to a reduction in myocardial contractility, rather than an isolated reduction in preload. In contrast, when a lower threshold was used to define cardiotoxicity (change in LVEF >5%), a minority of patients attained these changes solely due to a reduction in preload (13.2%); however, these small changes would not trigger interventions for cardiotoxicity. Age, BNP, as well as LVEF at baseline were predictors of LV-cardiotoxicity by 5 months.

6.3 Right ventricular – Cardiotoxicity

Right ventricular -cardiotoxicity by five months was seen in 2.4% of the patients. The reduction in RVEF was associated with a significant increase in RVESV in both patients (100%), suggesting that the primary mechanism of reduction from cancer therapy in RVEF is also due to a reduction in myocardial contractility. One of these two patients, however, also had significant reduction in RVEDV, which could be attributed to concomitant reduction in preload. Nevertheless, reduction in preload was not an isolated phenomenon in RV dysfunction.

Unlike in patients with LV dysfunction, where mean LVEF significantly dropped at both 2 and 5 months, significant RVEF reduction only occurred at 5 months. RV dysfunction is less common than LV dysfunction during the first 5 months of sequential anthracycline and trastuzumab therapy in HER2+ breast cancer. Changes in LVEF and RVEF appear to mirror each other when

examined as a continuous variable. This suggests that toxicity from anthracyclines and trastuzumab is not limited to the left ventricle as commonly described. However, a significant drop in RVEF as defined in this study occurs later than that of the left ventricle and seems to occur mostly during trastuzumab therapy. This may reflect the fact that our definition of RV dysfunction was too conservative or that significant injury first occurs to the LV prior to the RV. This needs to be studied further in larger studies with longer term follow-up. Our work however does suggest that when patients are followed sequentially during cancer therapy it is important to concomitantly assess changes to the LV and the RV.

6.4 Relationship Between Ventricular Volumes and Cardiotoxicity

Using a well-established criterion for cardiotoxicity, 10 out of 10 (100%) patients in our study developed cardiotoxicity associated with an increase in LVESV. None had an isolated reduction in LVEDV. Our findings were further confirmed by the univariable logistic regression analysis, where a change in LVESV by 2 months but not LVEDV as a continuous parameter showed significant association with development of cardiotoxicity. Our work therefore suggests that currently used definitions for cardiotoxicity based on LVEF appear to identify patients who have a reduction in contractility. Small reductions in LVEF (>5% and <10%) can occur in a subset (13.2%) of patients due to isolated reduction in preload, however larger LVEF reductions (>10%) appear invariably associated with reduced contractility. Our work also suggests that an increase in LVESV may be an early measure of myocardial injury and identify patients who are at risk of subsequent cardiotoxicity. Given the small number of cardiotoxicity events in our cohort, multivariable analysis was not feasible. However, as the current study continues to finish recruitment and complete follow-up, we believe that there will be many more cardiotoxicity events to perform a multivariable adjustment.

High sensitivity troponin-I and BNP did not show an association with LV-cardiotoxicity using univariate logistic regression models, questioning again the utility of these markers in predicting cardiotoxicity.

6.5 LV ejection fraction, contractility, and global longitudinal strain

Using echocardiography, multiple studies have demonstrated that changes in deformation (strain) precedes changes in ejection fraction in cancer patients during cancer therapy. Myocardial strain imaging is likely the best surrogate imaging marker of contractility available. Amongst strain methods GLS is the measure with the smallest variability and appears to have the best sensitivity in predicting subsequent cardiotoxicity (defined by threshold change in LVEF) by echocardiography (149). This observation is most likely because 2D LVEF measurements are not sensitive enough to detect small changes in LVEF, due to its intrinsic measurement variability as discussed previously (14). Recently, using cardiac MRI, Ong et al have shown that changes in GLS and LVEF measured by cardiac MRI mirror each other during cancer therapy (190). This finding has also been shown previously by other groups using MRI strain (182). Therefore, similar to our findings with LVEF, these recently described association between reduction in LVEF and GLS in patients receiving cancer therapy also supports the notion of a reduction in myocardial contractility as the primary mechanism of reduction in LVEF.

6.6 Comparison to Prior Work

Recent studies from Greg Hundley's group has raised concern that reduction in LVEF may occur secondary to reduction in preload in the context of cancer therapy (176,177). This was based on the demonstration that a fall in LVEF measured by CMR was driven by a reduction in LVEDV with preserved LVESV in 16-19% of patients. Furthermore, a reduction in CMR measured circumferential and global longitudinal strain were associated with a reduction in LVEDV. These data raised the possibility that a subset of patients diagnosed with cardiotoxicity have normal myocardial contractility and hence should not be subject to the usual management of cancer therapy adjustment and/or addition of cardiac medications. These findings have important clinical consequences, as one would need a robust method to accurately measure concomitant changes in LVEF and LVEDV during cancer therapy. Most commonly available clinically, 2D and 3D echocardiography techniques have test-re-test variability for LVEDV measurement

between 21-38ml making it challenging to reproducibly identify a 10ml reduction in LVEDV. The use of CMR in routine clinical practice would be challenging due to availability and cost.

Our finding that cardiotoxicity was primarily due to a reduction in LVESV (i.e. contractility) differed from recent work described above. This may be due to the following main reasons: 1) There were differences in the types of cancers included (100% breast cancer in our study versus <50% in prior studies) which are associated with different treatment regimens; 2) Our study included ~2 and ~5-month follow-up while the prior study included only ~3-month follow-up; 3) the definition of cardiotoxicity in the prior studies (>10% decrement in LVEF or any absolute LVEF value <50% between baseline to 3-month) differs from our definition. The definition of LVEF reduction to <50% used by Hundley's group may include patients with a small change in LVEF (e.g. from 54% to 49%); our data also shows that such small changes in LVEF can in fact occur due to isolated reduction in pre-load. However, based on current guidelines such small changes in LVEF would not constitute cardiotoxicity or require changes to cancer treatment.

6.7 Novelty

The EMBRACE-MRI study is the largest and first contemporary multicenter study using comprehensive quantitative CMR volumetric quantification of both Left and Right ventricles and biomarkers in patients and healthy volunteers for early detection of cancer-therapy-related cardiotoxicity (18). This advances the field from reliance on echo-based LV function methods to more sensitive methods capable of identifying pre-clinical changes of cardiotoxicity (i.e., LVESV and LVEDV) directly. Our study also provides an appreciation of the temporal variability of CMR volumetric parameters and blood markers in healthy people reflecting true physiologic changes that physicians need to account for in every patient during follow up to determine disease progression. Our study identifies for the first time that changes in LVESV are an early predictor (by 2 months) of future development (by 5 month) of LV-cardiotoxicity.

6.8 Strengths and Limitations

The strengths of our study include uniform patient population with uniform treatment, consecutive recruitment, prospective follow-up, use of contemporary definitions of cardiotoxicity, use of contemporary CVD-related biomarkers, and validating changes in patients with matched healthy volunteers. Limitations include a relatively small number of outcomes. However, our sample size and event rate are consistent with contemporary studies in cardio-oncology. Furthermore, each patient had repeated cardiac imaging at three separate time points with the acquisition of CMR within 2 hours of blood collection. We used CMR for volumetric measurements as it the modality that was most accurate and reproducible for volumetric analysis. Furthermore, we showed for the first time that reproducibility for RV parameters are quite similar to that of the LV and provided a detailed illustration of CMR postprocessing protocol used in our lab. Our protocol demonstrated in this thesis might be limited by using 2 dimensional images for illustration, however we emphasise the importance of utilizing the dynamic window when contouring difficult slices. We used hs-TnI and BNP as the biomarkers of interest as they are the most widely used and correlated with cardiovascular disease. We, therefore, believe our findings are clinically relevant. However, since our cohort was limited to a specific group of HER2+ breast cancer patients, the results may not be extrapolated to non-breast cancer populations treated with other regimens. We made the assumption that a reduction in LVEDV represents reduced preload, however other causes (e.g. diastolic dysfunction secondary to cardiotoxicity) have not been excluded. Unfortunately, a reference standard for assessment of preload does not exist. Similarly, we assumed that an increase in LVESV reflects reduction in contractility. However, there are no non-invasive ways to measure contractility in a reliable manner. Furthermore, caution is warranted when discussing myocardial contractility as end-systolic elastance or end-systolic wall stress were not measured. A small subset of our patients had LVEF<55% at baseline, which may have impacted cardiotoxicity incidence. However, only one of these patients developed LV-cardiotoxicity. Inclusion of these patients was based on the fact that baseline screening was performed by echocardiography as per clinical practice.

6.9 Future Directions

This EMBRACE-MRI study will be continued beyond the first three patient visits (5-month follow-up) to include total of 5 time points to examine the process of cardiac remodeling throughout the 15 months of cancer therapy. Sample size will also increase to a projected 136 patients (each with 5 time points) to increase power of analysis further. Examining CMR-volumetric changes longitudinally over the entire cancer therapy duration will serve two primary purposes: First, to test if there is an association between cardiotoxicity through the entire treatment period and early changes in LV volumes (ESV, EDV) or mass. If found, this association could be used to test various strategies to prevent LV remodeling, dysfunction, or HF in patients who develop these changes early on, such as 1) addition of dexrazoxane, beta-blockers, ACE inhibitors, 2) instituting a formal exercise program, 3) more aggressive treatment of existing cardiac risk factors, 4) increasing the frequency of cardiac imaging / follow-up, and 5) extending the time lag between anthracyclines and trastuzumab. Second, to examine other novel parameters including quantitative CMR tissue characterization (T1, T2 and ECV maps) and CMR strain for early detection of chemotherapy-related cardiotoxicity. If our EMBRACE-MRI study's primary purpose is achieved then, CMR can be used to identify high-risk patients who can then continue on with their current imaging surveillance as per clinical practice (6 studies), while those at low risk may be able to have less frequent follow-up. Since ~75% of the patients do not experience cardiotoxicity, by reducing the number of repeated MUGAs or echos in this low-risk group, there may be overall cost savings. The complete EMBRACE-MRI study is going to be the first to include clinical assessment, echo, CMR, biomarkers in a single study to determine ability to predict cardiotoxicity. Therefore, this study will describe which one of these biomarkers alone or in combination would be the best to predict cardiotoxicity. Since cardiotoxicity is determined prospectively using a gold standard technique (CMR), the associations will be more robust. This would have tremendous clinical impact by allowing optimal risk stratification of patients.

6.10 **Conclusion**

In a uniform cohort of women with HER2+ breast cancer receiving anthracyclines followed by trastuzumab this work demonstrates that left and right ventricular remodeling occurs during treatment. Left ventricular cardiotoxicity is more common than right ventricular cardiotoxicity and appears to occur earlier during treatment. The primary mechanism for cardiotoxicity is an increase in left ventricular end-systolic volume as opposed to a reduction in left ventricular end-diastolic volume. Although requires confirmation, our findings suggest that the primary mechanism of cardiotoxicity is likely a reduction in contractility and not a reduction in pre-load. Although there has been a growing interest in using serum biomarkers to identify early myocardial injury, these were not found to be valuable in our patient population.

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Copyright Acknowledgements

Figure 2.1.

Canadian Cancer Statistics Advisory Committee. Canadian Cancer Statistics 2017. Toronto, ON: Canadian Cancer Society; 2017. Available at: cancer.ca/Canadian-Cancer-Statistics-2017-EN (accessed Sep 2018)

Table 2.1.

Modified from open access: no copyright required. Publication: Journal of the National Cancer Institute. Title: Cardiotoxicity of Anticancer Drugs: The Need for Cardio-Oncology and Cardio-Oncological Prevention (2010)

Table 2.2.

Order Number: 4437171252881. Publication: Journal of Pathology. Title: Refinement of breast cancer classification by molecular characterization of histological special types (2008)

Figure 2.2

Order Number: 4437661505753. Publication: Canadian Journal of Cardiology. Title: Shared Risk Factors for Cardiovascular Disease and Cancer: Implications for Preventive Health and Clinical Care in Oncology Patients

Figure 2.3

Modified from the original – no copyright required. Publication: Expert Review of Cardiovascular Therapy. Title: Cardio-oncology Part I: chemotherapy and cardiovascular toxicity

Appendix

List of peer-reviewed abstracts from this work:

1- 2018, Oct. **Mustafa A. Altaha**. Left and Right Ventricular Remodeling in Patients with Early Stage Breast Cancer Receiving Sequential Anthracycline and Trastuzumab Therapy - A Cardiac MRI Study. Presented e-poster at the Canadian Cardiovascular Congress (**CCC**) in Toronto, ON

Left and Right Ventricular Remodeling in Patients with Early Stage Breast Cancer Receiving Sequential Anthracycline and Trastuzumab Therapy - A Cardiac MRI Study

Mustafa A Altaha, MBBS; Mark Nolan, MD; Kim Connelly, MD, PhD; Eitan Amir, MD, PhD; Christine Brezden-Masley MD, PhD; Bernd J Wintersperger, MD; Paaladinesh Thavendiranathan, MD, MSc, SM, on Behalf of the EMBRACE-MRI Investigators

Introduction:
Cardiac dysfunction induced by anthracycline and trastuzumab sequential therapy is an adverse prognostic marker in women with early-stage breast cancer. There is currently limited description of the patterns of ventricular remodeling during treatment in these patients. Also, much of the focus in cardiotoxicity has been on left ventricular dysfunction. Understanding changes to cardiac function beyond the left ventricle will provide a broader appreciation of cardiac impact of cancer therapy and can have implications for screening of cardiotoxicity.

Methods/Results:
Eighty-three consecutive women (age: 51 ± 9.5 years) with early stage HER2+ breast cancer, receiving sequential anthracycline and trastuzumab therapy, were prospectively recruited. The mean dose of epirubicin used was 301.8 mg/m^2 ± 12.3. All patients had cardiac MRI (CMR) pre-anthracycline, within three weeks post-anthracycline (but before trastuzumab, ~2 months), and at five months (~three months into trastuzumab therapy) on a 1.5T Siemens Avanto Fit magnet. Short axis SSFP cines with 8mm slice thickness (2mm gap) were acquired for ventricular function analysis. Left and right ventricular (LV/RV) volumes and ejection fraction (EF), and LV mass were measured by an observer blinded to patient identification and imaging time point. LV cardiotoxicity was defined as a >10% drop in LVEF to <55%, while RV cardiotoxicity was defined as a >10% drop in RVEF to <51%. A paired t-test was used for comparison. Ten patients (12%) developed LV dysfunction, 1 (10%) at 2 months and 9 (90%) at 5 months. Two patients (2.4%) developed RV dysfunction, both of which occurred at 6 months. One patient had concomitant LV and RV dysfunction. In patients with LV dysfunction, mean LVEF significantly dropped at both 2 and 5 months; whereas significant RVEF reduction only occurred at 6 months figure-1, table-1. Changes in RVEF mirrored that of LVEF in the patients who did not develop LV cardiotoxicity figure-2, table-1. Mean (± SD) LVEF and RVEF at baseline, ~2-month, and ~5-month for all patients, patients with LV dysfunction, and patients without LV dysfunction are summarized in table-1.

Conclusions:

RV dysfunction is less common than LV dysfunction during the first 6 months of sequential anthracycline and trastuzumab therapy in HER2+ breast cancer. Changes in LVEF and RVEF appear to mirror each other when examined as a continuous variable. However, a significant drop in RVEF appear to occur later than that of the left ventricle and seems to occur only during trastuzumab therapy.

Figure-1: Comparison between mean changes in LVEF and RVEF in patients with LV dysfunction at pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

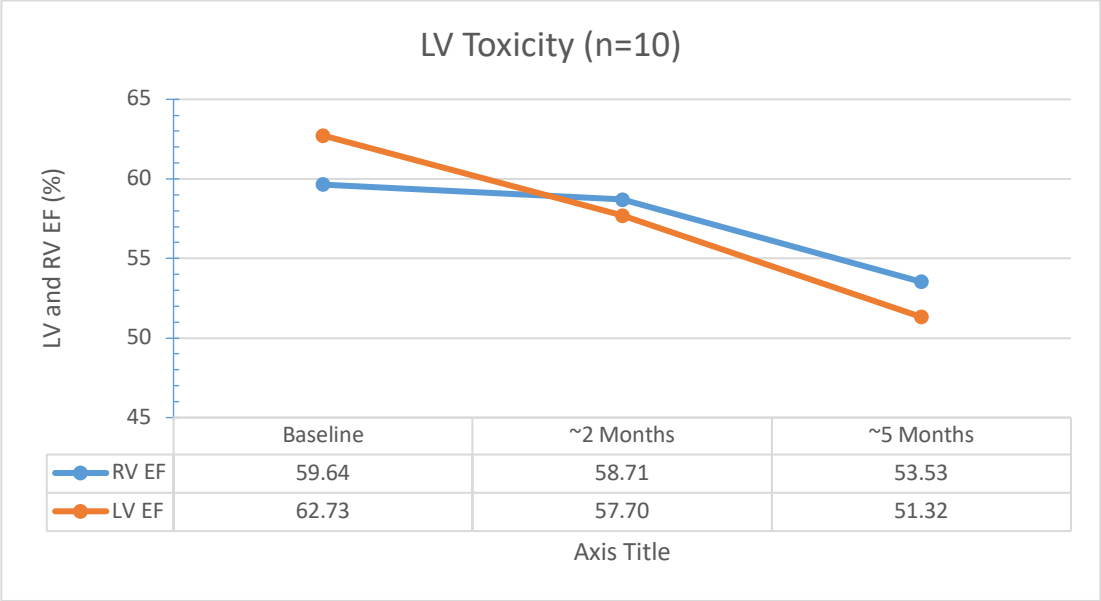


Figure-2: Comparison between mean changes in LVEF and RVEF in patients with no LV dysfunction at pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

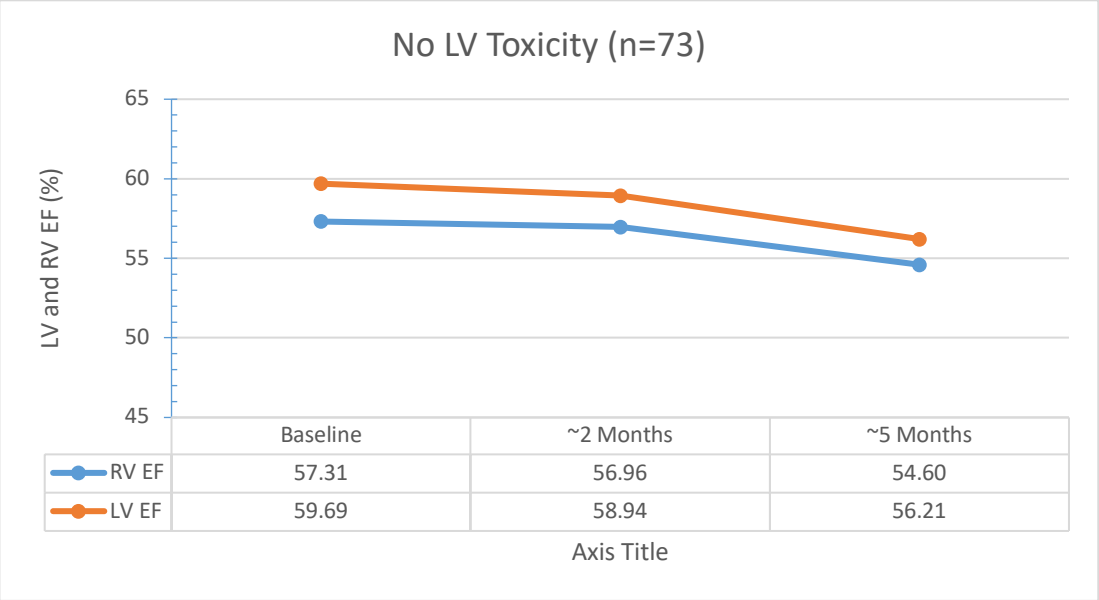


Table-1: Summary of changes in left and right ventricular ejection fraction between pre-anthracycline, within 3 weeks post anthracycline, and 3 months during trastuzumab therapy

Characteristics	Pre-Cancer therapy	~2 Months*	P-value (0-3)	~5 Months†	P-value (0-6)
Left Ventricular Ejection fraction, (%)					
All patients (n=83)	60.06 ± 4.4	58.79 ± 4.3	.003	55.62 ± 4.1	<.001
In pts with LV dysfunction (n=10)	62.73 ± 3.7	57.70 ± 4.1	.001	51.32 3.5	<.001
In pts with No LV dysfunction (n=73)	59.69 ± 4.3	58.94 ± 4.3	.076	56.21 ± 3.9	<.001
Right Ventricular Ejection fraction, (%)					
All patients (n=83)	57.59 ± 4.5	57.17 ± 4.4	.299	54.47 ± 4.0	<.001
In pts with LV dysfunction (n=10)	59.64 ± 4.6	58.71 ± 5.1	.339	53.53 ± 3.4	.002
In pts with No LV dysfunction (n=73)	57.31 ± 4.5	56.96 ± 4.3	.429	54.60 ± 4.0	<.001

Data presented as frequency mean ± standard deviation,
*Within 1 month of completion of anthracycline,
†Within 3 months of initiation of trastuzumab
‡paired t-test.

2- 2018, Mar. **Mustafa A. Altaha**. Ventricular Remodeling in Early Stage Breast Cancer Patients Receiving Sequential Anthracycline and Trastuzumab Therapy – A Cardiac MRI Study. Abstract is submitted to the American College of Cardiology (**ACC**) 67th Annual Scientific Sessions in Orlando, FL, USA

Ventricular Remodeling in Early Stage Breast Cancer Patients Receiving Sequential Anthracycline and Trastuzumab Therapy – A Cardiac MRI Study

Mustafa A. Altaha, MBBS; Eitan Amir, MD, PhD; Christine Brezden-Masley MD, PhD; Kim Connelly, MD, PhD; Maria Michalowska, BSc; Bernd J Wintersperger, MD; Paaladinesh Thavendiranathan, MD, MSc, SM on behalf of the EMBRACE-MRI Investigators

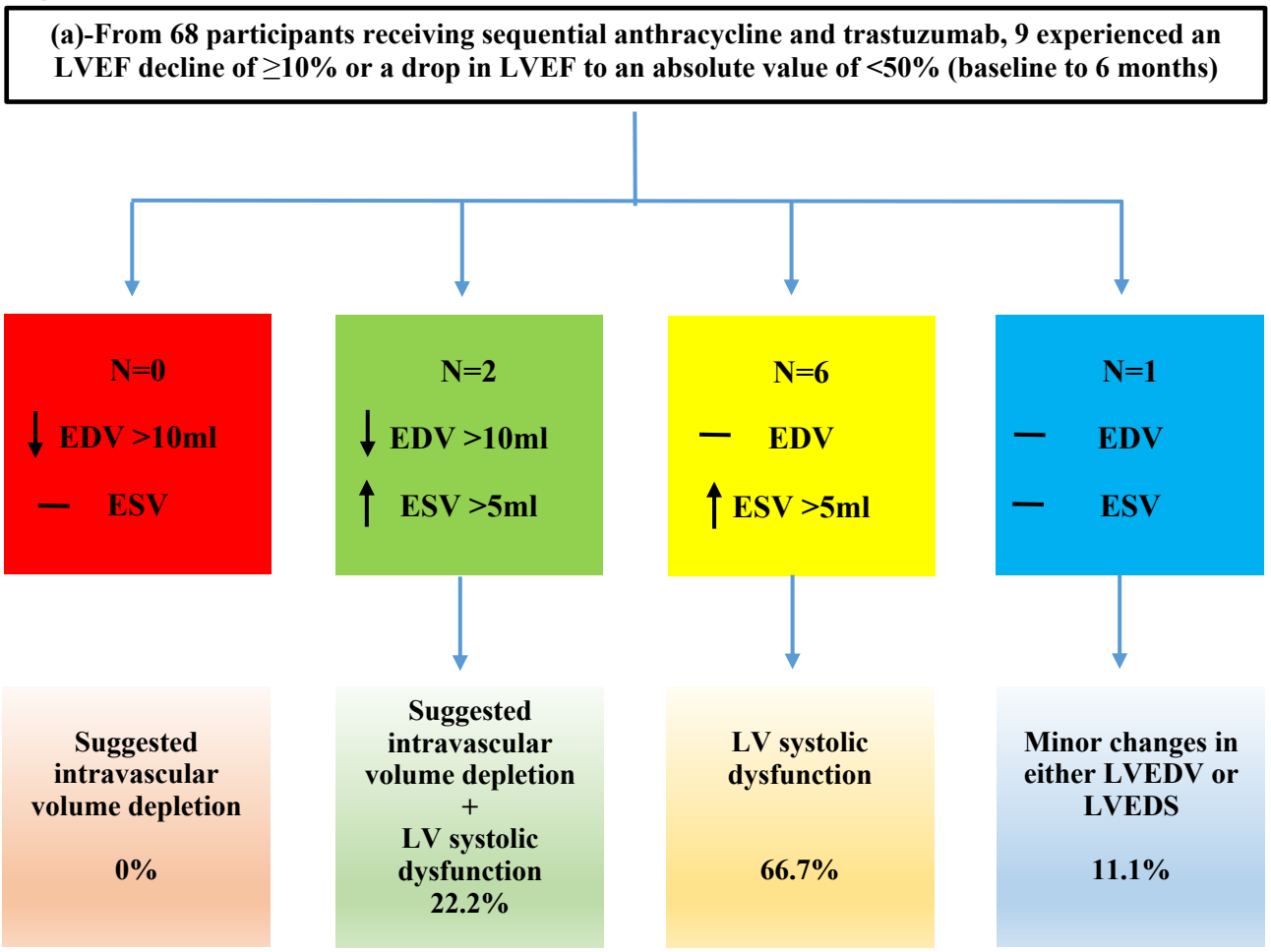
Background
Recent work raised concern that intravascular volume depletion (i.e. reduced pre-load) may be responsible for the observed fall in left ventricular (LV) ejection fraction (EF) in some patients receiving cancer therapy. We examined potential mechanisms for fall in LVEF in women with breast cancer receiving chemotherapy.

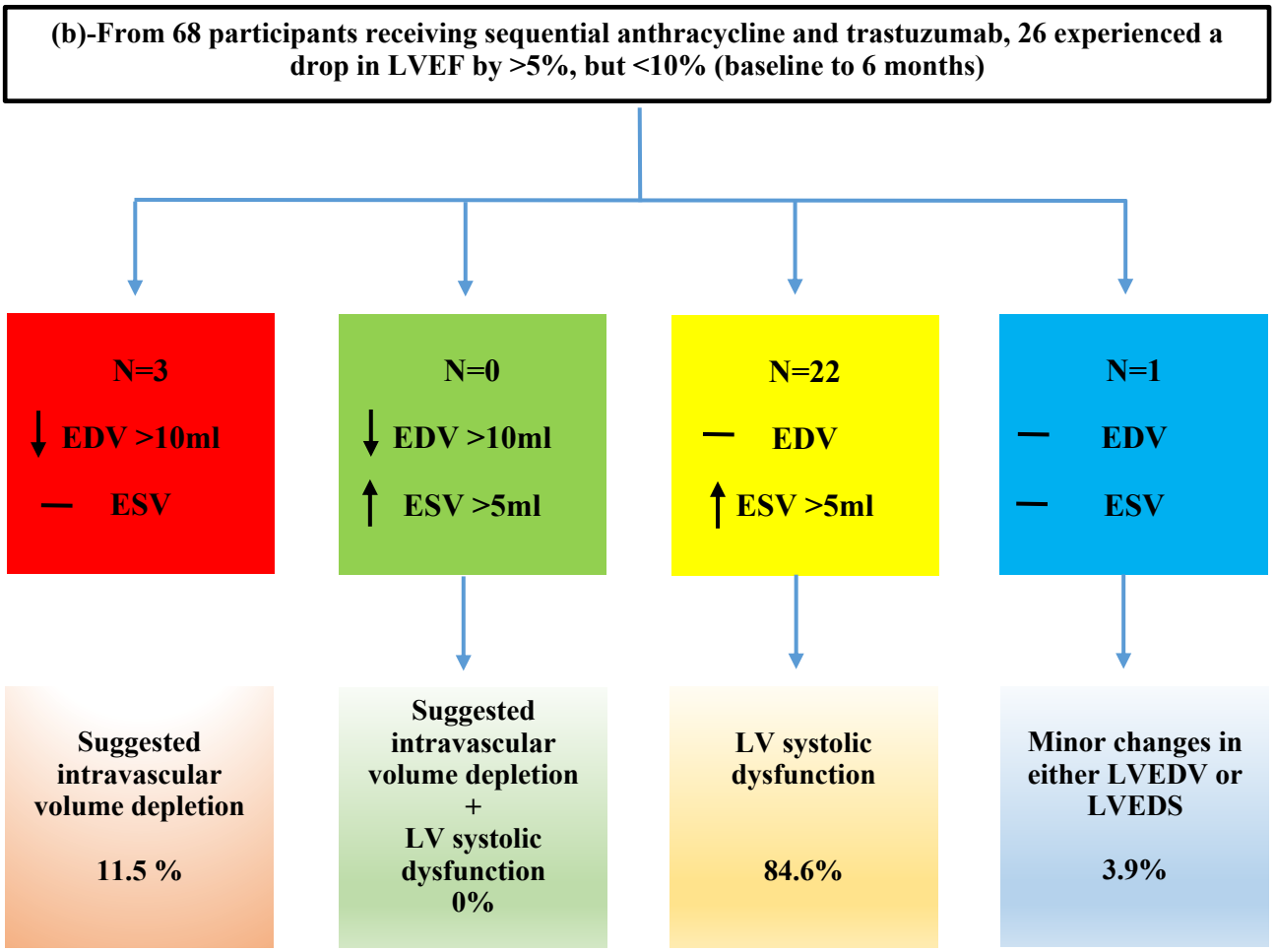
Methods
Sixty-eight consecutive women (50.9 ± 9.4 years) with early stage HER2+ breast cancer, receiving cancer therapy, were recruited prospectively. All had cardiac MRI pre-treatment, post-anthracycline, and at 5 months (3 months after trastuzumab initiation). LV volumes and EF were measured blinded to all patient's data. Cardiotoxicity was defined as a fall in LVEF by ≥10% or a drop to <50%. A change in LV end diastolic volume (LVEDV) >10ml or LV end-systolic volume (LVESV) >5ml was considered significant. A sensitivity analysis was performed for any drop in LVEF by ≥5%.

Results
Nine patients (13.3%) developed cardiotoxicity. The LVEF fall was primarily due to a rise in LVESV (Fig 1a) and not to isolated drop in LVEDV. Using the more sensitive definition, 26 patients had LVEF fall of ≥5% (but <10%). This fall occurred due to isolated drop in LVEDV in 3 of 26 patients, but was mainly due to a rise in LVESV (n=22, p<0.001) (Fig 1b).

Conclusion
The dominant mechanism of cardiotoxicity in breast cancer therapy relates to a rise in LVESV most likely reflecting altered myocardial contractility. Volume depletion may play a minor role in small falls in LVEF not meeting cardiotoxicity criteria.

Figure-1


(a)-From 68 participants receiving sequential anthracycline and trastuzumab, 9 experienced an LVEF decline of ≥10% or a drop in LVEF to an absolute value of <50% (baseline to 6 months)
N=0
↓ EDV >10ml
— ESV
N=2
↓ EDV >10ml
↑ ESV >5ml
N=6
— EDV
↑ ESV >5ml
N=1
— EDV
— ESV
Suggested intravascular volume depletion
0%
Suggested intravascular volume depletion + LV systolic dysfunction
22.2%
LV systolic dysfunction
66.7%
Minor changes in either LVEDV or LVEDS
11.1%


(b)-From 68 participants receiving sequential anthracycline and trastuzumab, 26 experienced a drop in LVEF by >5%, but <10% (baseline to 6 months)
N=3
↓ EDV >10ml
— ESV
N=0
↓ EDV >10ml
↑ ESV >5ml
N=22
— EDV
↑ ESV >5ml
N=1
— EDV
— ESV
Suggested intravascular volume depletion
11.5 %
Suggested intravascular volume depletion + LV systolic dysfunction
0%
LV systolic dysfunction
84.6%
Minor changes in either LVEDV or LVEDS
3.9%

3- 2017, Feb. **Mustafa A. Altaha**. Ventricular Remodeling and Right Ventricular Involvement in Patients with Early Stage Breast Cancer Receiving Anthracycline Chemotherapy. Abstract was accepted for walking poster (**oral**) presentation at the 20th annual Society of Cardiovascular Magnetic Resonance (**SCMR**) scientific sessions in Washington, DC, USA

Ventricular Remodeling and Right Ventricular Involvement in Patients with Early Stage Breast Cancer Receiving Anthracycline Chemotherapy

Mustafa A Altaha, MBBS; Kim Connelly, MD, PhD; Eitan Amir, MD, PhD; Christine Brezden-Masley MD, PhD; Bernd J Wintersperger, MD; Paaladinesh Thavendiranathan, MD, MSc, SM, on Behalf of the EMBRACE-MRI Investigators

Introduction:
Chemotherapy induced cardiac dysfunction is an adverse prognostic marker in women with early stage breast cancer. Patterns of ventricular remodeling and frequency of bi-ventricular involvement during cancer therapy in these patients have not been described. Understanding ventricular remodeling will provide novel knowledge as to the mechanism by which patients eventually develop heart failure (HF). This can help design targeted methods to prevent HF. Describing changes to cardiac function beyond the left ventricle will provide a broader appreciation of cardiac impact of cancer therapy and can have implications for screening of cardiotoxicity.

Methods:
Thirty-eight consecutive women (age: 49.6 ± 9.1 years) with early stage HER2+ breast cancer, receiving cancer therapy (anthracyclines followed by trastuzumab) were prospectively recruited. All patients had cardiac MR studies performed pre and post-anthracycline treatment on a 1.5T Siemens Avanto Fit magnet. Short axis SSFP cines with 8mm slice thickness (2mm gap) were acquired for ventricular function analysis. Left and right ventricular (LV/RV) volumes and ejection fraction (EF), and LV mass were measured by an observer blinded to patient identification and imaging time point. A significant change in EF was prospectively defined as a >5% drop after anthracycline therapy based on known reproducibility of cardiac MR measured EF. A paired t-test was used for comparison.

Results:
All patients received epirubicin (515.2 mg/m^2 +/- 49.9). Ventricular function and volumetric parameters pre and post epirubicin are summarized in the Table below. Eight (21.0%) patients had a fall in LVEF by >5%, and the primary cause was an increase in end-systolic volume (ESV) as opposed to a change in end-diastolic volume (EDV); Similarly, ten (26.3%) patients had a fall in RVEF by >5% primarily due to an increase in ESV. LVEF and RVEF reduction by >5% occurred concomitantly in four (10.5%) patients. Hypertension was more common in the 14 patients who had a significant drop in LVEF and/or RVEF compared to those who did not (36% versus 17%).

Conclusions:
In this study anthracycline exposure resulted in concomitant reduction in LV and RVEF by >5.0% in 10.5% of patients with breast cancer. The main mechanism of reduction in ventricular function was an increase in ESV, which is likely due to a reduction in contractility, as opposed to

a fall in EDV which may occur from intravascular volume depletion. The incidence of significant RVEF reduction was similar to LVEF reduction highlighting the importance of following RV function during anthracycline treatment.

Table: Summary of changes in ventricular volume, ejection fraction, and mass between pre-anthracycline and within 3 weeks post anthracycline.

Parameter	Pre-Treatment	Post Anthracycline	P value ‡	Mean change in all 38 patients, %	Mean change in patients without >5% drop in LVEF and/or RVEF (n=24), %	Mean change in patients (with >5% drop in LVEF (n=8), %	Mean change in patients with >5% drop in RVEF (n=10), %	Mean change in patients with >5% concomitant drop in LVEF and RVEF (n=4), %
$LVEDV_i$ (ml/m^2)	75.7 (9.5)	77.4 (12.9)	0.23	2.2	1.2	2.6	4.9	3.4%
$LVESV_i$ (ml/m^2)	29.3 (5.3)	30.2 (6.2)	0.18	3.5	-1.8	16.4	12.1	19.2%†
LVEF (%)	61.5 (3.6)	60.1 (4.4)	0.02	-1.4	0.1	-6.1	-2.9	-6.2%*
$LVmass_i$ (g/m^2)	42.7 (6.1)	40.3 (5.7)	<0.001	-5.4	-5.8	-4	-3.3	0.6%
$RVEDV_i$ (ml/m^2)	79.1 (12.5)	79.5 (13.4)	0.76	1	2.7	-1.5	-2.4	-2.3%
$RVESV_i$ (ml/m^2)	35.3 (6.9)	36.4 (7.3)	0.12	3.8	0.1	7.8	13.7	14.1%†
RVEF (%)	55.4 (4.2)	54.2 (4.4)	0.09	-1.2	1.1	-4.1	-6.9	-7.2%*

$LVEDV_i$, left ventricular end-diastolic volume index; $LVESV_i$, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; $LVmass_i$, left ventricular mass index; $RVEDV_i$, right ventricular end-diastolic volume index; $RVESV_i$, right ventricular end-systolic volume index; RVEF, right ventricular ejection fraction; data presented as mean and standard deviation.
*Significant drop in LVEF or RVEF was defined as an absolute reduction of > 5%;
†Significant change in indexed end-diastolic and end-systolic volume was defined as a relative increase >5%; ‡paired t-test.