EVALUATION OF CARDIOTOXICITY USING BLOOD BIOMARKERS

EVALUATION OF CARDIOTOXICITY USING BLOOD BIOMARKERS IN BREAST CANCER AND LYMPHOMA PATIENTS UNDERGOING CURATIVE TREATMENT

By: KATHARINE MACKETT, B.Sc. Hon.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements

for the Degree Master of Science

Descriptive Note

McMaster University MASTER OF SCIENCE (2019) Hamilton, Ontario, (Medical Sciences)

TITLE: Evaluation of Cardiotoxicity Using Blood Biomarkers in Breast Cancer and Lymphoma Patients Undergoing Curative Treatment

AUTHOR: Katharine Mackett, B.Sc. Hon. (McMaster University)

SUPERVISOR: Dr. Peter A. Kavsak, PhD, FCACB, FAACC, FCCS

NUMBER OF PAGES: xvi, 97

Lay Abstract

Objective:

To evaluate whether changes in proteins (i.e., biomarkers) in the blood of cancer patients undergoing treatment can predict who is at risk for subsequent heart damage by imaging scans.

Materials and Methods:

Blood samples were obtained from breast cancer and lymphoma patients (study participants) on treatment for their cancers. These blood samples were tested with biomarkers that provide information on inflammation, injury, and poor function in the heart. The number of abnormal tests was determined in all patients with a comparison made between those patients with versus those without heart damage.

Results:

The blood test that exhibited the most abnormal results was high-sensitivity cardiac troponin with >50% of patients having evidence of biochemical injury. However, <20% of the patients had heart damage by imaging. There was no difference in biomarker levels between groups.

Conclusion:

Different biomarker interpretations and/or combinations are needed to properly identify cancer patients at risk for heart damage.

Abstract

Objective:

To evaluate whether abnormal concentrations in cardiac and inflammatory biomarkers could predict reductions in left ventricular ejection fraction (LVEF) for cancer patients undergoing curative treatment.

Materials and Methods:

Longitudinal testing was performed for high-sensitivity cardiac troponin I (hs-cTnI), N-terminal pro-B-type natriuretic peptide (NT-proBNP), heart-type fatty acid binding protein (H-FABP) and C-reactive protein (CRP) in HER2+ breast cancer (BC) patients receiving adjuvant trastuzumab treatment (n=22) and in lymphoma patients treated with radiotherapy (n=4). Sex-specific and overall upper limit of normal (ULN) cutoffs were used to identify abnormal results with a reduction in LVEF (<50% and decrease of ≥10% from baseline) indicative of cardiotoxicity. A secondary analysis was performed on the BC patients with normal LVEFs (n=12 with baseline prior to chemotherapy through to 6-months on trastuzumab) with 15 blood collections spaced between 6- and 254-days post-baseline LVEF measurement.

Results:

A majority of the BC patients had evidence of myocardial injury (hs-cTnI >female ULN=90%) or myocardial dysfunction (NT-proBNP >overall ULN=91%) at any timepoint with fewer patients having abnormal CRP or H-FABP concentrations (H-FABP >ULN=14%; CRP >ULN=45%). Myocardial injury and dysfunction were most evident during the first two cycles of trastuzumab treatment, with myocardial injury also evident during this early timeframe in the female lymphoma patients (3 with hs-cTnI >ULN). In the 12 patients who completed trastuzumab with normal LVEFs (median=60% at 6-months), myocardial injury (hs-cTnI >ULN)

and dysfunction (NT-proBNP >ULN) was evident in >50% of patients. Four of the 22 patients did develop cardiotoxicity, but there was no difference in biomarker concentrations between patients with or without cardiotoxicity.

Conclusion:

The use of the recommended ULN cutoffs identified myocardial injury and dysfunction in a majority of cancer patients in this setting. Biomarker assessments did not relate to cardiac functional imaging studies. Future studies are warranted to assess different cutoffs or biomarker combinations for predicting cardiotoxicity.

Acknowledgements

I would like to express my utmost gratitude to my supervisor and mentor, Dr. Peter Kavsak, for without his guidance, knowledge and patience, this would not have been possible. Thank you for providing me with unbelievable opportunities and believing in my capabilities.

Thank you to my supervisory committee, Dr. Peter Kavsak, Dr. Bindi Dhesy-Thind, and Dr. Hal Hirte for their intellect, advice, and guidance throughout my Masters. Their insight is truly appreciated.

Thank you to the following researchers who designed the MEDICATE study: Dr. Elysia Donovan, Dr. Darryl Leong, Dr. Bindi Dhesy-Thind, Dr. Sachi Voruganti, Dr. Anand Swaminath, Dr. Jonathan Sussman, Dr. Jim Wright, Dr. Hisham Dokainish, Dr. Gordon Okawara, Dr. Graham Fraser, Dr. Peter Kavsak, Dr. Gregory Pond, and Dr. Stephen Sagar.

Thank you to the following researchers who designed the CABOT study: Dr. Bindi Dhesy-Thind, Dr. Mark Levine, Dr. Peter Ellis, Dr. Richard Tozer, Dr. Som Mukherjee, Dr. Andrew Arnold, Dr. Louise Bordeleau, Dr. Pierre Major, and Dr. Peter Kavsak. These two studies provided the samples for testing of biomarkers for my thesis work.

Thank you to the Juravinski Cancer Centre and Hospital Foundation, the clinical research lab at the Juravinski Hospital and Cancer Center, Hamilton Health Sciences (HHS), McMaster University, the Clinical Research Laboratory and Biobank (CRLB), and the Jay Kuhn Fund for their support, resources and funding.

Additionally, I would like to thank Dr. Mark Inman, Joe Bottos, Dr. Josko Ivica, Brenda DeMedeiros, Carol Ehler, Dr. Elysia Donovan, and Dr. Colleen Strain for their mentorship and support. Lastly, I would like to thank my parents, Anne and Robert, and my brothers, Michael and Stephen, for their unwavering love, support, and encouragement.

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List of Abbreviations

i. ACS = Acute Coronary Syndrome

ii. AI = Aromatase Inhibitor

iii. BNP = B-type Natriuretic Peptide

iv. CABOT = CArdiac Biomarkers On Trastuzumab study

v. CRF = Case Report Form

vi. CRP = C-Reactive Protein

vii. cTn = Cardiac Troponin

viii. CV = Coefficient of Variation

ix. C#D# = Cycle number, Day number

x. Echo = Echocardiography

xi. ED = Emergency Department

xii. EDTA = Ethylenediaminetetraacetic Acid

xiii. Gy = Gray (dosage of radiation)

xiv. HER2+ = Human Epidermal Growth Factor Receptor 2, positive

xv. H-FABP = Heart-type Fatty Acid Binding Protein

xvi. hs-cTnI = High-Sensitivity Cardiac Troponin I

xvii. hs-cTnT = High-Sensitivity Cardiac Troponin T

xviii. IQR = Interquartile Range

xix. LoD = Limit of Detection

xx. LoQ = Limit of Quantification

xxi. LVEF = Left Ventricular Ejection Fraction

xxii. MEDICATE = MEDiastinal Irradiation and CArdioToxic Effects study

xxiii. MI = Myocardial Infarction

xxiv. MUGA scan = Multigated Acquisition scan

xxv. NT-proBNP = N-terminal pro-B-type Natriuretic Peptide

xxvi. RCT = Randomized Control Trial

xxvii. RT = Radiotherapy

xxviii. ULN = Upper Limit of Normal

Declaration of Academic Achievement

I, Katharine Mackett, declare this thesis to be my own work. I am the sole author of this document. This thesis has not been published or submitted for publication.

To the best of my knowledge, the content within this thesis does not infringe on anyone's copyright.

My supervisor, Dr. Peter Kavsak, and my supervisory committee, Dr. Bindi Dhesy-Thind and Dr. Hal Hirte, have provided support and guidance throughout this project.

Chapter 1: Introduction

1.1 Cardiotoxicity and Cancer Therapies

1.1.1 Cardiotoxicity in the Cancer Setting

Numerous cancer survivors are leading longer lives as advancements are made towards the greater efficacy and variety of therapies [1]. With this improvement, new challenges emerge, such as short- and long-term effects from cancer therapies. Unfortunately, emerging data demonstrate an increasing association between cancer therapies and damage to the heart, with cardiotoxicity becoming the leading cause of long-term mortality among cancer survivors [2]. Cardiotoxicity is defined as a ≥10%-point decline in left ventricular ejection fraction (LVEF), with a final LVEF of less than 50% [3]. Cardiotoxicity can be characterized by hypertension, arrhythmias, acute coronary syndromes, left ventricular dysfunction, heart failure, and potentially cardiac death [3, 4]. These forms of treatment-induced cardiovascular disease are often asymptomatic, thus delaying diagnostic assessment [3]. Additionally, when chemotherapies affect the heart, therapy might be reduced to be less aggressive, and potentially less effective, resulting in worse patient healthcare outcomes [4]. Two cancers where such cardiotoxic therapies are used include breast cancer, which is one of the most prevalent cancers diagnosed in females worldwide (accounting for 25% of female cancer cases), and non-Hodgkin's Lymphoma, with North America having one of the highest incidence rates, with an estimated >385,000 new cases worldwide in 2012 [5].

1.1.2 Anthracycline Chemotherapy

Anthracyclines such as doxorubicin are an effective form of chemotherapy commonly used in cancer treatments [6]. By inhibiting topoisomerase II (and thus inhibiting DNA repair), doxorubicin has been associated with a 10% reduced risk of relapse and death in breast cancer

patients [6, 7]. However, this comes at a risk of cardiotoxicity, specifically cardiac fibre loss and dilated cardiomyopathy [8]. Mechanisms of cardiac damage include the binding of anthracyclines to the isozyme of topoisomerase II that is expressed on cardiomyocytes, along with free radical generation, lipid peroxidation, and the release of toxic metabolites and inflammatory cytokines, resulting in the damage and death of myocardial cells [6, 7, 8, 9, 10]. Anthracycline-induced cardiac dysfunction is considered to be dose dependent and irreversible [3, 9]. There are some factors that put patients at a higher risk for anthracycline-induced cardiotoxicity than others, including cumulative dose, older age, female sex, pre-existing cardiac disease, and mediastinal radiation [9, 11]. Approximately 7% of patients will experience anthracycline-induced cardiotoxicity, however this risk increases exponentially with dosage [9].

1.1.3 Trastuzumab Therapy

In patients with Human Epidermal Growth Factor Receptor 2 positive (HER2+) breast cancer, the HER2 gene is amplified and overexpressed, resulting in more invasive and metastatic cell growth [12]. This occurs in approximately 25-30% of all breast cancer diagnoses [12]. Expressed on myocytes, the HER2 gene has cardioprotective properties [6]. Trastuzumab ("Herceptin®") is a humanized monoclonal antibody that targets the extracellular domain of HER2, thus reducing the proliferation of breast cancer cells that overexpress HER2 [6, 12]. Many patients will receive trastuzumab in an adjuvant setting, as it has been associated with improved overall and disease-free survival in HER2+ breast cancer patients [13]. Unfortunately, patients receiving trastuzumab are put at risk for cardiotoxicity. It has been suggested that the interaction between trastuzumab and the HER2 signaling pathway in cardiomyocytes interferes with cell survival mechanisms, creating ultrastructural changes (as detected by electron microscopy) such as enlarged vacuoles, pleomorphic mitochondria, along with widened and split

Z-bands [14, 15]. Unlike anthracycline-induced cardiotoxicity, trastuzumab-induced cardiotoxicity results in cell dysfunction (not cell death) and is considered reversible [16, 17]. The risk factors associated with trastuzumab-induced cardiotoxicity include reduced post-anthracycline LVEF, older age, and hypertension [16, 18]. Approximately 2-7% of patients will experience trastuzumab-induced cardiotoxicity [17]. It has been noted that there is a 7-fold increased risk for heart failure or cardiomyopathy when monoclonal antibodies such as trastuzumab are used in combination with anthracyclines such as doxorubicin [19]. Therefore, it is vital to address signs of early cardiotoxicity in cancer patients receiving trastuzumab, as this could minimize and potentially reverse life-threatening cardiac damage from their treatment.

1.1.4 Taxanes

In combination with anthracyclines and trastuzumab, cancer patients may receive taxanes, such as paclitaxel or docetaxel, which are microtubule-stabilizing agents (also known as microtubule inhibitors) [20]. Taxanes work to cause cell arrest and apoptosis by disrupting spindle microtubule dynamics [20]. Although they have proven to be beneficial in terms of overall and disease-free survival in breast cancer patients, taxanes may increase the risk of anthracycline-induced cardiotoxicity by interfering with the metabolism and excretion of anthracyclines [6, 20]. Paclitaxel may increase the cardiotoxic effects of anthracyclines, however this risk is not excessive [21, 22]. As for docetaxel, the risk of heart failure is low, however this is dependent on the dose [21]. Ultimately, this risk is dependent upon the combination of chemotherapeutic agents used, and the cumulative dose of anthracyclines [16].

1.1.5 Endocrine Therapy

For the treatment of breast cancers, endocrine therapies such as hormonal agents may be administered to the patient. Such agents include tamoxifen and aromatase inhibitors (AI) such as

letrozole, anastrazole and exemestane [23]. These have been associated with improved overall and disease- free survival in post-menopausal women with breast cancer, with tamoxifen reducing the risk of recurrence by 47% [23, 24]. The anti-estrogenic effects of tamoxifen are mediated by binding to estrogen receptors, thus reducing their activity [23]. In contrast, AIs block the conversion of androgens to estrogens, thus reducing the levels of estrogen [23]. Although rare, tamoxifen has been associated with endometrial cancer and thromboembolism [23]. While ischemic heart disease is uncommon with tamoxifen, it has been noted in studies with AIs [24]. In a study that compared tamoxifen to letrozole, tamoxifen was noted to have some cardioprotective effects, such as decreasing serum low density lipoprotein cholesterol, whereas AIs did not alter plasma lipoproteins [24].

1.1.6 Radiotherapy

Radiotherapy is an important part of many cancer treatment regimens. In particular, radiotherapy for breast cancer patients has been shown to reduce the risk of local recurrence while improving disease-free survival [16, 25]. However, patients may develop radiation-induced heart disease. Breast cancer patients receiving radiation to the left breast or chest wall are more likely to acquire cardiac damage due to the close proximity of the radiation to the heart [16, 25]. Additionally, patients with Hodgkin's and non-Hodgkin's lymphoma who receive mediastinal radiation are at risk for micro and macro vascular damage [25]. Radiation damage affects coronary endocytes, which can result in myocyte ischemia, fibrosis and constrictive pericarditis, as well as triggering an inflammatory response which can result in atherosclerosis in these patients [16]. The severity of heart damage depends on the dose of radiation administered [25]. Often patients receiving radiotherapy will receive chemotherapy as well, and studies have demonstrated an association between mediastinal radiation and increased susceptibility to

anthracycline damage [22]. Additionally, subclinical radiation-induced cardiomyopathy is believed to be progressive [17].

1.1.7 Left Ventricular Ejection Fraction and Imaging Techniques

Left ventricular ejection fraction (LVEF) is defined as the percentage of blood that is pumped from the resting left ventricle with each systolic contraction [16]. A normal LVEF is 50% or more, with declines indicative of cardiac damage or dysfunction [16]. Cancer patients receiving curative treatment will typically have their heart function monitored in order to detect the presence of cardiotoxicity, often experienced as a reduction in LVEF [3]. The monitoring of cardiac function is assessed clinically using echocardiography (echo) and/or multiple-gated acquisition (MUGA) scans, also known as radionucleotide angiocardiography [16]. Echos are widely available, but interpretation may vary, leading to variability in the results [4, 16]. MUGA scans are reproducible, but expose the patient to radiation [6, 16]. Both echos and MUGA scans lack the sensitivity to detect subclinical changes in LVEF, which is a vital characteristic that cancer patients need when determining whether to adjust their treatment regimens due to left ventricular dysfunction [3, 6, 16, 19]. These scans can only measure changes in LVEF once a significant amount of myocardial damage has occurred, thus rendering them insufficient for early detection of cardiotoxicity and difficult for physicians to initiate preventative measures [3, 16]. Therefore, having sufficient methods to detect myocardial injury and early identification of subclinical cardiac dysfunction would allow for earlier intervention for patients at risk of cardiotoxicity, reducing the cardiovascular morbidity to cancer patients [19].

1.2 Blood Biomarkers

1.2.1 Using Cardiac and Inflammatory Blood Biomarkers to Identify Therapy-Induced Cardiotoxicity

Cardiac biomarkers are an invaluable tool for identification and evaluation of cardiac injury, especially in the setting of anticancer therapy-induced cardiotoxicity [4, 26]. The measuring of biomarkers is cost-effective, minimally invasive and reproducible, with a smaller chance of error as compared to imaging techniques [4]. Biomarkers like cardiac troponin are already widely used in the emergency department (ED) setting for the diagnosis of acute myocardial infarction (MI) in patients with chest pain, and an elevated cardiac troponin separates those at higher risk for an imminent major cardiac event from those who have a lower risk [27]. Studies have demonstrated the reliability of biomarkers like cardiac troponins (cTnI and cTnT) and natriuretic peptides (B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide) in the detection of myocardial dysfunction caused by anthracyclines like doxorubicin [3, 4, 19, 28]. Particularly, a relationship has been established between the level of cardiac troponin elevation, cumulative dose of anthracyclines administered and amount of cardiac damage in patients [3]. It is suggested that the focus of future biomarker research should be on deciding the optimal timepoint for measuring, in order to identify patients at highest risk for developing cardiac damage from potentially cardiotoxic chemotherapy agents [2, 3].

1.2.2 High- Sensitivity Cardiac Troponin (hs-cTn)

Cardiac troponins (cTn) are regulatory proteins that play an important physiological role in the of relaxation and contraction of the heart. The “troponin complex” has three protein subunits: C, T, and I [29]. Troponin C (TnC) is expressed in cardiac and skeletal muscle. The cardiac-specific isoforms are troponin I and T (TnI, TnT), which are located within cardiac

muscle, specifically in the sarcomeres of myocardial cells where is it bound to the contractile apparatus of the myofibril [3, 29]. A small percentage of cTnI and cTnT are free within the cytoplasm, though this is an area of continued interest in the literature [29]. When myocytes become damaged, plasma cardiac troponin concentrations rise due to the initial release of cytoplasmic cardiac troponin, which is followed by the release of the cardiac troponin that was bound [29]. Elevations of plasma cardiac troponin may be detected within 2-4 hours after the onset of myocardial injury, and may remain elevated for 4-7 days for cTnI and 10-14 days for cTnT [29]. Evaluation of cardiac troponin is widely used in patients with acute coronary syndromes (ACS) and other cardiac injury-related events [3, 27]. As a highly sensitive and specific biomarker, elevated concentrations of cardiac troponin in the blood can identify the severity of myocardial injury and may be used for cardiac risk stratification [3]. High-sensitivity cTn (hs-cTn) assays are becoming prominent in clinical laboratories, as the analytical sensitivity and precision in these assays can reliably detect and measure lower concentrations compared to contemporary cTn assays [3, 30]. The term "high-sensitivity" is related to the assay itself and not the biomarker in question [31, 32]. The hs-cTn assays are defined by parameters such as their ability to measure concentrations at or above the limit of detection (LoD) in at least 50% of individuals in a healthy reference group and by having an imprecision (i.e., percent coefficient of variation; %CV) of ≤10% at the 99th percentile upper limit of normal (ULN) [32]. These assays are beneficial in the cardio-oncological setting as they have high sensitivity for detecting small concentrations of cardiac troponin, such that elevations indicative of cardiac injury are detectable before changes in LVEF are observed on traditional scans [3, 19]. With this potential to detect the presence of myocardial injury much earlier, strategies to reduce toxicity may be initiated earlier, thus reducing morbidity and improving the outcome for cancer patients [3, 19].

1.2.3 Heart-Type Fatty Acid Binding Protein (H-FABP)

There are at least 9 different types of fatty acid binding proteins throughout the body, one of which is the heart-type fatty acid binding protein (H-FABP), also known as FABP3, predominately found in heart and skeletal muscle [33, 34]. H-FABP has a role in mitochondrial β- oxidation and is abundant in the cytoplasm of myocardial cells [34]. Because of this, elevated concentrations of H-FABP in the blood are an excellent indicator of myocardial injury, as such injury causes H-FABP to leak through the porous membranes of myocardial cells without the need for cardiomyocyte necrosis [34]. Typically, levels will increase 1-2 hours after the onset of symptoms, peaking after 5-10 hours [34]. The rapid release of H-FABP makes it an ideal marker for detection of injury in cancer patients at risk for cardiotoxicity.

1.2.4 C-Reactive Protein (CRP)

C-reactive protein (CRP) is a circulating acute phase protein that is synthesized by hepatocytes in the liver in response to inflammation [25, 35]. CRP binds to macromolecular ligands, resulting in the activation of the classical complement pathway [35]. Increasing concentrations of CRP are associated with tissue injury, infection, and inflammation, with its expression regulated by interleukins (IL-1, IL-6) and tissue necrosis factor α (TNF-α) [25, 35]. Along with its sensitivity, CRP is a good clinical marker for adverse non-physiological stress, with a plasma half-life of 19 hours [35]. High concentrations of CRP in patients with ACS have proven to be an effective prognostic marker [35, 36]. Elevations in CRP are also indicative of reductions in LVEF, which is associated with myocardial injury [25, 35]. In the cancer setting, tumour growth can cause tissue inflammation, resulting in elevated concentrations of plasma CRP, which has been associated with early death [37]. Additionally, CRP may also be a part of the host immune response to tumours [37].

1.2.5 N-Terminal Pro-B-type Natriuretic Peptide (NT-proBNP)

Natriuretic peptides are involved in cardiovascular and cardiorenal homeostasis [3, 38]. Specifically, B-type natriuretic peptide (BNP) is synthesized in the myocardium by ventricular cardiomyocytes [25, 38]. Starting as pre-proBNP, it is processed into proBNP, then cleaved into BNP and the N-terminal proBNP fragment (NT-proBNP) [25, 38]. In response to ventricular wall stress, pressure and volume overload in the heart, proBNP is rapidly produced and secreted, as well as BNP and NT-proBNP, with elevations evident within 4 hours [25, 38, 39]. BNP binds to the natriuretic peptide receptor type A, which causes the increased production of intracellular cyclic guanosine monophosphate (cGMP) [38, 40]. This leads to diuresis, vasodilation, inhibition of renin and aldosterone production, as well as the inhibition of cardiac and vascular myocyte growth [38, 40]. High concentrations of NT-proBNP in plasma are associated with acute heart failure, such that this peptide can be used to identify and diagnose subclinical cardiac dysfunction in patients who may be at a higher risk [38, 39, 41].

1.3 Analytical Aspects of Clinical Laboratory Assays

Some terms used to define and classify clinical laboratory assays include the limit of detection (LoD), which is the lowest concentration at which an analyte can be detected, and the limit of quantification (LoQ), which is the lowest concentration at which signals are reliably produced, meeting the predetermined target for bias and imprecision [42]. The precision of the assay as well as a healthy cohort of the population of interest help determine the 99th percentile cutoff value that is used as reference [32, 43]. It is recommended that high-sensitivity assays for markers such as cardiac troponin use sex-specific upper limit of normal cutoffs (ULN), however contemporary cTn assays do not possess the analytical sensitivity required for use of sex-specific cutoffs [32, 44]. The use of sex-specific ULNs for evaluating biomarkers is important, as females

and males respond differently to cardiac stressors [45]. Specifically, females tend to have lower levels of cardiac troponin and higher levels of NT-proBNP than males, as a result of different body compositions and left ventricular mass between females and males [45]. The interpretation of results may be affected if an overall ULN cutoff is employed when conducting analyses. The analytical characteristics and performance of the assays used to measure the biomarkers analyzed in this thesis have all been validated [36, 38, 46, 47, 48].

1.4 Hypothesis and Rationale

The main hypothesis for this thesis is that cardiac and inflammatory biomarkers measured from blood can predict reductions in LVEF in lymphoma and breast cancer patients receiving curative treatment. The rationale for this is that changes and elevations in biomarkers may be evident early after the initiation of potentially cardiotoxic treatment, and thus will be detected prior to evidence of structural changes in the heart that result in the decrease of LVEF, as observed by MUGA scans.

1.5 Objectives

i) To determine the temporal pattern of various cardiac-related blood biomarkers in cancer patients receiving potentially cardiotoxic therapies.

&

ii) To assess the prevalence of abnormal biomarker concentrations using the ULN and whether differences in these biomarkers are evident in those with versus without cardiotoxicity.

Specifically, it remains unclear if hs-cTnI assays, especially in females using sex-specific ULNs (as recommended for the detection of myocardial injury), are useful in this setting. Thus, this work was performed with the intention of critically evaluating the current criteria and

clinical measures used in the detection of cardiotoxicity in the female cancer population, and to evaluate early timepoints for assessing patients who are at risk of developing cardiotoxicity.

Chapter 2: Cardiac Troponin I in Patients Receiving Adjuvant Trastuzumab Therapy

2.1 Introduction

Trastuzumab is a commonly used therapy for the treatment of breast cancer, however many patients face the risk of asymptomatic changes to their LVEF or heart failure [26]. The literature suggests that this left ventricular dysfunction due to trastuzumab therapy may be reversible if detected at an early stage [3]. Clinical cardiac troponin assays have excellent analytical precision at low concentrations, with the availability of hs-cTnI assays possibly being a new tool for monitoring acute injury in the cancer setting [3, 4]. However, routine use of high-sensitivity troponin assays for identifying myocardial injury in cancer patients remains unclear [3]. Thus, the purpose of the following analyses was to evaluate hs-cTnI concentrations associated with myocardial injury in breast cancer patients to see if the evaluation of cardiac troponin concentrations in this setting is appropriate for the early identification of damage [49]. More specifically, this chapter focuses on the analyses of hs-cTnI in breast cancer patients, divided into 3 subgroups.

2.2 Methods

2.2.1 Assessment of Myocardial Injury in Female Breast Cancer Patients using hs-cTnI

Subgroup 1 involved female patients with human epidermal growth factor receptor 2 positive (HER2+) breast cancer, chosen from the CABOT study (Cardiac Biomarkers on Trastuzumab: Determining the cardiac biomarker profile in breast cancer patients receiving adjuvant trastuzumab therapy) (i.e., study participants) [50]. The goal of the CABOT study was to create biomarker profiles on patients with HER2+ breast cancer receiving adjuvant trastuzumab therapy [50]. Ethylenediaminetetraacetic acid (EDTA) plasma samples were

collected at multiple timepoints over the course of treatment from 23 participants with stage I-III, HER2+ breast cancer receiving adjuvant trastuzumab therapy, from October 2010 through to November 2016. The first was a baseline sample, prior to administration of chemotherapy. The next sample (approximately 3 months later) was collected following completion of anthracycline treatment and immediately before the first cycle of trastuzumab treatment, with another sample collected the day after the administration of trastuzumab. This process continued for cycles 1-5, 7, and 9 of trastuzumab (equating to 6 months), depending on the ability of the participant to participate, with the time between cycles approximately 3 weeks. Along with the blood collection, the participants' cardiac risk factors were also documented, such as cardiac history, blood pressure, cholesterol levels, smoking status, medications used, family history and age. Some eligible participants received radiotherapy in addition to the adjuvant trastuzumab therapy. The following analyses used EDTA plasma samples (stored below -70^0C) from 20 participants. Participants were included in these analyses if they had complete hs-cTnI measurements at baseline and cycle 1 (Fig. 2.1).

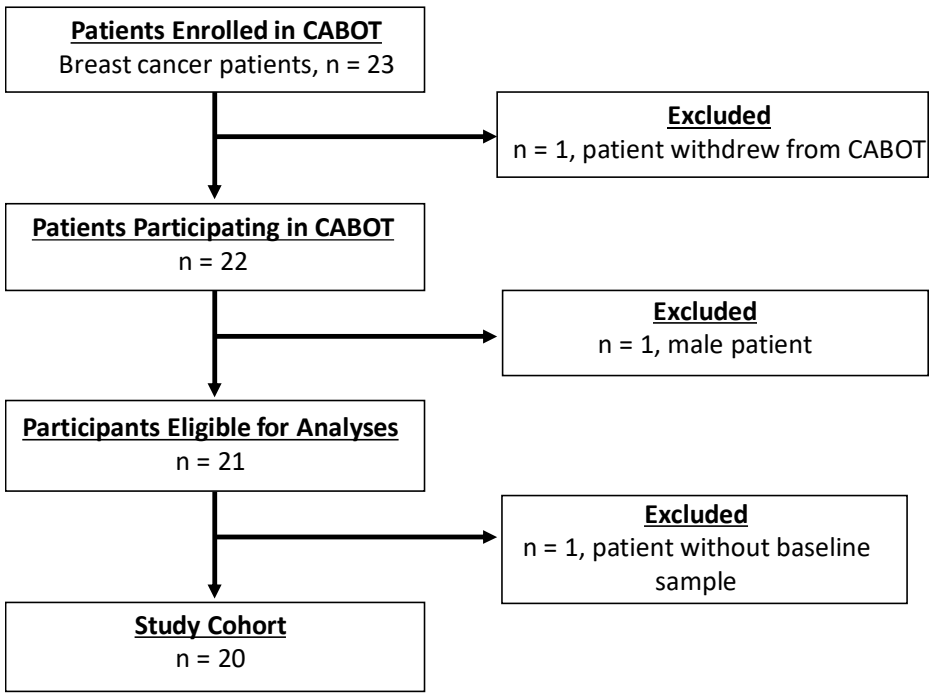

Figure 2.1. **Subgroup 1 Participant Inclusion Criteria**. Flow diagram of participants included in chapter 2.2.1 analyses.

Hs-cTnI was measured using the Beckman Coulter ACCESS System hs-TnI assay (20% CV LoQ = 2.2 ng/L; 99th percentile ULN: Female = 11.6 ng/L (rounded to 12 ng/L)), and Abbott Diagnostics ARCHITECT STAT hs-TnI assay (20% CV LoQ = 4.0 ng/L; 99th percentile ULN: Female = 15.6 ng/L (rounded to 16 ng/L)) [51]. The analyses were based on measurements collected at baseline (chemotherapy-naive), after anthracycline chemotherapy but prior to the first trastuzumab treatment (Cycle1/Day1, C1D1), and then the next day (Cycle1/Day2, C1D2) [49]. Spearman's correlation was used to assess the correlation between the two assays at baseline and both days of cycle 1 [49]. Sex-specific 99th percentile ULN values and an absolute change criterion of ≥12 ng/L (reported as a significant change in patients with potential acute coronary syndrome (ACS)) were employed to assess the prevalence of myocardial injury [52]. As the data did not follow a normal distribution, non-parametric tests were employed. Descriptive statistics and Spearman's correlation (rho) were performed via MedCalc version

17.9.7 (p <0.05 considered significant). Data was compiled using Microsoft Excel spreadsheets to create an overall database containing all the information on participant biomarker concentrations and medical history for the CABOT study. The lead investigator on the study provided participant case report forms (CRFs) for any information that was missing in order to complete the datasets.

2.2.2 Longitudinal Assessment of Cardiotoxicity in Female Breast Cancer Patients using hs-cTnI

Subgroup 2 included female breast cancer patients from the CABOT study, as outlined in 2.2.1. In order to assess any longitudinal damage in these participants, this subgroup must have had complete LVEF measurements prior to the initiation of trastuzumab therapy. Thus, 18 of the 20 participants described in 2.2.1 were included in the following analyses (Fig. 2.2) [53].

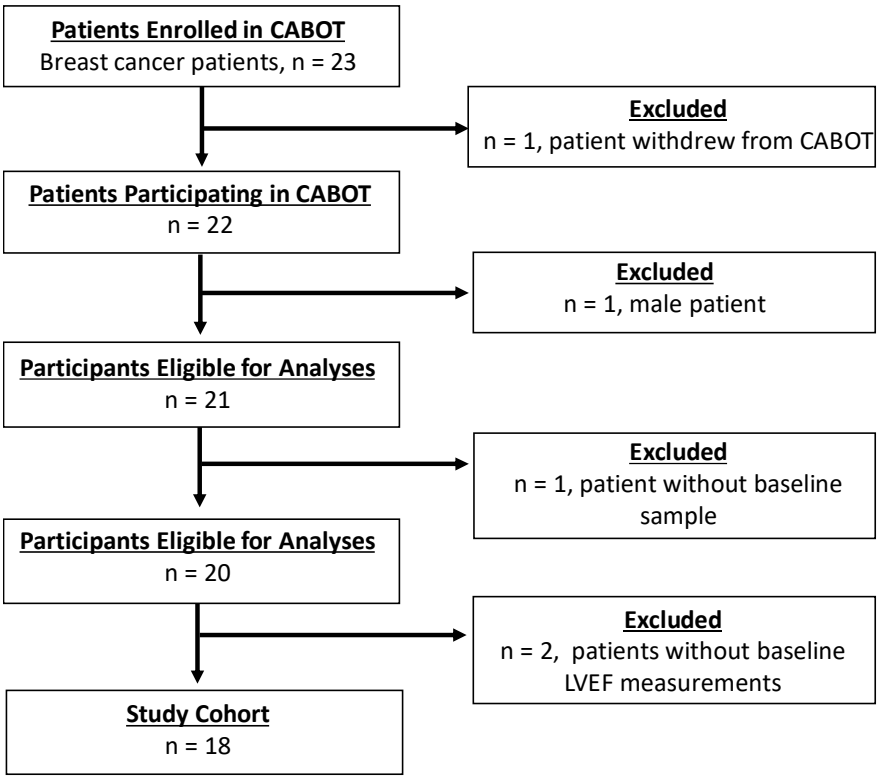

Figure 2.2. Subgroup 2 Participant Inclusion Criteria. Flow diagram of participants included in chapter 2.2.2 analyses.

In addition to cycle 1 plasma collections, 5 more cycles of trastuzumab treatment (following the same day 1, day 2 timing) were analyzed. The cycles were named as cycle 1 (trastuzumab initiation, $n = 18$), cycle 2 (3 weeks on trastuzumab, $n = 18$), cycle 3 (6 weeks on trastuzumab, $n = 18$), cycle 4 (9 weeks on trastuzumab, $n = 18$), cycle 5 (12 weeks on trastuzumab, $n = 16$), and cycle 7 (18 weeks on trastuzumab, $n = 16$) [53]. Sex-specific 99th percentile ULN cutoffs were employed to assess the prevalence of myocardial injury [51]. LVEF was assessed via MUGA scan prior to chemotherapy, prior to the initiation of trastuzumab, and after 3 months on trastuzumab. The criteria used for classifying the participants in this subgroup as having cardiotoxicity was a decline of at least 10% in LVEF with a final LVEF <50%, also discontinuing trastuzumab treatment [3]. Descriptive statistical analyses were also performed. Again, as the data did not follow a normal distribution, non-parametric tests were employed using MedCalc version 17.9.7 (p <0.05 considered significant). Data was compiled using Microsoft Excel spreadsheets to create an overall database containing all the information on participant biomarker concentrations and medical history for the CABOT study.

2.2.3 Overall Myocardial Injury Prevalence and Assessment of Cardiotoxicity in Breast Cancer Patients using hs-cTnI

The third subgroup included breast cancer patients from the CABOT study as outlined in 2.2.1 and 2.2.2. The following analyses are not sex-specific, as one male participant was included, and thus the use of overall cutoff ULN values were employed. The same cohort of participants were analyzed, as well as the method of blood sample collection and LVEF measurement [50, 53]. An additional 2 participants were included in these general analyses ($n = 22$), (Fig. 2.3).

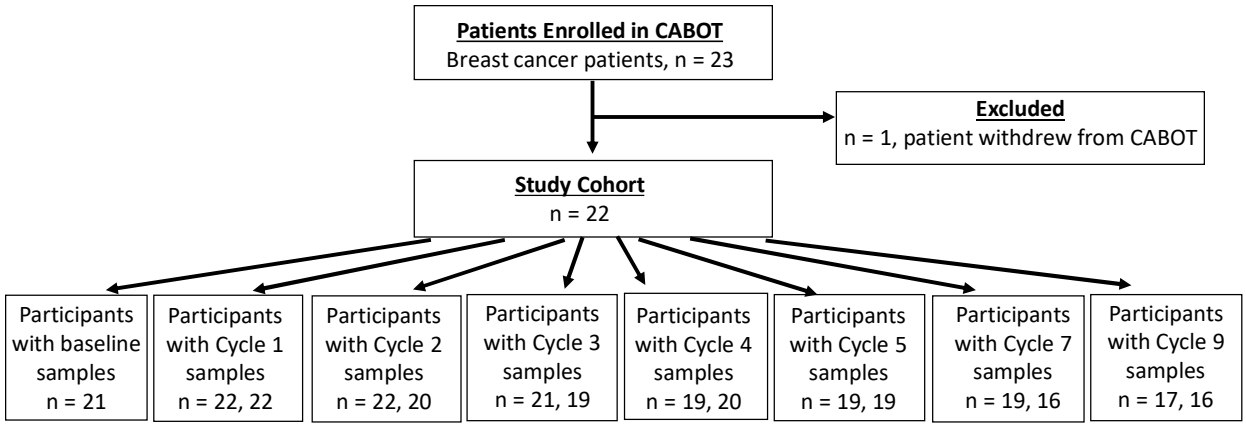

Figure 2.3. **Subgroup 3 Participant Inclusion Criteria**. Flow diagram of participants included in chapter 2.2.3 analyses, during days 1 and 2 for each cycle on trastuzumab treatment.

With the one male included in the CABOT study, it was of interest to see how this participant related to the females, and if a higher overall ULN provided a similar prevalence of myocardial injury in these participants. The 99th percentile ULN values were employed to assess the prevalence of myocardial injury (Beckman Coulter ACCESS System hs-TnI assay; 99th percentile overall ULN = 17 ng/L, and Abbott ARCHITECT System STAT hs-TnI assay; 99th percentile overall ULN = 26 ng/L) [51]. Participants in the third subgroup were classified as having cardiotoxicity if they discontinued treatment and thus left the study due to a decline in LVEF, or if they were still part of the study and had a decrease in LVEF greater than or equal to 10%, with an absolute LVEF less than 50% after 3 on trastuzumab [3]. Mann Whitney tests were used to compare the change from day 1 to 2 during cycle 1 on trastuzumab treatment, to compare the difference in concentration from baseline to the timepoint at which the median concentration was highest, and to compare the participants with the outcome versus those who did not experience cardiotoxicity at the timepoint of highest median concentration. Non-parametric statistical analyses were performed using MedCalc version 17.9.7 (p <0.05 considered significant). Data was compiled using Microsoft Excel spreadsheets to create an overall database

containing all the information on participant biomarker concentrations and medical history for the CABOT study.

2.3 Results

2.3.1 Assessment of Myocardial Injury in Female Breast Cancer Patients using hs-cTnI

The mean (SD) age of these 20 participants was 52 (10) years. The median concentration (interquartile range, IQR) for hs-cTnI at each timepoint is listed in Table 2.1.

Timepoint	**Beckman hs-cTnI (ng/L) (ULN = 12 ng/L)**	**Abbott hs-cTnI (ng/L) (ULN = 16 ng/L)**
Baseline; n = 20 - median (IQR)	1 (0.7- 2)	3 (3- 5)
Cycle 1, Day 1; n = 20 - median (IQR)	17 (8- 29)	16 (12- 30)
Cycle 1, Day 2; n = 20 - median (IQR)	20 (12- 43)	22 (13- 43)

Table 2.1. **Subgroup 1 Median (IQR) hs-cTnI Concentrations.** Listed in ng/L for Beckman and Abbott assays during the three timepoints assessed in chapter 2.2.1.

Prior to chemotherapy, less than 50% of participants had hs-cTnI above the LoQ, with no concentrations >99th percentiles (rho = 0.01 between the assays; p = 0.96). However, during the first cycle of trastuzumab treatment, all participant concentrations exceeded the LoQ and thus were measurable. During the first cycle, the hs-cTnI concentrations were highly correlated between the assays (day 1 rho = 0.97, day 2 rho = 0.99; p <0.0001) (Figs. 2.4 A, B).

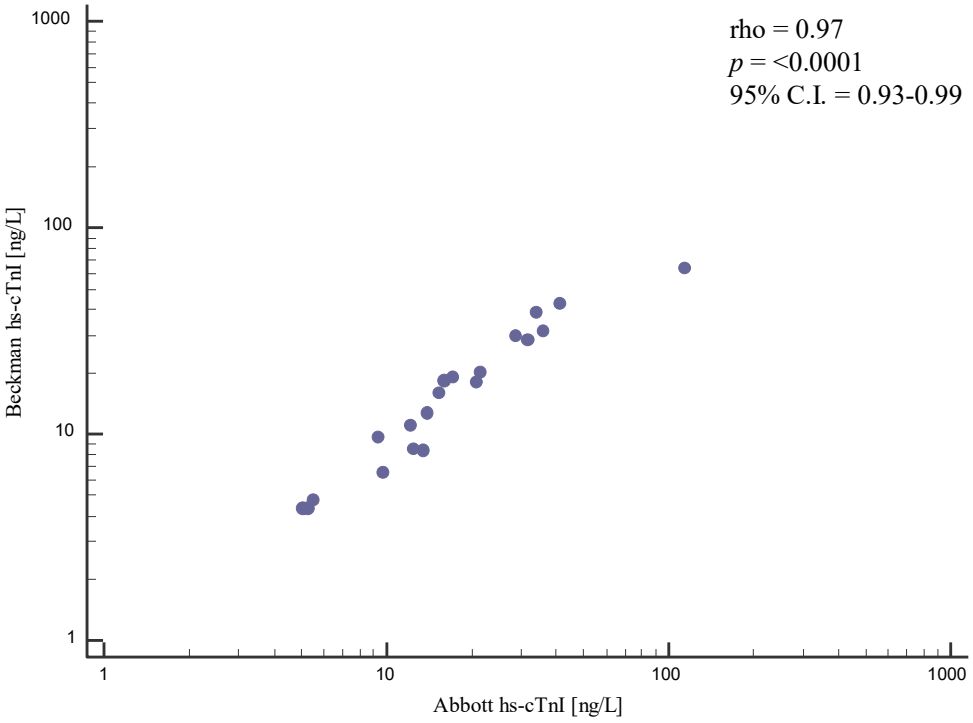

Figure 2.4A. **Correlation for hs-cTnI**. Plot between Beckman and Abbott assays during C1D1.

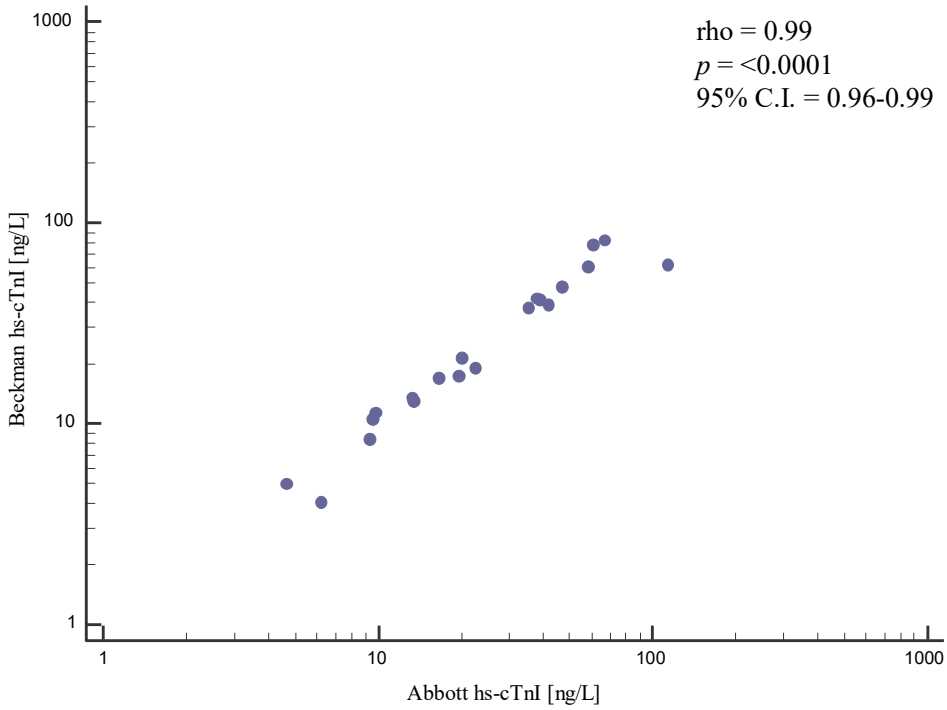

Figure 2.4B. **Correlation for hs-cTnI**. Plot between Beckman and Abbott assays during C1D2.

Using the 99th percentile cutoff, no participants were >ULN at baseline. But during cycle 1, 60% (12/20) (Beckman) and 50% (10/20) (Abbott) (C1D1), 75% (15/20) (Beckman) and 65% (13/20) (Abbott) (C1D2) of participants were identified as having myocardial injury (Table 2.2) [49].

Timepoint	**>ULN hs-cTnI Beckman assay (12 ng/L)**	**>ULN hs-cTnI Abbott assay (16 ng/L)**
Baseline	0% (0/20)	0% (0/20)
Cycle 1, Day 1	60% (12/20)	50% (10/20)
Cycle 1, Day 2	75% (15/20)	65% (13/20)

Table 2.2. **Subgroup 1 ULN to assess Myocardial Injury**. Incidence of myocardial injury using sex-specific ULN cutoffs for Beckman and Abbott hs-cTnI assays.

In applying the absolute change criterion as proposed in patients with potential ACS, 55% (11/20) (Beckman) and 60% (12/20) (Abbott) (C1D1 concentration – baseline concentration), 40% (8/20) (Beckman) and 35% (7/20) (Abbott) (C1D2 concentration – C1D1 concentration) of participants were identified with evolving or acute myocardial injury (Table 2.3) [49].

Timepoint	**# participants with ≥12 ng/L hs-cTnI change (Beckman assay)**	**# participants with ≥12 ng/L hs-cTnI change (Abbott assay)**
Cycle 1, Day 1 from Baseline	55% (11/20)	60% (12/20)
Cycle 1, Day 2 from C1D1	40% (8/20)	35% (7/20)

Table 2.3. **Change Criterion to assess Evolving or Acute Injury**. Prevalence of participants with a ≥12 ng/L change in hs-cTnI for Beckman and Abbott assays.

2.3.2 Longitudinal Assessment of Cardiotoxicity in Female Breast Cancer Patients using hs-cTnI

The mean (SD) age of these 18 participants was 51 (9) years. Median (IQR) hs-cTnI concentrations for each cycle are detailed below (see Table 2.4). After 3 weeks on trastuzumab, the median hs-cTnI concentrations were the highest (C2D2; Beckman median = 42 ng/L Abbott median = 44 ng/L), and lowest after 18 weeks on trastuzumab (C7D1; Beckman median = 5 ng/L, Abbott median = 6 ng/L) (Fig. 2.5, Table 2.4) [53]. Just before 3 months into trastuzumab treatment, 2 participants had to stop due to LVEF decline (participant #9 with LVEF of 42% and a 11% decline from baseline, and participant #17 with LVEF of 49% and a 12% decline from baseline) (Table 2.5). Participant #9 had hs-cTnI above the ULN during cycles 1 and 2 (C1D1: Beckman = 63 ng/L, Abbott = 115 ng/L; C1D2 = 61 ng/L, 114 ng/L; C2D1 = 76 ng/L, 128 ng/L; C2D2 = 67 ng/L, 118 ng/L), while participant #17 only had hs-cTnI elevation during cycle 1 (C1D1: Beckman = 18 ng/L, Abbott = 21 ng/L; C1D2 = 37 ng/L, 35 ng/L) (Table 2.6). The 16 participants that remained on treatment had a LVEF median (IQR) of 58% (54-62%) and a median difference from baseline of only 5% after 3 months on trastuzumab (Table 2.5) [53].

Timepoint (# of participants)	**Baseline** $n = 18$	**Cycle 1** $n = 18, 18$	**Cycle 2** $n = 18, 16$	**Cycle 3** $n = 18, 16$	**Cycle 4** $n = 17, 18$	**Cycle 5** $n = 16, 16$	**Cycle 7** $n = 16, 14$
Beckman hs-cTnI assay (ng/L) - Median (IQR)	1 (0.7- 2)	Day 1: 17 (8-26) Day 2: 20 (13-42)	Day 1: 32 (16-54) Day 2: 42 (15-64)	Day 1: 18 (10-36) Day 2: 14 (9-32)	Day 1: 8 (6-17) Day 2: 8 (6-17)	Day 1: 8 (5-12) Day 2: 7 (5-11)	Day 1: 5 (4-9) Day 2: 7 (5-9)
Abbott hs-cTnI assay (ng/L) - Median (IQR)	3 (3-5)	Day 1: 16 (10-29) Day 2: 22 (13-41)	Day 1: 35 (15-82) Day 2: 44 (16-96)	Day 1: 18 (12-42) Day 2: 17 (12-46)	Day 1: 12 (7-20) Day 2: 11 (7-23)	Day 1: 12 (6-15) Day 2: 10 (5-12)	Day 1: 6 (5-12) Day 2: 9 (6-12)

Table 2.4. Subgroup 2 Median (IQR) hs-cTnI Concentrations. Listed in ng/L for Beckman and Abbott assays during the timepoints assessed in chapter 2.2.2.

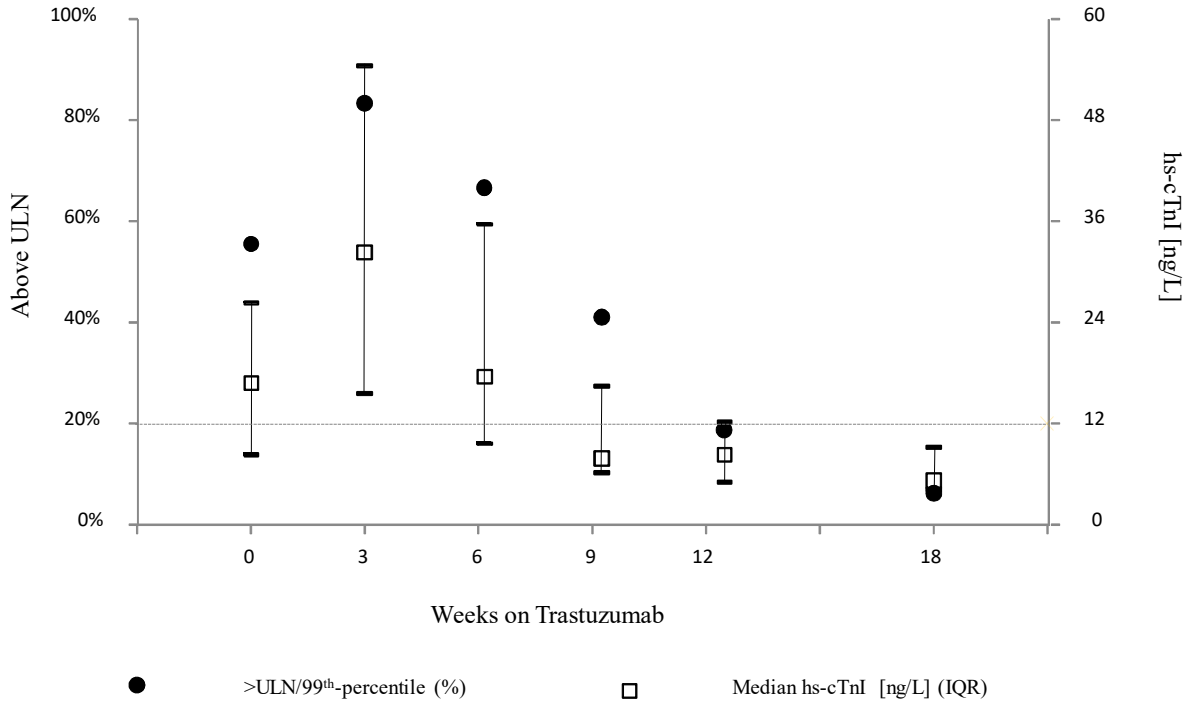

Figure 2.5. ULN to assess Myocardial Injury using the Beckman hs-cTnI assay. The longitudinal profile on the prevalence of concentrations greater than the ULN (%) and hs-cTnI (median, IQR) concentration (the dashed line is the ULN of 12 ng/L (Beckman assay)). Reprinted from [53]. *Per Elsevier, as an author of this article I have retained the right to include this figure in my thesis.

LVEF measurement	**Baseline**	**Prior to Trastuzumab**	**3 months on Trastuzumab (change from baseline)**
Participants that continued treatment post- 3 months (median (IQR)) n = 16	64% (60-67%)	61% (58-63%)	58% (54-62%) (-5%)
Discontinued Participant #9	53%	55%	42% (-11%)
Discontinued Participant #17	61%	56%	49% (-12%)

Table 2.5. Subgroup 2 LVEF to assess Cardiotoxicity. Assessment based on LVEF measurements during baseline, prior to trastuzumab, and 3 months on treatment.

Discontinued Participants hs-cTnI Concentrations (ng/L)	Cycle 1 Trastuzumab	Cycle 2 Trastuzumab	Cycle 3 Trastuzumab
Participant #9 - Beckman assay - Abbott assay	Day 1 = 63, Day 2 = 61 Day 1 = 115, Day 2 = 114	Day 1 = 76, Day 2 = 67 Day 1 = 128, Day 2 = 118	Day 1 = 40, Day 2 = 36 Day 1 = 66, Day 2 = 64
Participant #17 - Beckman assay - Abbott assay	Day 1 = 18, Day 2= 37 Day 1 = 21, Day 2 = 35	Day 1 = 10, Day 2 = 10 Day 1 = 10, Day 2 = 11	Day 1 = 7, Day 2 = 7 Day 1 = 6, Day 2 = 7

Table 2.6. **Discontinued Participants hs-cTnI Concentrations**. Initial hs-cTnI concentrations (ng/L) of participants that discontinued trastuzumab treatment due to LVEF decline.

2.3.3 Overall Myocardial Injury Prevalence and Assessment of Cardiotoxicity in Breast Cancer Patients using hs-cTnI

The mean (SD) age of these 22 participants was 52 (9) years, with other demographic information listed in Table 2.7.

Variable	CABOT (n = 22)
Median Age (range)	54 (36-68)
Cancer Diagnosis - Invasive carcinoma, ductal - Invasive carcinoma, lobular	 22 1 (one with both)
Location of Cancer - Left breast - Right breast - Bilateral breast cancer	 10 11 1
Systemic Therapy - AC[1] - AC-T[2] - FEC-D[3] - DC[4]	 1 19 1 1
Radiation Therapy (at any timepoint) - Left breast - Left chest wall - Right breast - Right chest wall	 4 3 6 3
Endocrine therapy - Tamoxifen - Letrozole	 2 1

[1]Doxorubicin, Cyclophosphamide (AC), [2]Doxorubicin, Cyclophosphamide, Paclitaxel (AC-T), [3]Fluorourcil/ Epirubicin, Cyclophosphamide, Docetaxel (FEC-D), [4]Docetaxel, Cyclophosphamide (DC).

Table 2.7. Demographics table. Demographic information on the CABOT study participants included in subgroup 3, *n* = 22.

All participants had hs-cTnI below the ULN prior to chemotherapy. Median (IQR) concentrations are listed below (see Table 2.8). The hs-cTnI concentrations increased from baseline to cycle 1 of trastuzumab, with persistent elevations on day 2 (Beckman hs-cTnI C1D1 median = 17 ng/L, C1D2 median = 20 ng/L, $p = 0.16$; Abbott hs-cTnI C1D1 median = 16 ng/L, C1D2 median = 22 ng/L, $p = 0.24$) (Table 2.8, Figs. 2.6 A, B). The timepoint that yielded the highest median concentration of hs-cTnI was C2D2, with >50% of participants exceeding the overall ULN (Beckman hs-cTnI median = 42 ng/L, Abbott hs-cTnI median = 44 ng/L) (Figs. 2.6 A, B). The elevation of hs-cTnI from baseline (Beckman median = 1 ng/L, Abbott median = 3 ng/L) to C2D2 was statistically significant ($p = <0.0001$, Beckman and Abbott). Three months after the initiation of trastuzumab, 4 participants (including the male participant) had a LVEF of less than 50% and decline greater than 10%, fulling the criteria for cardiotoxicity (Table 2.9). No significant difference was observed in the concentrations of hs-cTnI at C2D2 between participants with a significant change in LVEF versus those without (Beckman $p = 0.45$, Abbott $p = 0.57$).

Timepoint (# of participants)	**Baseline** $n = 21$	**Cycle 1** $n = 22, 22$	**Cycle 2** $n = 22, 20$	**Cycle 3** $n = 21, 19$	**Cycle 4** $n = 19, 20$	**Cycle 5** $n = 19, 19$	**Cycle 7** $n = 19, 16$	**Cycle 9** $n = 17, 16$
Beckman hs-cTnI assay (ng/L) - Median (IQR)	1 (0.7-2)	Day 1: 17 (9-29) Day 2: 20 (13-46)	Day 1: 32 (14-71) Day 2: 42 (10-72)	Day 1: 19 (9-37) Day 2: 16 (8-33)	Day 1: 11 (6-18) Day 2: 10 (6-18)	Day 1: 6 (5-12) Day 2: 6 (5-11)	Day 1: 5 (4-9) Day 2: 7 (5-10)	Day 1: 5 (4-7) Day 2: 5 (4-6)
Abbott hs-cTnI assay (ng/L) - Median (IQR)	3 (3-5)	Day 1: 16 (12-31) Day 2: 22 (13-46)	Day 1: 35 (13-82) Day 2: 44 (15-96)	Day 1: 19 (12-43) Day 2: 18 (11-47)	Day 1: 12 (8-26) Day 2: 12 (7-27)	Day 1: 11 (5-15) Day 2: 10 (5-13)	Day 1: 5 (4-12) Day 2: 9 (5-12)	Day 1: 6 (4-9) Day 2: 7 (4-8)
# participants >ULN (17 ng/L) Beckman assay	0	Day 1: 11 Day 2: 12	Day 1: 14 Day 2: 12	Day 1: 11 Day 2: 9	Day 1: 5 Day 2: 5	Day 1: 1 Day 2: 1	Day 1: 1 Day 2: 1	Day 1: 0 Day 2: 0
# participants >ULN (26 ng/L) Abbott assay	0	Day 1: 7 Day 2: 10	Day 1: 14 Day 2: 11	Day 1: 8 Day 2: 7	Day 1: 5 Day 2: 5	Day 1: 1 Day 2: 2	Day 1: 1 Day 2: 1	Day 1: 1 Day 2: 1

Table 2.8. Subgroup 3 Median (IQR) hs-cTnI Concentrations with >ULN Incidence. Listed in ng/L for Beckman and Abbott assays during the timepoints assessed in 2.2.3, along with the number of participants whose concentrations exceeded the overall ULN.

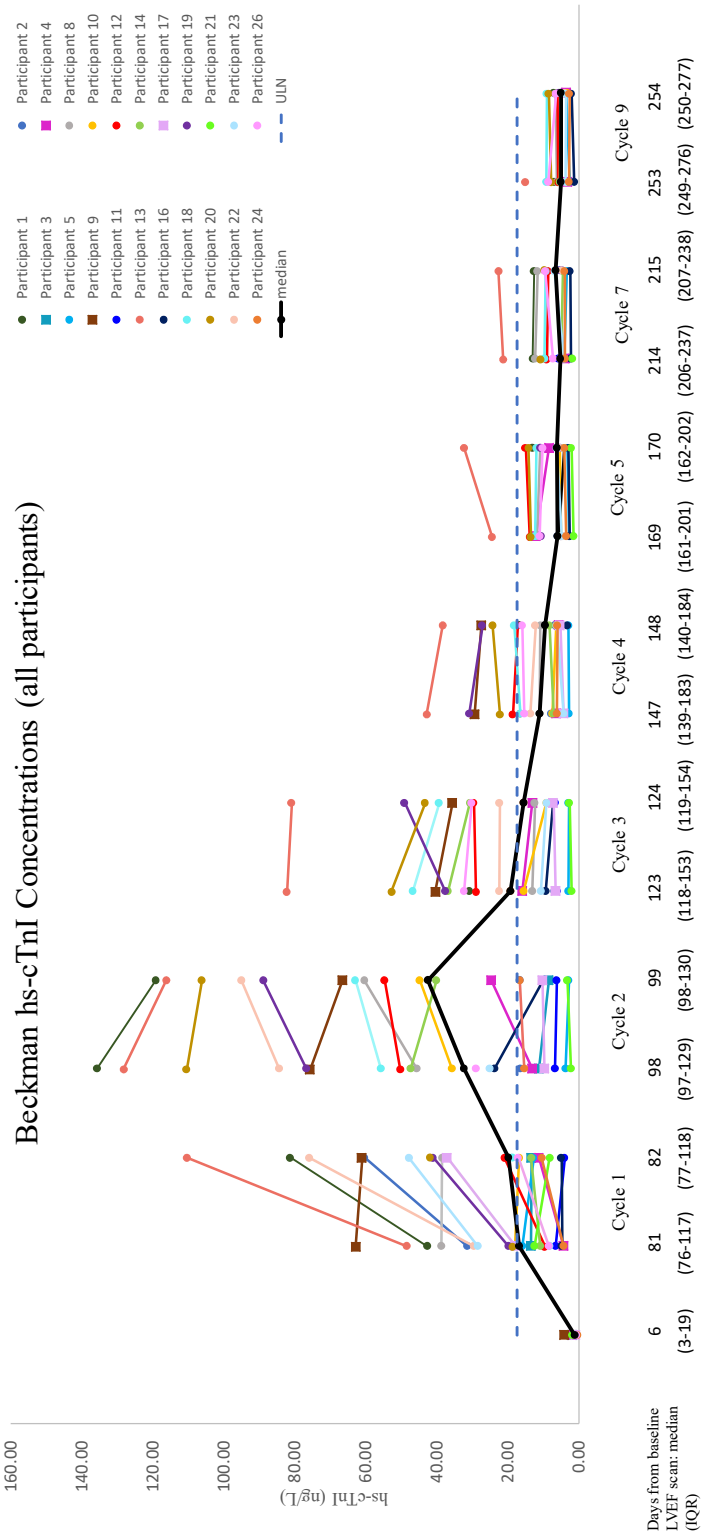

Figure 2.6A. **Beckman hs-cTnI Concentrations (all participants).** Plot of hs-cTnI concentrations (ng/L) for the Beckman assay during each timepoint assessed in 2.2.3. Available data for days 1 and 2 of each cycle are connected. Listed below the X axis are the median (IQR) days from which the baseline LVEF was measured. Participants with cardiotoxicity are squares, those without are circles. Dashed line is overall ULN.

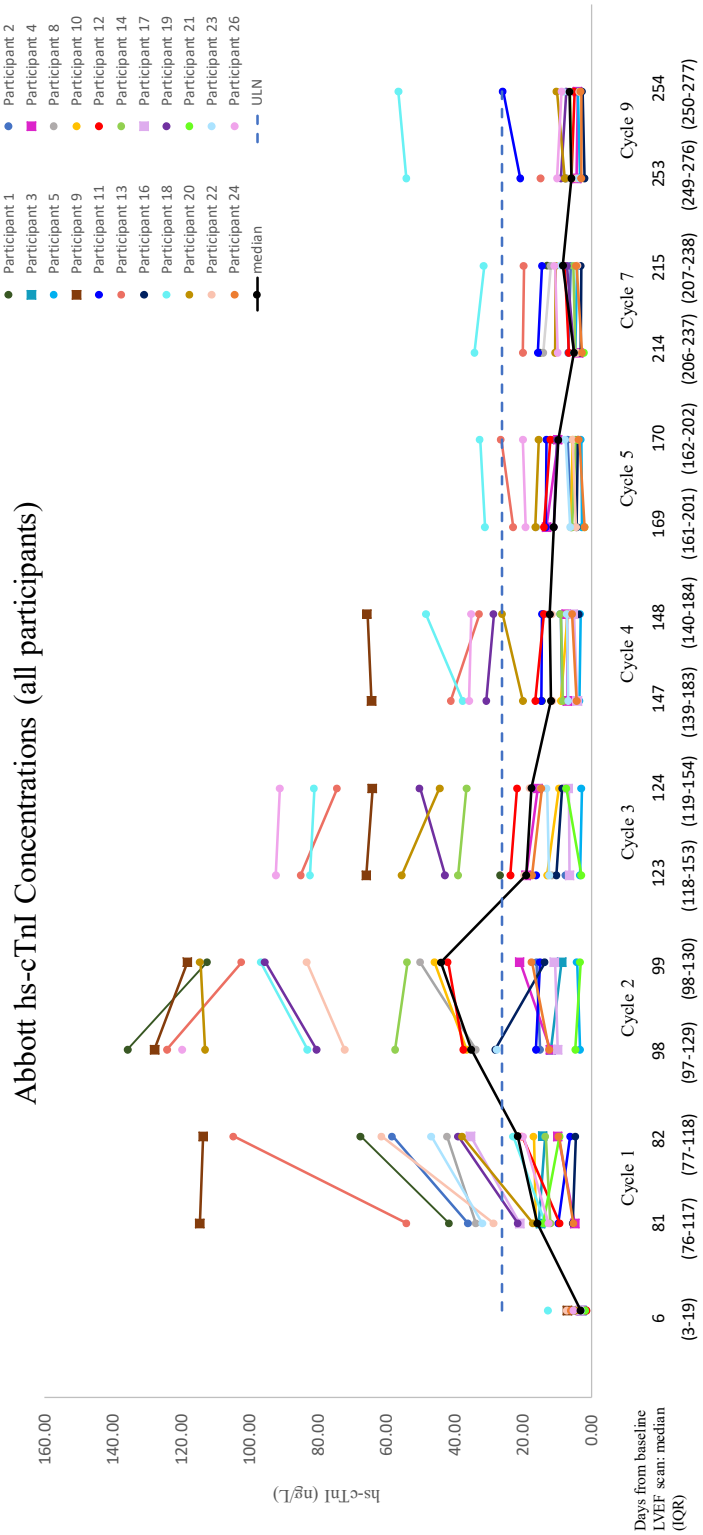

Figure 2.6B. **Abbott hs-cTnI Concentrations (all participants).** Plot of hs-cTnI concentrations (ng/L) for the Abbott assay during each timepoint assessed in 2.2.3. Available data for days 1 and 2 of each cycle are connected. Listed below the X axis are the median (IQR) days from which the baseline LVEF was measured. Participants with cardiotoxicity are squares, those without are circles. Dashed line is overall ULN.

LVEF measurement	**Baseline**	**Prior to Trastuzumab**	**3 months on Trastuzumab (change from baseline)**
Non-outcome Participants (median (IQR))	67% (62-69%) $n = 18$	61% (59-64%) $n = 15$	59% (55-65%) $n = 18$
Outcome Participant # 9	53%	55%	42% (-11%)
Outcome Participant #17	61%	56%	49% (-12%)
Outcome Participant #3	57%	51%	43% (-14%)
Outcome Participant #4	56%	56%	45% (-11%)

Table 2.9. Subgroup 3 LVEF to assess Cardiotoxicity. Assessment based on LVEF measurements during baseline, prior to trastuzumab, and 3 months on treatment, comparing the participants who experienced cardiotoxicity to the median of those who did not experience cardiotoxicity.

2.4 Discussion

2.4.1 Assessment of Myocardial Injury in Female Breast Cancer Patients using hs-cTnI

Many studies utilize a more conventional approach to obtain biomarker measurements in study participants, such as measuring before each new cycle of treatment (approximately once every 3 weeks). By only measuring once before a new cycle of treatment, clinicians may miss early myocardial injury after the commencement of treatment. Additionally, by 3 weeks the biomarker concentrations may have already normalized, leaving little to no trace of the potential acute injury. Thus, by having biomarker measurements the day following trastuzumab initiation, it is believed that acute cardiac events would not be missed. The median hs-cTnI concentrations from this subgroup increased (from baseline to C1D2) by 19 ng/L (Beckman and Abbott hs-cTnI assays) (Table 2.1), demonstrating a large elevation in troponin after the initiation of curative treatment. It has been documented that breast cancer patients have a 5-10% risk of developing cardiotoxicity from trastuzumab therapy [12]. However, by employing the sex-specific 99th percentile cutoffs, more than 50% of participants were identified as having myocardial injury throughout cycle 1, and thus at risk for future cardiac damage (Table 2.2). As for the absolute change criterion, 35-60% of participants were identified as having evolving acute myocardial injury during cycle 1 (Table 2.3). This was much higher than the anticipated 5%. There was poor correlation between the Beckman and Abbott hs-cTnI assays at baseline, as the participants' concentrations were all normal (rho = 0.01). However, there was a strong correlation after the commencement of trastuzumab treatment (C1D2 rho = 0.99), suggesting that both assays are identifying the same trends in these participants in cycle 1 (Fig. 2.4B). This leads to the question of whether the increases in cardiac troponin concentrations are a result of trastuzumab exposure

acting as an initial stressor to the heart, or is the prior anthracycline exposure acting in concert with trastuzumab resulting in the increasing cardiac troponin concentrations.

2.4.2 Longitudinal Assessment of Cardiotoxicity in Female Breast Cancer Patients using hs-cTnI

As so many participants were identified as having myocardial injury early after the commencement of trastuzumab therapy (and some prior to trastuzumab), it was of interest to assess subsequent cycles of therapy and LVEF to inquire if these participants actually developed cardiotoxicity after 3 months on trastuzumab treatment. Surprisingly, only 2 of these participants experienced significant LVEF decline causing them to withdraw from the study and thus terminate their trastuzumab treatment. For participant #9, their hs-cTnI concentrations early after the initiation of trastuzumab treatment were well above the ULN, followed by a decline during cycle 3 (Table 2.6). As for participant #17, their hs-cTnI concentrations were elevated during cycle 1 but decreased by cycle 2 (Table 2.6). Both participants were included in the cohort that had a change ≥12 ng/L from baseline to cycle 1, as analyzed in subgroup 1 (Table 2.3). Using published 99th percentile ULN cutoffs obtained from healthy populations identified a significant prevalence of myocardial injury in the breast cancer population. This is not unique to hs-cTnI, as similar findings of persistently elevated hs-cTnT during the first 12 weeks on trastuzumab have been documented as well [54]. The issue may lie in clinical specificity with emphasis on adhering to 99th percentile ULN values, and not analytical specificity. Perhaps a different set of hs-cTn criteria are needed for assessing early cardiotoxicity in the female breast cancer population, as using the sex-specific ULN from a healthy population was not a predictor of myocardial injury in this subgroup. From a different perspective, we are unaware of possible

myocardial injury or hs-cTnI trends during the anthracycline chemotherapy prior to trastuzumab due to lack of serial measurements during that timeframe. This is a limitation for the study.

2.4.3 Overall Myocardial Injury Prevalence and Assessment of Cardiotoxicity in Breast Cancer Patients using hs-cTnI

To evaluate the biomarker concentrations from a broader perspective, all participants who enrolled and remained in CABOT were included for analyses in this subgroup. The increase in concentrations from baseline to C1D1 was expected, as anthracyclines are known to be a cardiotoxic agent. It should be noted that we do not have information on the hs-cTnI trends for these participants between the end of anthracycline treatment and the commencement of trastuzumab treatment in order to definitively state that the hs-cTnI concentration increase is solely from trastuzumab. But after the addition of trastuzumab, the concentrations continued to rise well above the overall cutoff, suggesting that trastuzumab does have an effect on the hs-cTnI concentration in these participants (Figs. 2.6A, B). Even with this higher ULN, many participants still exhibited concentrations well above the cutoff. By cycle 3 (C3D1), Abbott hs-cTnI median concentrations were below the ULN (Abbott hs-cTnI median = 19 ng/L), with Beckman hs-cTnI slightly above the ULN (median = 19 ng/L) (Figs. 2.6A, B). Since data outside of this setting indicate that higher cTnI result in a worse outcome, C2D2 was selected for the analysis because the data demonstrated the highest hs-cTnI concentrations [27]. With no distinctive or significant difference in hs-cTnI concentrations between the participants with the decline in LVEF and those without at this timepoint (C2D2), it remains unclear which participants are at highest risk for subsequent cardiotoxicity. Based on this pilot study, the results do not support the routine use of hs-cTnI alone for early detection of cardiac toxicity in this breast cancer treatment setting, as

myocardial injury is very prevalent when using healthy population-based ULN cutoffs, demonstrating a lack of optimal specificity in the breast cancer setting.

2.5 Conclusion

Early, longitudinal and overall assessments of cardiac troponin revealed that elevations are evident after the initiation of trastuzumab treatment with concentrations peaking before the third cycle of treatment. However, this injury may be transient in nature as the median concentrations for hs-cTnI drop by the third cycle, regardless of the assay or subgroup analyzed. Using the hs-cTnI change criterion and the 99th percentiles resulted in a high prevalence of myocardial injury in the breast cancer participants. Therefore, the role of high- sensitivity cardiac troponin in the identification of early cardiotoxicity in the breast cancer population needs further investigation, perhaps with focus on risk cutoffs or in combination with another variable/test and additional timepoints to assess cardiac injury during anthracycline chemotherapy and after administration of trastuzumab.

Chapter 3: NT-proBNP, H-FABP, and CRP in Patients Receiving Adjuvant Trastuzumab Therapy

3.1 Introduction

As outlined in chapter 1, NT-proBNP, H-FABP and CRP are excellent biomarkers used in the detection and evaluation of myocardial injury and heart failure. In this chapter, these biomarkers were analyzed in a similar fashion to hs-cTnI, using participants from the CABOT study, with the purpose of identifying participants at risk for cardiotoxicity before damage becomes evident via imaging as indicated by a reduction in the LVEF.

3.2 Methods

For these analyses, EDTA plasma samples were obtained from 22 HER2+ breast cancer participants from the CABOT study, as outlined in Chapter 2 (Fig. 3.1).

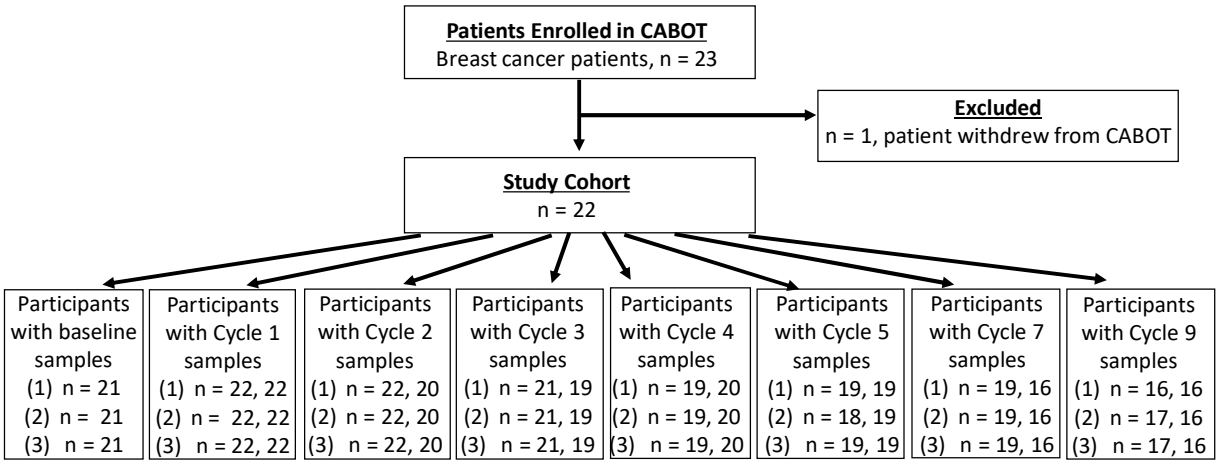

Figure 3.1. **Participant Inclusion Criteria**. Flow diagram of the participants included in chapter 3 analyses for days 1 and 2 during each cycle on trastuzumab. (1) NT-proBNP, (2) H-FABP, (3) CRP.

The samples were drawn at baseline, prior to initiation of trastuzumab, again the following day, for 5 subsequent cycles. Overall ULNs were used to assess the prevalence of acute injury, using the respective biomarker cut-off values: NT-proBNP = Roche proBNP II assay (97.5th percentile): 125 ng/L; H-FABP = Randox H-FABP assay (99th percentile): 6.32 µg/L; and CRP =

Beckman Coulter SYNCHRON System hs-CRP assay (95th percentile): 7.44 mg/L [36, 47, 55]. LVEF was measured at baseline then after 3 months on trastuzumab, using the outcome criteria of a decline of at least 10% with a final LVEF of <50%, whether they discontinued or remained on treatment, as explained for subgroup 3 in chapter 2 [3, 53]. Mann Whitney tests were used to compare the change from day 1 to 2 during cycle 1 on trastuzumab treatment, to compare the difference in concentration from baseline to the timepoint at which the median concentration was highest, and to compare the participants with cardiotoxicity versus those who did not experience cardiotoxicity at the timepoint of highest median concentration. Descriptive statistical analyses were also performed. Non-parametric statistical analyses were performed in MedCalc version 17.9.7 (p <0.05 considered significant). Data was compiled using Microsoft Excel spreadsheets to create an overall database containing all the information on participant biomarker concentrations and medical history for the CABOT study. The lead investigator on the study provided participant CRFs for any information that was missing in order to complete the datasets.

3.3 Results

3.3.1 Overall Assessment of Cardiotoxicity in Breast Cancer Patients using NT-proBNP

Participant demographics are detailed in chapter 2, table 2.7. Prior to the initiation of chemotherapy, 2 participants had NT-proBNP concentrations above ULN. Prior to the commencement of trastuzumab (Cycle 1, Day 1, C1D1), 10 participants had NT-proBNP >ULN. The following day (C2D2), the number of participants with NT-proBNP >ULN rose to 18 (Table 3.1). The median concentrations for NT-proBNP increased from baseline (60 ng/L) to cycle 1 of trastuzumab, with the following day yielding significantly higher concentrations (C1D1 median

= 108 ng/L, C1D2 median = 214 ng/L; $p = 0.01$) (Table 3.1). The timepoint that yielded the highest median concentration for NT-proBNP was C1D2 (214 ng/L) with 82% (18/22) of participants exceeding the ULN, which was a significant increase from baseline ($p = 0.0001$) (Table 3.1). By cycle 3, NT-proBNP concentrations were below the ULN (C3D1 median = 60 ng/L) (Fig. 3.2). Three months after the initiation of trastuzumab, 4 participants had a significant reduction in LVEF (Table 3.2). There was no significant difference in the concentrations of NT-proBNP at C1D2 between participants with the outcome versus those without ($p = 0.8$).

Timepoint (# of participants)	**Baseline** *n* = 21	**Cycle 1** *n* = 22, 22	**Cycle 2** *n* = 22, 20	**Cycle 3** *n* = 21, 19	**Cycle 4** *n* = 19, 20	**Cycle 5** *n* = 19, 19	**Cycle 7** *n* = 19, 16	**Cycle 9** *n* = 16, 16
NT-proBNP (ng/L) - Median (IQR)	60 (46-85)	Day 1: 108 (78-141) Day 2: 214 (145-381)	Day 1: 79 (43- 103) Day 2: 96 (52-177)	Day 1: 60 (41-133) Day 2: 53 (37-136)	Day 1: 52 (33-84) Day 2: 66 (51-81)	Day 1: 56 (37-69) Day 2: 54 (39-90)	Day 1: 61 (43-91) Day 2: 70 (58-106)	Day 1: 46 (33-73) Day 2: 54 (41-86)
# patients >ULN	2	10, 18	5, 7	6, 7	2, 4	1, 3	3, 4	3, 3

Table 3.1. Median (IQR) NT-proBNP Concentrations with >ULN Incidence. Listed in ng/L, during the timepoints assessed in chapter 3 along with the number of participants whose concentrations exceeded the ULN at that timepoint.

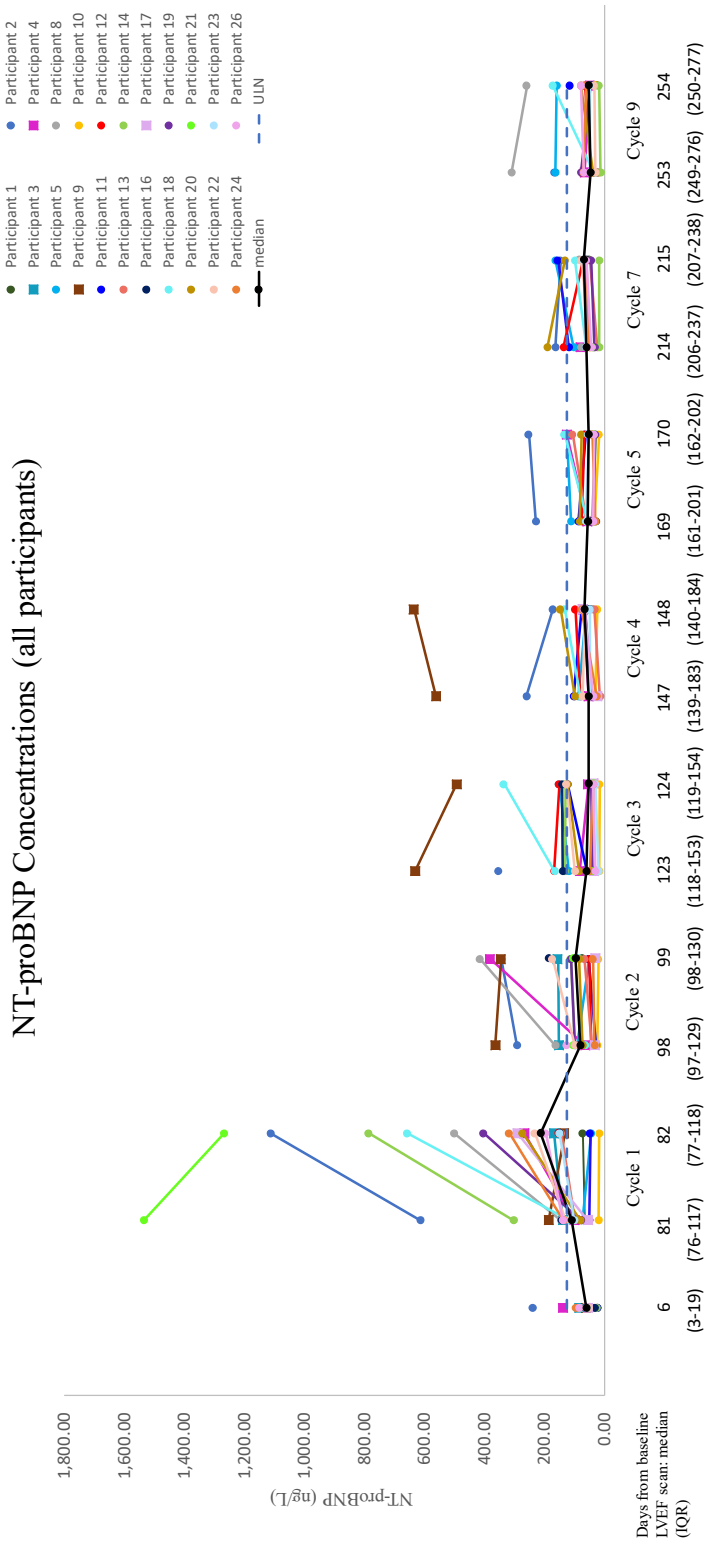

Figure 3.2. NT-proBNP Concentrations (all participants). Plot of NT-proBNP concentrations (ng/L) during each timepoint assessed in 3.3.1. Available data for days 1 and 2 of each cycle are connected. Listed below the X axis are the median (IQR) days from which the baseline LVEF was measured. Participants with cardiotoxicity are squares, those without are circles. Dashed line is overall ULN.

LVEF measurement	**Baseline**	**Prior to Trastuzumab**	**3 months on Trastuzumab (change from baseline)**
Non-outcome Participants (median (IQR))	67% (62-69%) $n = 18$	61% (59-64%) $n = 15$	59% (55-65%) $n = 18$
Outcome Participant # 9	53%	55%	42% (-11%)
Outcome Participant #17	61%	56%	49% (-12%)
Outcome Participant #3	57%	51%	43% (-14%)
Outcome Participant #4	56%	56%	45% (-11%)

Table 3.2. **LVEF to assess Cardiotoxicity**. Assessment based on LVEF measurements during baseline, prior to trastuzumab, and 3 months on treatment, comparing the participants who experienced cardiotoxicity to the median of those who did not experience cardiotoxicity.

3.3.2 Overall Assessment of Cardiotoxicity in Breast Cancer Patients using H-FABP

Prior to the initiation of chemotherapy, 1 participant had H-FABP >ULN. Prior to the commencement of trastuzumab, still only 1 participant had H-FABP >ULN. The following day, the number of participants with H-FABP >ULN rose to 3. The median concentrations for H-FABP remain below the ULN for each timepoint, with the first cycle yielding the lowest and highest concentrations (C1D1 median = 1.62 µg/L, C1D2 median = 3.24 µg/L; $p = 0.005$) (Table 3.3). However, only 3 of the 22 participants had concentrations >ULN at C1D2. Compared to the median baseline concentration (2.15 µg/L), the elevation observed during C1D2 was not significant ($p = 0.1$) (Table 3.3, Fig. 3.3). There was no significant difference in the concentrations of H-FABP at C1D2 between participants with the outcome versus those without ($p = 0.9$).

Timepoint (# of participants)	**Baseline** *n* = 21	**Cycle 1** *n* = 22, 22	**Cycle 2** *n* = 22, 20	**Cycle 3** *n* = 21, 19	**Cycle 4** *n* = 19, 20	**Cycle 5** *n* = 18, 19	**Cycle 7** *n* = 19, 16	**Cycle 9** *n* = 17, 16
H-FABP (µg/L) - Median (IQR)	2.15 (1.41-3.29)	Day 1: 1.62 (0.93-2.85) Day 2: 3.24 (2.33-3.72)	Day 1: 2.35 (1.57-2.71) Day 2: 2.24 (1.77-3.23)	Day 1: 2.07 (1.35-3.30) Day 2: 2.02 (1.45-3.01)	Day 1: 1.86 (1.41-2.97) Day 2: 2.08 (1.40-3.60)	Day 1: 1.99 (1.24-3.19) Day 2: 2.13 (1.41-3.26)	Day 1: 2.35 (1.59-3.50) Day 2: 2.29 (1.82-3.74)	Day 1: 2.20 (1.52-4.02) Day 2: 2.07 (1.62-3.62)
# patients >ULN	1	1, 3	1, 1	1, 1	2, 2	0, 1	0, 0	0, 0

Table 3.3. Median (IQR) H-FABP Concentrations with >ULN Incidence. Listed in µg/L, during the timepoints assessed in chapter 3 along with the number of participants whose concentrations exceeded the ULN at that timepoint.

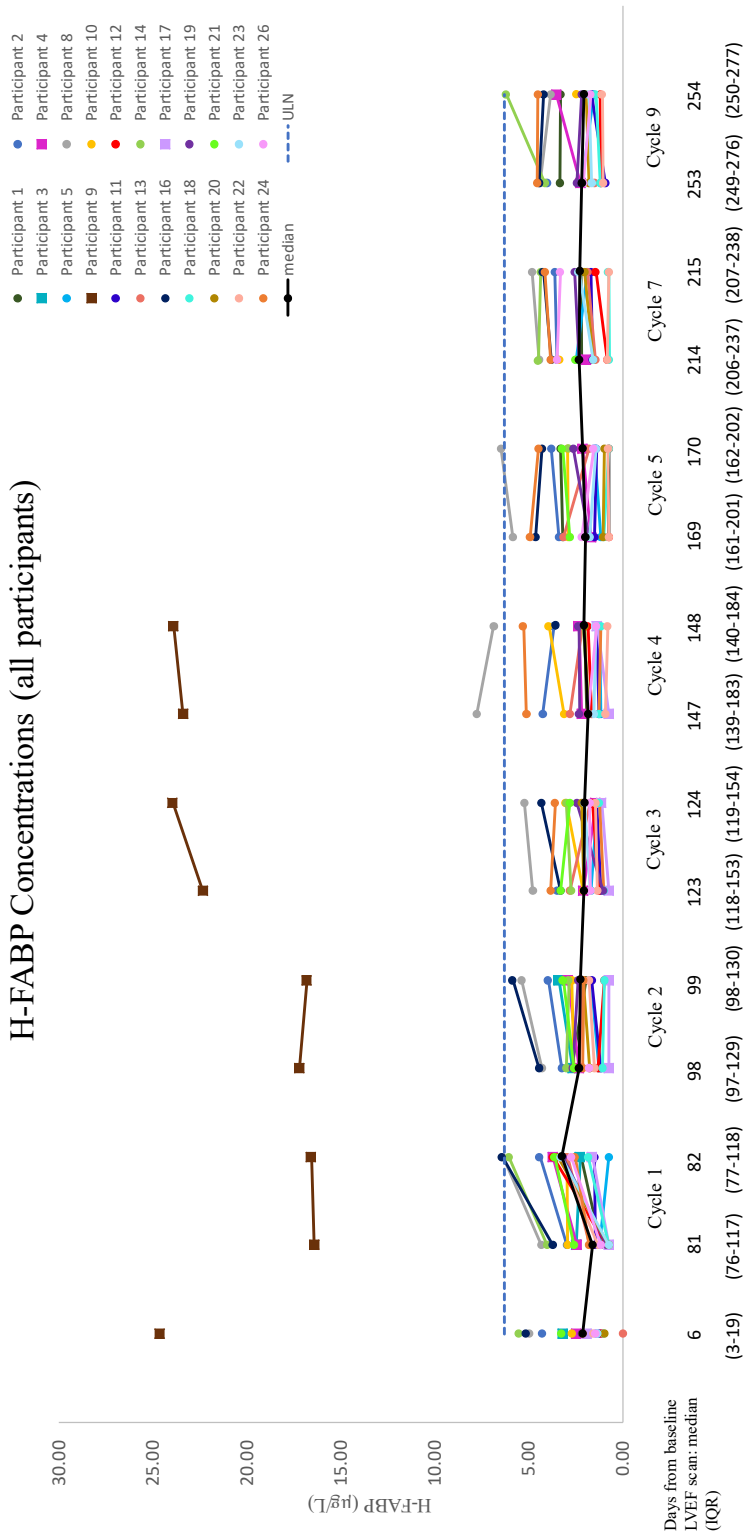

Figure 3.3. H-FABP Concentrations (all participants). Plot of H-FABP concentrations (µg/L) during each timepoint assessed in 3.3.2. Available data for days 1 and 2 of each cycle are connected. Listed below the X axis are the median (IQR) days from which the baseline LVEF was measured. Participants with cardiotoxicity are squares, those without are circles. Dashed line is ULN.

3.3.3 Overall Assessment of Cardiotoxicity in Breast Cancer Patients using CRP

Prior to the initiation of chemotherapy, 4 participants had CRP >ULN. Prior to the commencement of trastuzumab, 5 participants had CRP >ULN. The following day, 4 participants had CRP >ULN. The median concentrations for CRP also remain below the ULN for each timepoint, with no significant elevations during the first cycle on trastuzumab ($p = 0.8$) (Table 3.4). Cycle 2 yielded the highest median concentration (C2D1 median = 3.35 mg/L), with only 3 participants >ULN. Compared to the median baseline concentration (2.38 mg/L), the elevation to C2D1 was not significant ($p = 0.3$) (Table 3.4, Fig. 3.4). There was no significant difference in the concentrations of CRP at C2D1 between participants with the outcome versus those without ($p = 0.5$).

Timepoint (# of participants)	**Baseline** $n = 21$	**Cycle 1** $n = 22, 22$	**Cycle 2** $n = 22, 20$	**Cycle 3** $n = 21, 19$	**Cycle 4** $n = 19, 20$	**Cycle 5** $n = 19, 19$	**Cycle 7** $n = 19, 16$	**Cycle 9** $n = 17, 16$
CRP (mg/L) - Median (IQR)	2.38 (0.92-4.67)	Day 1: 3.19 (1.47-6.29) Day 2: 2.98 (1.83-6.29)	Day 1: 3.35 (1.64-5.81) Day 2: 3.08 (1.35-5.92)	Day 1: 2.50 (1.00-6.13) Day 2: 1.93 (0.97-6.68)	Day 1: 1.03 (0.54-3.56) Day 2: 1.03 (0.54-3.01)	Day 1: 0.83 (0.70-3.31) Day 2: 1.02 (0.65-3.95)	Day 1: 1.21 (0.66-2.40) Day 2: 1.12 (0.51-2.00)	Day 1: 1.06 (0.70-1.85) Day 2: 1.36 (0.60-2.45)
# patients >ULN	4	5, 4	3, 3	4, 4	1, 3	3, 4	2, 2	2, 2

Table 3.4. Median (IQR) CRP Concentrations with >ULN Incidence. Listed as mg/L, during the timepoints assessed in chapter 3 along with the number of participants whose concentrations exceeded the ULN at that timepoint.

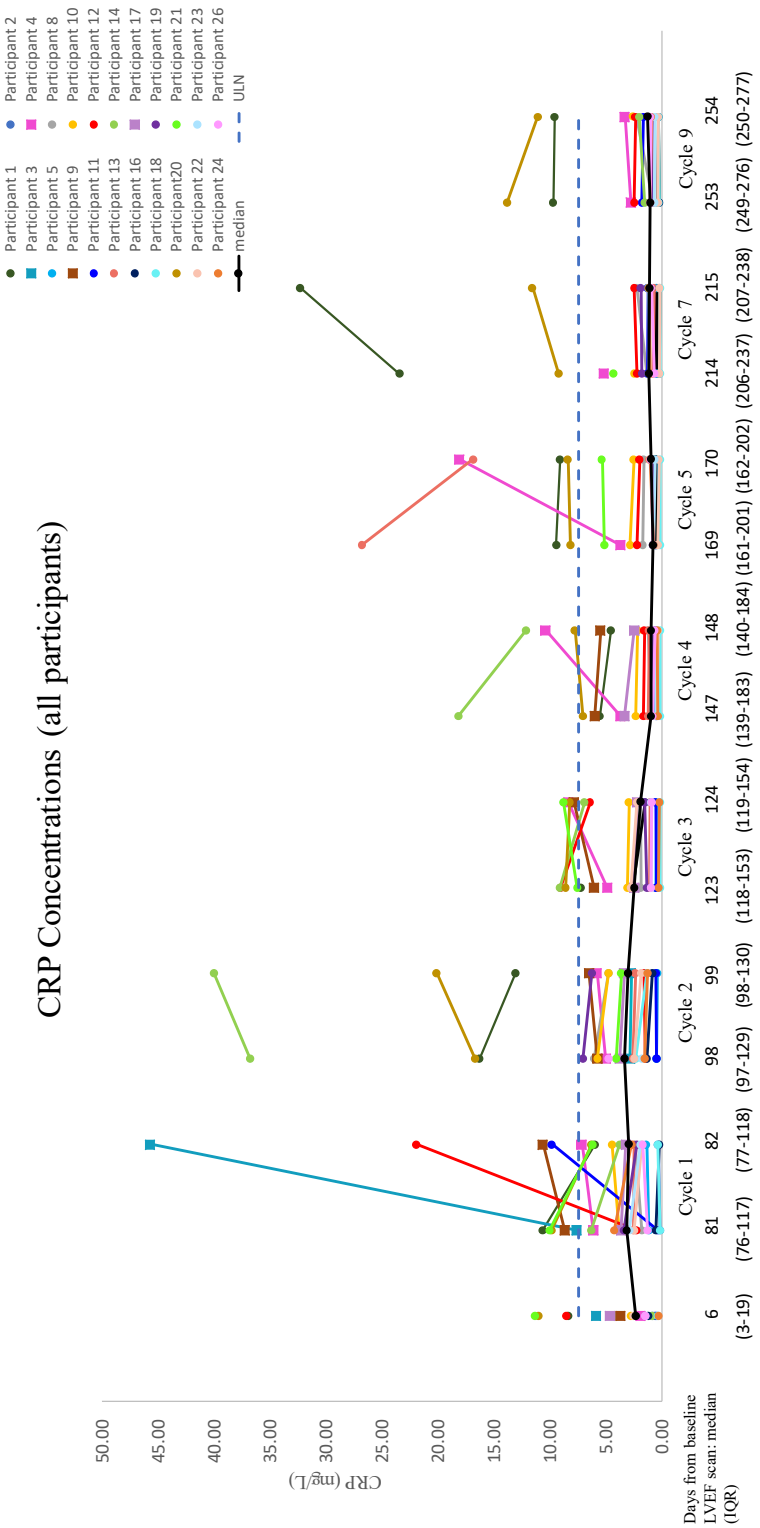

Figure 3.4. CRP Concentrations (all participants). Plot of CRP concentrations (mg/L) during each timepoint assessed in 3.3.3. Available data for days 1 and 2 of each cycle are connected. Listed below the X axis are the median (IQR) days from which the baseline LVEF was measured. Participants with cardiotoxicity are squares, those without are circles. Dashed line is ULN.

3.4 Discussion

As in chapter 2, the median concentrations of biomarkers involved with acute cardiotoxicity between those with a decline in LVEF versus those who did not experience cardiotoxicity were compared to observe if the difference between the two groups were significant. This would inform on whether these biomarkers would be a better tool for aiding in the prediction of cardiotoxicity in the breast cancer population. There was no difference in NT-proBNP, H-FABP or CRP between those with versus those without a decline in LVEF. Thus, these data do not support the routine use of these biomarkers alone in the detection of possible cardiotoxicity in this setting. Like hs-cTnI, concentrations of NT-proBNP rose significantly early after the initiation of trastuzumab, signifying an effect that trastuzumab has on NT-proBNP concentrations (Fig. 3.2). Also, use of the overall ULN cutoff (as stated in the ESC guidelines for NT-proBNP) identified more participants as having early myocardial dysfunction than those who subsequently developed cardiotoxicity, as 3 of the 10 participants with NT-proBNP >ULN at C1D1 and 4 of the 18 participants with NT-proBNP >ULN at C1D2 actually had a LVEF decline. Further studies should evaluate the use of the sex-specific ULN for NT-proBNP to observe whether similar results are found, as presently only age (and not sex) is used for the diagnosis of heart failure in the cardiac setting [41]. H-FABP is a marker commonly used for myocardial injury, however in this setting the prevalence of injury was lower compared to hs-cTnI seen in chapter 2. Notably, for participant #9 who experienced cardiotoxicity, their H-FABP concentrations were extremely high at baseline (Fig. 3.3). It remains unclear as to why this participant had such elevations. Besides this participant, all but 2 other participants had H-FABP concentrations below the ULN cutoff at all timepoints, suggesting that H-FABP may be more specific in these analyses, but not very sensitive, as 3 of the 4 outcome participants were

missed using the ULN cutoff (Fig. 3.3). As for CRP, cycle 1 did not produce a significant change in median concentrations from day 1 to day 2 like H-FABP and NT-proBNP. Although for participant #3 (who happened to have cardiotoxicity) their CRP concentration significantly increased after the initiation of trastuzumab. Three of the 4 participants with cardiotoxicity did have CRP concentrations >ULN at least once during trastuzumab treatment, perhaps making it more sensitive in this setting (Fig. 3.4). However, the prevalence of CRP >ULN in participants without cardiotoxicity was 39% (7 of the 18 participants without LVEF decline), and thus not very specific. A majority of the participants who experienced cardiotoxicity had to stop treatment just before the 5th cycle, and based on the longitudinal profile of CRP, it is unclear when the most optimal time would be to measure the protein from this indication.

3.5 Conclusion

Elevations were evident in NT-proBNP early after the initiation of trastuzumab treatment with concentrations peaking by the 2nd day of cycle 1, however this was experienced by a majority of the participants, not just those with cardiotoxicity. CRP might be more informative than H-FABP in this setting, however additional strategies and perhaps a unique set of cutoff criteria and different combinations of biomarkers may prove to be more useful for predicting cardiotoxicity.

Chapter 4: Assessment of the Biomarker Profile in Patients who Completed Trastuzumab Therapy with Normal LVEFs: A Sub-group Analysis

4.1 Introduction

As discussed in chapters 2 and 3, using ULN from a healthy population alone for various biomarkers was suboptimal for identifying cardiotoxicity. However, the ULN is related to the population where it is derived. Therefore, this may not be the most optimal approach for cancer patients who successfully complete adjuvant treatment and have normal LVEF. This suggests that additional methods or metrics are needed in this setting. To this end, participants from the CABOT study who completed trastuzumab treatment and had normal LVEF measurements at each timepoint where selected to form a "treatment complete, normal LVEF" group. As the number of participants is too small to establish a ULN, a ratio of the participants' biomarker concentrations to the healthy population ULN was derived with a ratio >1 indicating that different cutoffs may be needed.

4.2 Methods

There were 20 female participants that had plasma collections at the times when LVEF was measured (prior to chemotherapy, prior to trastuzumab treatment initiation, 3 months on trastuzumab, and 6 months on trastuzumab) [56]. Specifically, EDTA plasma was collected on the first day of each cycle before trastuzumab and again the next day for a total of 7 cycles, equating to approximately 6 months of trastuzumab treatment [56]. Samples were tested for hs-cTnI (Abbott ARCHITECT STAT hsTnI assay with ULN = 16 ng/L and Beckman Coulter Access hsTnI assay with ULN = 12 ng/L), NT-proBNP (Roche proBNP II assay ULN = 252 ng/L), CRP (Beckman Coulter assay with ULN = 7.44 mg/L) and H-FABP (Randox assay with ULN = 6.3 µg/L) [36, 47, 51, 55] (see International Federation of Clinical Chemistry and

Laboratory Medicine's Committee on Clinical Applications of Cardiac Bio-Markers website for specific sex-specific cutoffs for hs-cTn and NT-proBNP: http://www.ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-bio-markers-c-cb). For these analyses, the female sex-specific cutoff for NT-proBNP was used, consistent to what has been proposed for hs-cTnI [32]. The cohort for these analyses consisted of 12 participants, as 4 participants had a missing LVEF and 4 participants had a LVEF <50% at one of the 3 LVEF measurement timepoints (Fig. 4.1) [56].

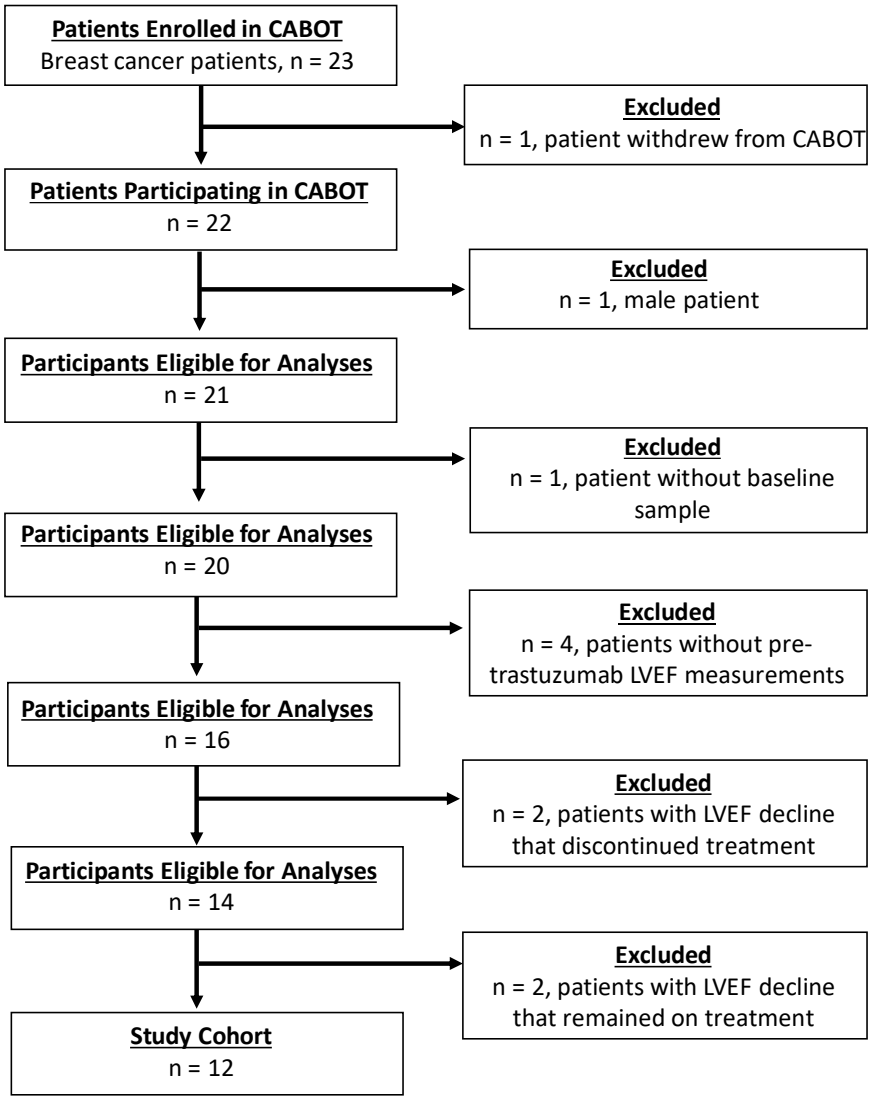

Figure 4.1. Participant Inclusion Criteria. Flow diagram of participants included in the "treatment complete, normal LVEF" group for chapter 4 analyses.

These 12 participants completed trastuzumab treatment without experiencing a decline in ejection fraction. ULN cutoffs were used to derive the ratio ([biomarker]/[ULN]) and to assess the prevalence of myocardial injury in this cohort, as well as in those who experienced cardiotoxicity. Specifically, to calculate the ratios, each of the biomarkers were divided by their respective ULN (concentration/ULN ratio) to normalize and plot the results from the "treatment complete, normal LVEF" group over the 15 different timepoints on one graph [56]. Here, ratios < 1 would indicate that the healthy population ULN may be appropriate, with ratios >1 suggesting a higher cutoff may be useful. After these analyses, the participants with the outcome in CABOT (decline of 10% with a final LVEF of <50%; $n = 4$) were analyzed against this 12-participant cohort also using the ULN. Differences in the concentration of each biomarker during each cycle on trastuzumab (Day 2- Day 1) was also calculated to determine the time at which the greatest change in biomarker concentration was experienced, as well as to assess how the changes in concentrations differ between the participants in CABOT with cardiotoxicity versus those without (ie. this 12-participant cohort), with the hypothesis that participants with LVEF decline will experience greater changes in their biomarker concentrations. Mann Whitney testing was used during the first cycle (C1D2) on trastuzumab treatment to compare the outcome participants against the "treatment complete, normal LVEF" group in order to analyze the initial effects of treatment, due to the previous observations of such high elevations during this time. Descriptive statistical analyses were also performed, along with non-parametric tests being employed via MedCalc version 17.9.7. Data was compiled using Microsoft Excel spreadsheets to create an overall database containing all the information on participant biomarker concentrations and medical history for the CABOT study.

4.3 Results

4.3.1 "Treatment Complete, Normal LVEF" Participants Results

The mean (SD) age of this 12-participant cohort was 50 (10) years. Prior to the initiation of chemotherapy, concentrations of hs-cTnI and NT-proBNP in all 20 female CABOT participants were below the sex-specific ULNs, with 1 participant >ULN for H-FABP and 4 participants >ULN for CRP, suggesting that the ULN for hs-cTnI, NT-proBNP and H-FABP are appropriate for these cancer patients before treatment (as it is required to have 18 of 20 results fall below the ULN to accept per laboratory recommendations) [56]. As the "treatment complete, normal LVEF" group number is smaller than 20, the median of the 12 participants was determined to provide a distribution (see Table 4.1). After chemotherapy but prior to the initiation of trastuzumab treatment, nearly half of the participants had hs-cTnI >ULN (C1D1), with >50% of the participants having concentrations >ULN for hs-cTnI and NT-proBNP the next day after trastuzumab treatment (C1D2) (Table 4.1, Fig. 4.2) [56]. This trend for hs-cTnI remained for two more subsequent cycles, before dropping at cycle 4 with steady concentrations for the rest of the cycles (Fig 4.2), whereas for NT-proBNP the median concentration decreased by the next cycle (cycle 2) [56]. The median/ULN ratio was >1 on C1D2 for NT-proBNP and hs-cTnI, with the ratio being >1 for hs-cTnI until cycle 4 (Fig. 4.2). As for H-FABP and CRP, the median/ULN ratios were <1 at all timepoints, with no more than 2 participants yielding H-FABP >ULN and no more than 3 patients yielding CRP >ULN over the course of the treatments (Fig. 4.2). The timepoint that yielded a majority of the participants with concentrations exceeding the ULN for NT-proBNP was C1D2 (n = 7), while for hs-cTnI it was on C2D1 (Beckman n = 11 and Abbott n = 9) [56]. The median (range) change from day 1 to day 2 for all biomarkers was highest during the first cycle on trastuzumab (NT-proBNP median = 186 ng/L (-30 to 540);

Abbott hs-cTnI median = 8 ng/L (-2 to 22); Beckman hs-cTnI median = 7 ng/L (-3 to 29); CRP median = 0.15 mg/L (-3.55 to 19.69); H-FABP median = 1.79 µg/L (-0.51 to 2.87) (Table 4.2).

Median Concentration (n = 12)	**Baseline**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**	**Cycle 7**	**Cycle 9**
CRP (mg/L)	1.41	Day 1: 2.07 Day 2: 2.33	Day 1: 3.60 Day 2: 1.63	Day 1: 1.66 Day 2: 1.57	Day 1: 0.97 Day 2: 0.78	Day 1: 0.82 Day 2: 0.96	Day 1: 1.10 Day 2: 116	Day 1: 1.06 Day 2: 1.04
H-FABP (µg/L)	2.18	Day 1: 1.52 Day 2: 3.33	Day 1: 2.36 Day 2: 2.36	Day 1: 2.08 Day 2: 2.44	Day 1: 1.86 Day 2: 2.23	Day 1: 2.18 Day 2: 2.77	Day 1: 3.45 Day 2: 3.32	Day 1: 2.44 Day 2: 2.18
Beckman hs-cTnI (ng/L)	0.95	Day 1: 13 Day 2: 18	Day 1: 41 Day 2: 45	Day 1: 24 Day 2: 30	Day 1: 11 Day 2: 10	Day 1: 8 Day 2: 8	Day 1: 5 Day 2: 6	Day 1: 6 Day 2: 6
Abbott hs-cTnI (ng/L)	3	Day 1: 15 Day 2: 20	Day 1: 37 Day 2: 46	Day 1: 21 Day 2: 22	Day 1: 12 Day 2: 11	Day 1: 9 Day 2: 9	Day 1: 6 Day 2: 7	Day 1: 6 Day 2: 6
NT-proBNP (ng/L)	60	Day 1: 126 Day 2: 294	Day 1: 88 Day 2: 86	Day 1: 101 Day 2: 123	Day 1: 46 Day 2: 66	Day 1: 51 Day 2: 51	Day 1: 61 Day 2: 72	Day 1: 69 Day 2: 65

Table 4.1. Median of CRP, H-FABP, hs-cTnI and NT-proBNP Concentrations. For the "treatment complete, normal LVEF" group during all timepoints assessed in chapter 4.

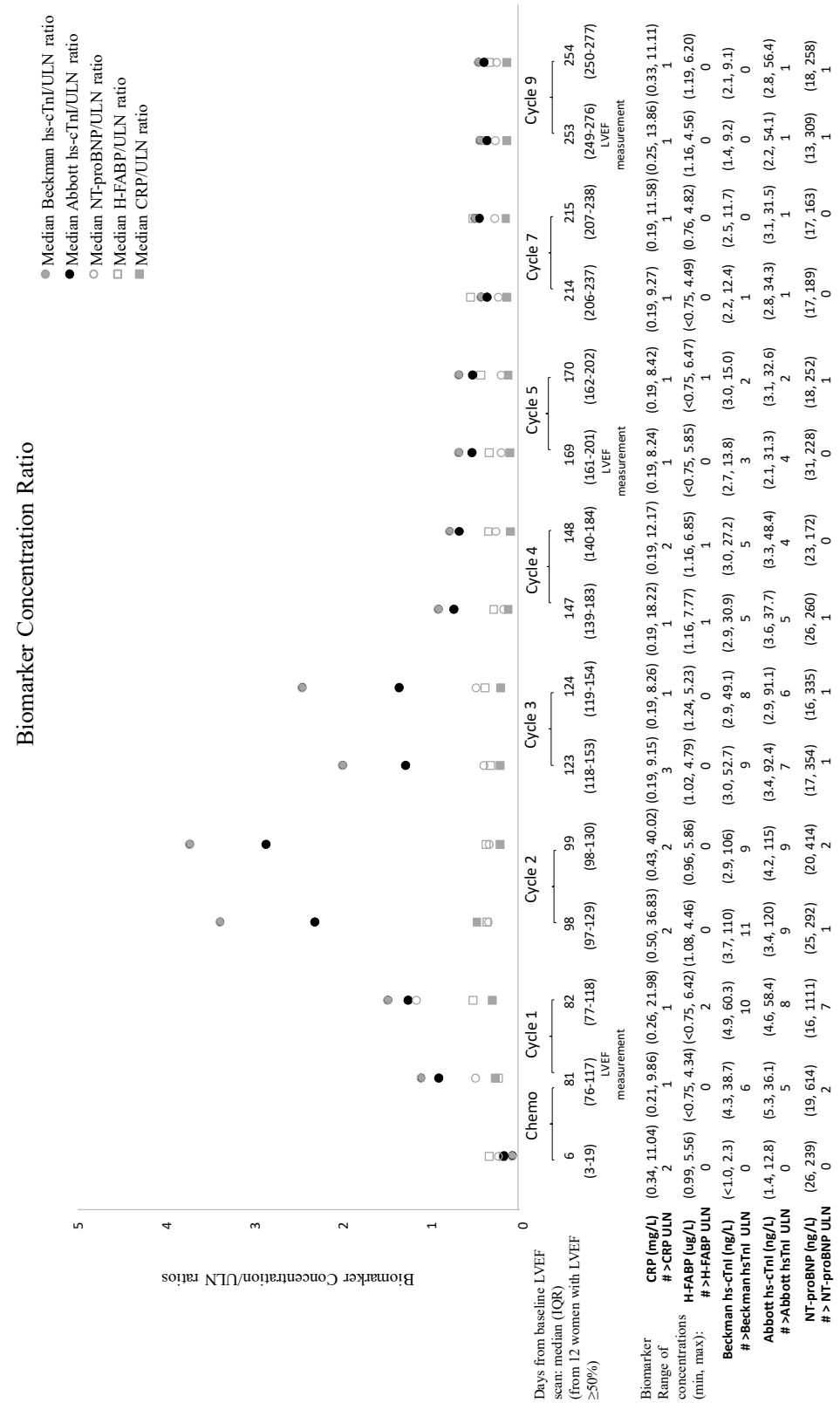

Figure 4.2. **Biomarker Concentration Ratios**. The profile of CRP, H-FABP, hs-cTnI and NT-proBNP as expressed by the median concentration/ULN for the "treatment complete, normal LVEF" group. Range of concentrations and number of women >ULN is below the graph [56].

Cycle # (D2-D1)	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 7	Cycle 9
CRP (mg/L) - Median (range)	0.15 (-3.55 to 19.69)	-0.28 (-1.33 to 3.46)	-0.06 (-2.66 to 0.18)	0 (-6.05 to 0.67)	-0.04 (-0.36 to 0.33)	0.09 (-0.08 to 2.31)	-0.02 (-2.75 to 1.52)
H-FABP (µg/L) - Median (range)	1.79 (-0.51 to 2.87)	0.13 (-0.37 to 1.40)	0.26 (-0.34 to 1.39)	0.06 (-0.92 to 0.80)	0 (-0.63 to 0.83)	0.21 (-0.59 to 0.61)	-0.15 (-0.66 to 2.05)
Beckman hs-cTnI (ng/L) - Median (range)	7 (-3 to 29)	1 (-14 to 15)	-2 (-9 to 11)	-0.1 (-4 to 2)	0.3 (-2 to 1)	-0.3 (-0.9 to 2)	0.4 (-2 to 0.8)
Abbott hs-cTnI (ng/L) - Median (range)	8 (-2 to 22)	4 (-14 to 16)	-2 (-11 to 7)	0.4 (-3 to 11)	-0.6 (-4 to 2)	-0.07 (-3 to 2)	0.2 (-2 to 2)
NT-proBNP (ng/L) - Median (range)	186 (-30 to 540)	13 (-39 to 250)	3 (-18 to 168)	19 (-89 to 52)	-5 (-14 to 77)	8 (-65 to 60)	6 (-51 to 137)

Table 4.2. Median Difference between Day 1 and Day 2 Concentrations for "Treatment Complete, Normal LVEF" Participants. For each cycle, day 1 concentrations were subtracted from day 2 concentrations for CRP, H-FABP, hs-cTnI and NT-proBNP to determine the median change within each cycle. Range is listed underneath.

4.3.2 Outcome Participants' Results

When looking at the CABOT cohort as a whole, there were 4 participants with significantly reduced LVEF. Two patients completed trastuzumab treatment, while 2 participants had to discontinue treatment due to their LVEF decline. The range in LVEF values for the 4 outcome participants were: 53-63% (baseline), 55-64% (after chemo, prior to trastuzumab), 42-58% (3 months on trastuzumab), and 46-50% (6 months on trastuzumab) (Table 4.3).

LVEF Measurement (%)	**Baseline**	**Prior to Trastuzumab**	**3 months on Trastuzumab**	**6 months on Trastuzumab**
12- Participant Median (IQR)	65% (55-70%)	61% (50-66%)	59% (52-67%)	60% (52-67%)
Outcome participant #1	63%	64%	58%	46%
Outcome participant #4	56%	56%	45%	50%
Outcome participant #9	53%	55%	42%	-
Outcome participant #17	61%	56%	49%	-

Table 4.3. LVEF to assess Cardiotoxicity. Assessment based on LVEF measurements during baseline, prior to trastuzumab, 3 and 6 months on treatment; comparing the participants who experienced cardiotoxicity to the median of those who did not experience cardiotoxicity (ie., "treatment complete, normal LVEF" group).

Three of these participants have been discussed in chapters 2 and 3. However, when comparing them against the "treatment complete, normal LVEF" group in this chapter, all 4 participants had CRP greater than the median of this group at multiple times, 3 of which (participants #1, 4, 9) exceeded the ULN during at least one timepoint (Fig. 4.3A). As for H-FABP, only participant #9 drastically exceeded the ULN, while the other 3 participants maintained similar concentrations to the median of the "treatment complete, normal LVEF" group (Fig. 4.3B).

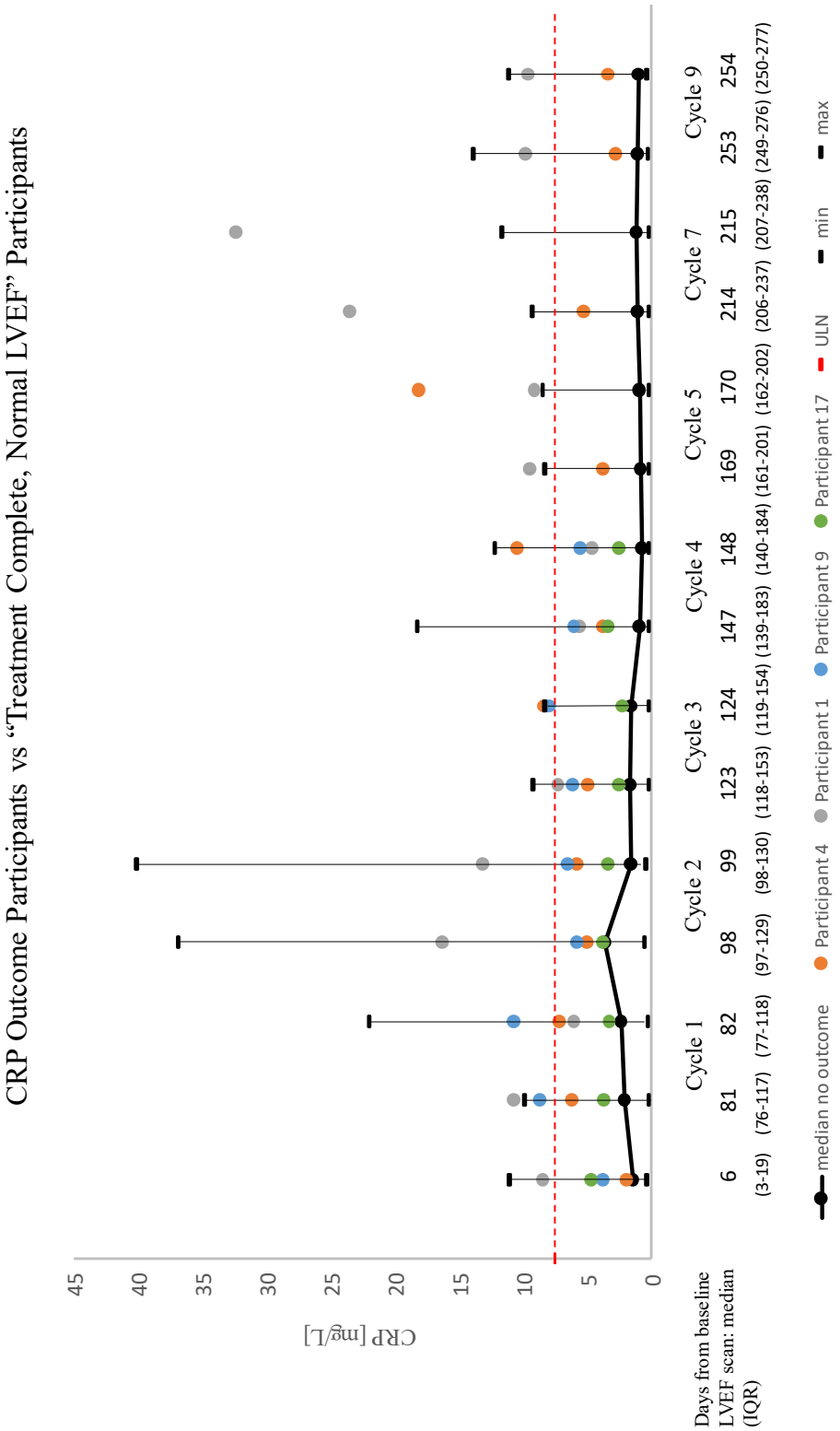

Figure 4.3A. CRP Outcome Participants vs "Treatment Complete, Normal LVEF" Participants. Plot of CRP concentrations from the participants who experienced cardiotoxicity with the median and range of those who did not. Red dashed line is the ULN.

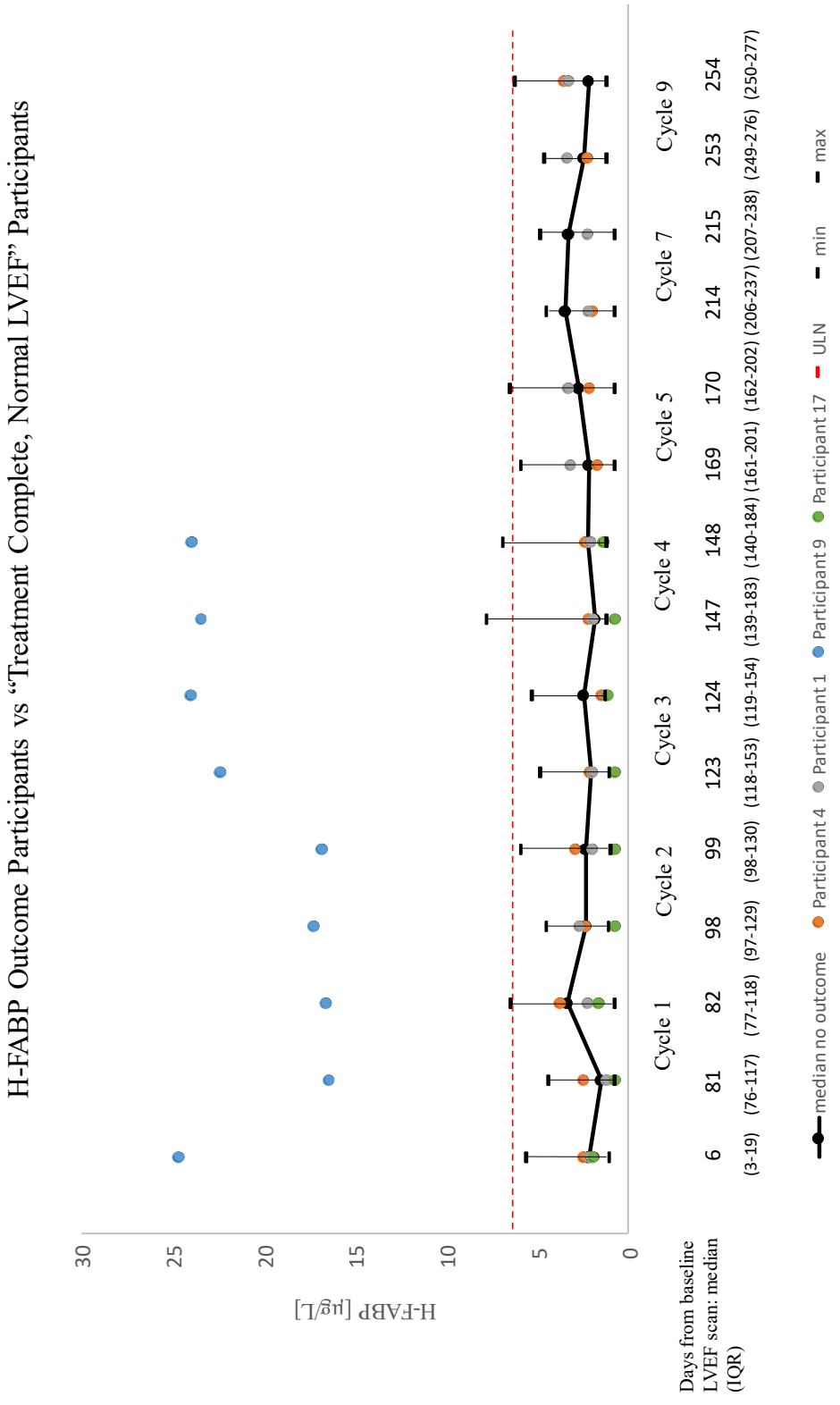

Figure 4.3B. **H-FABP Outcome Participants vs "Treatment Complete, Normal LVEF" Participants.** Plot of H-FABP concentrations from the participants who experienced cardiotoxicity with the median and range of those who did not. Red dashed line is the ULN.

For Beckman and Abbott hs-cTnI, a similar trend was observed for participants #1 and 9, with elevations evident during cycles 1 through 3, declining by cycle 4, but still greater than the "treatment complete, normal LVEF" group median at all timepoints (Figs. 4.3C, D). As for participants #4 and 17, they maintained concentrations below the "treatment complete, normal LVEF" group median and ULN for a majority of the timepoints. All 4 participants exceeded the ULN at least once during treatment for both Beckman and Abbott assays (Figs. 4.3C, D). For NT-proBNP, 3 of the 4 participants with cardiotoxicity exceeded the ULN at least once during treatment (Fig. 4.3E). Participants #1 and 17 followed a similar trend as the treatment complete normal LVEF" group. Participant #4 had a noticeable concentration above the group median and ULN during cycle 2, but was similar to the group median for the remainder of testing, while participant #9 exceeded the ULN and "treatment complete, normal LVEF" group median concentrations for the majority of testing while on trastuzumab treatment (Fig. 4.3E).

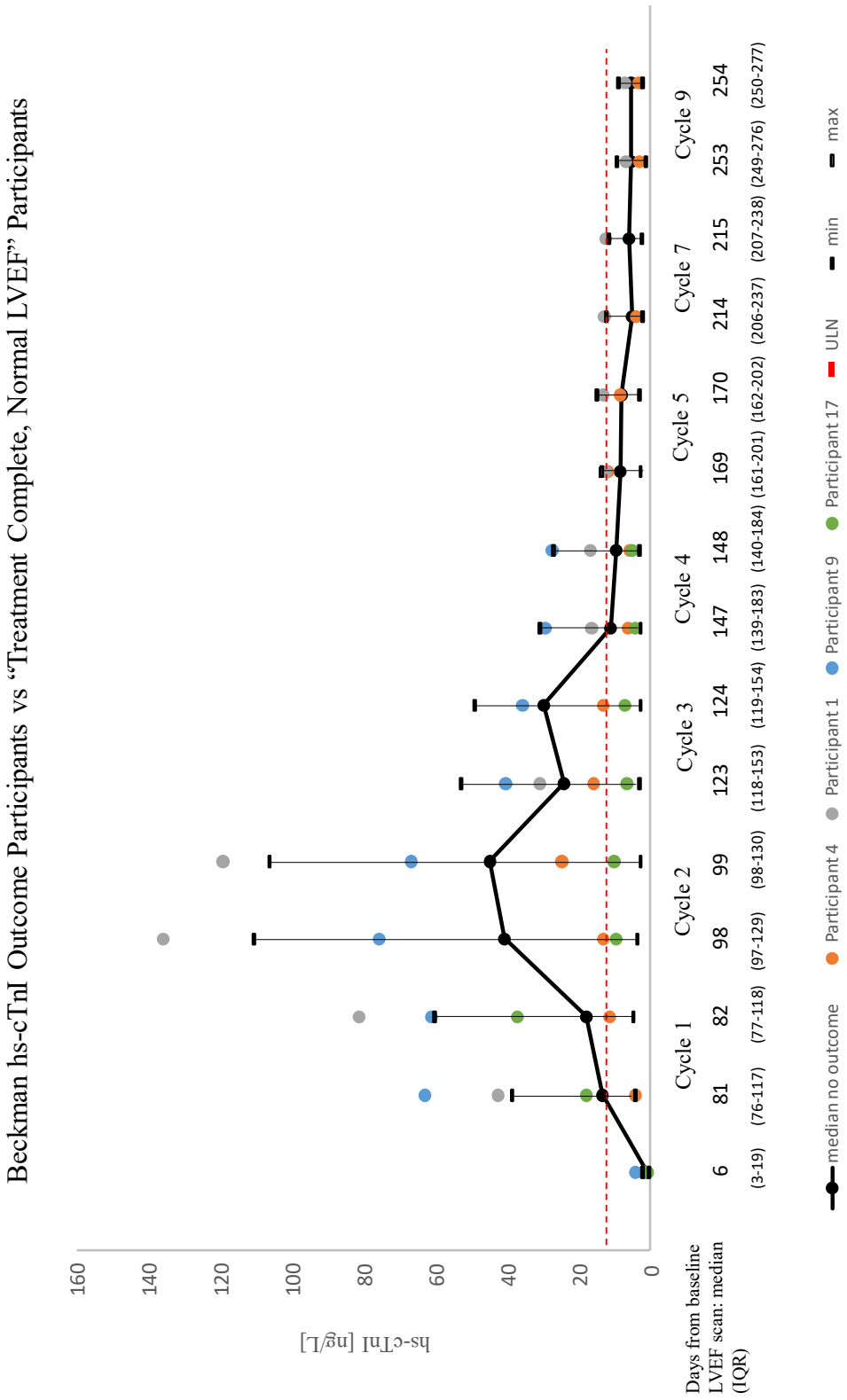

Figure 4.3C. Beckman hs-cTnI Outcome Participants vs "Treatment Complete, Normal LVEF" Participants. Plot of hs-cTnI concentrations from participants who experienced cardiotoxicity with the median and range of those who did not. Red dashed line is the ULN.

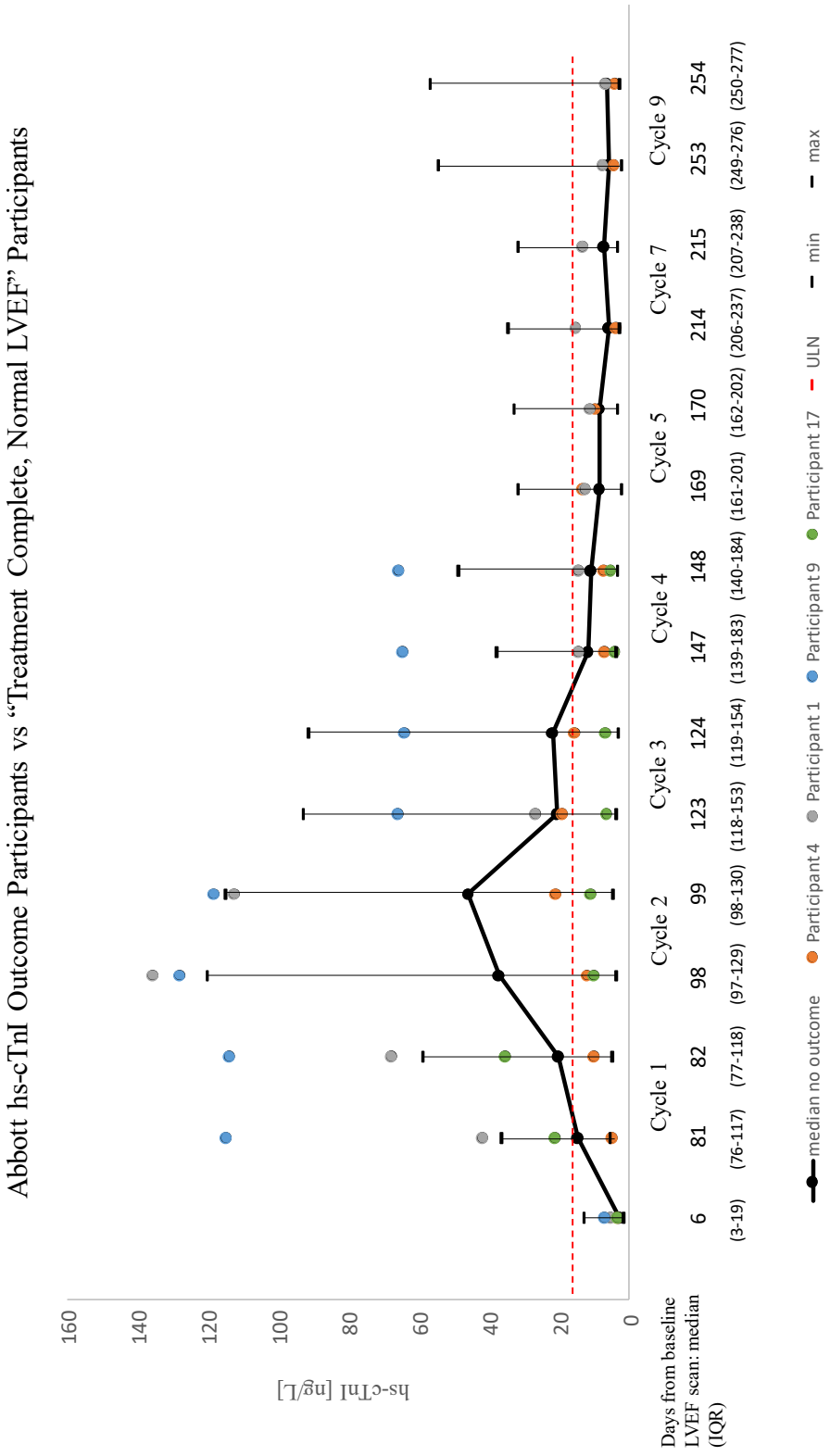

Figure 4.3D. Abbott hs-cTnI Outcome Participants vs "Treatment Complete, Normal LVEF" Participants. Plot of hs-cTnI concentrations from participants who experienced cardiotoxicity with the median and range of those who did not. Red dashed line is the ULN.

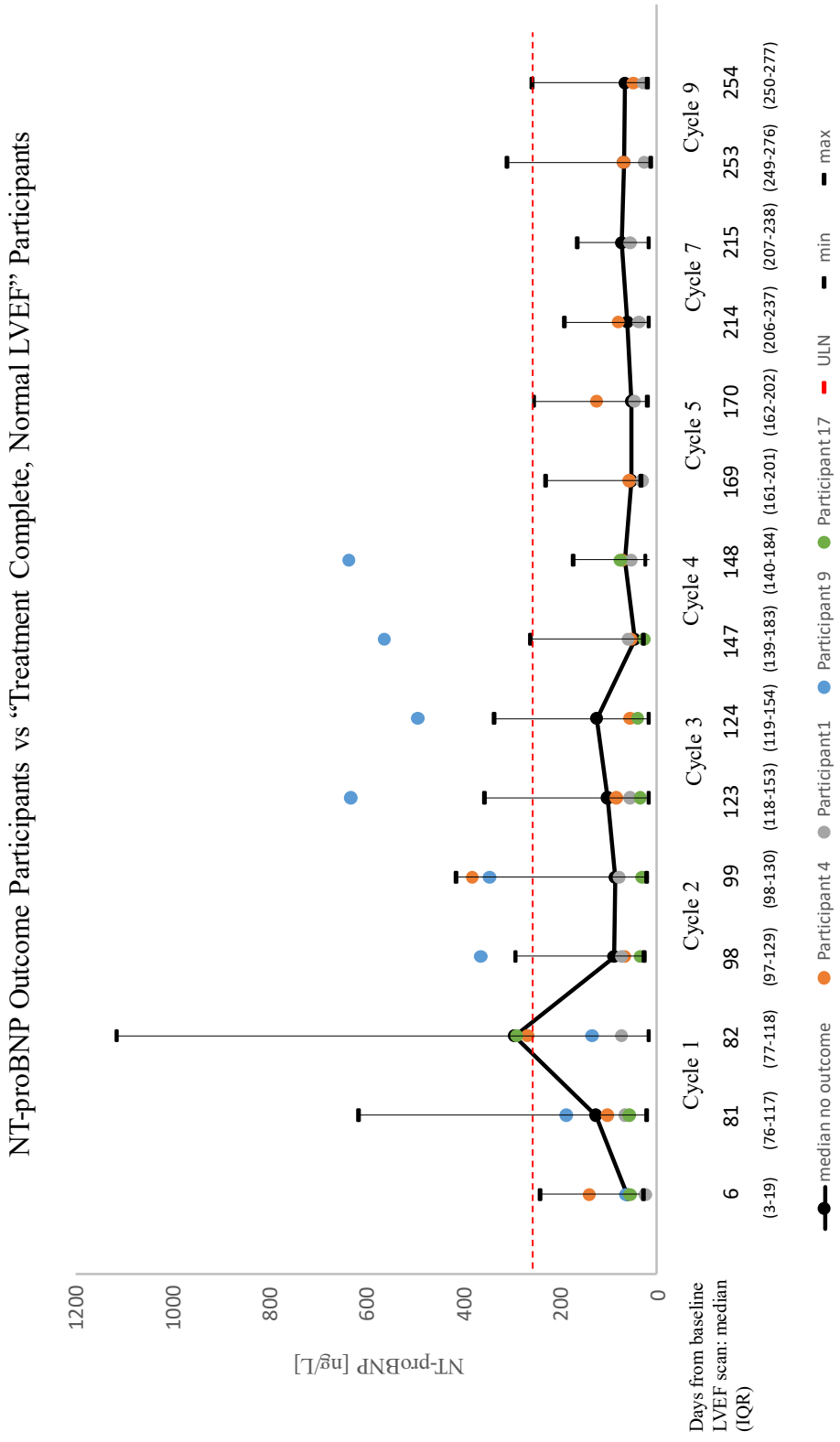

Figure 4.3E. NT-proBNP Outcome Participants vs "Treatment Complete, Normal LVEF" Participants. Plot of NT-proBNP concentrations from participants who experienced cardiotoxicity with the median and range of those who did not. Red dashed line is the ULN.

The changes from day 1 to day 2 were significant during the first two cycles on trastuzumab, but only for approximately one participant per biomarker (Table 4.4). During cycle 1, participant #9 experienced a large change in CRP, participant #1 had a large change in hs-cTnI, while participant #4 had a large change in their NT-proBNP concentration (Table 4.4).

Mann Whitney testing during C1D2 between the "treatment complete, normal LVEF" group and outcome participants revealed that the biomarkers were not significantly different between the two groups (CRP, $p = 0.06$; H-FABP, $p = 0.9$; hs-cTnI, $p = 0.3$ for both Beckman and Abbott assays; and NT-proBNP, $p = 0.3$).

Cycle # (D2-D1)	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**	**Cycle 7**	**Cycle 9**
CRP (mg/L)	-4.68, 1.02, 38.05, 1.95	-3.25, 0.74, -0.13, 0.70	*, 3.44, *, 1.77	-1.00, 6.65, *, -0.54	-0.36, 14.39, *,*	8.82, *, *, *	-0.12, 0.53, *, *
H-FABP (µg/L)	1.05, 1.28, -0.23, 0.18	-0.74, 0.56, 0.71, -0.41	*, -0.65, *, 1.61	0.13, 0.15, *, 0.51	0.10, 0.45 *, *	0.04, * *, *	-0.07, 1.29, *, *
Beckman hs-cTnI (ng/L)	39, 7, 0, -2	-17, 12, -3, -9	*, -3, *, -5	0, -1, *, -2	1, -4, *, *	0, *, *, *	0, 0, *, *
Abbott hs-cTnI (ng/L)	26, 5, -1, -1	-23, 9, -3, -10	*, -3, *, -2	0, 0, *, 1	-1, -3, *, *	-2, *, *, *	-1, 0, *, *
NT-proBNP (ng/L)	7, 167, 30, -54	3, 316, 3, -19	*, -30, *, -140	-8, 17, *, 74	16, 68, *, *	17, *, *, *	2, -19, *, *

Table 4.4. Difference between Day 1 and Day 2 Concentrations for Outcome Participants. For each cycle, day 1 concentrations were subtracted from day 2 concentrations for CRP, H-FABP, hs-cTnI and NT-proBNP to determine the change within each cycle. 1) Participant #1, 2) Participant #4, 3) Participant #9, 4) Participant #17. *Indicates a missing value for that participant.

4.4 Discussion

Early after the initiation of trastuzumab in this seemingly healthy cohort of female breast cancer participants (i.e., no evidence of cardiotoxicity from the treatment) there was an increase in concentrations for NT-proBNP on cycle 1 and hs-cTnI on cycle 2, which trended lower by

cycles 2 and 3, respectively (Fig. 4.2). Whereas for H-FABP and CRP, no significant elevations were apparent (median/ULN ration <1), with at maximum 3 participants exceeding the ULN at one timepoint (Fig. 4.2). These elevations above the ULN in female participants with normal LVEF were evident for hs-cTnI from cycles 1-3, but not the other biomarkers (NT-proBNP, H-FABP and CRP) [56]. By cycle 7 in these 12 participants, (approximately 4.5 months after starting trastuzumab), all biomarker concentrations trended below the healthy population ULN, with no elevations evident for NT-proBNP and H-FABP, and 11 of the 12 women having normal CRP and hs-cTnI concentrations (Fig. 4.2).

By assessing the concentration change from day 1 to day 2 in each cycle, it was expected that large differences in concentrations were to be evident in only the participants who experienced LVEF decline. However, not every participant with cardiotoxicity experienced large changes in their biomarker concentrations. In comparison, in the "treatment complete, normal LVEF" group (i.e. no outcome), there were large changes in the median biomarker concentrations during the first cycle on trastuzumab, especially in NT-proBNP and hs-cTnI (Table 4.2). A confounding variable is the treatment with anthracycline prior to trastuzumab, and it remains unclear whether the changes are due to anthracycline or trastuzumab or a combination of both, as there are no available timepoints to assess during the anthracycline treatment.

In this setting, sex-specific ULN may provide higher specificity for NT-proBNP, as 78% (14 of 18) of the participants without cardiotoxicity assessed in chapter 3 had concentrations greater than the overall ULN at C1D2, compared to 58% (7 of 12) of participants without cardiotoxicity from these analyses that had concentrations greater than the sex-specific ULN at the same timepoint (Fig. 4.2). More studies would need to be conducted in order to assess this further.

The fact that all 4 participants with a LVEF decline had CRP concentrations above the "treatment complete, normal LVEF" group median suggests that this model design might be useful in identifying participants with myocardial injury using CRP as a biomarker (Fig. 4.3A). However, the same findings were not observed for H-FABP (Fig. 4.3B). Three of the 4 outcome participants also exceeded the "treatment complete, normal LVEF" group median hs-cTnI concentration (for both Beckman and Abbott) early after the initiation of trastuzumab treatment, suggesting that this model may achieve greater specificity than using the healthy population sex-specific ULN cutoffs alone (Fig. 4.3C, D). In this regard, it may be of interest to try another method, such as multiple of median (MoM), which is used in maternal serum screening, but would require a significant larger sample size to derive appropriate medians over the course of treatment [57]. Similar to findings in chapter 3, NT-proBNP concentrations peaked during cycle 1 on trastuzumab treatment, even for participants that did not experience LVEF decline (Fig. 4.3E). As with hs-cTnI, the "treatment complete, normal LVEF" group had NT-proBNP concentrations above the sex-specific ULN. Perhaps subclinical cardiotoxicity is being detected with more sensitive measures of myocardial injury. Overall, the median of the "treatment complete, normal LVEF" was below the ULN for CRP and H-FABP, suggesting that this approach may be useful to explore in future studies. As for hs-cTnI and NT-proBNP, further analyses are warranted to understand why so many HER2+ breast cancer patients experience elevations, regardless of the presence of cardiotoxicity.

4.5 Conclusion

This was a secondary analysis that evaluated the biomarker profiles in only those breast cancer patients that completed treatment without cardiotoxicity (i.e., "treatment complete, normal LVEF" group). Using these participants as a reference group permitted not only an

assessment of the appropriateness of the healthy population ULNs but also enabled descriptive approaches using the biomarkers comparing those patients with cardiotoxicity versus those without. The finding that early after initiation of trastuzumab treatment the median concentration/ULN ratios were >1 during cycle 1 for NT-proBNP and cycles 1 through 3 for hs-cTnI further supports the finding that myocardial injury and dysfunction using hs-cTnI and NT-proBNP, respectively, are common in female breast cancer patients who have normal LVEF throughout and at the end of treatment [56].

Chapter 5: MEDICATE and CABOT Study Comparison

5.1 Introduction

It remains unclear whether different modalities of curative cancer therapy differ in the development of myocardial injury in female cancer patients exposed to these potentially cardiotoxic treatments. Several studies have demonstrated the significant impact of having minimally invasive diagnostic techniques (such as sensitive and specific cardiac biomarkers) with the ability to identify myocardial injury [4, 46]. Thus, the objective of these analyses was to assess the early temporal relationship of acute injury biomarkers (CRP, H-FABP, and hs-cTnI) between different potentially cardiotoxic curative therapies in female cancer patients [58]. In chapters 2 and 4, the purpose was to determine whether sex-specific ULN cutoffs were appropriate. In this chapter, the biochemical profiles of cancer patients are explored to determine whether the trends are dependent on the treatment administered. By comparing the participants in CABOT (as previously analyzed in chapters 2 through 4), it was of interest to assess if the early increase in biomarker concentrations were specific to the CABOT female cohort, or whether the same observations were evident in a different subset of female cancer patients (ie. MEDICATE cohort).

5.2 Methods

5.2.1 Overview

A post-hoc subgroup analysis was conducted, with the focus on only female participants from the MEDICATE and CABOT studies. Mediastinal Irradiation and Cardiotoxic Effects (MEDICATE study) asks the question, "*Does modern mediastinal irradiation cause acute subclinical cardiac damage?*". This study assessed radiation-induced heart disease in Hodgkin's and non-Hodgkin's Lymphoma patients receiving radiotherapy (RT) after chemotherapy [59].

Serum samples were collected from December 2014 through to December 2016 from 19 participants prior to radiotherapy, after 2 weeks, and again after 4 weeks on radiotherapy. In addition, echocardiograms were completed prior to and one-year post RT, which assessed LVEF [59]. Descriptions of the cancer diagnosis and RT techniques were also recorded. Both the MEDICATE and CABOT studies were prospective and observational, with the common element being baseline blood collection prior to initiation of curative therapy. The MEDICATE cohort in these analyses included lymphoma participants who received mediastinal RT after anthracycline chemotherapy (n = 4) (Fig. 5.1A).

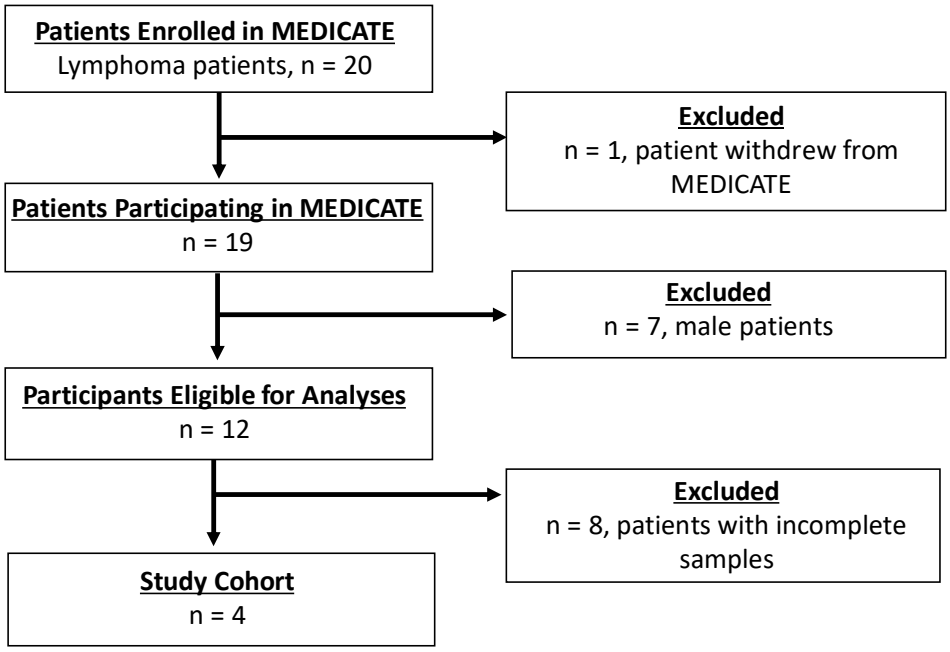

Figure 5.1A. **Participant Inclusion Criteria - MEDICATE**. Flow diagram of participants included in chapter 5 analyses, MEDICATE study.

The CABOT cohort for these analyses included HER2+ breast cancer participants who received adjuvant trastuzumab therapy. Some participants received radiotherapy within the first 6 weeks of trastuzumab treatment, and thus were categorized into a separate group for analyses: 1) breast cancer participants treated with adjuvant trastuzumab without RT during this timeframe (n = 16);

and 2) breast cancer participants treated with adjuvant trastuzumab and RT (n = 5) (Fig. 5.1B) [58].

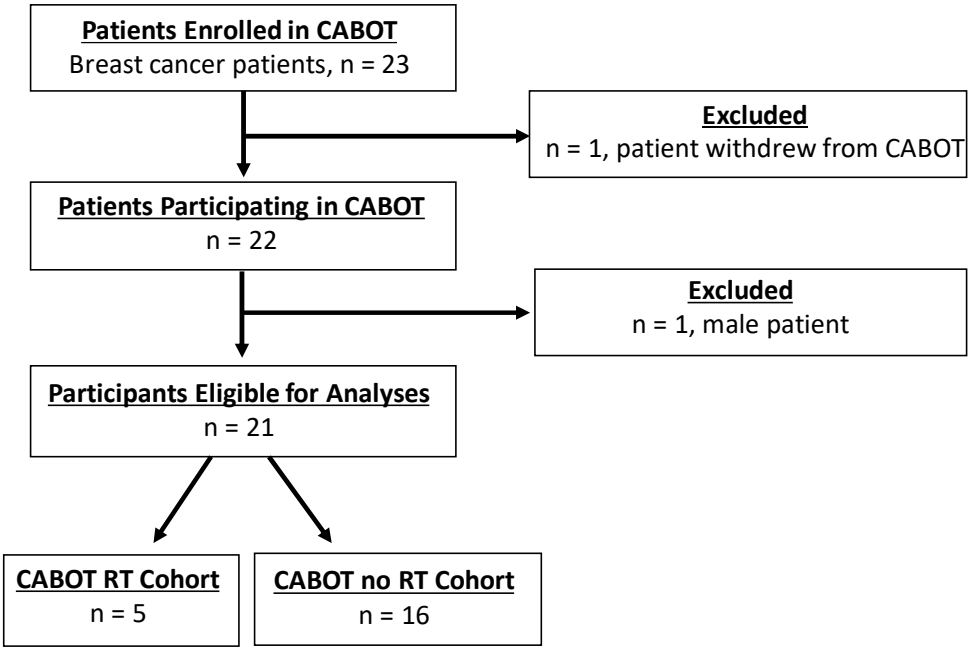

Figure 5.1B. Participant Inclusion Criteria – CABOT. Flow diagram of participants included in chapter 5 analyses, CABOT study. "CABOT RT" refers to participants who received radiotherapy in addition to trastuzumab treatment, whereas "CABOT no RT" refers to those who did not receive radiotherapy.

Similar timepoints were chosen for analyses between the two studies. The blood samples (serum) that were analyzed were collected at pre-RT, mid-RT (2 weeks after 1st treatment) and during the last week of RT (4 weeks after 1st treatment) for MEDICATE. For CABOT, blood samples (EDTA plasma) were collected prior to the initiation of trastuzumab treatment, before the 2nd treatment (3 weeks after 1st treatment), and before the 3rd treatment (6 weeks after 1st treatment) [53, 59]. As the objective of our study was to determine the early biochemical profiles, only those participants that had complete measurements within 4 weeks of RT and 6 weeks of trastuzumab were selected to be in the cohorts [58]. All samples were stored below -70^{0}C. Three biomarkers were measured using the following assays: Beckman Coulter SYNCHRON System

hs-CRP assay (CRP for CABOT), Roche CRP Modular P800 Immunoturbidimetric assay (CRP for MEDICATE), Randox H-FABP Immunoturbidimetric assay (measured on the Modular P platform), and Abbott ARCHITECT STAT i2000SR hs-TnI assay, with the following ULNs = 7.44 mg/L (CRP), 6.3 µg/L (H-FABP), and 16 ng/L (hs-cTnI) [36, 47, 51]. These biomarkers were chosen for the following analyses as they were measured in both studies during similar timepoints. Stability and analytical performance of these assays have been established [36, 47, 48, 60, 61]. The imprecision of CRP (on both analyzers) and H-FABP across different concentration ranges was <11%, with hs-cTnI imprecision being 14% at the normal range (quality control (QC) mean = 5 ng/L) [62]. ULN cutoff values were employed to assess the prevalence of myocardial injury in the participants. The changes in the biomarker concentrations were evaluated visually and descriptively, along with unadjusted non-parametric analyses using the ULN for the biomarkers [58]. LVEF was assessed at 3 months for CABOT and 12 months for MEDICATE.

5.2.2 Statistical Tests

As the data was not normally distributed, non-parametric tests were employed. Mann Whitney testing for independent samples was performed with Friedman tests performed for each biomarker within each cohort to evaluate the changes between the three timepoints. MedCalc statistical software (version 18.2.1) was used for analyses (p <0.05 considered significant). Data was compiled using Microsoft Excel spreadsheets to create an overall database containing all the information on participants biomarker concentrations and medical history for both CABOT and MEDICATE studies. The lead investigator on the studies provided participant CRFs for any information that was missing in order to complete the datasets.

5.3 Results

5.3.1 Descriptive Results

The MEDICATE study cohort was younger (median = 27 years) as compared to the CABOT cohort that received radiotherapy (median = 49 years) ($p = 0.02$) and the CABOT cohort that did not receive radiotherapy (median = 54 years) ($p = 0.008$) (Table 5.1) [58]. In MEDICATE, half of the participants were diagnosed with mediastinal diffuse large B-cell lymphoma (DLBL), the other half were diagnosed with Hodgkin's Lymphoma. For CABOT the diagnosis was primarily invasive ductal carcinoma with no significant difference in the incidence of left or right sided breast cancer (Table 5.1). All participants received chemotherapy prior to the commencement of radiation or trastuzumab therapy, and all but one participant received an anthracycline in the regimen. For MEDICATE, all participants had two-field RT using an anterior-posterior beam arrangement, whereas for CABOT, 2 of the RT participants received radiation to the left side chest wall or breast, and 3 received radiation to the right side (Table 5.1). Two of the breast cancer patients also received a radiation boost dose. For CABOT, all participants had trastuzumab therapy, with 2 participants also receiving anti-estrogen therapy (i.e., one with tamoxifen and one with letrozole) (Table 5.1) [58].

Variable	MEDICATE mediastinal radiotherapy (RT) (*n* = 4)	CABOT trastuzumab + RT (*n* = 5)	CABOT trastuzumab (*n* = 16)
Median Age (range)	27 (20-42)	49 (42-66)	54 (36-68)
Cancer Diagnosis			
-Diffuse Large B-cell Lymphoma	2		
-Hodgkin's Lymphoma	2		
-Invasive Ductal Carcinoma		5	16
-Invasive Lobular Carcinoma		0	1 (one with both)
Location of Cancer			
-Mediastinum	4		
-Left breast		2	7
-Right breast		3	8
-Bilateral breast cancer			1
Systemic therapy			
-R-CHOP[1]	2		
-ABVD[2]	2		
-AC[3]		1	0
-AC-T[4]		3	15
-FEC-D[5]		0	1
-DC[6]		1	0
-Trastuzumab	0	5	16
-Endocrine therapy	0	2	0
Radiation Therapy			
-3500 cGy/20 fractions(#)	3		0
-3060 cGy/17#	1		
-4256 cGy /16#		2 (1 boost1000cGy/5)	
-5000 cGy /25#		3 (1 boost1000cGy/5)	

[1]Rituximab, Cyclophosphamide, Doxorubicin Hydrochloride, Vincristine Sulfate, Prednisone (R-CHOP), [2]Adriamycin (Doxorubicin), Bleomycin, Vinblastine, Dacarbazine (ABVD), [3]Doxorubicin, Cyclophosphamide (AC), [4] Doxorubicin, Cyclophosphamide, Paclitaxel (AC-T), [5]Fluorourcil/ Epirubicin, Cyclophosphamide, Docetaxel (FEC-D), [6] Docetaxel, Cyclophosphamide (DC). Reprinted from [58]. *Per Elsevier, as an author of this article I have retained the right to include this table in my thesis.

Table 5.1. **Demographics table**. Demographic information for the MEDICATE and CABOT study cohorts.

5.3.2 Biomarker Profile Results

In MEDICATE, there were no significant changes in the concentrations of CRP, H-FABP or hs-cTnI across the three timepoints (p = 0.7, 0.8, 0.5, respectively) (Figs. 5.2A, 5.3A, 5.4A). The CABOT participants who received trastuzumab therapy without radiation experienced significant changes in the concentrations of CRP (p = 0.03), H-FABP (p = 0.01) and hs-cTnI (p = 0.001) (Figs. 5.2B, 5.3B, 5.4B) [58]. For all three biomarkers, concentrations were highest 3 weeks post-commencement of trastuzumab, followed by a decrease. Notably, for hs-cTnI, the change in median concentration went from 19 ng/L to 65 ng/L, followed by a drop to 25 ng/L after 6 weeks (p = 0.001) (Fig. 5.4B). In contrast, the CABOT participants who received radiotherapy in addition to the trastuzumab therapy did not experience any significant changes in the concentrations of CRP, H-FABP nor hs-cTnI (p = 0.6, 0.9, 0.3, respectively) (Figs. 5.2C, 5.3C, 5.4C) [58].

When comparing the two CABOT cohorts (those with RT versus those without), a significant difference was observed only in hs-cTnI during the second and third timepoints (p = 0.03, p = 0.02) (Figs. 5.4B, C).

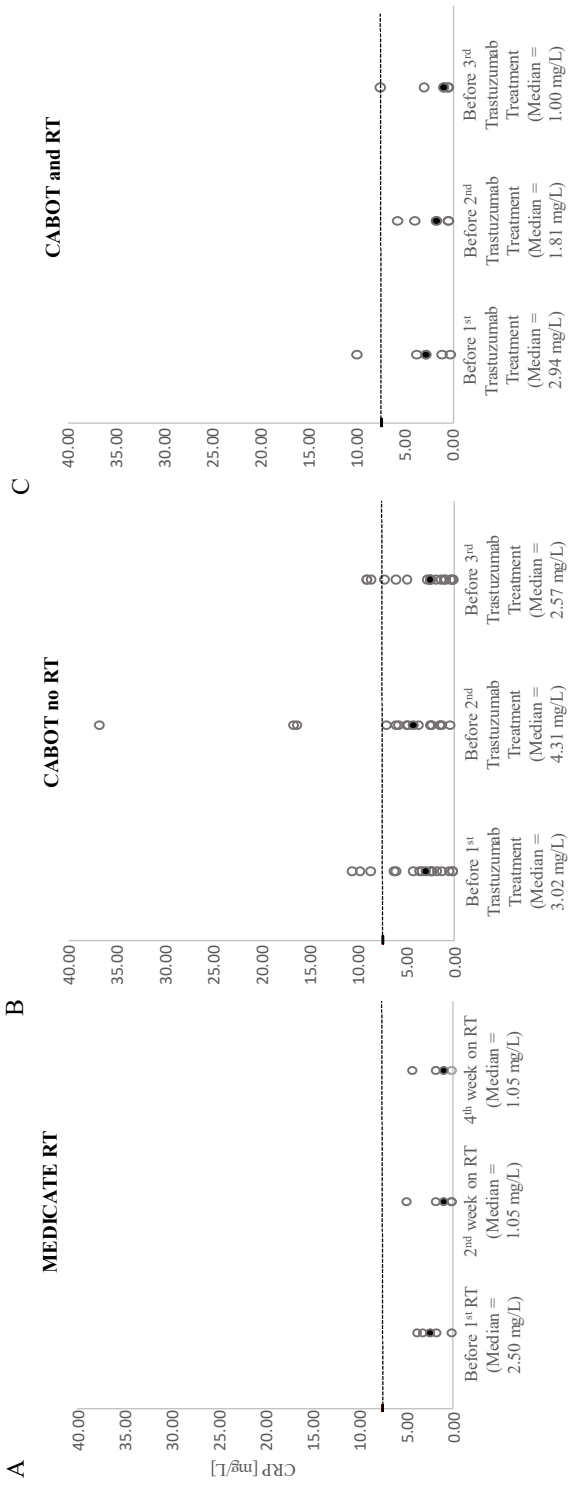

Figure 5.2 A, B, C. CRP concentrations; (A) MEDICATE RT (B) CABOT no RT, (C) CABOT and RT. Listed as mg/L for all 3 timepoints analyzed. Note: the solid black circle represents the median concentration at each timepoint. Dashed line is the ULN.

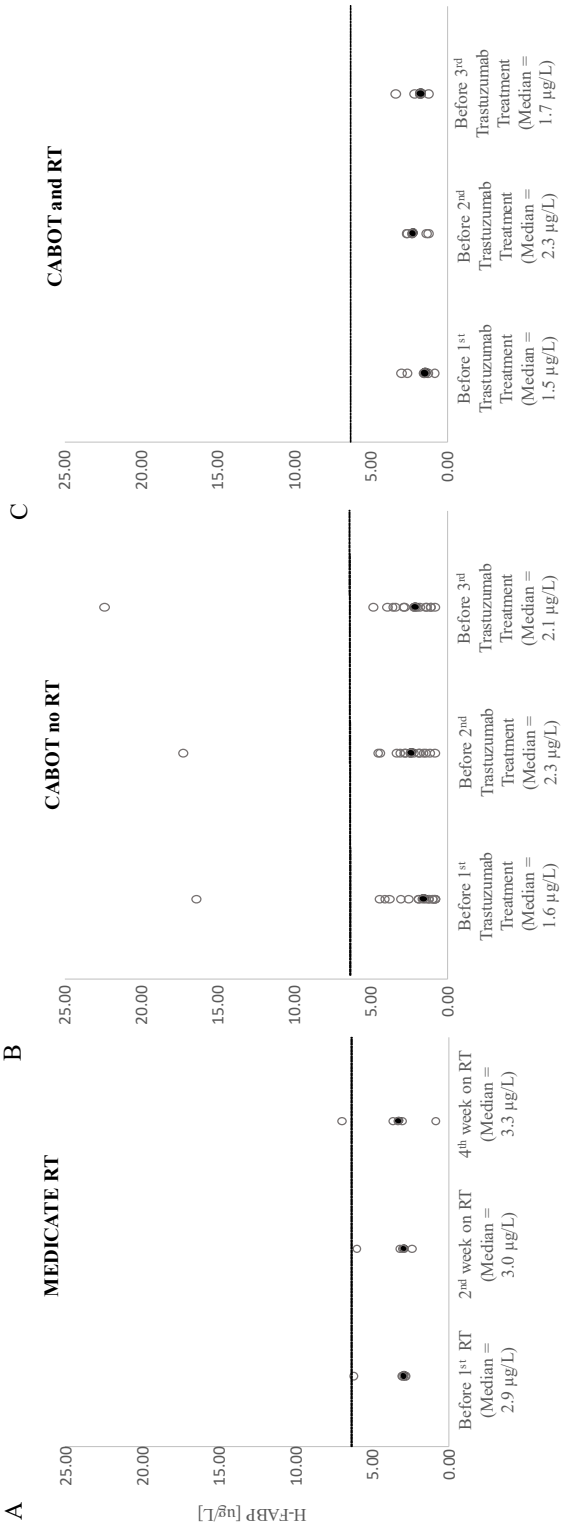

Figure 5.3 A, B, C. H-FABP concentrations; (A) MEDICATE RT, (B) CABOT no RT, (C) CABOT and RT. Listed as µg/L for all 3 timepoints analyzed. Note: the solid black circle represents the median concentration at each timepoint. Dashed line is the ULN. Reprinted from [58]. *Per Elsevier, as an author of this article I have retained the right to include this figure in my thesis.

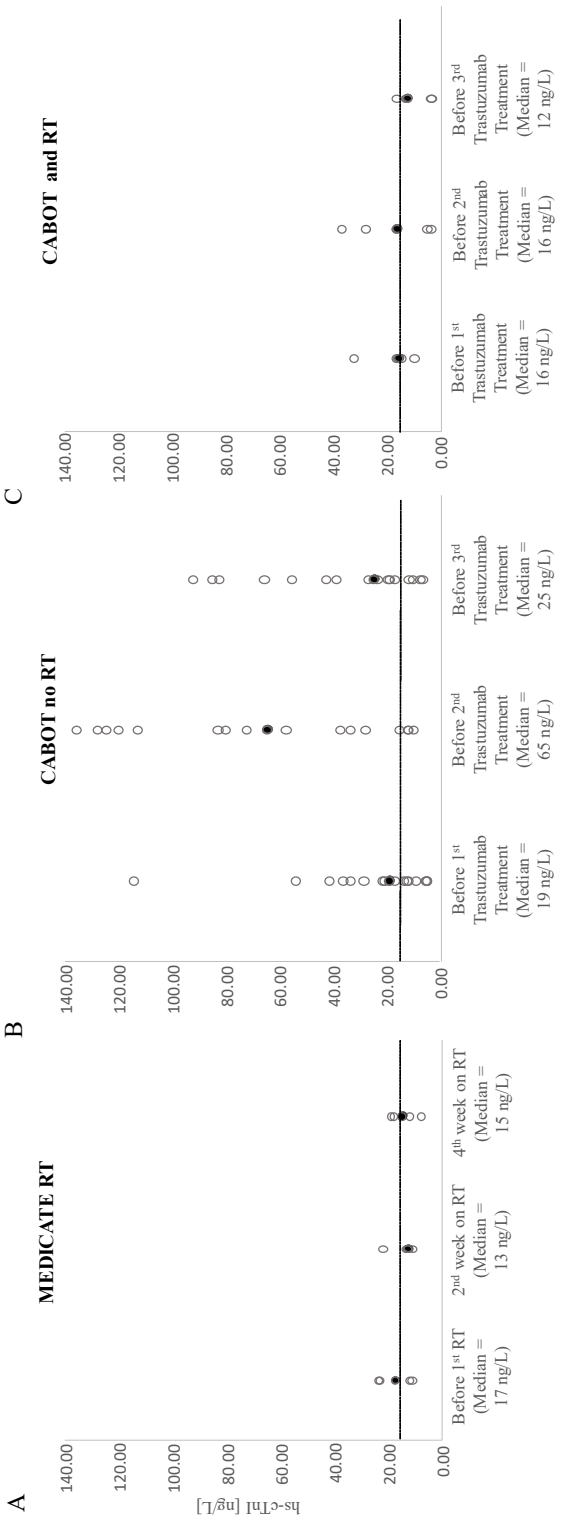

Figure 5.4 A, B, C. Hs-cTnI concentrations; (A) MEDICATE RT, (B) CABOT no RT, (C) CABOT and RT. Listed as ng/L for all 3 timepoints analyzed. Note: the solid black circle represents the median concentration at each timepoint. Dashed line is the ULN. Reprinted from [58]. *Per Elsevier, as an author of this article I have retained the right to include this figure in my thesis.

In MEDICATE there were no participants with CRP >ULN, 1 participant with H-FABP >ULN and 3 participants with hs-cTnI >ULN in total using accepted cutoffs for myocardial injury at any point during this timeframe (Table 5.2). In CABOT (without RT) there were 5 participants with CRP >ULN, 1 participant with H-FABP >ULN, but all 16 participants with hs-cTnI >ULN at any point during this timeframe (Table 5.2). As for those who received radiotherapy in CABOT, 1 participant had CRP >ULN, no participants had H-FABP >ULN, while a total of 3 of the 5 had hs-cTnI >ULN at any point during this timeframe (Table 5.2) [58].

# Participants >ULN	Before 1st treatment	Before 2nd treatment	Before 3rd treatment
CRP			
- MEDICATE	0/4	0/4	0/4
- CABOT no RT	3/16	3/16	3/16
- CABOT RT	1/5	0/5	1/5
H-FABP			
- MEDICATE	0/4	0/4	1/4
- CABOT no RT	1/16	1/16	1/16
- CABOT RT	0/5	0/5	0/5
Hs-cTnI			
- MEDICATE	2/4	1/4	2/4
- CABOT no RT	9/16	12/16	12/16
- CABOT RT	2/5	3/5	1/5

Table 5.2. Incidence of Concentrations >ULN. Number of participants whose CRP, H-FABP, and hs-cTnI concentrations exceeded the ULN for each of the three timepoints.

5.3.3 LVEF Results

After 1 year, all MEDICATE participants had LVEF >50% (median = 56%, range: 55-59) (Table 5.3). However in CABOT, after 3 months on trastuzumab therapy, several participants experienced a LVEF below what is classified as normal (ie. LVEF <50%) (median (no RT) = 58%, range: 42-67%) and (median (RT) = 60%, range: 52-66%) (Table 5.3). Two participants had to discontinue treatment due to cardiotoxicity, and one participant remained on treatment with LVEF of 45% at 3 months (Table 5.3) [58]. These 3 participants did not receive

RT. Participant #4 (who completed their trastuzumab treatment with cardiotoxicity) had an increase in hs-cTnI concentrations, yielding a concentration above the 99th within 6 weeks on trastuzumab (before 1st treatment = 5 ng/L, before 2nd treatment = 12 ng/L, before 3rd treatment = 19 ng/L). They also had stable and normal H-FABP and CRP concentrations (Table 5.4) [58]. Their LVEF was 56% at baseline (Table 5.3). Participant #9 discontinued treatment and had persistently elevated hs-cTnI (115 ng/L, 128 ng/L, 66 ng/L) and H-FABP (16.4 µg/L, 17.2 µg/L, 22.3 µg/L) at all three timepoints [58]. Only before the first trastuzumab treatment did participant #9 exhibit a slightly elevated CRP concentration (Table 5.4). Their LVEF was 42% at 3 months (baseline 53%) (Table 5.3). In contrast, participant #17 who also discontinued treatment, had hs-cTnI slightly above the ULN at first timepoint (21 ng/L) with normal values for the two subsequent timepoints, undetectable H-FABP concentrations at all timepoints (<0.75 µg/L), and CRP concentrations all within the normal range (Table 5.4) [58]. Their LVEF was 49% at 3 months (baseline 61%) (Table 5.3). Of the 13 participants in CABOT who had LVEF >50% at 3 months (without RT) and thus did not experience cardiotoxicity, 4 participants had a CRP concentration >ULN, H-FABP concentrations were all below the ULN, but all 13 participants had hs-cTnI >ULN, with some measurements several-fold higher than the ULN. Specifically, for hs-cTnI in these 13 remaining participants, 7 of them had a hs-cTnI concentration 4-times higher than the ULN (>64 ng/L) during this timeframe.

LVEF Measurement	Baseline LVEF	Post-treatment LVEF (1 year MEDICATE; 3 months CABOT)
MEDICATE (n = 4) - Median % (range)	58% (54-62)	56% (55-59)
CABOT no RT (n = 16) - Median % (range) - Outcome participant #4 - Outcome participant #9 - Outcome participant #17	64% (53-74) 56% 53% 61%	58% (42-67) 45% 42% 49%
CABOT RT (n = 5) - Median % (range)	66% (55-70)	60% (52-66)

Table 5.3. LVEF to assess Cardiotoxicity. Assessment based on LVEF measurements during baseline and after the participants have been on treatment (3 months for CABOT, 1 year for MEDICATE); comparing the participants who experienced cardiotoxicity to the median of those who did not experience cardiotoxicity.

Outcome Participant Concentrations	CRP (mg/L)			H-FABP (µg/L)			Hs-cTnI (ng/L)		
Timepoint	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**
Participant #4	6.15	5.06	4.95	2.4	2.4	2.1	5	12	19
Participant #9	8.71	5.81	6.13	16.4	17.2	22.3	115	128	66
Participant #17	3.70	3.80	2.50	<0.75	<0.75	<0.75	21	10	6

Table 5.4. Biomarker Profile of Participants with Cardiotoxicity. CRP, H-FABP, and hs-cTnI concentrations of the 3 participants with cardiotoxicity at the 3 timepoints analyzed.

5.4 Discussion

The findings from these analyses demonstrate that adjuvant therapy with trastuzumab (CABOT) elicits different acute biomarker concentration changes early after initiation of

treatment compared to radiation therapy (MEDICATE). As revealed by the Mann Whitney test, there was only a significant difference observed later on in cardiac troponin concentrations between the CABOT participants who received RT versus those who did not. The participants who received RT had lower concentrations of hs-cTnI, suggesting that RT did not have an additive effect on hs-cTnI in this population, though this cannot be stated with confidence as the RT cohort was much smaller (Figs. 5.4A, C). Additionally, employing the female sex-specific 99th percentile to define myocardial injury (fourth universal definition of myocardial infarction) [63], identified more than 85% of women with myocardial injury in either treatment setting during this timeframe (i.e., 3 of the 4 women had hs-cTnI >99th with mediastinal radiotherapy, all 16 women had hs-cTnI >99th with trastuzumab therapy and 3 of 5 women had hs-cTnI >99th with trastuzumab + RT) (Figs. 5.4A, B, C) [58]. Collectively, only 3 women experienced early cardiotoxicity (a significant decline in LVEF), yet 22 of these 25 women had myocardial injury, suggesting that additional variables (besides an elevated hs-cTnI concentration) may need to be considered when assessing which women are at risk for cardiotoxicity early after treatment (Tables 5.2, 5.3) [58]. This is not unique to the Abbott hs-cTnI assay, as a recent study using a similar cohort, persistent elevations of hs-cTnI (using a different assay than the one presented in this chapter) and hs-cTnT have been observed in a majority of the participants over the course of 3 months on trastuzumab [53, 54]. These studies urge caution to avoid over-interpretation of such elevations in cardiac troponin, as do the results from the analyses in this thesis. In a different study using a stepped-wedge, cluster-randomized controlled trial (RCT) design assessing hs-cTnI in the emergency department setting, the authors found that the implementation of sex-specific 99th percentiles with hs-cTnI identified more participants with myocardial injury than actually having type 1 myocardial infarction [64]. The hs-cTnI assay used

in the RCT (Abbott ARCHITECT hs-cTnI) was the same hs-cTnI assay used in this chapter, suggesting that elevations of hs-cTnI concentrations slightly above the sex-specific 99th percentile are not predictive of acute cardiotoxicity during short-term observations.

For participants #4 and 17 with evident cardiotoxicity, their concentrations of H-FABP and CRP were normal. It was only in participant #9 that H-FABP was very elevated, with a slightly high CRP. With only 2 participants in total having H-FABP above the ULN (1 of which experienced LVEF decline), measuring this biomarker using the overall ULN could aid in the specificity in identifying myocardial injury, but additional variables would be needed, (ie. measuring H-FABP alone would not be sufficient in this setting) (Table 5.2) [58]. Notably, approximately 25% (6 of 25) had elevated CRP above the ULN, however only one of which experienced cardiotoxicity, suggesting this biomarker alone is not specific enough to determine cardiotoxic outcomes when comparing different potentially cardiotoxic therapies (Table 5.2).

One confounding variable in these analyses is the use of the anthracycline called doxorubicin, which is known to be a dose-dependent cardiotoxic agent [8]. Nearly all participants in MEDICATE and CABOT had doxorubicin as part of their chemotherapy regimen (Table 5.1). Following chemotherapy administration, the median hs-cTnI concentrations in the mediastinal radiotherapy group (17 ng/L), trastuzumab group (19 ng/L) and trastuzumab with RT group (16 ng/L) (Figs. 5.4A, B, C) were significantly higher than the documented median concentration of healthy individuals (≤ 4 ng/L) [58, 65]. However, despite this biochemical indication of injury prior to commencement of additional therapy, no participant had a LVEF <50% nor exhibited signs related to cardiotoxicity. It is possible that there is some lingering effect from the anthracycline chemotherapy in these participants before they commence trastuzumab treatment, however with elevations in hs-cTnI evident the very next day following

trastuzumab during the first few cycles, it can be concluded that trastuzumab does have an effect on the myocardial cell integrity in these participants.

The severity of cardiac injury is often dependent on irradiation dosage and proximity to the heart, with left sided breast cancer patients at a higher risk for cardiotoxicity [16, 25]. However, when considering the CABOT cohort, none of the participants who had left sided radiation experienced a decline in ejection fraction, nor significant changes in biomarker concentrations. Additionally, the other participants who received radiotherapy did not have any significant changes in their biomarker profiles. Keeping in mind the small sample size, it cannot be concluded that radiotherapy has no negative cardiac effect in these participants.

The two participants who received endocrine therapy did not experience LVEF decline. As for taxanes, all but 1 participant in the CABOT cohort received it in their chemotherapy regimen. (Table 5.1). Taxanes and endocrine therapies were not extensively studied in these analyses. It would be of interest to evaluate their effect in future studies.

The small sample size of the study cohorts warrant caution in over interpretation of the findings. Some other limitations are that only short-term cardiotoxicity and LVEF decline was assessed, and post 1-year follow up is not available in the CABOT study [58]. Larger studies and long-term outcomes may clarify if these elevations in hs-cTnI are prognostic of additional cardiac outcomes over several years, as it has been demonstrated for other populations [66]. The participants in the MEDICATE study had relatively low RT doses to the heart (median cardiac dose was 8 gray (Gy) or 3 Gy LV dose) as compared to other cancer populations, such as those with lung cancer, who typically receive much higher doses of radiation (standard 60 Gy) (Table 5.1) [59, 67]. This may affect the interpretation of RT on cardiac function, as there is a greater

risk for radiation-associated cardiotoxicity with doses >30 Gy [17]. Additionally, the therapies employed were not uniform in these analyses [58].

5.5 Conclusion

Notwithstanding the small sample size, post-hoc design, and different cancer populations, these results suggest that analyzing H-FABP, CRP and hs-cTnI using the sex-specific 99th percentile may not be sufficient to detect early cardiotoxicity in female patients undergoing curative therapy [58]. To establish and follow the progression of myocardial injury with patients receiving cancer therapies, it was suggested that obtaining baseline hs-cTnI concentrations prior to the initiation of cardiotoxic therapies is beneficial, since even small changes outside of the oncological setting are important indicators of cardiac damage [58, 68, 69]. Additional studies assessing alternative cutoffs for biomarkers with additional timepoints are needed to assess if early biomarker measurements can predict subsequent treatment-induced short- and long-term cardiotoxicity [58].

Chapter 6: Overall Discussion and Summary

The overall goal of this thesis was to determine if elevations in cardiac and inflammatory biomarkers could predict reductions in LVEF, in order to identify cancer patients who are at risk of developing cardiotoxicity from their treatment. In particular, the main focus was on female breast cancer patients, with additional analyses on lymphoma patients. Prior to the initiation of treatment in the CABOT participants, all baseline hs-cTnI, NT-proBNP and a majority of CRP, H-FABP concentrations were within the reference interval (or below the ULN). This suggests that the ULN for the healthy population can be used for cancer patients prior to treatment.

In chapter 2, the focus was on changes in hs-cTnI concentrations while on trastuzumab treatment and LVEF measurements within the first 3 months. It was found that for both hs-cTnI assays (Beckman and Abbott) using the sex-specific cutoff values identified >50% of participants as having myocardial injury, whereas the absolute change criterion identified 35-60% of participants as having myocardial injury. However, not all of these participants experienced cardiotoxicity. The highest concentrations were observed after the 2nd cycle on trastuzumab treatment, and by 3 months only 4 participants (including the one male participant) had a decline in LVEF, 2 of which had persistently elevated hs-cTnI. With both assays yielding strong correlations during the first cycle of treatment for these female breast cancer participants, this suggests that elevated cardiac troponin I concentrations are being measured and that perhaps there is an issue with clinical specificity. Accordingly, different cutoffs or a different set of criteria could be used in future analyses like these, as there was no significant difference in hs-cTnI concentrations between the participants who experienced cardiotoxicity and those who did not.

In chapter 3, the focus was on changes in NT-proBNP, H-FABP and CRP concentrations while on trastuzumab treatment, and LVEF measurements within the first 3 months. A similar trend to hs-cTnI was observed with NT-proBNP, as 82% of participants were identified as having myocardial dysfunction, using ULN criteria. The highest concentrations were expressed earlier than hs-cTnI, during cycle 1, which significantly increased from baseline concentrations. As for H-FABP and CRP, few participants were identified as having abnormal concentrations using these biomarkers, with cycles 1 and 2 experiencing the highest concentrations, respectively. Just as in hs-cTnI, there was no significant difference in the concentrations of NT-proBNP, H-FABP nor CRP in the 4 participants who experienced cardiotoxicity versus those that did not.

In chapter 4, the data was assessed from a different perspective. The "treatment complete, normal LVEF group" consisted of 12 female breast cancer participants who completed trastuzumab treatment, without acquiring cardiotoxicity by the end of 6 months. The participants with cardiotoxicity were compared against this group. From the "treatment complete, normal LVEF" group, >50% of these participants had hs-cTnI and NT-proBNP >ULN early after the initiation of trastuzumab. Few elevations were observed with CRP and H-FABP. The median change from day 1 to day 2 for any cycle was highest during cycle 1 for all biomarkers, indicating a strong response to the initiation of trastuzumab in these participants. Of the 4 participants that had LVEF <50% with a change >10% from baseline after at least 3 months of trastuzumab treatment, all had at least one CRP concentration >ULN and >median concentration from the "treatment complete, normal LVEF" group, suggesting this biomarker might have clinical utility in this setting. As seen in previous chapters, hs-cTnI and NT-proBNP were both elevated in the outcome participants and in the group median, thus unable to prove the

hypothesis that these two biomarkers possess sufficient clinical specificity to only target those who are at risk for early cardiotoxicity. Additional studies are warranted in this setting, including re-evaluating what is deemed "abnormal" in cancer populations.

In the previous chapters, the participants were a part of the CABOT study. In chapter 5, those participants were compared to lymphoma patients from the MEDICATE study. In these analyses, the CABOT participants were divided into two groups, depending on whether they received radiotherapy in addition to trastuzumab treatment. The purpose was to evaluate whether the biomarker changes observed in the previous analyses were cancer or treatment specific. In all 3 cohorts, nearly all participants had hs-cTnI >ULN at least once during their treatment. Few participants experienced CRP or H-FABP >ULN, much like in the previous chapters. For participants who received RT (MEDICATE and CABOT RT), there were no significant changes in any of the biomarker concentrations. Additionally, these participants had LVEF measurements >50%. In contrast, the participants who did not receive RT experienced significant changes in all biomarker concentrations, with 3 participants experiencing cardiotoxicity, defined by a LVEF <50% and change >10% from baseline. This may be just a coincidence that those who received RT did not experience drastic changes in their biomarker profiles, as the sample size was small, and cannot be said with confidence that RT has no effect on these participants. Of the 3 participants with the outcome, only one participant had persistently elevated hs-cTnI, indicative of myocardial injury. Concentrations of CRP and H-FABP were relatively normal, as anticipated from the trends in previous chapters. The remaining 13 participants in the "CABOT no RT" cohort also experienced elevations of hs-cTnI, making it difficult to evaluate hs-cTnI as a predictive marker of injury in this setting. Hs-cTnI elevations >ULN were evident in all cohorts, but particularly prominent in CABOT participants without RT.

Collectively, the biomarkers and timepoints at which they were measured did not successfully predict reductions in LVEF. Elevated concentrations of biomarkers, mainly hs-cTnI and NT-proBNP, were apparent in both participants who experienced cardiotoxicity, as well as those who did not. It was expected that only participants with a significant drop in their LVEF to experience significant elevations of hs-cTnI and NT-proBNP. It has been demonstrated that cardiac biomarkers are very effective in other settings, such as in the evaluation of ACS and acute HF patients, however that was not the case in breast cancer patients early after exposure to trastuzumab.

Moving forward, anthracycline therapy typically precedes trastuzumab treatment, so having serial measurements during this time would be beneficial in understanding biomarker concentrations in these participants. At this point, it remains unclear as to what effect anthracyclines had on the cardiomyocyte integrity in these participants, and if these effects were compounded during the radiotherapy and trastuzumab therapy. With these additional biochemical profiles and sampling, the cause of myocardial injury may be more apparent. If no elevations were observed during anthracycline therapy, then one could reason that the rise in cardiac troponin is purely from trastuzumab. Additionally, by having three days of blood collections per cycle; for example: day before trastuzumab, day of trastuzumab, day following trastuzumab, one might be able to assess if an increasing biomarker pattern is evident before trastuzumab or only present following trastuzumab. It would also be useful to have measurements a week prior to the initiation of trastuzumab. However desirable these additional blood collections may be, it may be difficult to enroll and maintain participants in the study due to the burden of extra participant visits and blood collections. Furthermore, these studies were purely observational on a relatively homogeneous group of patients, so for future projects,

having a comparative group would aid in the analyses of biomarker and LVEF changes in cancer patients. Such groups may include different ages (i.e. young versus older), including more males (as the one male included did experience cardiotoxicity), comparing different comorbidities in participants to observe how that might affect their biomarker profile, or have a group of participants undergoing treatments that have cardiotoxic effects, but not for cancer. It is critical to continue blood-based biomarker research in the field of cardio-oncology, if early detection and prevention of cardiotoxicity and subsequent cardiovascular disease is to be realized.

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