DOCTOR OF MEDICINE

Early Detection of Anthracycline Induced Cardiotoxicity

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Abbreviations

ABVD	Adriamycin, Bleomycin, Vinblastine, Dacarbazine
ACEi	Angiotensin Converting Enzyme inhibitor
AI	Artificial Intelligence
AIC	Anthracycline Induced Cardiotoxicity
ARB	Angiotensin Receptor Blocker
ASCO	American Society of Clinical Oncology
ASE	American Society of Echocardiography
ATP	Adenosine Triphosphate
AUC	Area under the curve
BMT	Bone Marrow Transplant
BP	Blood Pressure
BPM	Beats Per Minute
BSA	Body Surface Area
BSE	British Society of Echocardiography
CBR3	Carbonyl Reductase 3
CCB	Calcium Channel Blocker
CEOP	Cyclophosphamide, Etoposide, Prednisolone, Vincristine
CHOP	Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone
C.I	Chief Investigator
CI	Confidence Interval
Cr	Serum creatinine
CRF	Case Report Form
CTIA	Cancer Treatment Induced Arrhythmia
cTnC	Cardiac Troponin C
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
CTRCD	Cancer Therapy Related Cardiac Dysfunction
CVA	Cerebrovascular Accident
DNA	Deoxyribonucleic Acid
EACVI	European Association of Cardiovascular Imaging
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
ESC	European Society of Cardiology
ET	Ejection Time
FBC	Full Blood Count
FEC 75	Fluorouracil, Epirubicin, Cyclophosphamide
FEC-T	Fluorouracil, Epirubicin, Cyclophosphamide, Docetaxel
FPS	Frames Per Second
GCS	Global Circumferential Strain
GDF-15	Growth differentiation factor-15

GLMM	Generalised Linear Mixed Effect Model
GLS	Global Longitudinal Strain
GRS	Global Radial Strain
Hb	Haemoglobin
HL	Hodgkin's Lymphoma
Hs-cTnI	High Sensitivity Cardiac Troponin I
Hs-cTnT	High Sensitivity Cardiac Troponin T
ICT	Isovolumic Contraction Time
ID	Identifier
IHD	Ischaemic Heart Disease
IQR	Interquartile Range
IT	Information Technology
IVRT	Isovolumic Relaxation Time
LA	Left Atrium
LAScd	Left Atrial Strain during Conduit phase
LASct	Left atrial Strain during Contraction phase
LASr	Left Atrial Strain during Reservoir phase
LV	Left Ventricle
LV EDV	Left Ventricular End-diastolic Volume
LVEF	Left Ventricular Ejection Fraction
LVESV	Left Ventricular End-Systolic Volume
LVIDd	Left Ventricular Internal Diameter at end-diastole
LVIDs	Left Ventricular Internal Diameter at end-systole
LV RWT	LV Relative Wall Thickness
MI	Myocardial Infarction
MPI	Myocardial Performance Index
MPO	Myeloperoxidase
MRA	Mineralocorticoid
MRI	Magnetic Resonance Imaging
MuRF1	Muscle Ring Finger-1
MV DecT	Mitral Valve Deceleration Time
NA	Not Applicable
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NHL	Non-Hodgkin's Lymphoma
PACS	Picture Archiving and Communication System
P.I	Principle Investigator
PVD	Peripheral Vascular Disease
RA	Right Atrium
RAScd	Right Atrial Strain during Conduit phase
RASct	Right Atrial Strain during Contraction phase
RASr	Right Atrial Strain during Reservoir phase
R-CHOP	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone
RCF	Relative Centrifuge Force
RIMP	Right Ventricular Index of Myocardial Performance

ROC	Receiver Operating Characteristics
ROI	Region of Interest
ROS	Reactive Oxygen Species
RT	Radiotherapy
RV	Right Ventricle
RV EDA	Right Ventricular End-Diastolic Area
RV ESA	Right Ventricular End-Systolic Area
RV FAC	Right Ventricular Fractional Area Change
RVFWS	Right Ventricular Free Wall Strain
RV GLS	Right Ventricular Global Longitudinal Strain
RV IVRT	Right ventricular Isovolumic Relaxation Time
SD	Standard Deviation
SST	Serum Separation Tube
STE	Speckle Tracking Echocardiography
TAPSE	Tricuspid Annular Plane Systolic Excursion
TBI	Total Body Irradiation
TDI	Tissue Doppler Imaging
TGF-ß1	Transforming growth factor beta 1
U&Es	Urea and Electrolytes
WLE	Wide Local Excision
2D	2 Dimensional
3D	3 Dimensional

Abstract

Background:

Anthracyclines are highly effective chemotherapy agents which have revolutionised the treatment of breast and haematological malignancies. However, one of the well-recognised associated risks with their use is dose-dependent cardiotoxicity which can lead to heart failure and poor prognosis. So far, most studies have focussed on the effects of anthracyclines on the left ventricle (LV) with relatively little known regarding the other cardiac chambers.

Aims and Methods:

The purpose of this thesis was to assess the effects of anthracyclines on all four cardiac chambers using 2-dimensional echocardiography and speckle tracking echocardiography (STE) alongside measurement of cardiac biomarkers. Patients with a new diagnosis of lymphoma or breast cancer undergoing anthracycline chemotherapy were included in two separate studies conducted for the purpose of this thesis. In the retrospective study, echocardiograms that were performed at baseline (T0), mid-chemotherapy (T1) and post completion of chemotherapy (T2) were analysed. In the prospective study, echocardiograms were performed at baseline (V1) and 1-month post completion of chemotherapy (V2). High sensitivity troponin T (hs-cTnT) was measured at different time points.

Results:

A total number of 106 patients were included in this thesis. Amongst all the echocardiographic measures obtained, LV global longitudinal strain (GLS) and RV free wall strain (FWS) were the only two measures to demonstrate a consistent decline during treatment. However, in contrary to previously published data, a reduction in LV GLS was not seen to precede a decline in LVEF. hs-cTnT levels showed an increase from the V1 to V2 in all patients and these changes were statistically significant in patients with reduced LVEF and good LVEF.

Conclusion:

Adverse effects of anthracyclines are not solely confined to the left ventricle. Comprehensive assessment of all cardiac chambers with particular focus on the left and right ventricles should be taken into consideration during the assessment of patients undergoing chemotherapy treatment.

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Chapter 1: Introduction

1. Brief history of cancer

Cancer is one of the leading causes of death worldwide with an annual incidence of 3.7 million new cases, in Europe alone.[1] Lymphoma, characterised by an abnormal growth of the lymphatic system, and breast cancer are both frequent forms of cancer affecting individuals across the world.[2-4]

Prior to the sixteenth century, very little knowledge and theories of cancer existed, and the understanding of the human body and its circulatory system remained a huge mystery.[5, 6] With the initial works of Galileo and Newton using a scientific approach, and the later works done by Harvey in 1628, a stepping stone in the understanding of the human body and the disease processes affecting it, was placed.[5] However, it was not until 1761, when Giovanni Morgagni, an Italian physician considered the father of modern anatomical pathology, became the first to lay the groundwork of clinical oncology by attempting to relate deceased patients' diseases to pathological findings during autopsies.[6] Shortly later, John Hunter, a famous Scottish surgeon, introduced the concept of surgery as a possible cure for certain types of cancer. With this theory in mind and the development of anaesthesia a century later, surgery was performed and operations such as radical mastectomy for the treatment of breast cancer were developed.[5] In the nineteenth century, with the provision of scientific basis for the better comprehension of cancer pathology, and the correlation of microscopic pathology to disease processes by Rudolf Virchow, a German pathologist and politician of his time, a more sophisticated and refined approach to the surgical treatment of cancer was developed.[5, 6] Up until the mid-twentieth century, surgery and the later development of radiation treatment, were predominantly the most adept treatments in the management of early stage cancer.[6, 7] However, despite these advances cure rates for cancer remained below 50% and this was later on attributed to the presence of micro-metastases.[7]

2. Discovery of chemotherapy and anthracyclines

The modern era of the development of cancer chemotherapy can be traced back to the beginning of the twentieth century with the introduction of mustard gas during World War I.

The consequences of using this chemical weapon of mass destruction were so devastating that this led to banning of its use by the Geneva Protocol in 1925.[8] Concerns regarding the re-introduction of this chemical warfare agent during the World War II, guided the researchers to investigate the underlying mechanisms of this poisonous gas to help facilitate the development of antidotes against its use.[5, 6, 9] After observing low levels of immune cells with the later development of leukaemia and lymphoma in individuals who had been exposed to mustard gas, Louis Goodman and Alfred Gilman from Yale University, were the first to hypothesise a potential role for the use of a related compound called nitrogen mustard, in targeting cancerous cells.[7, 9] With successful results from their experiments on mice, this agent was trialled on an individual with advanced non-Hodgkin's lymphoma in 1942. Despite promising results, the findings of this incredible discovery were only released a few years later. This was due to the associated secrecy with the war gas program.[7] Goodman and Gilman's work, created a monumental era in the history of medicine. Their ground-breaking research led to the birth of chemotherapy and the basis for the development of future similar compounds called alkylated agents found to damage rapidly growing cells through interference with their DNA.[5, 9] Furthermore, their work sparked a great deal of research interest amongst the international scientists in identifying other agents with anti-tumour properties which could potentially be used in the treatment of cancer. This led to the later development of anti-folates, anti-metabolites and Vinca alkaloids.[5] With the rapid advance in the field of chemotherapy, a strong desire for discovering more natural and less toxic anti-cancer agents arose.

In the late 1950s, another group of drugs under the name of anthracyclines were discovered after the extraction of daunorubicin from the bacterium Streptomyces peucetius, found in a soil sample collected from India.[10, 11] Although original research on the early-identified anthracyclines revealed associated potent antimicrobial properties, their role as anti-tumour agents later became apparent in animal studies.[10-12] It was not until the late 1960s, when their excellent anti-cancer characteristics, led to their clinical use in the treatment of leukaemia and lymphomas.[10, 13, 14] This breakthrough discovery, stimulated an intensive effort in developing numerous similar analogues with improved therapeutic applications.[15] Since then, anthracycline chemotherapy has revolutionized the treatment of a wide range of cancers ranging from haematological to solid organ tumours leading to improvement in cancer prognosis and survival.[13, 14, 16]

3. Anti-tumour mechanisms of anthracyclines

To better understand their outstanding anti-tumour role, scientists were inspired to undertake research into the pharmacological structure and underlying mechanistic properties of these agents. It was not long, until the basic chemical structure of anthracyclines consisting of an aglycone ring combined with an amino sugar, were understood (Figure 1). This aided the creation of similar drugs with better anti-tumour and less toxic profiles (Table 1).[17, 18]

3.1 DNA intercalation

To this date, studies into the underlying mechanistic actions of anthracyclines have revealed that these agents primarily exert their anti-tumoural activity through intercalation of their aglycone portion between the adjacent base pairs of DNA leading to disruption of DNA and RNA synthesis in highly replicating cells.[10, 19, 20] This effect is believed to impair the transcription and replication processes, resulting in cell apoptosis and death.

3.2 Topoisomerase-II inhibition

Another widely explained mechanism for the action of anthracyclines has been attributed to their inhibitory role of the enzyme topoisomerase II.[19] In normal conditions, this enzyme is largely involved in the formation of temporary double-stranded DNA breaks during DNA supercoil. With the introduction of anthracyclines, these agents have shown to intercalate into the DNA, forming DNA-anthracycline-topoisomerase II complexes, impeding the underlying processes required for the re-ligation of DNA breaks and DNA repair mechanisms, cultivating in subsequent programmed cell death.[21-23]

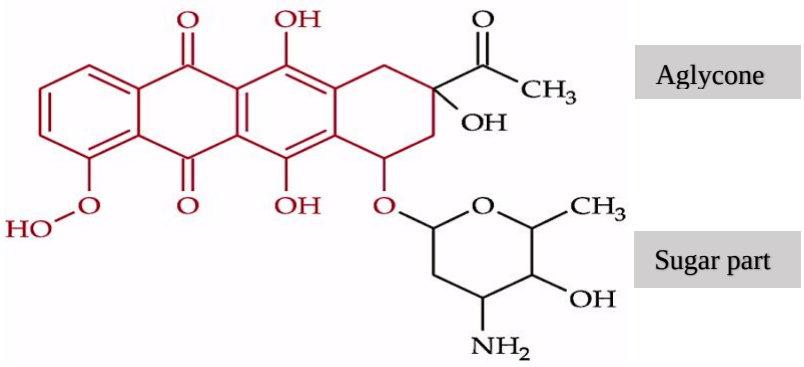


Figure 1. Anthracycline basic structure (doxorubicin)

Anthracycline type	Cancer	Relative cardiotoxicity	Incidence of HF exceeds >5% when cumulative dose exceeds (mg/m2)
Doxorubicin	Solid organ tumours/lymphomas	1	400
Epirubicin	Solid organ tumours/lymphomas	0.7	900
Idarubicin	Leukaemias	~0.75	800
Daunorubicin	Leukaemias/Kaposi's sarcoma	0.53	150

Table 1. Common anthracyclines and equivalence dose with doxorubicin as a reference[24]

4. Anthracycline side effects

4.1 General toxicity

Despite continuing to form the backbone of modern chemotherapy regimens in daily clinical practice, the benefits of anthracyclines have come at a cost of adverse events.[25] Myelosuppression, in particular neutropenia has been considered a common side effect from these agents affecting approximately 80% of patients treated at lower doses, with nearly all patients affected at higher cumulative doses, suggesting a narrow therapeutic index for anthracyclines. [23, 26] Furthermore, stomatitis, nausea and vomiting, and reversible alopecia have commonly been reported in individuals treated with these agents.[23]

4.2 Cardiotoxicity

One of the major limiting factors in the use of anthracyclines is the associated dose-dependent cardiotoxicity, a complication first recognized in 1971.[27] Cardiomyocyte apoptosis and cell death, previously commonly known as type 1 cardiotoxicity, occurs as a continuous, dose-dependent phenomenon with anthracycline treatment.[28, 29] In a proportion of patients, irreversible left ventricular dysfunction can occur with the later development of symptomatic heart failure.[30] Anthracycline cardiotoxicity affects 4.7% of cases at doses of 400mg/m^2 doxorubicin increasing to a rate of 48% at doses of 700mg/m^2.[24, 31] Once a patient is diagnosed with congestive heart failure, the 5-year survival rate is considered to be as low as 50%.[25] Although the adverse effects of these agents are more pronounced with higher cumulative

doses, histopathological changes have been evident in the endomyocardial biopsies of patients receiving lower doses, suggesting there is no safe dose.[13, 32-35]

The cardiotoxicity of anthracyclines is classified into three different categories of acute, subacute or chronic cardiotoxicity.[36-38] In acute/subacute cardiotoxicity, a myo-pericarditis type of picture is usually observed with non-specific repolarisation ECG changes, arrhythmias and LV impairment. This type of cardiotoxicity, considered extremely rare, occurs immediately to several weeks post administration of the first anthracycline dose, and is believed to be transient and self-limiting.[37, 38] In contrast, chronic cardiotoxicity, manifests insidiously with asymptomatic LV systolic impairment most commonly within the first year post anthracycline treatment, leading to subsequent dilated cardiomyopathy years later. This type of cardiotoxicity is the most common type and, in most cases irreversible.[38, 39]

With growing cancer survivorship and the recognised association of cardiotoxicity secondary to anti-cancer treatment, also known as cancer therapy-related cardiac dysfunction (CTRCD), the need for a more collaborative approach amongst the cardiologists, haematologists, and oncologists has risen. This has led to the formation of the field of cardio-oncology allowing a better understanding of the underlying mechanistic causes for cardiotoxicity, with better provision of monitoring, prevention and treatment of cardiac complications of cancer and cancer therapies.

5. Cardiotoxic mechanisms of anthracyclines

In addition to attempting to understand the underlying anti-tumour properties of anthracyclines, scientists have undertaken a great deal of research to further evaluate the principle cardiotoxic mechanisms of these agents. To date, the exact mechanism remains uncertain, though a number of pathways have been suggested.

Given the purpose of this thesis is not to explore the underlying mechanisms, only a brief description of these has been provided.

5.1 Molecular mechanisms

5.1.1 Oxidative stress

Oxidative stress has been one of the main proposed molecular mechanisms involved in anthracycline induced cardiotoxicity (AIC). This is thought to be secondary to the release of reactive oxygen species (ROS) via redox-cycling of the quinone component of the anthracyclines which can subsequently lead to generation of anthracycline-iron complexes, causing oxidative stress and subsequent DNA damage and cell apoptosis.[40-42]

5.1.2 Mitochondrial disruption

Another suggested contributing mechanism in the development of AIC is mitochondrial disruption. Mitochondria which have a crucial importance in cell physiology and are considered the "powerhouses of cells", can also be directly affected by anthracyclines through the activation of caspase cascade secondary to oxidative stress, resulting in cell injury and death. This is thought to be a consequence of anthracycline retention within the inner membrane of the mitochondria and formation of irreversible complexes with the mitochondrial phospholipid cardiolipin, causing loss of architectural integrity and cytochrome C release into the cytosol in response to oxidant stress.[41-44]

5.1.3 Fe^{2+} and Ca^{2+}homeostasis disruption

Disruption to iron homeostasis and consequent iron loading is a further proposed pathway for ROS generation and cell death in the pathogenesis of AIC. Anthracycline-iron complexes are capable of causing a Fenton reaction, generating hydroxyl radicals which subsequently result in the formation of ROS. Furthermore, anthracyclines are capable of exhibiting their effects through dysregulation of calcium homeostasis and ATP-synthesis which can lead to difficulties in maintaining energy production and metabolic demands.[42, 44-46]

5.1.4 Topoisomerase IIβ inhibition

As described earlier, anthracyclines exert their anti-tumour properties through inhibition of topoisomerase II, promoting DNA damage and subsequent programmed cell death. However, these agents have also shown to have a high affinity for the non-proliferating cardiomyocytes

which express topoisomerase IIβ, as opposed to the α-isoform predominantly expressed in cancer cells. Anthracyclines can bind to topoisomerase IIβ impeding the re-ligation of double stranded DNA break leading to eventual cardiomyocyte injury and apoptosis.[41, 42, 47]

5.1.5 Alterations in sarcomeric structure

In addition to the more common pathways discussed, other mechanisms such as doxorubicin-induced degradation of titin, a protein involved in the maintenance of the structural and functional integrity of cardiac sarcomeres, have been reported.[48-50] This effect is believed to lead to the destruction and total disarray of sarcomeric myofilaments resulting in AIC and the development of dilated cardiomyopathy.[42, 49-51]

5.2 Genetic factors

Genetic factors are also believed to have a contributory role in increasing individual susceptibility to AIC. Over-expression of cardiac-specific type-3 carbonyl reductase (CBR3) can increase the production of secondary alcohol metabolites in response to anthracyclines which subsequently leads to rapid development of cardiac dysfunction.[52-54] Additionally, polymorphisms in genes that encode for certain proteins such as NADPH oxidase can lead to ROS generation and subsequent cardiotoxicity.[55] Recently, a study investigating doxorubicin effects on the cardiomyocytes of rats, demonstrated that dose-dependent-atrophy and reduced cardiac mass is evident starting from lower dose doxorubicin exposure. This study further showed that mice who lack the muscle ring finger-1 (MuRF1) enzyme, a ubiquitin ligase expressed in cardiac and skeletal muscles involved in mediating muscle protein degradation and atrophy, can be resistant to the adverse effects of doxorubicin.[56]

Additionally, more recently, studies have confirmed that modulation in the microRNAs, important in the electrical signal conductance of the heart and cardiac function, could also lead to AIC.[57-59] A number of animal studies have revealed that a group of microRNAs, such as miR-208b, miR-34a, miR-34c, miR-216b, and miR-367, are upregulated in the cardiac cells with increasing doses of doxorubicin leading to cardiomyocyte apoptosis.[42, 57-59]

5.3 Contributory factors

The presence of patient-specific risk factors further potentiates the risk of cardiotoxicity development. These include: extremes of age (age>60 years or <18 years), female gender, mediastinal radiation, and the presence of conventional cardiovascular risk factors (Table 2).[24, 60] Furthermore, the combination of non-anthracycline chemotherapy in particular HER2 monoclonal antibodies, with anthracyclines can further aggravate the risk of cardiotoxicity and associated cardiac dysfunction.[61]

	ESC position statement paper 2016(24)	**ASCO clinical guidelines 2017[60]**
Risk factors for development of AIC	Female sex	-
	Age > 65 years or < 18 years	Age > 60 years
	Cumulative anthracycline dose	- High dose anthracycline (doxorubicin ≥ 250mg/m^2, epirubicin ≥ 600mg/m^2) - Lower dose anthracycline (eg, doxorubicin < 250mg/m^2, epirubicin < 600mg/m^2 in combination with lower dose radiation therapy (< 30 Gy) involving the heart
	Concomitant chemotherapy - Alkylating/antimicrotubule agents - Targeted therapies	Concomitant chemotherapy such as trastuzumab
	Previous or concomitant radiation therapy involving the heart	High dose radiation therapy (≥ 30 Gy) involving the heart
	Pre-existing cardiovascular conditions: - Arterial hypertension - Cardiac diseases associated with increased wall stress - Genetic factors	Cardiovascular risk factors (≥ risk factors): - Smoking - Hypertension - diabetes - Dyslipidaemia - Obesity
	Renal failure	Compromised cardiac function: - Borderline low LVEF - Previous MI - ≥ moderate valvular heart disease

Table 2. Risk factors for AIC development

Given this complex clinical, molecular and genetic interplay in the genesis of anthracycline related cardiomyocyte cell death and apoptosis, identification of individuals who will go on to develop future LV systolic impairment remains a big challenge for clinicians. Therefore, better

strategies for screening, prevention and/or early treatment of cardiotoxicity is required to improve cardiovascular outcomes in survivors of cancer.

Although, ROS production appears to be the most predominant causative mechanism for inducing anthracycline mediated cardiotoxicity, the extent of ROS involvement in AIC is unknown.[62] Studies have been undertaken to assess the role of different neurohormonal antagonists in the treatment and prevention of AIC (**Table 3**).[63-72]

Through their promotion of nitric oxide, and inhibition of angiotensin II production which leads to a reduction in ROS generation, ACEis have thought to have potential protective role against cardiac cell apoptosis and oxidative stress.[73-78] Additional studies are underway assessing the role of these medications in AIC prophylactically, hoping to prevent the future development of LV systolic impairment and subsequent cardiac failure.[79, 80]

Authors	Total no. of patients	Design	Cancer type	Anthracycline type and dose	Cardio-protective treatment assessed	Average daily dose	Timing of therapy	Primary clinical endpoint	Follow-up period	Findings
Kalay et al. (2006)[65]	50	Single - centre, Prospective, Randomised, Single-blind, Placebo controlled	Breast (68%) Lymphoma (18%) Other (14%)	Doxorubicin (525.3mg/m^2) Epirubicin (787.9mg/m^2)	Carvedilol	12.5mg/day	Prior to chemotherapy up until 6 months	Drop in EF (<50%) using echocardiography	6 months	Carvedilol showed some protective effects on systolic and diastolic function. Impaired systolic (p=0.0001) and diastolic function (p=0.008) in the control arm
Cadeddu et al. (2010)[81]	49	Single-centre, Prospective, Randomised, Placebo-controlled	Endometrial (43%) Breast (37%) Ovarian (1%) Non-Hodgkin lymphoma (<1%) Salivary gland (<1%) Lung (NSLC) (<1%)	Epirubicin (400±20mg/m2)	Telmisartan	40mg/day	1 week prior to chemotherapy until the end of treatment	Drop in LVEF (<55%) and a change in strain and strain-rate using TDI	1 week post epirubicin chemotherapy	No significant abnormalities detected in LVEF in both arms. Strain-rate normalised in telmisartan compared to placebo. Also, a significantly higher interleukin 6 and ROS species in placebo
Georgakapolouse et al. (2010)[72]	125	Single-centre, Prospective, Parallel-group, Randomised, Open-label, Controlled	Lymphoma	Doxorubicin (383.2mg/m^2)	Metoprolol Enalapril	Metoprolol (88.8mg/day) Enalapril (11mg/day)	Commenced concomitantly with chemotherapy	Change in echocardiographic variables from baseline	36 months	Less frequent heart failure in the intervention arm (more in the metoprolol group) but not statistically significant
Salehi et al. (2011)[82]	66	Single-centre, Prospective, Randomised,	Breast (72%) Lymphoma (29%)	Doxorubicin (540.28±31.17 mg/m^2)	Carvedilol	Carvedilol (12.5mg/day) Carvedilol (25mg/day)	24 hours prior to chemotherapy up until 4 months	Change in echocardiographic variables from baseline	4 months	No statistically significant difference seen in the systolic and diastolic measures

		Placebo-controlled		Epirubicin (768.44±26.87 mg/m^2)						between the control arm and both treatment arms but results favoured carvedilol 25mg
Kaya et al. (2013)[66]	45	Single-centre, Prospective, Randomised, Placebo-controlled	Breast	Epirubicin (361±88 mg/m^2) Doxorubicin (257±29 mg/m^2)	Nebivolol	5mg/day	7 days prior to chemotherapy up until 6 months	Change in echocardiographic measurements and NT-proBNP from baseline	6 months	No statistical significance between the two arms in the echocardiographic and NT-proBNP levels but results favoured nebivolol
Bosch et al. (2013)[67]	90	Single-centre, Prospective, Randomised, Open-label, Controlled	Malignant haemopathies	Type unknown (290±189mg/m^2) +/- HSCT	Enalapril **and** Carvedilol	Enalapril (8.2±5.9mg/day) + Carvedilol (23.8±17mg/day)	24hr prior to the first cycle of chemotherapy up until 6 months	Drop in LVEF (≥ 10% to <50%) using echocardiography and cardiac MRI	6 months	Combination therapy showed some protective effects on LVEF. Intergroup difference -3.1% (p=0.04)
Elitok et al. (2014)[83]	80	Single-centre, Prospective, Randomised, Open-label, Placebo-controlled	Breast	Doxorubicin (523.3mg/m^2)	Carvedilol	12.5mg/day	Prior to chemotherapy, up until 6 months	Change in LVEF, FS (measured by m-mode) and strain parameters from baseline (using TDI)	6 months	A reduction in septal and lateral systolic strain and strain-rate values in the control group compared to the carvedilol group.
Akpek et al. (2015)[84]	83	Single-centre, Prospective, Randomised, Placebo-controlled, Double-blind	Breast	Doxorubicin (430.2±52.2mg/m^2) Epirubicin (688.9±136mg/m^2)	Spironolactone	25mg/day	1 week prior to chemotherapy up until 3 weeks after chemotherapy	Drop in LVEF >10% from baseline using echocardiography	3 weeks post chemotherapy	Protective effects on LVEF in the treatment arm compared to the control group (p<0.001) & diastolic measures. Higher TnI levels in the control arm (p=0.006)

Gulati et al. (2016)[63]	130	Single-centre, Prospective, 2x2 factorial, Randomised, Placebo-controlled, Double-blind	Breast	Epirubicin 240mg/m^2 (56%), 360mg/m^2 (18%), 400mg/m^2 (22%) +/- trastuzumab	Candesartan ± metoprolol	Candesartan (23±11mg/day) + metoprolol (68±34mg/day) Candesartan-placebo (26±9mg/day) Metoprolol-placebo (78±32mg/day)	Prior to chemotherapy up until 10-61 weeks	Change in LVEF (>5%) using cardiac MRI	3-5 months for patients receiving anthracyclines only. 15 months for those receiving additional trastuzumab (22%)	Candesartan showed some protective effects on LVEF (p=0.026). No benefit seen with metoprolol.
Beheshti et al. (2016)[85]	70	Single-centre, Prospective, Randomised, Placebo-controlled, Double-blinded	Breast	Doxorubicin (240mg/m^2)	Carvedilol	6.25mg twice a day	10 days prior to chemotherapy up until 10 days after last dose of chemotherapy	Change in LVEF and strain and strain-rate (using TDI)	1-week post chemotherapy	No significant change in LVEF between the intervention and control arms. Statistically significant decrease in all strain and strain-rate parameters in the control arm (p<0.001)
Jhorawat et al. (2016)[86]	54	Single-centre, Prospective, Randomised, Placebo-controlled, Single-blind	Malignant haemopathies	Doxorubicin (427.96 ± 124.36mg/m2)	Carvedilol	12.5mg/day	Prior to chemotherapy up until 6 months	The presence of any of the criteria highlighted by CREC for definition of CTRCD	6 months	No significant difference in LVEF seen at follow up between the intervention and control arm. But results favoured carvedilol with statistically significant drop in LVEF in the control arm from baseline.
Nabati et al. (2017)[71]	91	Single-centre, Prospective, Randomised,	Breast	Doxorubicin	Carvedilol	?	?	Changes in LVEF, LVEDV, LVESV, Land diastolic measures from baseline	6 months	Carvedilol showed protective effects on LVEF with a statistically significant drop in

		Placebo-controlled, Single-blind								LVEF in the control arm from baseline ($p<0.001$). Also, higher TnI level in control arm compared to the interventional arm ($p<0.036$)
Janbabai et al. (2017)[87]	69	Single-centre, Prospective, Randomised, Placebo-controlled, Single-blind	Breast (93%) Lymphoma (1%) Lung Ca (<1%) Bone sarcoma (<1%) Wilms tumour (<1%)	Doxorubicin ($363.34\pm34.87mg/m^2$)	Enalapril	17.94 ± 4.1mg/day	24hr prior to chemotherapy up until 6 months	change in LVEF from baseline	6 months	Enalapril showed protective effects on LVEF with a statistically significant lower LVEF in the control arm from baseline ($p<0.001$). Also, higher LVESV and LA diameter in the control arm compared to the intervention arm. Higher TnI and CK-MB levels in the control arm
Avila et al. (2018)[68]	192	Single-centre, Prospective, Randomised, Placebo-controlled, Double-blind	Breast	Doxorubicin ($240mg/m^2$)	Carvedilol	18.50 ± 17.60mg/day	First day of chemotherapy up until 20 weeks	Change in LVEF $\geq$10% from baseline	6 months	Carvedilol showed no protective effect on LVEF ($p=1.0$). Some protective effects on diastolic function (p0.039) and troponin elevation ($p=0.003$).
Cardinale et al (2018)[88]	273	Multi-centre. Prospective, Randomised, Controlled,	Breast (76%)	Epirubicin Doxorubicin	Enalapril	5mg/day	Prevention group: first day of	Troponin elevation above the threshold	12 months	No difference in troponin elevation or LVEF

		Open-label	Lymphoma (13%) Acute leukaemia (11%) Sarcoma/other (<1%)	Idarubicin Daunorubicin Doxorubicin (liposomal) (doxorubicin equivalent) 240 [189-252]mg/m^2)			chemotherapy cycle Troponin triggered group: only when troponin elevated			between both groups (note 22% of patients received bisoprolol for different reasons)

Table 3. Prospective studies assessing neurohormonal antangonist treatment in prevention of AIC

6. Detection of anthracycline-induced cardiotoxicity

6.1 Endomyocardial biopsy

In the 1970s, anthracycline induced cardiotoxicity was diagnosed by means of obtaining endomyocardial biopsies from left and right ventricles of patients who had been receiving these agents.[89, 90] Histological analysis of these biopsies proved that anthracyclines can affect both ventricles.[89, 90] This technique was once considered gold standard in aiding the diagnosis of cardiotoxicity.[91] However, given the invasive nature of endomyocardial biopsy and associated complication risk, non-invasive methods for diagnosing and monitoring the effects of the anthracyclines replaced this technique, and have been preferred in the modern era.

6.2 Electrocardiography

Although LV dysfunction is the most concerning cardiovascular complication of anthracyclines, cancer treatment-induced arrhythmia (CTIA) either as a primary issue, resulting from a direct effect of anthracyclines, or secondary to cardiomyopathy has been suggested as a possible associated problem with these agents.[92, 93] However, electrocardiographic changes with anthracycline exposure are usually non-specific. ST-T wave changes, or lengthening of the QTc interval have been commonly reported in the literature.[94-98] Furthermore, sinus tachycardia, supraventricular arrhythmias such as atrial fibrillation, premature atrial or ventricular complexes, and rarely ventricular tachycardia have also been described.[92, 99, 100] Nevertheless, it is somewhat difficult to confidently ascertain the direct association of these changes with anthracyclines in these studies due to the lack of baseline ECG monitoring prior to the commencement of treatment.[92] Additionally, if ECG changes are seen this does not necessarily reflect the existence, or the risk of future development of underlying anthracycline mediated cardiomyopathy.[24, 101]

Although the ESC recommends ECG monitoring during cancer treatment, this is not incorporated into the ASCO clinical guidelines due to its associated low diagnostic profile in detecting subsequent cardiomyopathy.[24, 60] It is also worth noting that the new guidelines are based on small clinical trials and therefore limited evidence. Hence better means of detection are required to help identify patients at risk of CTRCD.

6.3 Imaging modalities

Detection of AIC has been dependent on serial cardiac imaging to identify a reduction in the left ventricular ejection fraction (LVEF).[14, 24, 60]A number of different imaging modalities have been used in practice for identification and monitoring of cardiotoxicity in patients undergoing cancer treatment. Unfortunately, due to the lack of interchangeability between the measurements used with each technique, the utilisation of the same imaging modality for serial comparisons in patients undergoing cancer treatment monitoring has been advised.[24, 102-104] However, some controversy remains around the best method of surveillance for these patients.

6.3.1 Echocardiography

Given its non-invasive nature, lack of ionising radiation use, and its ability in providing valuable structural and functional information, two-dimensional echocardiography (2DE) is the first line investigation in clinical practice.[14, 24, 60, 91, 105] Three-dimensional echocardiography (3DE) is yet another more advanced imaging method when compared to 2DE due to its lack of dependence on geometrical assumptions, making this a more accurate and reproducible imaging modality in providing volumetric and functional information.[106-108] However, its lower spatial and temporal resolution considered key for optimal image quality, its susceptibility to stitch artefacts caused by patient movement, rhythm disturbances and respiratory motion, and its lack of widespread availability and operator experience has limited its routine use in clinical practice.[24, 109, 110] Therefore, 2DE has remained the mainstay imaging modality for both CTRCD and other clinical scenarios.

Several consensus statements and guidelines focusing on cardio-oncology have recommended the use of transthoracic echocardiography surveillance as the first line of investigation, owing to its wide availability, cost effectiveness and evidence base.[2-4, 13, 14, 24]

6.3.1.1 Systolic function

6.3.1.1.1 LV Ejection Fraction

Evaluation of LV systolic function has significant implications in the diagnosis, risk stratification, monitoring and management of patients with cardiovascular disease. So far, the most accepted and validated measure of LV systolic function is LVEF, which is defined as a

volumetric fraction of blood ejected during systole in relation to the LV end-diastolic volume.[111] With this measurement considered a powerful predictor of adverse cardiovascular events and mortality, LVEF has and continues to serve as a selection criteria in major cardiovascular clinical trials that have constituted the evidence-base for clinical practice guidelines.[24, 112] In cardio-oncology, several definitions have been used to define cardiotoxicity, reflecting the different LVEF criteria used across cancer studies and real-world clinical practice.[2, 14, 24, 113-115] Conventionally, a reduction in LVEF of ≥5% to <55% with symptoms of heart failure[4, 116]or an asymptomatic drop in the LVEF of ≥10% and to below the normal range (<53%)[2, 14, 115] or (<50%)[24] has been regarded as echocardiographic evidence of cardiotoxicity. This measurement has to be confirmed by repeat echocardiography after a few weeks of the initial scan, before a decision on chemotherapy is made, such as stopping a cardiotoxic agent or starting treatment for LV dysfunction.[2, 115]

However, it is well established that the use of LVEF has major limitations in this setting.[2, 4, 30, 117-119] This measurement is highly reliant on good image quality and delineation of the endocardial border for accurate assessment, and can be vulnerable to foreshortening. Additionally, recapturing the exact same imaging planes for follow-up purposes can be challenging, limiting the use of LVEF as a suitable measure for assessment of minor changes in LV systolic function, and identification of CTRCD during chemotherapy follow-up.[116] Furthermore, LVEF is sensitive to physiological factors creating variability in the loading conditions of the heart, masking the true underlying contractility of the left ventricle.[14, 120] Other shortcomings of the technique include the associated moderate level of inter- and intra-observer variability[14, 32-34, 116, 121] with a temporal variability of ~10%.[111, 116] These factors are of particular concern given the definition of cardiotoxicity relies on a decline in LVEF by 5-10%.[14, 35, 122] Crucially, when a true reduction in LVEF is seen, cardiotoxicity is already established, the chance of full recovery is low and the opportunity for early intervention has already been missed.[122-126] Clearly with this degree of variation in measurement, and the late manifestation of LVEF reduction in the pathophysiology of cardiotoxicity, better methods of detection are required.[119]

6.3.1.1.2 Myocardial deformation

With the absence of robust models in risk prediction, a number of studies conducted in cardio-oncology have focused on more advanced echocardiographic-derived measures of myocardial mechanics, namely strain and strain-rate, providing an insight into more accurate measurements of cardiac function.[30, 114, 125] "Strain" denotes a dimensionless index of deformation, measuring local shortening and thickening of the myocardium during stress at end-systole compared to its original length at end-diastole **(Figure 2)**.[111, 127] Due to the fractional change in the myocardial length and thickness, this is expressed as a percentage which can be negative or positive indicating shortening and thinning, or lengthening and thickening, respectively.[2, 30, 119, 127-129] "Strain-rate" is the speed at which deformation occurs with respect to time and has a unit of 1/s.[2, 30, 119, 128-132] Strain measurements focus on myocardial velocity, displacement and deformation, and have a remarkable ability to differentiate active versus passive movements within the myocardial tissue.[119] This key advantage provides a unique opportunity for quantification of both regional and global systolic and diastolic function, independent of the heart's translational motion.[30, 119]

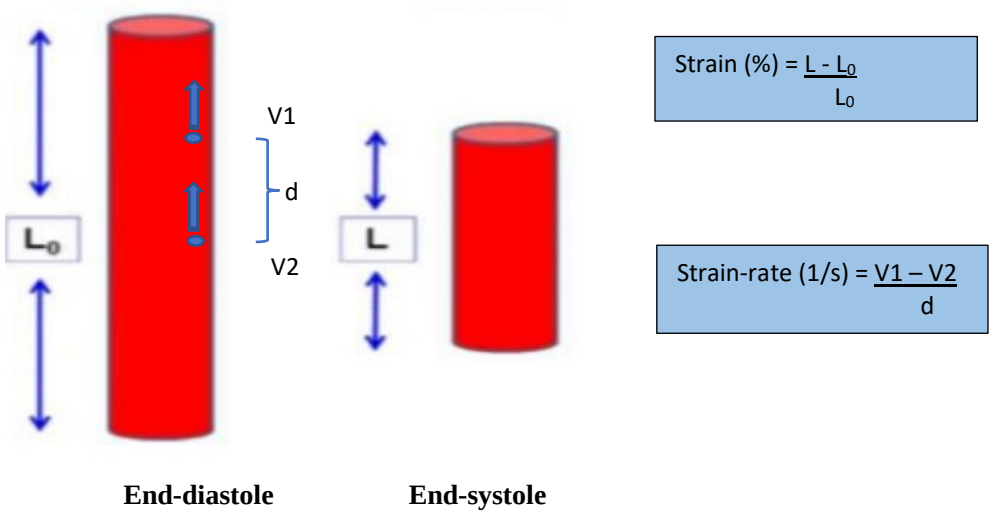


Figure 2. Strain and strain-rate pattern in longitudinal direction

6.3.1.1.3 Tissue Doppler Imaging

Originally, Mirsky et al. were the first to publish on the concept of myocardial strain in 1972.[133] Two years later, this theory was put into practice using M-mode echocardiography,[134] but never became part of routine clinical practice due to its cumbersome technique.[135] However, in 1998, after a great deal of research and work, strain imaging was enabled as an extension of Tissue Doppler Imaging (TDI).[136] Through the measurement of myocardial motion (velocity) at certain locations in the myocardium in relation to the transducer, strain and strain-rate measurements were made possible.[136, 137] TDI was accepted as a useful echocardiographic measure for the assessment of cardiac function, providing the ability to diagnose and predict outcomes in a variety of cardiovascular diseases.[138-141] Despite TDI providing a promising tool for the evaluation of LV function, several major limitations were found to be associated with this technique. One of the main disadvantages relates to the dependency of this measurement on the Doppler angle of incidence; only velocities parallel to the ultrasound beam can be measured with this technique.[111, 142] Additionally, with imaging at high temporal resolution to aid a better signal-to-noise ratio, major limitations are imposed on spatial resolution.[137, 142, 143] This methodology of strain analysis is also complex and time-consuming, and requires expert readers for its use.[131, 142] Clearly in considering these aspects, it is of no surprise that TDI-derived strain measurements are prone to high levels of interobserver variability of 10-15%, limiting its routine use in clinical practice.[142]

6.3.1.1.4 Speckle Tracking Echocardiography

Given the limitations of TDI-based measurements of strain, new improved non-Doppler techniques for strain and strain-rate measurements were developed.[144] This was achieved using Speckle Tracking Echocardiography (STE) which has been validated using sonomicrometry and tagged MRI (tMRI).[139, 145-150] “Speckles” are natural acoustic markers formed by the grey scale ultrasound interference patterns within the myocardial tissue.[30, 127, 129, 131, 132] The movement of these speckles, identified in discrete sections of the myocardium, can be followed (“tracked”) frame by frame throughout the cardiac cycle enabling the differentiation between active thickening and passive wall motion.[30, 130, 142] Using this method, STE has the ability to track speckles in two dimensions, along the direction of the wall rather than along the ultrasound beam.[2, 124, 127, 130, 142, 151] Additionally this technique, has better spatial resolution, and less sensitivity to signal-noise when acquiring the images.[128] Based on

these advantages, TDI has therefore been superseded by STE, allowing a more comprehensive assessment of myocardial deformation, independent of the Doppler angle.[2, 117, 129, 130] Moreover, STE is semi-automated, and as a consequence has better measurement reproducibility, is quick to perform and user friendly with straightforward data processing.[128]

However, one of the drawbacks to STE is the requirement for high resolution image quality for accurate measurements which can be a limitation in some patients.[117, 128] Also, strain measurements (both for STE and TDI) are vendor-specific with a lack of consistency in strain values amongst different vendors.[152, 153] The reasons for this variability are explained by several factors including: differences in the types of stored data required for analysis, varied terminology in the description of myocardial mechanics and parameters, and the output of results.[154-158] Clearly, this can pose a major barrier in the ability to ascertain whether an interval change in strain is a true effect, or secondary to software variation. Recognising this issue, has led to the gathering of multiple technical representatives from different vendor companies with the aim of identifying a solution in reducing inter-vendor variability. This has resulted in the formation of the first document in standardisation of deformation imaging attempting to reduce the hurdles surrounding this issue.[152]

Despite these issues, a number of validation studies have proven the consistency of STE when compared to other modalities with reasonable intra- and inter-observer variability (<8% and <6% respectively).[13, 117, 124] Since its introduction, STE (also known as two-dimensional strain analysis) has been widely applied in the assessment of different cardiovascular conditions, established as a valid measures of strain and is proposed as more objective, when compared to traditional methods, in quantifying cardiac function.[13, 114, 117, 127] More recently, STE has gained increasing recognition in the field of cardio-oncology owing to its potential to detect subclinical cardiac dysfunction before changes in LVEF are established.[124]

6.3.1.1.5 Types of Strain

Francisco Torrent-Guasp was the first anatomist to demonstrate that the heart is composed of a single intertwined muscular band, giving the heart its rotational movement and its ability to contract in several directions.[159] Taking this theory into consideration, with the different orientation of the myocardial fibres and the complex multi-dimensional deformation that the left ventricle (LV) undergoes during the cardiac cycle, three principle types of LV strain are

described by STE: longitudinal, radial and circumferential.[4] Longitudinal strain represents the shortening of the LV along its long axis, radial strain denotes the thickening of the LV wall along its radius and circumferential strain relates to the reduction in the LV cavity circumference during the cardiac cycle (

Figure 3). Beyond these linear deformation measurements, peak systolic LV torsion by STE, is a further measurement that focuses on the myocardial rotational deformation owing to the helical orientation of the myocardial fibres.[124, 147, 151, 160]

Global Longitudinal Strain

Abnormalities in strain and strain-rate, have shown an association with chronic heart failure prognosis.[118, 119, 129, 161] This finding is particularly strongly observed for global longitudinal strain (GLS), which is a combined measure of LV regional longitudinal strains.[35, 162] GLS represents the longitudinal muscle fibres located in the sub-endomyocardial layer of the heart.[126] The ability of GLS in providing additional information beyond LVEF in a variety of disease processes, has made this measurement a prognostically more valuable method of quantifying LV function when compared to LVEF.[163-168] This advantage over LVEF is explained by the ability to detect regional deterioration in myocardial function; in the early stages, the normal segments of the myocardium are able to compensate for this regional decline leading to a preserved LVEF.[169, 170]

In cardio-oncology, GLS has shown to precede and therefore predict subsequent declines in LVEF and hence has been the most studied and validated measurement so far.[13, 35, 115, 118, 119, 122, 162] Some studies have also revealed the usefulness of GLS systolic and diastolic strain-rates in this setting.[171, 172] An altered GLS is an independent and robust predictor of later cardiotoxicity, with a 10%-20% reduction observed amongst studies during treatment.[35, 122, 124, 171, 173] A relative decrease of >15% in GLS has been deemed to represent AIC.[4, 35, 115, 124, 129, 174] Therefore, based on these findings, the use of GLS for monitoring patients during cancer treatment has been recommended. [14, 24, 129]

Although, the American Society for Clinical Oncology (ASCO) has acknowledged the advantages in the use of this measure in early detection of subclinical LV systolic dysfunction,

it has not incorporated its use in the latest clinical guidelines due to the lack of evidence surrounding the clinical significance of early intervention based on changes in strain only.[60] Furthermore, a recent meta-analysis by Oikonomou et al. has highlighted that the despite the strong prognostic value of GLS, more prospective multicentre studies are required to assess the clinical utility of this measurement during cancer monitoring; this is due to the current clinical heterogeneity and publication bias seen in the studies done so far.[175]

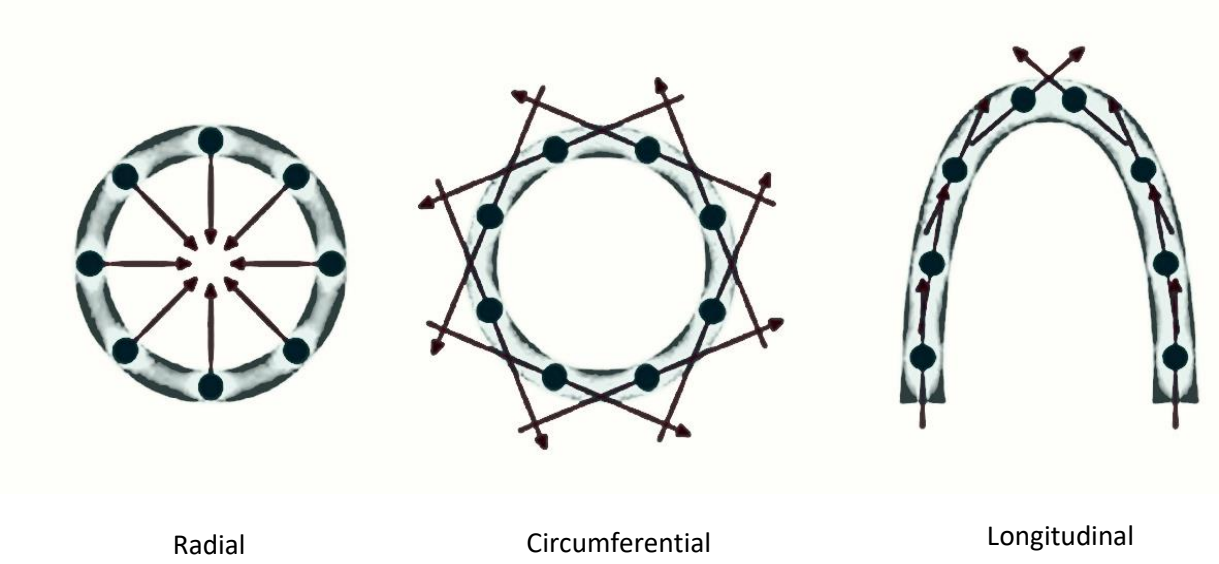


Figure 3. Different types of LV strain

Global Radial and Circumferential Strain

In the context of CTRCD, a number of studies have assessed the application of global radial (GRS) and global circumferential (GCS) strain in addition to GLS.[13, 118, 119, 122, 124, 126, 160, 176] Of these, some identified that global radial strain could be seen to reduce after 1 week to 3 months of administration of anthracyclines and could potentially be considered a robust parameter in detecting early myocardial damage during chemotherapy.[126, 177-180] However, other studies have been conflicting, failing to prove such findings and have demonstrated GRS to not be predictive of future toxicity.[119, 120, 160, 181] This has also been the case for GCS, with studies revealing contradictory results.[13, 118, 119, 125, 160, 176, 181] These results have been attributed to a lower reproducibility of these measurements,[13, 35, 122] small sample-size in the studies undertaken, and short follow-up periods (**Table 4**).

Therefore, the use of these parameters in routine clinical practice has not yet been validated and larger prospective studies are required to assess their role in this setting.[13]

Authors	Total no. of patients	Cancer Type	Treatment	Strain measurement	Follow-up period	Results
Ganame et al. (2007)[182]	13	Leukaemia Lymphoma Sarcoma	Anthracycline	2D TDI GRS and strain-rate	End of third cycle	Decline in radial systolic strain ($p<0.05$) after third dose, and radial strain-rate ($p<0.05$) after first cycle
Jurcut et al. (2008)[179]	16	Breast	Anthracycline	2D TDI GRS and strain-rate	End of sixth cycle	Decline in GRS ($p=0.001$) and strain-rate ($p=0.046$) after 3 cycles of chemotherapy
Sawaya et al. (2011)[170]	43	Breast	Anthracycline + Trastuzumab	2D STE GRS, GCS	6 months	Decline in GRS at 3 months $p=0.02$: predictive of cardiotoxicity, no change in GCS
Fallah-Rad et al. (2011)[180]	42	Breast	Trastuzumab (post anthracycline)	2D STE GRS	12 months	Decline in GRS at 3 months $p<0.001$: predictive of cardiotoxicity
Stoodley et al. (2011)[177]	52	Breast	Anthracycline	2D STE GRS, GCS, and strain-rates	1-week (post chemotherapy)	Decline in GRS ($p<0.01$) at 1-week post chemotherapy, no change in GCS
Tsai et al. (2011)[183]	47	Lymphoma	Anthracycline + radiotherapy (n=27) Radiotherapy alone +/- non-anthracycline regimens (n=20)	2D STE GCS	Recruited 22 years post chemotherapy	Reduced GCS in both treatment groups compared to control ($p<0.001$)
Sawaya et al. (2012)[173]	81	Breast	Anthracycline + Trastuzumab	2D STE GRS, GCS	15 months	Decline in GRS ($p<0.03$) and GCS ($p<0.005$) at 3months **but** not predictive of cardiotoxicity
Mornos et al. (2013)[169]	74	Breast Lymphoma	Anthracycline	2D STE GRS	12 months	Decline in GRS ($p<0.001$) at 6 weeks **but** not predictive of cardiotoxicity

		Leukaemia Osteosarcoma				
Negishi et al. (2013)[171]	81	Breast	Trastusumab +/- anthracycline, radiotherapy	2D STE GRS, GCS	12 months	No significant decline in GCS (p=0.18) and GRS (p=0.11)
Tarr et al. (2015)[126]	25 (plus 25 controls)	Breast Lymphoma Leukaemia Multiple myeloma Gastric Lung Hepatocellular Thyroid Lung Lingual RCC	Anthracycline, Platinum based chemotherapy, Kinase inhibitors, Alkylating agents,	2D and 3D STE GRS, GCS	3 months	Decline in 2D GRS (p<0.05) after 3 months, predictive of cardiotoxicity. No change in GCS and 3D GRS and GCS
Narayan et al (2016)[125]	135	Breast	Anthracycline +/- Trastuzumab	2D STE, GRS, GCS, and strain-rates	1.9 years	Decline in GCS, GRS and strain-rates post chemotherapy. GCS (and ventricular-arterial coupling) predictive of cardiotoxicity
Chang et al. (2016)[181]	35 (plus 10 controls)	Breast	Anthracycline	2D STE GRS, GCS and strain-rates	After third cycle of chemotherapy	No significant decline in GRS (p=0.07), GCS (p=0.53), LV radial strain-rate (p=0.4), LV circumferential strain-rate (p=0.74)
Narayan et al. (2017)[118]	165	Breast Lymphoma Leukaemia Sarcoma	Anthracycline	2D STE GRS, GCS and strain-rate	12 months	Decline in GCS (p=0.009) at 12 months but not predictive of cardiotoxicity, no change in GRS

Paraskevaidis et al. (2017)[184]	88	Leukaemia Lymphoma	BMT preceded by anthracyclines	2D STE GCS and strain-rate	12 months	No significant decline in GCS (p=0.10) and circumferential strain-rate (p=0.20) at follow up
Santoro et al. (2017)[124]	100	Breast	Anthracycline	3D STE GRS, GCS	After completion of anthracycline chemotherapy	Decline in GRS (p<0.002) and GCS (p<0.0001) post chemotherapy
Song et al. (2017)[174]	89	Lymphoma	Anthracycline	2D and 3D STE GCS	3 weeks post fourth cycle	Decline in GCS at follow up using both 2D STE (p=0.002) and 3D STE (p=0.0001)
Zhang et al. (2018)[185]	142 (plus 21 controls)	Breast	Anthracycline +/- Trastuzumab	3D STE GCS	2.1 years	Decline in GCS (p<0.001) compared to control and associated with subsequent LV systolic and diastolic impairment.
Alam et al. (2019)[186]	55	Breast Squamous cell carcinoma Osteosarcoma Ewings sarcoma	Anthracycline	3D STE GRS, GCS	After completion of anthracycline chemotherapy	Decline in GRS (p<0.001) and GCS (p<0.001) post chemotherapy

Table 4. Prospective studies assessing GRS and GCS in addition to other strain parameters in cardio-oncology

Torsion and Twist

During systole, owing to the helical myocardial fibre architecture, the base of the LV rotates in a clockwise pattern, with the apex demonstrating a counter-clockwise rotation.[147, 151, 160, 187, 188] Intuitively, this action is predominantly undertaken by the subepicardial fibres, whereas the subendocardial layers contract the LV base and apex in complete opposite directions. Despite this counterforce, the greater amount of power exerted by the outer located epicardial fibres prevents the nulling of LV rotation.[189] Twist, untwist and torsion are the most commonly used terminologies in describing LV systolic and diastolic rotational motion. "Twist" is the absolute angle difference between LV base and apex, measured in degrees.[30, 187] "Untwist" is the reverse of this phenomenon, during diastole.[187] As a consequence, a torsional deformation is created leading to a dynamic interaction between the opposing epicardial and endocardial myocardial fibre helices.[120, 147, 188] LV "torsion" is expressed in degrees/radians per centimetre, and is measured by dividing the twist angle by the distance between the cross-sectional planes of the LV base and apex.[187, 188]

Although LV twist measurements are load-dependent, any disease process affecting the normal myocardial geometry can lead to unwanted adverse effects on the LV twist mechanics.[190-194] Since the early 1990s, a number of studies have assessed torsion using tMRI.[151, 195-200] However due to the high cost and complex data analysis processing of tMRI, this technique has not gained widespread use.[151] However, with the recent development of STE, twist/torsion has once again gained interest and has been widely used in the assessment of different cardiac conditions offering a more comprehensive assessment of ventricular performance.[120, 160, 187, 201]

In patients with cardiomyopathy, abnormalities in the twist and untwist measurements have been clearly seen providing evidence that these measurements can deliver mechanistic information in the assessment of myocardial diseases.[189, 201, 202] This has also been the case in CTRCD, where the use of these measurements have shown some promising results in the detection of subclinical LV dysfunction (**Table 5**). In one study, a deterioration in LV torsion was observed with cumulative doses of anthracyclines, [160, 203] highlighting a potential role in the utilisation of torsion as a useful parameter in the early detection of anthracycline induced subclinical LV dysfunction.[120, 160, 203] In a further study, the addition of GLS to LV twist was shown to improve the prediction of cardiotoxicity in CTRCD.[120]

Authors	Total no. of patients	Cancer Type	Treatment	Strain measurement	Follow-up period	Results
Cheung et al. (2011)[204]	36 (plus 20 controls)	Leukaemia	Anthracycline	3D STE basal/apical rotation, peak apical/basal twisting rate, peak apical/basal untwisting rate, LV twist	Recruited 3.1-24.3 years post chemotherapy	Reduced apical rotation (p=0.03), peak apical untwisting rate (p=0.002), and twist (p=0.003) compared to control. Also reduced peak systolic twisting (p<0.001) and peak diastolic untwisting velocities (p=0.04)
Motoki et al (2012)[203]	25	Leukaemia Lymphoma	Anthracycline	2D STE torsion, twisting and untwisting rates	3 months	Early decline in torsion (p<0.0001), twisting rate (p<0.0001), and untwisting rate (p<0.0001) starting at 1month post chemotherapy
Yu et al. (2013)[205]	53 (plus 38 controls)	Leukaemia Lymphoma Sarcoma Neuroblastoma	Anthracycline	3D STE twist and torsion	Recruited 2.4-16.4 years post chemotherapy	Reduced twist (p<0.001) and torsion (p<0.001) compared to control
Mornos et al. (2013)[169]	74	Breast Lymphoma	Anthracycline	2D STE basal/apical rotation, LV twist, GLS x LV twist	12 months	Decline in LV apical rotation (p<0.001), LV twist (p=0.001), and GLS x LV twist (p<0.001) at 6 weeks. GLS x LV twist predictive of cardiotoxicity
Song et al. (2017)[206]	101	Lymphoma	Anthracycline	3D STE basal/apical rotation, twist, torsion, TTP	End of fourth cycle of chemotherapy	Progressive decline in apical rotation (P<0.01), basal rotation (p<0.01), twist (p<0.01) and torsion (p<0.01) starting from second cycle of chemotherapy. Torsion most significantly correlated with LVEF, no change in GLS

				basal rotation, apical rotation and twist		
Paraskevaidis et al. (2017)[184]	88	Leukaemia Lymphoma	BMT preceded by anthracycline	2D STE twist	12 months	Decline in LV twist at 3 months all the way through to 12 months (p=0.03)
Zhang et al. (2018)[185]	142 (plus 21 controls)	Breast	Anthracycline +/- Trastuzumab	3D STE twist and torsion	2.1 years	No significant decline in twist or torsion compared to control

Table 5. Studies assessing LV twist mechanics in addition to other strain parameters in cardio-oncology

Right Ventricular (RV) function and RV Strain

To this date, most studies of AIC have focussed on the LV with only limited number of these assessing the RV,[122, 174, 207-212] hence sometimes termed the "forgotten chamber".[213, 214] One potential reason for the relative lack of study could be due to the crescentic anatomic and morphological structure of the RV, which complicates its full assessment by conventional echocardiography.[174] However, with advancing imaging techniques such as STE, a more comprehensive assessment of the RV is made possible which has enabled the undertaking of more studies into this chamber highlighting the predictive significance of its structure and function in a variety of cardiovascular diseases.[215-223] Additionally, this has more recently supported the conduction of studies in cancer chemotherapy which have been able to detect changes in the RV in the context of anthracycline treatment emphasising the global effect of these agents.[174, 224, 225]

Despite scarce data, RV global longitudinal strain (RV GLS) and RV free wall longitudinal strain (RVFWS) are emerging as useful tools for the detection of subclinical myocardial dysfunction and their use in the full assessment of the RV function have been incorporated in an updated American and European guidelines on the chamber quantification.[114, 226] However, given the lack of data in AIC (Table 6) and the unknown prognostic implications of RV dysfunction in cancer treatment, the assessment of RV in the monitoring of patients undergoing chemotherapy has not yet been integrated into the latest clinical practice guidelines,[24, 60] but has been recommended by the ASE expert consensus statement paper.[14]

Authors	Total no. of patients	Cancer Type	Treatment	RV measurements	Follow-up period	Results
Ganame et al. (2007)[227]	56 (plus 32 controls)	Leukaemia Lymphoma Solid tumours	Anthracycline	2D TDI RVFWS	Recruited 2.0-15.2 years post chemotherapy	Decline in RV strain in basal RV free wall only in those treated with anthracyclines ($p<0.05$).
Tanindi et al. (2011)[211]	37	Breast	Anthracycline	2D TDI RV basal free wall (S'), RV FAC, TAPSE, tricuspid annular mean E'/A', and diastolic measures	Post second cycle of chemotherapy	Decline in RV FAC post second cycle (($p<0.001$) and TAPSE post first cycle of chemotherapy ($p=0.002$). Also decline in other RV diastolic measures. No change in RV S'.
Yağci-Küpeli et al. (2012)[228]	19 (plus 17 controls)	Lymphoma Sarcoma Neuroblastoma Hepatoblastoma Nasopharynx carcinoma Primitive neuroectodermal tumour	Anthracyclines	2D TDI RVFVWS and strain-rates	Recruited post chemotherapy	Reduced RVFWS and strain-rates compared to controls ($p<0.05$)
Calleja et al. (2015)[210]	30 (plus 30 controls)	Breast	Trastuzumab +/- anthracycline	2D STE RV GLS, RV FAC, TAPSE	Retrospective	Reduced RV GLS in 40% of patients with evidence of LV cardiotoxicity compared to controls. Concomitant RVSD at the time of LV cardiotoxicity is associated with reduced recovery of LVEF → statistically insignificant
Boczar et al. (2016)[229]	49	Breast	Anthracycline	2D STE RVFWS, RV FAC	Retrospective	Reduced RVFWS ($p=0.04$) and RV FAC ($p=0.01$) post chemotherapy compared to baseline.

Chang et al. (2016)[181]	35 (plus 10 controls)	Breast	Anthracycline	2D STE RVFWS and strain-rate, TAPSE, RV FAC & diastolic measures	After third cycle of chemotherapy	Decline in RVFWS (p=0.001) and TAPSE (p=0.01) after third cycle of chemotherapy
Christiansen et al. (2016)[230]	246 (plus 211 controls)	Leukaemia Lymphoma	Anthracyclines +/- mediastinal RT	2D STE RV GLS, RVFWS, RV S', TAPSE, RV FAC and diastolic measures	Recruited 21.7 years post chemotherapy	Reduced RVFWS (p<0.001), RV S' (p<0.001), TAPSE (p<0.001), and RV FAC (p<0.001) compared to controls
Murbraech et al. (2016)[231]	274	Lymphoma	Auto-HCT preceded by TBI, RT, anthracyclines	2D STE RV GLS, RVFWS, RV S', TAPSE, RV FAC	Recruited 6-20 years post chemotherapy	Reduced RV systolic function in 6.2% of patients compared to 0.7% of controls. Reduction in all RV systolic measurements in anthracycline-treated patients receiving high dose RT (>30Gy)
Paraskevaidis et al. (2017)[184]	80	Leukaemia lymphoma	BMT preceded by anthracyclines	2D STE RV GLS and strain-rate	12 months	Decline in RV GLS starting at 1 month follow up (p=0.02) and RV systolic strain-rate starting at 3months follow up (p=0.04)
Song et al. (2017)[174]	89	Lymphoma	Anthracycline	2D and 3D STE RV GLS	3 weeks post fourth cycle	No change in RV GLS at follow up using 2D STE (p=0.666) however significant decline in RV GLS using 3D STE (p=0.0001)
Calle et al. (2018)[232]	66	Breast	Anthracycline + Trastuzumab	2D STE RV GLS and strain-rate	Retrospective	Reduced RV GLS and RV systolic strain-rate post first cycle of chemotherapy (p<0.001) and post second cycle of chemotherapy (p<0.01). RV GLS combined with LV GLS strong predictor of cardiotoxicity (AUC 0.9; sensitivity 100%, specificity 83%; p<0.001)
Gripp et al. (2018)[233]	49	Breast	Anthracycline +/- Trastuzumab	2D STE RV GLS, RVFWS, TAPSE, RV S'	12 months	Minor non-significant changes in the RV GLS and RV FWS at 3 months and 6months follow-up with subsequent normalization. No change in TAPSE and RV S' during follow up.

Khairat et al. (2019)[234]	100 (plus 100 controls)	Osteosarcoma	Anthracycline	2D STE RV GLS, RV basal, mid and apical strain, TAPSE, RV FAC	3 months	Decline in RV GLS (p=0.001) and RV basal, mid and apical strain (p=0.001) in 7 patients at 10 weeks post chemotherapy increasing to 12 patients at 29 weeks. Decline in TAPSE only at 20 weeks (p=0.044) with decline in both TAPSE and RV FAC at 29 weeks Only 4 patients at 3 months with persistent RV dysfunction (reduced RV GLS, RV basal, mid and apical strain, TAPSE and RV FAC)

Table 6. Studies assessing RV strain using 2D echocardiography in AIC

Left Atrial Strain

The left atrium (LA) plays an important role in the cardiovascular function contributing to 20-30% of the total LV stroke volume, or even higher in the setting of LV dysfunction.[235, 236] It is now well established that enlargement of the LA, is an independent predictor of adverse cardiovascular outcomes and its function serves as a key prognostic factor in several cardiovascular diseases.[235, 237-248] Traditionally, cardiac catheterisation was considered the mainstay investigation for the assessment of LV filling pressures and diastolic function. However, with the development of Doppler echocardiography and more recently STE, a new non-invasive method of assessment has been made available.[249, 250]

Despite its initial purpose to supplement LV function quantification, STE has recently been further incorporated into the evaluation of LA function.[251] In principle, LA function comprises of three main phases which include LA "reservoir" (εR) occurring during systole, "conduit" (εCD) in early diastole, and "contraction" or "booster pump" (εCT) in late diastole, phases.[251, 252] A close interaction exists between the LA and LV during the cardiac cycle. LA reservoir phase denotes LA relaxation as the LV base descends during systole.[252, 253] In contrast, LA conduit function is dependent on the suction force of the LV. This is related directly to the underlying LV diastolic function, and the LA booster function is reliant on the contractility of the LA combined with LV end-diastolic pressures.[254-256] The recent application of STE, has enabled the assessment of all three phases, aiding the detection of minor alterations in LA deformation at these different stages of cardiac cycle, in spite of the presence of normal conventional echocardiographic measurements (**Figure 7**).[251, 257]

To date, most studies on LA strain and function have predominantly been in the setting of valvular heart disease, prediction of atrial arrhythmias and LV diastolic dysfunction.[249, 258-260] Data on the measurement of these parameters in the context of chemotherapy has been scarce. In one study investigating LA function in breast cancer patients post anthracycline administration, the intra- and inter mechanical delays, considered as electrophysiological features of the atrium prone to atrial fibrillation, were found to be prolonged.[261] This finding revealed a potential higher risk of atrial arrhythmia development leading to increased morbidity and mortality.[261] A further retrospective study in 100 patients with breast cancer receiving either anthracyclines, trastuzumab, or radiation treatment, revealed a reduction in LA

contraction strain post treatment which was associated with age. However, this was not predictive of cardiotoxicity.[262]

Given the close interplay between the LA and LV and the fundamental role of LA in maintaining cardiac function, its assessment could provide some insight into the effects of anthracyclines on this cardiac chamber.[261] Currently no other studies than the ones mentioned have assessed the role of this chamber in this clinical setting. Therefore, more research is required to evaluate the clinical utility of LA function in CTRCD.

Right Atrial Strain

The right atrium (RA) has also been relatively neglected in the assessment of cardiac function. This chamber only gained interest following 1979, after Bloomer et al. were the first to measure its dimensions.[263] However, even then its purpose was mainly studied in mass lesions or electrophysiological assessments. The role this chamber played in the right heart systolic and diastolic function was not fully explored.[263] Nevertheless, recently, some studies have assessed the RA, its function and strain, mainly in the context of pulmonary arterial hypertension (PAH).[264-266] Despite the sparse literature available, the results from these studies have been promising, demonstrating the usefulness of the RA measurements including strain, and the potential valuable information these could add in the assessment of right heart function aiding the prediction of clinical outcomes in PAH.[161, 265, 267-269] Furthermore, right atrial strain measurement as an adjunct to simultaneous strain assessment of the other chambers could provide new insight into inter-chamber relationships.[161]

As seen with the assessment of LA, the RA serves as three separate phasic roles during the cardiac cycle which include RA reservoir, conduit and contractile functions; their analysis has been enabled and considered feasible using STE.[270-274]

Right atrial function/strain has not yet been studied in the context of CTRCD. Given the evidence that anthracyclines could affect the right heart function in addition to the left ventricle, the use of this measurement could add supplementary information about the changes the right heart undergoes during treatment with these agents.

6.3.1.2 Diastolic function

In the general population, conventional echocardiographic measurements of diastolic dysfunction are well known to have a strong association with the future development of heart failure.[275-278] There have been suggestions that the assessment of diastolic function could assist the early detection of subclinical LV systolic dysfunction in those at risk of cardiac decompensation which could potentially facilitate the early initiation of cardioprotective treatments.[279] Therefore, we included detailed diastolic function assessment for the classification and prognostication of cardiovascular diseases.[280]

Despite its practicality, some associated limitations exist with diastolic function assessment requiring careful consideration. One of these include the susceptibility of its measures to the loading conditions affecting the heart (i.e. chemotherapy administration, hypertension, tachycardia, etc.).[281] Another limitation is the age-related physiological changes which can lead to altered diastolic measures considered normal for that specific age group, emphasising the importance of using age-adjusted reference values at the time of analysis.[281] Nevertheless, incorporating diastolic function assessment into practice has been recommended by the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[282]

In cardio-oncology, a number of studies have investigated the role of diastolic measurements in the context of CTRCD with variable findings.[169, 170, 262, 279, 280, 283-288] In one study investigating the effects of anthracycline chemotherapy in the treatment of cancer, a worsening diastolic function was observed at lower than normal anthracycline doses.[283] In a further pilot study conducted, asymptomatic diastolic dysfunction was found in 36% of patients 1 week post chemotherapy treatment.[286] However, despite the alterations in the diastolic function in some studies, the role of these measures in the prediction of subsequent risk of LV systolic impairment and adverse cardiovascular outcomes were not studied. Recently, a study which was the first largest prospective study assessing diastolic function and its association with LV systolic impairment in CTRCD, a worsening persistent diastolic dysfunction was observed starting early on with doxorubicin exposure.[280] This abnormality was found to have a modest association with LV systolic dysfunction highlighting a role in the importance of diastolic measures in cardio-oncology.[280]

6.3.2 Radionuclide Imaging

One of the other imaging modalities commonly used in cardio-oncology is multiple-gated acquisition (MUGA) imaging. Owing to its high reproducibility and availability, this imaging technique was once considered the backbone of cardiac functional assessment.[102, 103, 289, 290] It was the use of serial MUGA imaging that gave rise to the earliest definition of AIC in the late 1970s; a decline of >15% in LVEF to <45% using serial MUGA scans was considered as evidence of moderate cardiotoxicity.[291] This definition was later altered after the largest MUGA-based study on anthracycline chemotherapy defined cardiotoxicity as a >10% drop in LVEF to <50%.[290]

Despite its advantages and widespread clinical use, MUGA imaging was soon replaced by other imaging techniques such as echocardiography and cardiac MRI. This was mainly owing to the changes in equipment and technique, the associated repeated radiation exposure with this modality, its inability to provide additional structural and functional information beyond LVEF measurements, and its higher cost when compared to echocardiography.[14, 91, 292]

More recently, new emerging techniques such as positron emission tomography (PET) have been put to test in the context of CTRCD with promising preliminary results.[293-296] Given its high spatial and temporal resolution and diagnostic accuracy, PET has been able to assess cardiac metabolism and perfusion.[297] However, data regarding its role in AIC is limited and therefore more studies are required to assess its utility in this context.

6.3.3 Cardiac Magnetic Resonance Imaging

Currently, cardiac magnetic resonance imaging (CMR) is the gold standard imaging modality for the detection of minor changes in the ventricular volumes and function.[209, 289, 298] CMR has a better reproducibility when compared to echocardiography with a superior ability in aiding the diagnosis of cardiomyopathies.[299-304] However, CMR studies into CTRCD are limited and so far, have failed to prove a relationship between minor alterations in the myocardial function measurements and the future development of cardiotoxicity and heart failure.[91] Currently a prospective observational study is underway assessing the predictive role of CMR in anthracycline/trastuzumab related cardiotoxicity and LV impairment.[305]

The associated high cost and the lack of widespread availability, the long duration of each exam, and claustrophobia which some patients may experience, have limited the routine application of CMR and therefore, its use has not yet been incorporated into the clinical practice guidelines.[14, 60, 306, 307] Only when a lack of other imaging modalities is an issue, is where the use of this imaging technique has been advised.[14, 60]

6.4 Cardiac biomarkers

In addition to utilisation of imaging modalities, there has also been a growing interest in the concomitant use of cardiac biomarkers in the screening and monitoring of patients treated with anthracycline chemotherapy. These biological variables are capable of providing valuable information about the normal and pathological processes that occur during chemotherapy administration which may be useful in identifying, risk stratifying and monitoring patients treated.[308] Furthermore, early identification of cardiotoxicity allows the instigation of preventative therapeutic strategies before clinical cardiotoxicity has developed.[309]

6.4.1 Troponins

The use of biochemical markers for the detection of possible myocardial damage was initially introduced in the early 1950s by Karmen et al, when an increase in the levels of serum glutamate oxaloacetate transaminase (now aspartate transaminase) in those patients presenting with acute myocardial infarction was noted.[310] This finding led to more stimulating attempts to aid identify better markers of myocardial damage (e.g lactate dehydrogenase, creatinine kinase and their isoenzymes). However due to the lack of specificity and sensitivity of these biomarkers, it was not until the 1980s when the attention of the researchers shifted towards the myofibrillar proteins of the myocardium named cardiac troponins.[311]

Troponins are protein complexes involved in the modulation of contraction and relaxation of striated muscle through interaction with calcium ions and inhibition of ATPase activity of the actin-myosin filaments.[312] The majority of troponins lie within the contractile apparatus of the myocytes with only 3-8% found soluble within the cytosol under physiological conditions.[313, 314] Troponins consist of three different subunits: troponin I, T and C (cTnI, cTnT, cTnC).[69, 311, 315, 316] Amongst these, cTnI and cTnT are considered the most sensitive and specific biomarkers for detecting cardiac damage.[69, 311, 315, 316] Their clinical utility has been well

established in the evaluation of patients with suspected myocardial infarction and they are now considered "gold standard" for the biochemical diagnosis of myocardial necrosis in acute coronary syndromes.[69, 317-323]

The role of troponin in identifying cardiac damage, and therefore potentially predicting subsequent chemotherapy-induced cardiotoxicity has also been extensively investigated.[69, 316, 324-330] Seino et al. were the first to report cTnT as a biomarker of doxorubicin cardiotoxicity in spontaneously hypertensive rats in the early 1990s.[311] Later on, Auner et al. demonstrated a greater degree of LVEF reduction in those patients with positive cTnT levels when treated with high dose anthracyclines, compared to those without any troponin elevation.[331] Since then, a number of studies have demonstrated a positive correlation between raised troponin levels and risk of cardiotoxicity in patients receiving high dose chemotherapy.[311, 316, 329, 330, 332] Over a mean follow up of 20 months, a study conducted by Cardinale et al. demonstrated that elevated troponin levels 3 days post anthracycline administration and at one month post treatment were predictive of future development of LV dysfunction in patients suffering from cancer.[316, 333] Furthermore, the same group revealed that a negative troponin during and at one month of chemotherapy can essentially exclude significant cardiotoxicity in those patients treated with high dose anthracyclines.[333] However, a few studies conducted in childhood cancer survivors have failed to demonstrate this association which may partially be attributed to the use of different biomarker assays.[334-336]

In the recent years, the development of high sensitivity troponin assays has provided a more sensitive evaluation of subclinical myocardial reserve with the aim of improving the risk stratification for patients undergoing chemotherapy treatment for their cancer.[173, 337-340] Ky et al. demonstrated a positive correlation between raised high-sensitivity TnI from baseline to three months, and risk of future cardiotoxicity.[339] Despite these findings, when compared to contemporary measures of troponin, measurement of high sensitivity troponin has not offered any superior prognostic ability.[341]

The European Society of Cardiology (ESC) position statement and the American Society of Clinical Oncology (ASCO) guidelines have acknowledged the need for monitoring of patients for signs of cardiotoxicity using imaging modalities having agreed that utilisation of cardiac biomarkers such as troponin or natriuretic peptides may be useful in further cardiac surveillance.[24, 60] They have however recommended the use of the same assay for follow-up

purposes to increase comparability, and have advised careful attention to the timing of blood sampling.[24]

Currently, some studies are underway assessing the role of cardiac troponins in the early initiation of cardioprotective treatment with the hope of preventing cardiotoxicity.[79, 80, 342] ICOS-ONE (Prevention of anthracycline-induced cardiotoxicity) clinical trial was a recent study designed to use the well described relationship between cardiotoxicity and troponin in a randomised clinical trial.[343] The study was the first to investigate the effectiveness of enalapril either in a prophylactic setting or in response to troponin elevation. This study showed an increase of 23% in troponin levels in patients in the pre-treatment arm, compared to 26% in those receiving enalapril post troponin elevation ($p = 0.50$). In this trial, only 1.1% of patients developed cardiotoxicity during follow up by conventional echo criteria, with no significant difference between the two groups. More research into the use of these biomarkers in CTRCD is required before their use is incorporated into routine clinical practice.

6.4.2 Natriuretic peptides

Since their discovery almost three decades ago the role of natriuretic peptides alongside their inactive N-terminal amino acid fragment (NT-proBNP) has been well established in aiding heart failure diagnosis and predicting cardiovascular outcomes.[344-346] After troponins, these biomarkers have been the second most researched markers of cardiotoxicity in CTRCD. Their influence in detecting chemotherapy related cardiotoxicity has been extensively evaluated with some studies demonstrating promising results.[336, 347-351] In one study investigating the role of BNP measurement in anthracycline chemotherapy, elevated BNP levels were noted in all of those patients experiencing pre-specified cardiac events (10%).[352] A further study conducted in women with breast cancer, demonstrated a strong correlation between NT-proBNP levels and future development of cardiomyopathy.[353] Despite these studies, others have revealed contradictory results with some failing to demonstrate a predictive value in these biomarkers.[170, 173, 339, 354, 355] One explanation for this variation in results could be explained by the influence of factors including age, gender, weight, underlying cancer diagnosis, renal function and haemoglobin status on BNP levels. Furthermore, a lack of standardised biomarker reference range, as well as small study-sample sizes and different treatment schedules could be other crucial reasons for these contrasting results.[356-359]

6.4.3 Myeloperoxidase

The role of myeloperoxidase (MPO), an enzyme released by polymorphonuclear leukocytes mediating oxidative stress, has also been investigated in cardio-oncology. In evaluating the utilisation of this biomarker in breast cancer patients receiving doxorubicin and trastuzumab, Ky et al. were the first to prove the predictive value of MPO in AIC.[339] A subsequent study conducted by Putt et al. further supported these findings by proving the continued predictive value of MPO in cardiotoxicity in patients with elevated levels beyond three months post chemotherapy.[354] However, despite these positive findings, measurement of MPO has not yet been incorporated into routine clinical practice. More studies are required to prove its role in relation to predicting cardiovascular outcomes in patients with cancer.

6.4.4 Other biomarkers

In addition to cardiac troponins, BNPs, and MPO, the role of other emerging biomarkers including galactin-3, ST2, growth differentiation factor 15 (GDF-15), high-sensitivity CRP (hs-CRP), and microRNAs has also been investigated in the context of CTRCD.[173, 339, 360, 361] Findings from these studies have been variable with some failing to detect a correlation between some of these biomarkers and future risk of cardiotoxicity.[173, 339, 362] However, one study conducted on a group of paediatric cancer survivors was able to detect an association between GDF-15 and late onset cardiotoxicity.[363] Furthermore, microRNAs, involved in gene expression regulation, have recently shown encouraging results in their ability to predict cardiotoxicity.[364, 365] However, firm evidence regarding the use of these biomarkers is lacking highlighting the need for more studies prior to their utilisation in routine clinical practice.

Other biomarkers such as heart-type fatty acid-binding protein (H-FABP) and glycogen phosphorylase BB (GPBB), both secreted into the blood-stream in response to myocardial ischaemia and necrosis, are currently under investigation to evaluate their role in chemotherapy-induced cardiotoxicity.[366]

7. Research Objectives

With improving cancer survivorship and development of anti-cancer treatments, chemotherapy-induced cardiotoxicity has gained an increasing recognition amongst the oncology, haematology and cardiology specialties. Despite attempts in limiting the dose of anthracycline chemotherapy within treatment regimens for some cancers,[367] these agents continue to form the backbone of modern management protocols in daily practice. Given the associated cardiotoxicity with anthracyclines irrespective of dose, identifying the best method of early detection of AIC is crucial in allowing the instigation of appropriate therapeutic and surveillance measures before it is considered late.

Although a number of studies have explored the utilisation of either single or combined imaging and biomarker use aiming to establish better means of early recognition of AIC, most have focused their attention on the LV without much consideration other cardiac chambers. Furthermore, some advanced echocardiographic measurements have not been systematically assessed in cardio-oncology with limiting and conflicting evidence for other measurements. Currently guidelines regarding the best mode of surveillance and timing of cancer treatment remain unclear.[24, 60] Therefore, more research is required into this field before this is widely implemented in clinical practice.

This thesis aims to investigate the role of advanced STE and cardiac biomarkers, beyond those measurements of LVEF and GLS, in identifying better means of detecting early measures of AIC. A retrospective, followed by a prospective study have been conducted to assess the effects of anthracyclines on all four cardiac chambers.

7.1 Retrospective Study

7.1.1 Aims

The "*Detection of early anthracycline induced cardiotoxicity using speckle tracking echocardiography in patients with lymphoma*" study is the retrospective observational study of this thesis (*Appendix 1*). This study has been designed to explore the utility of advanced STE in the early detection of AIC in patients with lymphoma. This will allow the assessment of reliability and reproducibility of the findings, helping to inform the design of further prospective studies. Furthermore, if better means of early detection of cardiotoxicity are

identified, it will potentially enable the early appropriate cardiovascular treatment for those patients affected, without compromising cancer treatment.

7.1.2 Objectives

- To collect detailed information on a cohort of patients who have received anthracycline chemotherapy for the treatment of their lymphoma between January 2015 to January 2018
- To assess cardiac function in detail by STE measuring LV GLS, global circumferential strain (GCS) and global radial strain (GRS), LV twist and torsion, right ventricular free wall strain RV FWLS, left and right atrial strains, and strain-rates
- To determine which measure, or combination of measurements, are most sensitive and specific for early cardiac damage, in the subset of patients whose LVEF, measured by Simpson's biplane method, has declined by >10% after anthracycline treatment and whether this was evident at an earlier time point
- To assess which single or combination of measurements are better at detecting subclinical LV systolic dysfunction when compared to GLS
- To assess if any routinely available clinical or demographic factors are associated with echo changes following anthracycline chemotherapy
- To examine the inter and intra observer variability of LVEF, GLS and the novel strain parameters

7.2 Prospective Study

7.2.1 Aims

The prospective study of this thesis is the "*PROACT PLUS registry and echocardiographic sub-study*". The full study protocol with the relevant study forms are provided in *Appendices 4 and 5,* respectively. To further supplement the findings of the PROACT (Preventing cardiac damage in patients treated for breast cancer: a phase 3 Randomised, Open label, blinded endpoint, superiority trial of enalapril to prevent Anthracycline-induced CardioToxicity)[79] clincal trial, this parallel prospective observational cohort study has been designed. This will allow the assessment of the cardiac effects of anthracyclines on all the patients with a diagnosis of breast cancer and lymphoma who fall outwith the eligibility criteria for the PROACT trial.

Furthermore there is limited information on the effects of lower dose anthracyclines regimens and the utility of newer echocardiographic methods and cardiac biomarkers which will be studied in the PROACT PLUS registry.[176]

7.2.2 Objectives

- To assess troponin T release in the PROACT PLUS registry patients, during, and at one month post anthracycline chemotherapy
- To assess cardiac function using STE, measuring LV GLS, GRS and GCS, twist, torsion, right ventricular free wall strain, left atrial and right atrial strain and strain-rates at baseline and at one month post anthracycline chemotherapy
- To assess LA strain change with chemotherapy, and how this relates to LV function
- To assess RA strain change with chemotherapy, and how this relates to RV function
- To assess if any routinely available clinical or demographic factors are associated with echo changes following anthracycline chemotherapy
- To examine the inter and intra observer variability of LVEF, GLS and the novel strain parameters

7.2.3 Hypothesis

1. In this feasibility study, we hypothesise that a reduction in the LV strain values prior to a deterioration in the LVEF is seen in patients with breast cancer and lymphoma undergoing anthracycline chemotherapy, using advanced STE

2. Given the thinner wall structure of the RV and atria, we hypothesise that changes in the strain parameters of these chambers will detect cardiac damage sooner than the LV strain measures, using the same methods.

3. We hypothesise that troponin T and I release will be directly correlated with a reduction in strain parameters identifying patients at risk of future LV impairment

Chapter 2: Methods

Current literature and latest guidelines suggest a relative decrease of >15% in the GLS as evidence of subclinical LV dysfunction with a change of < 8% considered as clinically non-significant.[14, 24] However, no value has yet been assigned to other strain parameters as a marker of CTRCD due to the underlying inconsistencies and lack of sufficient evidence surrounding these measurements. Therefore, the aim of this thesis is to assess all strain parameters discussed earlier, in a retrospective followed by a prospective setting (with the addition of cardiac biomarkers in the prospective study). This will allow the evaluation of the role of advanced STE (and cardiac biomarkers) in the early detection of AIC and assess the extent of changes in these measurements, if any, in patients undergoing anthracycline chemotherapy treatment.

2.1 Retrospective Study

2.1.1 Ethical Approval

The "Detection of early anthracycline induced cardiotoxicity using speckle tracking echocardiography in patients with lymphoma: a retrospective cohort study" received a favourable opinion by the *South East Scotland Research Ethics Committee 02*, and was approved by *HRA and Health and Care Research Wales (HCRW)*, reference number *18/SS/0139* (*Appendix 2*).

2.1.2 Study Registration

For transparency purposes, this study is registered with "ISRCTN registry" under study identification number of *ISRCTN84544539.*

2.1.3 Study Design

This study was a retrospective observational study conducted at The James Cook University Hospital, in Middlesbrough. It evaluated which individual or combined strain measurements using 2D STE aided the early detection of AIC in the subset of patients whose LVEF had declined by >10% after anthracycline treatment, at an earlier time-point.

2.1.4 Study Population

In collaboration with the haematology team at The James Cook University Hospital we identified patients who had received anthracycline chemotherapy for the treatment of lymphoma between January 2015 to January 2018 through a computerised search of the haematology database. A list of patients deemed suitable for the study were identified and anonymized for data collection and analysis purposes. **Table 7** summarises the different anthracycline containing chemotherapy regimens used in the treatment of lymphoma at this hospital and in the United Kingdom (UK). All treatment, except for prednisolone, dacarbazine, and filgrastim had been administered intravenously. Each patient had undergone an echocardiogram before the commencement of chemotherapy (T0), mid-chemotherapy treatment (T1), and end of chemotherapy (T2) as part of standard care.

2.1.4.1 Inclusion criteria

- Patients with a new diagnosis of histopathologically confirmed lymphoma between January 2015 to January 2018
- Patients who had received anthracycline based chemotherapy for the treatment of their lymphoma between January 2015 to January 2018 (**Table 7**)

2.1.4.2 Exclusion criteria

- Inadequate echocardiographic imaging on Picture Archiving and Communication System (PACS) database
- Explicit dissent and unwillingness to participate in research detailed in the medical notes

2.1.5 Research Procedures

Data that were collected on patients deemed suitable for the study are included below. Each patient was allocated a unique study ID for confidentiality purposes.

2.1.5.1 Demographic information

The following demographic data were obtained and recorded from patients' medical records:

- Age
- Gender
- Ethnicity

2.1.5.2 Medical history

Information regarding patients' full medical history relevant for study purposes, including the list of medications were obtained from the medical records.

Regimen	Type of Cancer	Description	No. of cycles	No. anthracycline cycles	Dose of anthracycline	Total dose of anthracycline
R-CHOP	Advanced Non-Hodgkin's Lymphoma and Hodgkin's lymphoma (nodular lymphocyte type)	Rituximab 375mg/m2, Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (for 1 to 5 days)	6	6	50mg/m2	300mg/m2
CHOP	Advanced Non-Hodgkin's Lymphoma	Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (for 1-5 days)	6	6	50mg/m2	300mg/m2
ABVD	Advanced Hodgkin's Lymphoma	**Adriamycin (doxorubicin)** 25mg/m2, Bleomycin 10,000 IU/m2, Vinblastine 6mg/m2, Dacarabazine 375/m2	6*	12*	25mg/m2	300mg/m2
Escalated BEACOPP	Advanced Hodgkin's Lymphoma (based on PET scan results)	Bleomycin 10,000 IU/m2, Etoposide 200mg/m2, **Adriamycin (doxorubicin)** 35mg/m2, Cyclophosphamide 1250mg/m2, Oncovin	4-6**	4-6**	35mg/m2	140mg/m2-210mg/m2

		(vincristine) 1.4mg/m2 (max 2mg), Procarbazine 100mg/m2, Prednisolone 40mg/m2, Filgrastim 300mcg				

Table 7. Common chemotherapy regimens used in the treatment of lymphoma

**Doxorubicin 25mg/m2 given 2 weekly x 12 doses (6 cycles of treatment – each cycle is a 4-week block)*
***Esc BEACOPP either given from the outset due to high clinical risk (x6 Esc BEACOPP) or given due to a poor PET/CT after two cycles of ABVD (x2 ABVD, x4 Esc BEACOPPP)*

2.1.5.3 Echocardiograms

2D echocardiography images taken at T0, T1, and T2 time-points were obtained by two different commercially available ultrasonographic systems including *Epiq 7C (Philips Ultrasound Inc, Bothwell, USA)* **and** *Vivid E95 (GE Vingmed Ultrasound AS, Horten, Norway).* These were equipped with *X5-1 (1 to 5 MHz)* and *MS5c-D (1.5 to 4.5 MHz)* fully sampled matrix array transducers, respectively.

Although two different ultrasound machines were used, care was taken to ensure the same machine was utilised for follow-up purposes in the majority of patients to reduce the risk of vendor variation when obtaining strain measurements. Furthermore, due to the vendor-independent nature of TomTec™, the variation in strain measures between the two machines was further reduced.

Images were obtained in accordance with the recommendations of the British Society of Echocardiography (BSE) with superimposed ECG, and were optimised for angle, focus, depth, and sector size achieving a frame rate of 50-70 fps.[368] Images were obtained by BSE accredited sonographers. All echocardiographic images were digitally stored, and after anonymisation were analysed offline using vendor-independent software (TomTec Imaging Systems, 2D Cardiac Performance Analysis, Unterschleisshiem, Germany). Each scan had to contain a full cardiac cycle to enable analysis, with scans lacking a full cardiac cycle omitted from the study. All analysis was undertaken whilst blinded to patients' clinical data.

2.1.5.3.1 2D, M-Mode, and Doppler echocardiography analysis

All measurements were performed in accordance with the recommendations from ASE.[114] 2D linear internal measurements of the LV for wall thickness, cavity size, and fractional shortening were obtained using the parasternal long-axis view. This further allowed the automatic measurement of LV mass by TomTec™ software using the *cube* formula. This was then indexed for body surface area (BSA) to generate LV mass index. Additionally, LA dimension was also measured using the same imaging plane at end-systole.

For attaining volumetric measurements of the LV, non-foreshortened apical four- and two-chamber views with good endocardial definition were used in end-diastole and end-systole which allowed the subsequent measurement of LVEF using the biplane method of disks summation (modified Simpson's biplane rule). For the purpose of this thesis, a LVEF of < 53% was considered impaired.[14, 114] LA and RA volumes were also obtained by means of the biplane disk summation technique using the apical four- and two-chamber views for LA, and the apical four-chamber view for RA. Once again, all volumetric measurements were divided by BSA to achieve indexed values. An indexed LA volume of > 34 mL/m^2 was considered as abnormal.[114, 282]

For quantification of RV dimensions, the non-foreshortened apical four-chamber view was used enabling the measurement of RV basal- (RVD1) and mid-cavity (RVD2) dimensions. Furthermore, RV end-diastolic and end-systolic areas were measured from this imaging plane allowing the calculation of RV FAC, with a value of < 35% indicating impaired RV systolic function. For assessment of RV longitudinal function, TAPSE was determined using the M-Mode measurement of the cursor placed at the junction of the tricuspid valve plane with the RV free wall. A TAPSE of < 17 mm was considered as abnormal.

The assessment of diastolic function was also made possible using the transmitral pulsed-wave Doppler obtained from the mitral valve leaflet tips in the apical four-chamber view. This allowed the measurement of peak early (E) and late (A) diastolic filling velocities, E/A ratio, and E-wave deceleration time. Additionally, where the ultrasound beam appeared to be in parallel alignment with the lateral and medial (septal) mitral valve annulus in the apical four-chamber view, LV TDI measurement of the lateral and medial peak systolic annular velocity (S'), early (E') and late (A') annular diastolic velocities, and E/E' were made possible and averaged. Furthermore, a continuous-wave spectral Doppler through the tricuspid valve

enabled the measurement of tricuspid regurgitant peak (TR) velocity. This measurement combined with the estimated right atrial pressure and inferior vena cava calibre, provided the estimated value for the pulmonary artery systolic pressure. A lateral E'< 10 m/s, septal E'< 7 m/s, averaged E/E' > 14, and a TR velocity > 2.8 m/s in the presence of an abnormal indexed LA volume (> 34 mL/m^2) was considered as evidence of diastolic dysfunction.[282] Additionally, a continuous-wave Doppler measurement from the apical five-chamber view placed in the LV outflow tract enabling the visualisation of both the aortic ejection and onset of mitral inflow Dopplers traces, allowed the measurement of LV IVRT and the subsequent measurement of LV myocardial performance index (LV MPI, also known as Tei index).

TDI-derived S'-wave velocity at the lateral tricuspid annulus was assessed to further complement other measures of RV systolic function with a value of < 0.95 m/s indicative of RV systolic dysfunction. For evaluation of global RV performance, RIMP with RV IVRT, RV ICT, and RV ET were measured from TDI-velocity of the lateral tricuspid annulus, and a RIMP measurement of > 0.54 was suggestive of RV dysfunction.

2.5.1.3.2 2D strain analysis

The measurement of all strain parameters was performed in accordance with the recommendations from the ASE/EACVI/Industry task force.[153, 154, 251] Multilayer strain analysis of the LV and RV were performed providing strain values for endocardial and myocardial layers of both cardiac chambers.

TomTec™ automated tracking analysis (AutoSTRAIN©) allowed the measurement of LV endocardial GLS after selecting non-foreshortened LV apical four-, two-, and three-chamber views with reasonable image quality. Only images with a complete R-R cycle (end-diastole to end-diastole) were included in the analysis. With a specialised contour detection module for the respective apical views, the inner contour of the myocardium was traced automatically starting from the end-diastolic time-frame of the cardiac cycle ('beginning of cardiac cycle').[153] Adjustments to the endocardial border tracings were made if the automated tracing was deemed unsuitable. LV GLS was calculated by the software as global shortening of all the 16-segments of the LV at end-systole (**Figure 4** and **Figure 5**). For the purpose of this thesis, end-systolic strain was utilised as the measurement for LV GLS rather than peak-systolic strain, as recommended by the ASE and EACVI speckle tracking task force.[153, 251] Although end-

systole was automatically defined as the time-point of global peak strain, this was adjusted for each study according to the aortic valve closure time (AVC), measured by detecting the aortic valve closure click on the spectral Doppler of the aortic valve flow.[153] For additional measurements of LV strain such as LV myoGLS (myocardial strain), systolic and diastolic strain-rates, 2D CPA was used. With using the same three LV apical views, the complete myocardial region of interest (ROI) was determined at end-diastole by manually contouring the endocardial and epicardial borders. The middle ROI located between the endocardial and epicardial contours allowed the software to provide measurement of the myocardial GLS, in addition to quantifying endocardial GLS and strain-rate values. Given peak and end-systolic longitudinal strain-rate were provided as an average for each apical imaging plane, these values were averaged for all three apical views to postulate a single strain-rate measurement in peak-systole and end-systole.

LV GRS was also measured using TomTec™ 2D CPA. Although most studies have measured GRS using the parasternal short axis views, the use of this method proved difficult during this study due to limited number of short axis apical views that were available. Therefore, all three LV apical views were usedinstead by means of the same method described for the measurement of GLS.[153] This was automatically generated by the software when GLS measurement was performed using TomTec™ 2D CPA. Once again, the peak-systolic and end-systolic strain-rates were generated by the software for each imaging plane. These were averaged to provide a single value for all three apical views.

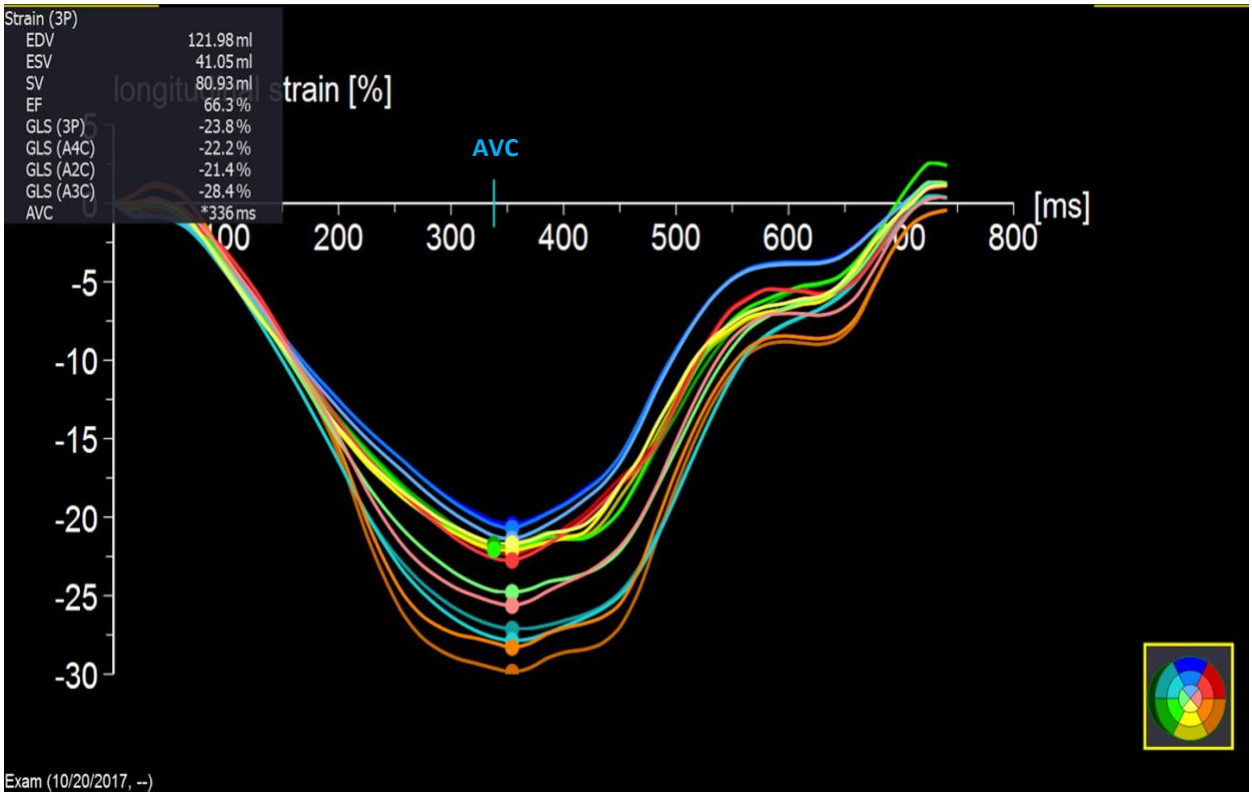


Figure 4. Regional and global GLS using AutoSTRAIN

AVC, aortic valve closure time coinciding with end-systole. Each colour-coded curve represents longitudinal strain throughout the cardiac cycle in each LV segment. GLS (a negative value) is an average of all 16-segment longitudinal strain values, highlighted in the box

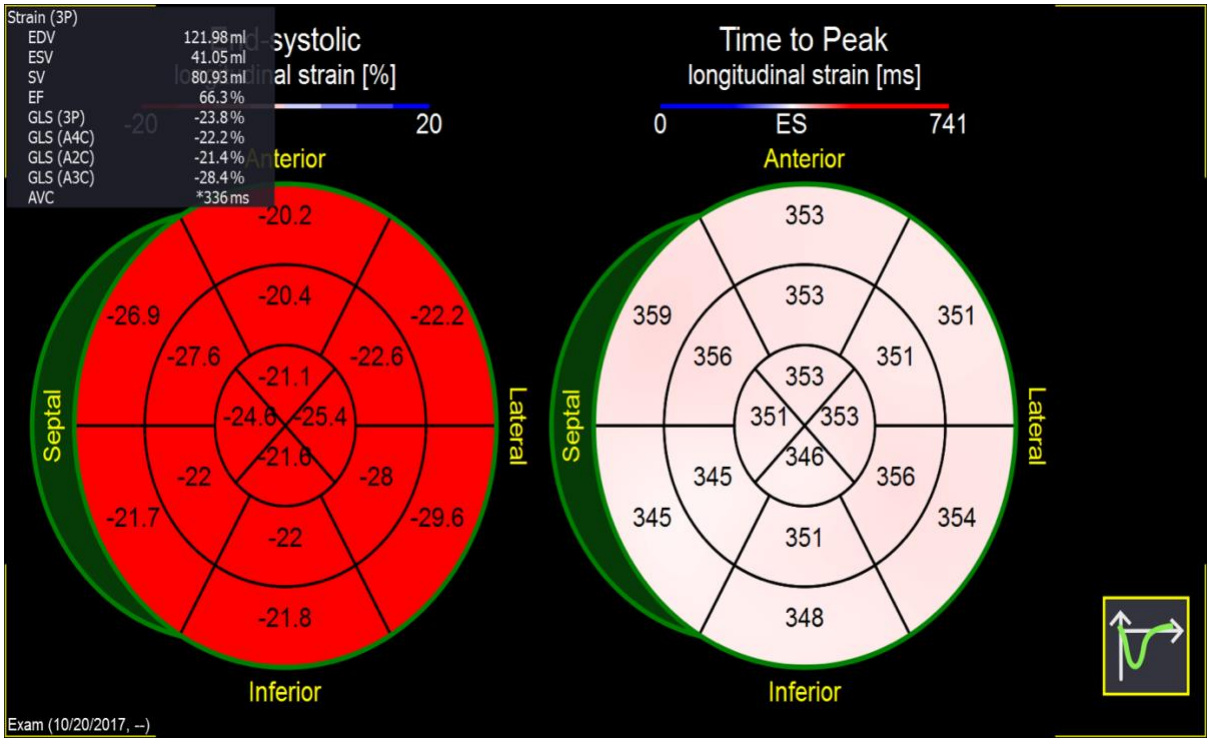


Figure 5. Bull's eye view for GLS using AutoSTRAIN

Image on the left showing regional longitudinal strain values in Bull's eye forma in the same patient discussed in figure 4. The red colour for each segment highlights the negative shortening for each segment. As the colour moves towards blue, there is lengthening of the segment meaning an impairment of longitudinal strain in that segment (a positive value). Imagine on the right shows the time it takes to reach the peak systolic strain in milliseconds (ms) for each LV segment measured from the R-wave (end-diastole).

Furthermore, peak GCS was obtained from the parasternal short-axis views including the LV at the mitral valve, papillary muscle, and apical levels. Using 2D CPA, the endocardial and epicardial borders were contoured and ROI determined allowing the software to provide a measurement for both LV endocardial GCS and myocardial GCS. The peak systolic, end-systolic, early- and late-diastolic strain-rates were all measured after averaging the values obtained from each LV imaging plane using the same method described earlier. Using these views, the software was further able to provide a measurement for LV twist. This value when divided by LV length, measured using the apical four-chamber view, allowed quantification of LV torsion.

Using the apical four-chamber view, RV GLS was also analysed. Images without clear view of the RV free wall and a full R-R cycle were excluded from the study. With 2D CPA, the inner and outer contours of the RV myocardium were manually traced to the medial and lateral tricuspid annulus allowing the definition of the ROI with an adjusted width of no more than 5 mm (**Figure 6**).[(251)] This allowed software tracking of the RV segments providing a value for

RV GLS, RV myoGLS (myocardial GLS), and RVFWS. The global peak-systolic and end-systolic RV and RVFW longitudinal strain-rates were provided by the software.

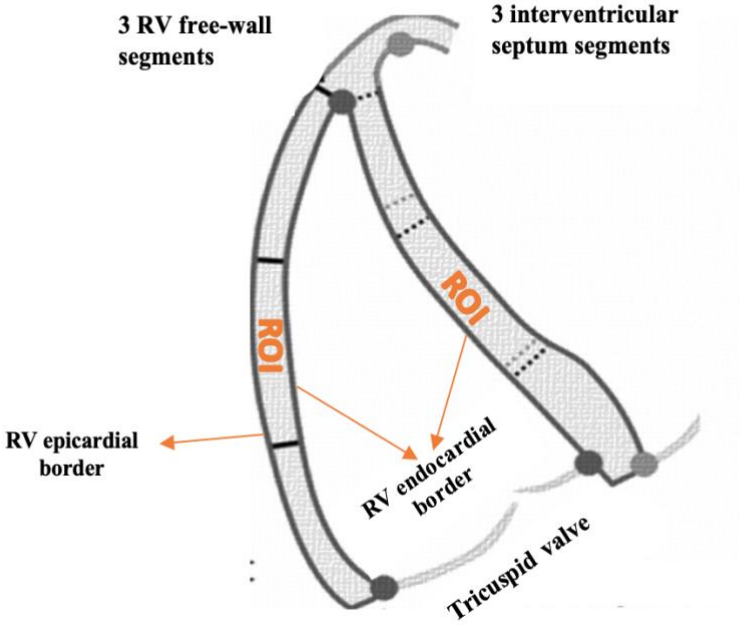


Figure 6. Contouring of the RV

ROI, region of interest highlighting RV myocardial layer. RV free-wall between the base and apex is divided into three segments including basal, mid, and apical free wall. The interventricular septum is also divided into base, mid and apical septum segments. 2D CPA not only provides strain values for each segment, but also provides RV GLS (global endocardial longitudinal strain of all 6 segments), RV myoGLS (global myocardial longitudinal strain of all 6 segments), and RV FWS (global longitudinal strain for all 3 segments of the RV free-wall).

The measurement of LA and RA strain were also performed in accordance with the latest EACVI/ASE/Industry task force recommendations.[251] Although, TomTec™ 2D CPA was licensed for the measurement of LA strain in the LV apical two-chamber view only, this package was further used for quantification of LA strain using the LV apical four-chamber imaging plane, enabling calculation of biplane LA strain measurement where possible. The software used for analysis purposes was not adapted for measurement of LA strain using the apical four-chamber view. Only studies with non-foreshortened LV apical four- and two-chamber views with a full R-R cycle and a good image quality were included in the final analysis. For quantification of LA GLS, the endocardial border of the LA was manually traced starting from one side of the mitral valve annulus to the opposite side, extrapolating across the pulmonary veins and the LA appendage. R-R gating was used for LA strain analysis, and end-diastole was defined manually by referring to the upslope of the R-wave on the superimposed ECG and closure of the mitral valve. This allowed the software to generate a LA strain curve

with a LA GLS value. Given the software was not able to provide measurements of the three LA reservoir, conduit, and contraction phases, these were manually calculated by referring to the LA strain curve (**Figure 7**).

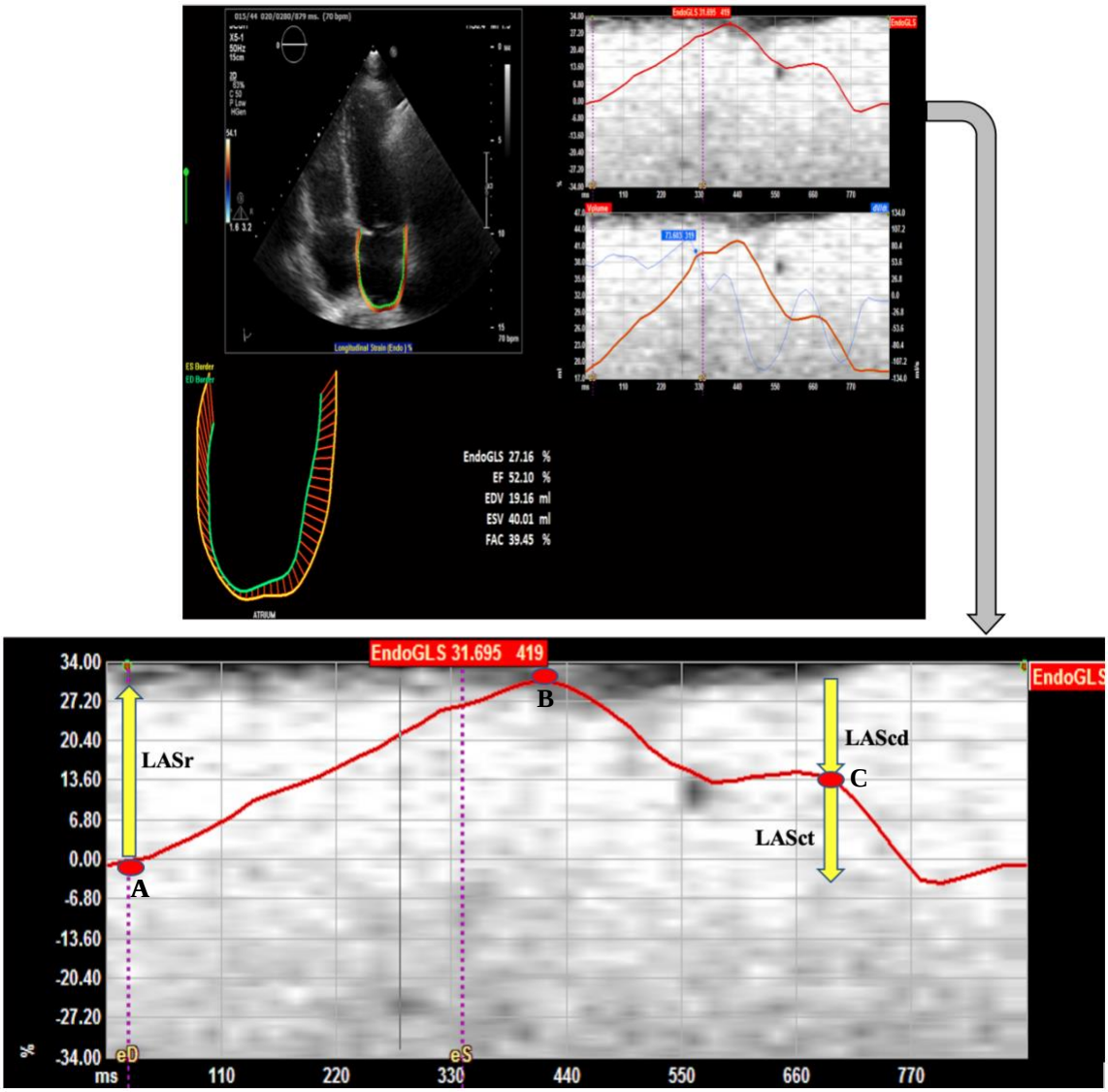


Figure 7. LA strain phases

Top panel showing LA strain measurement for a patient where the LA GLS value and strain curve is generated. The bottom panel is the magnified version of the LA strain curve highlighting the different phases of LA strain with the zero-reference set at end-diastole. LASr (positive value) is strain during LA reservoir phase measured as the difference of the strain value between points B and A (B-A, mitral valve opening minus ventricular end-diastole). LAScd (negative value) is strain during LA conduit phase measured as the difference of the strain value between points C and B (C-B, atrial contraction minus mitral valve opening). LASct (negative value) is strain during atrial contraction phase, measured as the difference of the strain value between points A and C (A-C, ventricular end-diastole minus onset of atrial contraction).

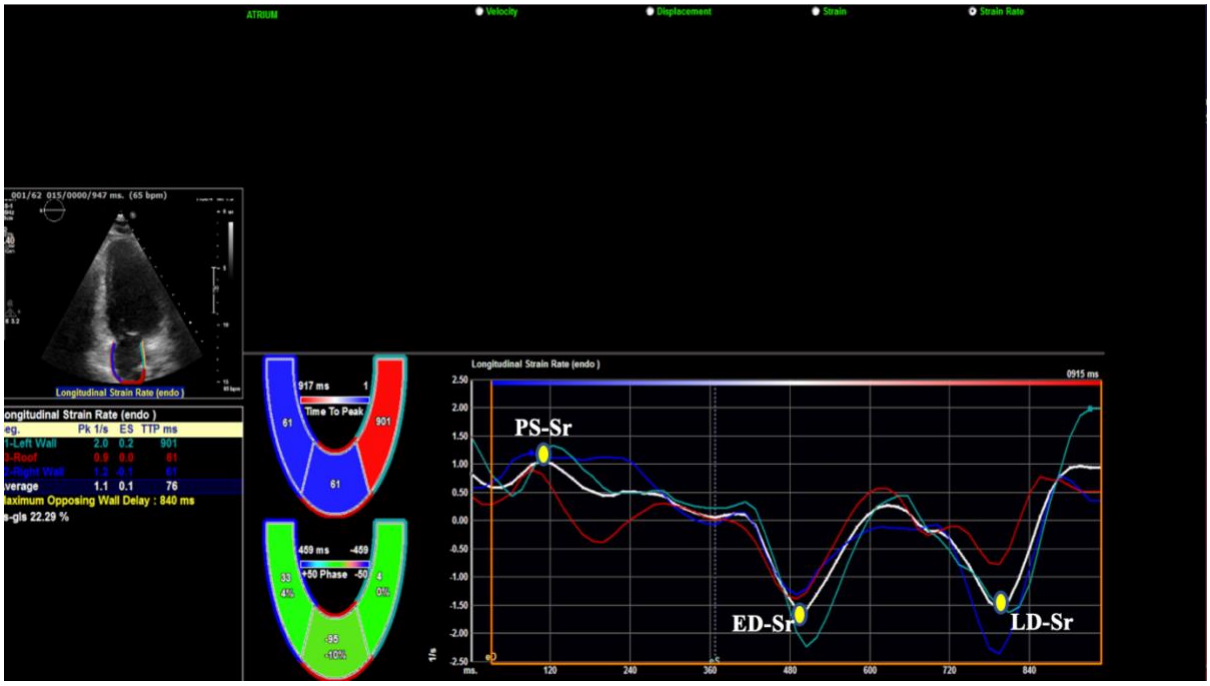


Figure 8. LA strain-rate using LV apical two-chamber view

LA strain-rate measurement using TomTec ™2D CPA. LA segmental (blue, red and green) and average (white) strain-rate curves have been generated by the software. By referring to the average strain-rate curve, LA PS-Sr, peak-systolic strain-rate, ED-SR, early-diastolic strain-rate, and LD-Sr, late-diastolic strain-rate values can be determined.

Once these values were obtained for both LV apical views, the results were averaged and used as biplane LA strain measurements. The peak-systolic, early- and late-diastolic strain-rates were determined by evaluating the average strain-rate curve generated by the software (**Figure 8**) and averaged between the two LV apical views.

For quantification of RA strain, the same method of analysis utilised for LA strain measurement was used. However, similar to the limitations for measurement of LA four-chamber strain, the software used was not adapted for measurement of RA strain and therefore the LA strain two-chamber package was used to obtain the RA strain values. Using the non-foreshortened LV apical four-chamber view, the endocardial RA border was delineated starting from one side of the tricuspid valve annulus to the other. Although, 2D CPA did not offer a platform for RA strain analysis, the LA strain package was used to enable this measurement. Once again, the software was able to produce a strain value for RA GLS. Using the longitudinal strain curve generated, the RASr, RAScd, and RASct were manually calculated using the end-diastolic, tricuspid valve opening, and atrial contraction time-points. The peak-systolic, early- and late-diastolic strain-rates were measured using the average strain-rate curve generated by the software for RA similar to the measurements obtained for LA strain-rate.

2.1.6 Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 24.0 software (SPSS Inc, Chicago, IL, USA). Continuous variables were assessed for normality of distribution using the Shapiro-Wilk test in combination with assessment of the skewness and kurtosis of the data. If normal distribution was confirmed, continuous variables were expressed as mean ± standard deviation. For measurement of group differences, homogeneity of variances was first assessed using the Levene's test for equality of variances. If the assumption of homogeneity of variances was not violated, group differences were measured via the independent student *t*-test, and for those variables with unequal population variances via the Welch's *t*-test. Non-normally distributed data were expressed as median and interquartile range (IQR) with group differences measured using the Mann-Whitney U test.

Categorical variables were expressed as percentages. Fisher's exact test was conducted due to a small sample size for the Chi-square test of homogeneity, to compare the baseline characteristics between groups, as established according to Cochran.[369]. A significance level (p-value) was used for all data to determine whether to accept or reject the null hypothesis. If the probability was sufficiently small ($p < 0.05$), it was concluded that equal group differences in the population were unlikely leading to an acceptance of the alternative hypothesis and rejection of the null hypothesis. Alternatively, the alternative hypothesis was rejected and the null hypothesis accepted if $p > 0.05$.

2.1.6.1 Outliers

For the purpose of the study, the presence of any outliers was retained in the data analysis to maintain the originality of the data. If Shapiro-Wilk test revealed a normal distribution of the continuous data but the assessment of boxplots revealed the presence of outliers, a non-parametric test such as the Mann-Whitney U test was once again used to express the group differences.[370, 371] If the distribution of each variable in different groups was similar by visual inspection, the group differences were measured by comparing the medians. However, if the distribution was different, then group differences were assessed by comparing mean ranks.

2.1.6.2 Missing data

Given the small number of patients, it was decided to not use multiple imputation for the missing data in this study. For the purpose of describing the data, pairwise deletion (available-case analysis) was used instead of listwise deletion, maximising all data available by an analysis-by-analysis basis increasing the power in the analysis of the variables. Additionally, in order to measure the changes in the markers of interest at T0, T1 and T2, the generalized linear mixed model (GLMM) was used instead which handled the missing data appropriately without causing bias when compared to other statistical methods such as analysis of variance (ANOVA).

2.1.6.3 Assessment of change in measures of interest

For the reasons described above, GLMM was the statistical method of choice for assessment of changes in the different echocardiographic measures. Using this method all echocardiographic parameters were measured at T0, T1 and T2 visits. These parameters were initially measured in all patients. This was then followed by a comparison of the echocardiographic parameters in two different sub-groups (G) based on whether or not they had developed cardiotoxicity by conventional criteria; those with a preserved LVEF at ≥ 53% at T2 (G1), and those with a drop of >10% in their LVEF to < 53% at T2 (G2).

2.1.6.4 Assessment of reproducibility

Intra-observer variability was assessed by myself, measuring LVEF and each strain parameter twice at three different time-points in 6 random patients with fair to good quality images. Using the two-way mixed model, the intraclass correlation coefficient (ICC) was then obtained for the assessment of the reliability of these measurements. For the assessment of inter-observer variability, one set of measurements was obtained by myself, and a second set by a BSE accredited sonographer, A.K, in 10 random patients. ICC for each measure was obtained using the same statistical method for intra-observer variability. An ICC coefficient of >0.9 meant an excellent agreement between the observers or intra-observer for the different measures. Meanwhile an ICC coefficient value between 0.75-0.90 demonstrated a good agreement. An ICC < 0.75 indicated a poor agreement.

2.2 Prospective Study

2.2.1 Ethical Approval

The "PROACT PLUS registry and echocardiographic sub-study: an observational, prospective, cohort study assessing the use of novel echocardiographic tools and measurement of troponin to help detect early signs of cardiotoxicity in patients treated for breast cancer and lymphoma" received a favourable opinion by the *East Midlands – Nottingham 1 Research Ethics Committee*, and was approved by *HRA and Health and Care Research Wales (HCRW)*, reference number *18/EM/0177 (Appendix 6).*

2.2.2 Study Registration

For transparency purposes, this study is registered with "ISRCTN registry" under study identification number of *ISRCTN11676341*.

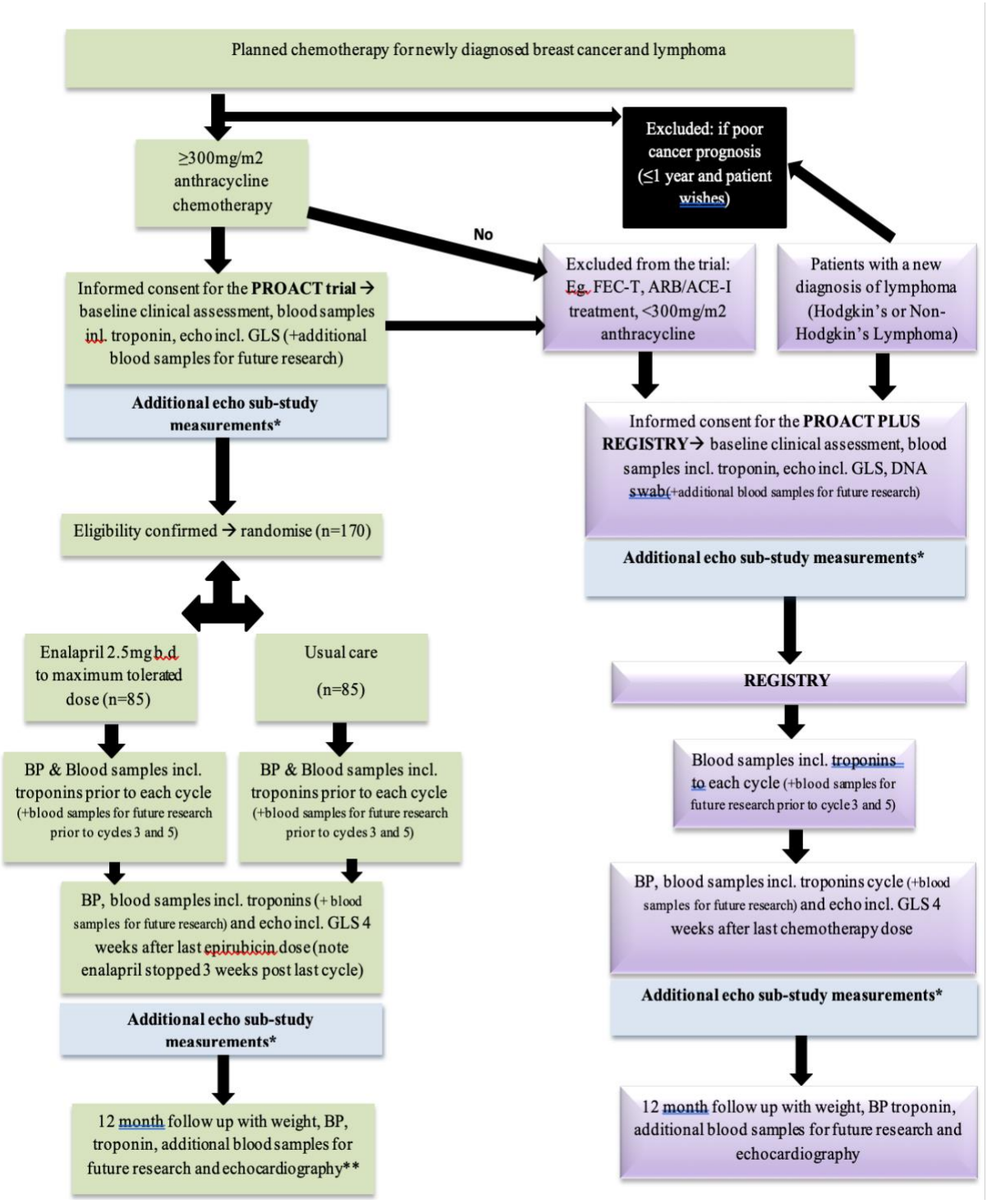
2.2.3 Study Design

This registry was a prospective, observational cohort study conducted at South Tees, and Durham and Darlington NHS Foundation Trusts. The rationale for study conduction is briefly discussed in **section 7.2** of Chapter **1** with full explanation in the *study protocol* attached in *Appendix 4*. This feasibility study evaluated the effects of anthracyclines on advanced STE measures and cardiac troponins in patients treated for breast cancer and lymphoma, hoping to identify patients at future risk of cardiotoxicity and LV dysfunction development (

Figure 9). Due to the time restrictions in undertaking this study and slow patient recruitment, the 12 months follow-up visit analysis of the data is beyond the scope of this thesis.

2.2.4 Research Setting

Patient recruitment for this study occurred at both South Tees, and Durham and Darlington NHS Foundation Trusts. Both sites were fully accommodated for research nurse support and undertaking of the relevant investigations and assessments required for the study purposes. Echocardiograms were undertaken by experienced sonographers with full BSE accreditation and were anonymized for offline analysis by myself in a blinded fashion.



* Note patients do not need to have additional scans - extra measurements wiill be done on the scans already taken

**Separate application has recently been put in for 12 months follow-up and investigations for PROACT trial

Figure 9. PROACT PLUS registry study design

The column on the right describes the PROACT PLUS registry and how it is complimentary to the ongoing PROACT clinical trial on the left.

2.2.5 Study Population

Patients with a new diagnosis of lymphoma or breast cancer planned to receive anthracycline based chemotherapy as part of their cancer treatment (**Table 7** and **Table 8**), and who did NOT meet the eligibility criteria for the PROACT clinical trial were included in the registry (**Table 9**).

Regimen	Description	No. of cycles	No. anthracycline cycles	Dose of anthracycline	Total dose of anthracycline	Cardio-toxic equivalent dose for doxorubicin**
EC 90*	**Epirubicin** 90mg/m2, Cyclophosphamide 600mg/m2	6	6	90mg/m2	540mg/m2	378mg/m2
FEC 75*	Fluorouracil 600mg/m2, **Epirubicin** 75mg/m2, Cyclophosphamide 600mg/m2	6	6	75mg/m2	450mg/m2	315mg/m2
FEC-T	Fluorouracil 500mg/m2, **Epirubicin** 100mg/m2, Cyclophosphamide 500mg/m2, Taxane (docetaxel) 100mg/m2	6	3	100mg/m2	300mg/m2	210mg/m2

Table 8. Most commonly used anthracycline based chemotherapy regimens used in the treatment of breast cancer in the North East of England

** Both EC 90 and FEC 75 chemotherapy regimens are not eligible for the PROACT PLUS registry and are **only** included if patients receiving treatment with these types of regimens fail to meet the eligibility criteria for the PROACT clinical trial.*
*** anthracycline toxicity equivalence ratio for assessment of cardiotoxicity calculated as per table 1.*

2.2.5.1 Inclusion criteria

- Adult patients with a new diagnosis of histopathologically confirmed breast cancer or lymphoma (Hodgkin's and non-Hodgkin's lymphoma)
- Age ≥ 18 years
- Planned to receive anthracycline based chemotherapy (adjuvant or neo-adjuvant) – any dose
- Written informed consent

2.2.5.2 Exclusion criteria

- Meets eligibility criteria for PROACT trial (**Table 9**)
- Known metastatic cancer
- Poor cancer prognosis of ≤ 1 year

2.2.6 Screening, Recruitment, and Consent

2.2.6.1 Screening

Patients meeting the eligibility criteria for the PROACT PLUS registry were identified by their clinical teams prior to commencing chemotherapy and approached for consideration of enrolment into the registry.

2.2.6.2 Recruitment and consent

Once patients had been identified as eligible candidates for the study, they were invited to participate in the registry. Potential participants were provided a patient information sheet outlining the main principles of the PROACT PLUS registry. All steps were taken to ensure that patients were afforded a reasonable time to consider enrolment into the registry, to ask questions, and have all their queries answered prior to consent. Once patients were happy with the information provided and were keen to participate, written consent was obtained by one of the delegated members of the research team. The consent form was then retained in the Study File, with a copy filed in the clinical notes and one given to the patient. Additional consent was obtained for DNA swab and storage of extra blood samples for translational research.

Inclusion criteria	Exclusion criteria
Histopathologically confirmed breast carcinoma who have received surgery for their breast cancer	Positive baseline cardiac troponin T (≥14 ng/L)
Planned to receive 6 cycles of EC90 (total planned dose of 540mg/m^2 epirubicin) or FEC 75 (total planned dose of 450mg/m^2 epirubicin) adjuvant chemotherapy	Known contraindication to ACE inhibitor e.g. renal artery stenosis, severe aortic stenosis
Adult patients with histopathologically confirmed non-Hodgkin's lymphoma planned to receive 6 cycles of R-CHOP or CHOP (total planned dose 300mg/m^2 doxorubicin) chemotherapy	Are taking, or having a previous intolerance to ACEi (e.g. angioedema)
Written informed consent	Patient already taking other agents acting on the renin-angiotensin-aldosterone system e.g. Aliskiren, ARBs, Entresto, spironolactone, epleronone
	LVEF <50%
	Estimated GFR < 30 mL/min/1.73m^2 at baseline
	Hyperkalaemia (≥5.5 mmol/L)
	Symptomatic hypotension, or systolic blood BP < 100 mmHg
	Poorly controlled hypertension (BP >160/100 mmHg, or ambulatory BP of >150/95 mmHg)
	Previous myocardial infarction
	Known metastatic breast cancer
	Previous exposure to anthracycline chemotherapy
	Pregnancy or breast feeding
	Previous Herceptin treatment or planned Herceptin treatment within four weeks following anthracycline chemotherapy
	Refusal to use adequate contraception in patients of childbearing age
	Any other invasive cancer diagnosed and treated in the past 5 years
	Symptomatic or severe radiation-induced cardiac disease
	Participation on other interventional medicinal trials in the past 6 months
	Prognosis of < 1 year or unlikely to complete 6 cycles of chemotherapy
	High risk of tumour lysis syndrome (NHL patients)
	Unlikely to comply with study procedures, restrictions, and requirements

Table 9. PROACT clinical trial eligibility criteria

Furthermore, consent was also obtained to seek permission for future contact of the registry patients if further follow-up including additional echocardiography was planned, beyond the final study visit. Finally, consent was sought for use and storage of patients' personal data for a total of 15 years *(Appendix 5)*.

2.2.7 Adherence and Withdrawals

2.2.7.1 Adherence assessment

Study visits were planned to coincide with routine clinical practice where possible to increase the likelihood of adherence to study-related procedures.

2.2.7.2 Withdrawal procedures

Patients were able to withdraw their consent to take part in the study at any point they wished to do so. Any data up to the point of participation was used for analysis purposes. No further data was collected beyond that time-point.

2.2.8 Study Procedures

The procedures required for the registry including their visit times have been highlighted in **Table 10**.

2.2.8.1 Demographic information

The following demographic data were obtained and recorded from patients' medical records at baseline:

- Month and year of birth
- Gender
- Ethnicity

2.2.8.2 Medical history

Information regarding patients' full medical and cancer history including a list of baseline medication were obtained from the medical notes or the patients at the time of consent and recorded in the Case Report Form (CRF) - *Appendix 8.*

2.2.8.3 Height and Weight

Patients' height and weight for the purpose of measuring BSA, which were already taken by their oncology/haematology team as part of standard care were obtained from the medical notes at baseline and recorded in the CRF (*Appendix 8)*. The weight will be re-measured at 12-months after the chemotherapy at the time of the final echocardiography appointment (which is not included in this thesis as explained earlier).

2.2.8.4 Heart rate and Blood pressure

Heart rate and blood pressure (BP) measurements were obtained from patients' medical notes at baseline and documented in the CRF. If this had not been done, a heart rate and BP measurement was taken at the time of consent to avoid unnecessary hospital admission. This was repeated at 4 weeks, and 12 months (not included in this thesis) post chemotherapy, and if not available taken at the time of echocardiography if possible.

2.2.8.5 Echocardiography and Core laboratory

Patients enrolled in the registry, underwent 2D echocardiography assessment before the initiation of chemotherapy (V1), 4-weeks post-chemotherapy (V2), and at 12-months end of treatment (V3, not included in this thesis). Comprehensive echocardiography examinations were performed by expert BSE accredited sonographers at all three time-points using *Epiq 7C (Philips Ultrasound Inc, Bothwell, USA)* ultrasound system equipped with *X5-1 (1 to 5 MHZ)* fully sampled matrix array transducer. Images were obtained in accordance with the recommendations of BSE with superimposed ECG. For each 2D image, three cardiac cycles were recorded and images were optimized for angle, focus, depth, and sector size achieving a frame rate of 50-70 fps. Digital loops were stored and analysed offline after anonymisation, using vendor-independent software (TomTec Imaging Systems, 2D Cardiac Performance Analysis, Unterschleisshiem, Germany). A designated echocardiography core laboratory previously established at the James Cook University Hospital, Middlesbrough, was used to allow blinded analysis of the studies by myself.

M-mode and 2D Doppler echocardiography analysis was performed in accordance with the ASE recommendations and as highlighted in section **2.1.5.3.1** of this chapter.[114] For

assessment of RV size and function, RV-focused apical four-chamber view, rather than standard apical four-chamber view was used as recommended by ASE and EACVI.[114, 226, 251] Additionally, comprehensive 2D strain analysis was undertaken on all four cardiac chambers in accordance with the latest ASE/EACVI/Industry task force recommendations using the best cardiac cycle with optimal tracking.[153, 251] The exact method of how these measurements were performed are described in detail in section **2.5.1.3.2** of this chapter. Additionally, Early- and late-diastolic longitudinal strain-rates were also measured. These were not generated by the software and therefore, were measured by referring to the average strain-rate curves of each imaging plane and averaging these for all three apical views providing a single measure of early- and late-diastolic longitudinal strain-rates.

LV GRS was measured using the LV apical four-, two-, and three-chamber views. The average peak systolic and end-systolic strain-rates were measured. The average early- and late-diastolic radial strain-rates were measured using the same technique described for longitudinal strain-rates. Furthermore, RV GLS, RVFWS, and strain-rates were measured using the RV-focused apical four-chamber view. Additionally, the early- and late-diastolic RV and RVFW longitudinal strain-rates were determined by referring to the average strain-rate curves.

Data obtained from the echocardiography analysis were recorded on an encrypted excel sheet, and later inputted into the CRF *(Appendix 8)*.

2.2.8.6 Blood sampling

Troponin T and I

Patients consented to participate in the PROACT PLUS registry underwent blood sampling to assess troponin T and troponin I (not included in this thesis) levels at different time-points (**Table 10**). Since troponin I analysis was not routinely available locally, the measurement of this cardiac biomarker was dependent on transportation of the samples to Queen's Medical Research Institute at the University of Edinburgh through a prior agreement with the PROACT clinical trial team. As the samples were planned to be sent close to the completion of the clinical trial, the decision was made to only include troponin T analysis for this thesis due to time constraints with completing this MD. These were drawn at the same time of blood samples taken as part of standard care to avoid unnecessary venipuncture and hospital admissions. It is

important to note that even though samples for troponin I were taken for the purpose of this study, these will not form part of this thesis and troponin T results will only be discussed.

At baseline (prior to initiation of chemotherapy), up to 5 mL of blood was taken in a serum-separation tube (SST) for troponin T and troponin I measurements. Immediately after collection, this sample was inverted 5-times and given 30-minutes to clot, followed by centrifuging for 10-minutes at 1000-1300 RCF (g) in a swing bucket centrifuge before being divided into two separate aliquots of serum. These were then stored at -80°C at each participating site's pathology lab for subsequent analysis. During the course of chemotherapy, further sampling of 5 mL of blood for measurement of troponin T and I was performed, up to 72-hours prior to the intended start of chemotherapy at cycles 2 and subsequent chemotherapy cycles. A further sample was taken using the same method at 4-weeks post last dose of chemotherapy. This 4-weeks post-chemotherapy sampling, typically coincided with the V2 echocardiography study-visit to avoid unnecessary hospital admissions. All these samples were once again stored at -80°C after splitting the serum into two aliquots using the same technique described above.

The samples stored were later sent for central analysis to Newcastle Upon Tyne NHS Foundation Trust Laboratories for measuring high-sensitivity cTnT (hs-cTnT) using the Elecsys cardiac Troponin T assay from Roche Diagnostics and Queen's Medical Research Institute at the University of Edinburgh, for measuring high-sensitivity cTnI (hs-TnI) using the Abbott ARCHITECT*STAT* high-sensitivity cardiac troponin I assay. A hs-cTnT <14ng/L and a hs-cTnI <5ng/L were considered as a normal result. Troponin T and I levels were batch-tested in a blinded manner and the results combined with the echocardiography data at the end of the study (not including the 12-month visit).

A further troponin T and I sampling occurred at 12-months post chemotherapy, and stored using the same method described above. These samples will be batch-tested once the 12-month visit has been achieved for all patients (the results of the 12-months samples fall outside the time-frame of this MD thesis, and have therefore not been included here).

The results of the patients' troponin results were later documented in the CRF (*Appendix 8*), by a delegated member of the research team. Additionally, blood tests taken as part of standard

care (**Table 10**), were also recorded in the CRF at baseline and at 4-weeks post-final chemotherapy dose.

The principal investigator (P.I) was responsible for the full traceability of the samples collected whilst in storage, until shipment of these samples. Records of shipment for each sample was kept by the P.I. The receiver would then acknowledge receipt of each sample and keep full traceability of the samples during storage and use until samples used or disposed of.

Additional blood sampling for further research

Although not included in this thesis, as part of the study protocol, further blood samples for future research were collected at different time-points highlighted in **Table 10**. These included a 5 mL sample in one SST tube and a further 5 mL sample in an Ethylenediaminetetraacetic acid (EDTA) tube. The SST tube was inverted 5-times, allowed to clot for 30-minutes, followed by centrifuging for 10-minutes at 1000-1300 RCF (g) in a swing bucket centrifuge before being spun and divided into four separate cryovials. The EDTA tube was inverted 8- to 10-times, centrifuged for 10-minutues at ≤ 1300 RCF (g), followed by being spun and divided into four separate aliquots of plasma. Once all these samples were divided into aliquots they were stored at -80°C at each site's pathology lab until subsequent analysis or for a maximum of 15-years following the patient's last registry visit.

As with the troponin samples, the P.I at the study site kept full traceability of collected samples whilst in storage at the site until shipment or disposal, and kept records of shipping for each sample. Furthermore, the receiver, acknowledged receipt of each sample and kept full traceability of the samples whilst in storage, during use and until disposed of.

2.2.8.7 DNA sampling

During the registry, a buccal swap for DNA analysis was taken if the patients had provided additional consent (**Table 10**) to facilitate a related project into cardiotoxicity at Newcastle University. These samples were processed into lysis buffer and stored at -20°C for up to one-month before being transported to the Newcastle University where DNA was extracted and stored at -80°C until analysis for a maximum of 15-years following the patient's final registry-visit after which time they will be destroyed. The results of DNA analysis will not be included in this thesis.

2.2.9 Data Collection

A specific CRF database was designed by myself and set up with the help of the Information Technology (IT) team at the James Cook University Hospital (South Tees NHS Foundation Trust) after local information governance approval was sought. No patient identifiable information was used; each patient was allocated a unique study ID. Only the month and year of birth was recorded to allow the calculation of age. Given the blinded nature of the study, the echocardiographic data was inputted into a password protected excel sheet by myself and saved on a password-protected Trust computer. This was later transcribed onto the CRF database (*Appendix 8*). The baseline medical information including the troponin levels was inputted into the paper CRF by a delegated member of the research team and later transcribed onto the database. This was checked at the end of the study by myself to reduce the risk of unblinding and bias.

	T0	Prior to each cycle of chemotherapy	T1	T2*
Demographics	x			
Medical history	x			
Eligibility check	x			
Height and weight	x			
BP and pulse	x			
Echocardiogram	x		x	x
Troponin T and I	x	x	x	x
Additional blood samples for future research	x	x**	x	x
Other blood tests as per usual care (FBC, U&Es)**	x		x	
Buccal swabs for future research***	x			

Table 10. Summary of registry procedures

** The T2 study-visit related procedures have not been included in this thesis*

***Additional blood samples for future research taken prior to cycles 3 and 5 of chemotherapy. (Results of the additional blood samples for future research are not part of this MD thesis).*
*** Other blood tests' (FBC, U&Es) results taken as part of standard care prior to chemotherapy will be obtained from patients' medical records at baseline. 4-weeks after the last dose of chemotherapy an FBC result taken as part of standard care will be recorded (note this blood sample may have been taken any time before the last dose of chemotherapy)*
****The buccal swab for DNA analysis was taken at any time during the study-period. Results of this analysis are not included in this thesis*

2.2.10 Statistical Analysis

2.2.10.1 Data analysis

Data analysis was performed using IBM SPSS Statistics version 28.0 software (SPSS Inc, Chicago, IL, USA).

Continuous variables were assessed for normality of distribution using the Shapiro-Wilk test in combination with assessment of the skewness and kurtosis of the data. If normal distribution was confirmed, continuous variables were expressed as mean ± standard deviation. For measurement of group differences, homogeneity of variances was first assessed using the Levene's test for equality of variances. If the assumption of homogeneity of variances was not violated, group differences were measured via the independent student *t*-test, and for those variables with unequal population variances via the Welch's *t*-test. Non-normally distributed data were expressed as median and interquartile range (IQR) with group differences measured using the Mann-Whitney U test. As two different types of anthracyclines were used for the purpose of this study (doxorubicin and epirubicin) and the associated different cardiotoxicity profile that exists for each anthracycline (see Table 1), the doxorubicin equivalent dose was calculated and used when discussing anthracycline dose administered.

Categorical variables were expressed as percentages. Fisher's exact test was conducted due to a small sample size for the Chi-square test of homogeneity, to compare the baseline characteristics between groups, as established according to Cochran.[369]. A significance level (p-value) was used for all data to determine whether to accept or reject the null hypothesis. If the probability was sufficiently small ($p < 0.05$), it was concluded that equal group differences in the population were unlikely leading to an acceptance of the alternative hypothesis and rejection of the null hypothesis. Alternatively, the alternative hypothesis was rejected and the null hypothesis accepted if $p > 0.05$.

2.2.10.2 Dealing with outliers

The presence of any outliers in the continuous data were initially examined for data entry or measurement errors. If the presence of outliers were not related to any of the forementioned reasons and were considered to be genuine unusual values, they were retained in the final data analysis to help maintain the originality of data. However, the non-parametric Mann-Whitney U test was used instead for analysis of the group differences, as described in the section above.

2.2.10.3 Dealing with missing data

Given the small number of patients, it was decided to not use multiple imputation for the missing data in this study. For the purpose of describing the data, pairwise deletion (available-case analysis) was used instead of listwise deletion, maximising all data available by an analysis-by-analysis basis increasing the power in the analysis of the variables. Additionally, in order to analyse the changes in the markers of interest at V1 and V2 the generalized linear mixed model (GLMM) was used instead which handled the missing data appropriately without causing bias when compared to other statistical methods such as analysis of variance (ANOVA).

2.2.10.4 Assessment of change in measures of interest

GLMM was the statistical method used to assess the changes in the different echocardiographic and biomarker measures. Using this method all echocardiographic parameters were measured at V1 and V2. These parameters were initially measured in all patients. This was then followed by a comparison of the echocardiographic parameters in two different sub-groups (G) based on whether or not they had developed cardiotoxicity by conventional criteria; those with a preserved LVEF at ≥ 53% at T2 (G1), and those with a drop of >10% in their LVEF to < 53% at T2 (G2).

2.2.10.5 Assessment of reproducibility

Intra-observer variability was assessed by myself, measuring each strain parameter twice at two different time-points in 10 random patients with fair to good quality images. Intraclass

correlation coefficient (ICC) was then used to assess the reliability of these measurements by using the two-way mixed model. For assessment of inter-observer variability, one set of measurements was obtained by myself, and a second set by a BSE accredited sonographer, B.T, in 10 random patients. These were then compared using ICC to assess the inter-rater reliability. Once again, the two-way mixed model was used for the purpose of obtaining the ICC result. An ICC coefficient of >0.9 meant an excellent agreement between the observers or intra-observer for the different measures. Meanwhile an ICC coefficient value between 0.75-0.90 demonstrated a good agreement. An ICC < 0.75 indicated a poor agreement.

Chapter 3: Retrospective Study Results

3.1 Study Population

From January 2015 to January 2018, a total of 131 patients with a new diagnosis of lymphoma requiring anthracycline chemotherapy were identified through a computerised search of the haematology database. A list of these patients was provided to myself by the haematology team at The James Cook University Hospital. A unique study ID was then allocated to each patient for anonymisation purposes. Patients' echocardiogram images were reviewed prior to any analysis to ensure study eligibility criteria was met. Those who had undergone an echocardiogram at T0, T1, and T2 or those who had echocardiograms at T0 and T2 were included in the study. 86 patients were excluded from the study for reasons highlighted in **Figure 10**. For the purpose of this study, two patient groups (G) were defined: those with a preserved LVEF at ≥ 53% at T2 (G1), and those with a drop of >10% to LVEF to < 53% at T2 (G2), see 2.1.6 Statistical analysis.

Out of 131 patients, 12 (9%) were found to have dropped their LVEF to <53% (G2) from T0 to T2, post completion of anthracycline chemotherapy. 119 patients (91%) had normal LVEF at T2 (G1) however, only 33 out of 119 (28%) patients' echocardiograms were analysable as illustrated in **Figure 10**.

3.2 Baseline Characteristics

Full description of the statistical methods used for this chapter have been provided in section 2.1.6 Statistical analysis. The baseline characteristics for patients with and without evidence of cardiotoxicity (G2 and G1, respectively) are outlined in **Table** 11.

The median age of the patients was 64 years with no statistically significant difference between patients in G1 and G2. A total number of 30 patients (67%) in this study were male. The median total anthracycline (doxorubicin) dose was 285.7 mg/m^2 and 294.1mg/m^2 in G1 and G2, respectively with no statistically significant difference between the two groups. The percentage of patients on ACE inhibitors, ARBs, betablockers, and statins were similar in both groups.

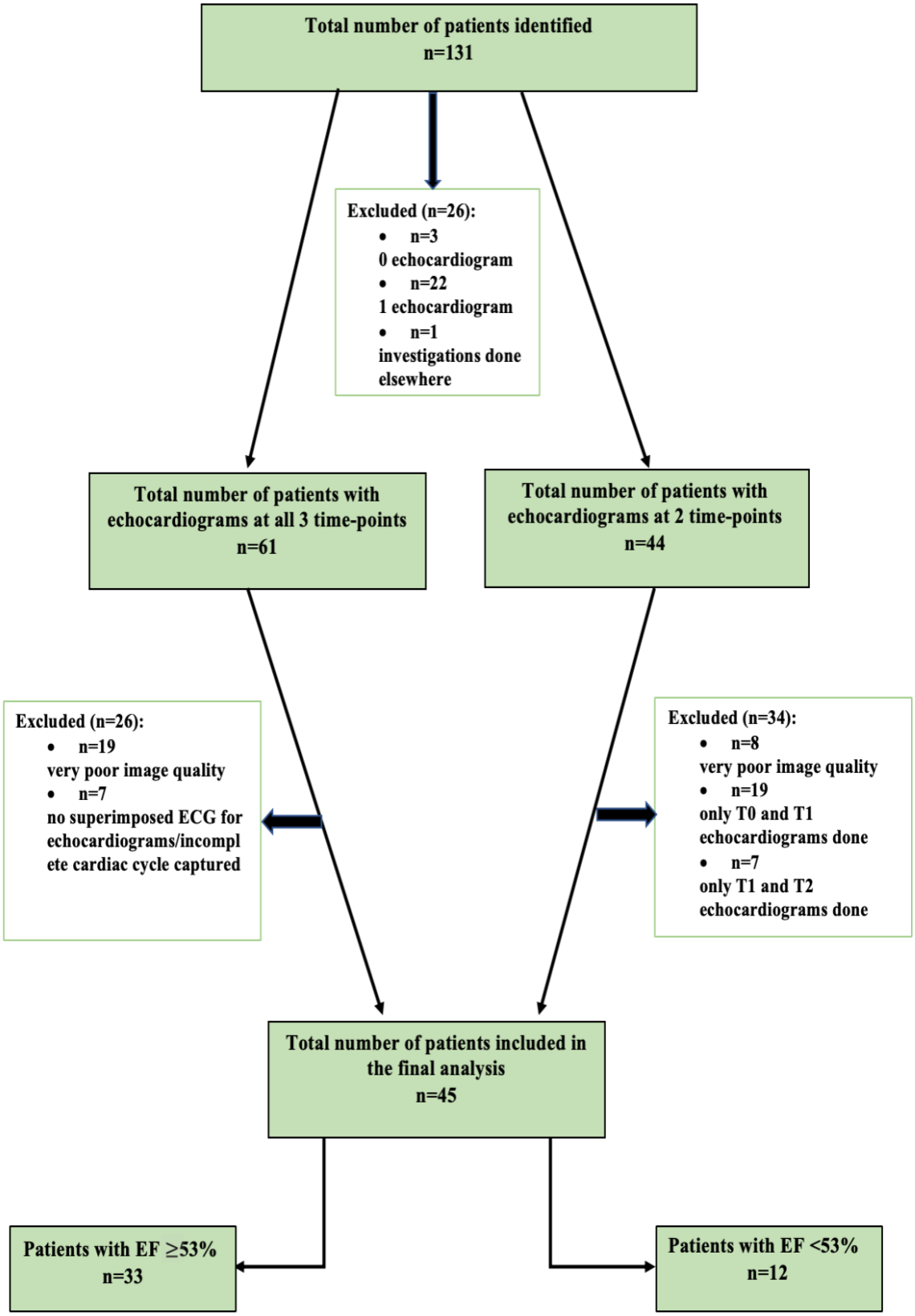


Figure 10. Consort diagram

Variable	N	All patients (n=45)	G1 (n=33)	G2 (n=12)	p-value
Age	45	64 [54 – 73]	66 [56-74]	61 [54-70]	0.35
Male sex (%)	45	30 (67)	24 (73)	6 (50)	0.17
BSA (m^2)	45	1.89 ± 0.19	1.89 ± 0.18	1.91 ± 0.22	0.71
Caucasian (%)	45	45 (100)	33 (100)	12 (100)	N/A
Cancer diagnosis (%) **DLBCL** **B Cell NHL** **Classical HL** **HL (nodule lymphocyte)** **T cell lymphoma**	45	 27 (60) 8 (18) 8 (18) 1 (2) 1 (2)	 17 (52) 7 (21) 7 (21) 1 (3) 1 (3)	 10 (83) 1 (8) 1 (8) 0 (0) 0 (0)	0.58
Cancer stage (%) **I** **II** **III** **IV**	45	 1 (2) 9 (20) 11 (24) 24 (53)	 1 (3) 7 (21) 7 (21) 18 (55)	 0 (0) 2 (17) 4 (33) 6 (50)	0.86
Chemotherapy treatment (%) **R CHOP (x6 cycles)** **R CHOP (x3 CHOP, x3 CEOP)** **CHOP** **ABVD**	45	 35 (78) 1 (2) 1 (2) 8 (18)	 24 (73) 1 (3) 1 (3) 7 (21)	 11 (92) 0 (0) 0 (0) 1 (8)	0.68
Anthracycline dose (mg/m^2)	45	291.9 [259 – 303]	285.7 [253.3 – 304.1]	294.1 [289.3 – 298.7]	0.54
Other current cancer diagnosis (%)	45	3 (6)	3 (9)	0 (0)	0.55
Previous anthracycline treatment (%)	45	2 (4)	2 (6)	0 (0)	1.00
IHD (%)	45	6 (13)	4 (12)	2 (17)	0.65
Previous LVSD (%)	45	4 (9)	3 (9)	1 (8)	1.00
Hypertension (%)	45	9 (9)	6 (18)	3 (25)	0.68
Diabetes (%)	45	3 (7)	2 (6)	1 (8)	1.00
Hypercholesterolaemia (%)	45	5 (11)	4 (12)	1 (8)	1.00
Smoking history (%) **Current smoker** **Ex-smoker** **Non-smoker** **Unknown**	45	 6 (13) 8 (18) 23 (51) 8 (18)	 5 (15) 3 (9) 19 (58) 6 (18)	 1 (8) 5 (42) 4 (33) 2 (17)	0.94
ACEi (%)	45	8 (18)	6 (18)	2 (17)	1.00
ARB (%)	45	3 (7)	1 (3)	2 (17)	0.16
Betablocker (%)	45	5 (11)	4 (12)	1 (8)	1
Statin (%)	45	11 (24)	8 (24)	3 (25)	1

Table 11. Baseline characteristics

G1: patients with LVEF ≥ 53%. G2: Group 2 representing patients with LVEF < 53%.
Data are presented as n (%) or mean ± SD or median [IQR]
Inter-group differences for categorical variables assessed using the Fisher's Exact Test.
For the measurement of inter-group differences for continuous variables the independent student t test or the Mann-Whitney U test (if not normally distributed) was used.

Table 12 highlights the conventional echocardiography measures in both patient groups at T0. No statistically significant difference was identified in most of these measures between G1 and G2, apart from LVEDV, LVESV, and RV FAC. The median indexed LVEDV and LVESV were higher in patients in G2 when compared to G1 ($p = 0.02$ and $p = 0.003$, respectively). Despite these findings, LVEF was similar in both groups without any significant difference. Additionally, patients in G2 showed to have a greater RV FAC in comparison to those patients in G1.

3.2.1 Baseline Conventional Echocardiography Measures

	Total N (and total n in each group)	All patients	G1	G2	p value
Sinus Rhythm	45 (G1=33, G2=12)	45	33 (100)	12 (100)	N/A
Heart rate (bpm)	45 (G1=33, G2=12)	77 [70-100]	75 [70-100]	81 [71-117]	0.20
LVIDd (cm)	44 (G1=33, G2=11)	4.5 [4.2-4.8]	4.4 [4.1-4.8]	4.8 [4.5-5.4]	0.11
LVIDs (cm)	44 (G1=33, G2=11)	2.55 [2.2-2.9]	2.5 [2.1-2.8]	2.9 [2.2-3.4]	0.07
Fractional shortening (%)*	44 (G1=33, G2=11)	42.8 ± 9.3	43.8 ± 9.1	39.5 ± 9.8	0.19
LV mass index (mg/m^2)	44 (G1=33, G2=11)	80 [70-96]	78 [69-94]	84 [75-98]	0.42
LV RWT (%)	44 (G1=33, G2=11)	0.41 [0.37-0.47]	43 [0.37-0.47]	40 [0.32-0.44]	0.20
LA diameter (cm)	44 (G1=	3.4 ± 0.51	3.4 ± 0.55	3.4 ± 0.38	0.34
LA volume biplane (ml/m^2)	32 (G1=24, G2=8)	21.5 [17.2-26]	21 [17-26]	22 [19-25]	0.68
LVEDV indexed (ml/m^2)	42 (G1=31, G2=11)	41 [36-48.2]	40 [35-43]	47 [41-56]	0.02**
LVESV indexed (ml/m^2)	42 (G1=31, G2=11)	14 [12-17]	13 [11-15]	18 [14-20]	0.003**
LVEF (%)*	45 (G1=33, G2=12)	65 ± 5.6	66 ± 5.7	63 ± 4.1	0.05
MV E/A	43 (G1=33, G2=10)	0.81 [0.67-0.89]	0.81 [0.67-0.88]	0.83 [0.58-0.96]	0.75
MV DecT (cm)	42 (G1=32, G2=10)	168 [141-203]	173 [144-211]	150 [130-172]	0.18
Lateral E/E'	40 (G1=30, G2=10)	6.8 [5.6-7.5]	6.8 [5.6-7.7]	6.8 [5.3-7.9]	0.90
Medial E/E'	29 (G1=22, G2=7)	9.7 [8.2-12.2]	9.8 [8.0-12.4]	9.5 [8.0-12.2]	0.94
Mean E/E'	42 (G1=22, G2=7)	7.5 [7.1-9.7]	8.2 [7.1-9.8]	8.6 [6.6-9.6]	0.98
TR Vmax (m/s) **TR Vmax ≤ 2.8 m/s** **TR Vmax > 2.8 m/s and ≤ 3.4 m/s** **TR Vmax > 3.4 m/s**	44 (G1=32, G2=12)	37 (84) 7 (16) 0 (0)	26 (81%) 6 (19%) 0 (0)	11 (92%) 1 (8%) 0 (0)	0.65
IVRT (cm)	31	85	86	83	0.32

	(G1=22, G2=9)	[69-99]	[79-99]	[51-103]	
Tei Index (LV)	31 (G1=22, G2=9)	0.54 [0.46-0.60]	0.54 [0.46-0.60]	0.53 [0.36-0.63]	0.66
RA volume indexed (ml/m^2)	37 (G1=25, G2=12)	16 [12-22]	15 [11-23]	17 [12-21]	0.99
RV basal-wall diameter (cm)	41 (G1=30, G2=11)	3.5 [3.1-4.0]	3.4 [2.9-4.2]	3.7 [3.5-3.9]	0.34
RV mid-wall diameter (cm)*	41 (G1=30, G2=11)	2.9 ± 0.6	2.9 ± 0.6	3.1 ± 0.4	0.40
RV free wall S' (m/s)	21 (G1=14, G2=7)	0.12 [0.1-0.14]	0.11 [0.1-0.12]	0.13 [0.1-0.15]	0.31
RV EDA (cm^2)	35 (G1=26, G2=9)	16.3 [13.1-18.7]	14.2 [13.0-18.6]	17.3 [14.4-15.0]	0.19
RV ESA (cm^2)	35 (G1=26, G2=9)	8.2 [7.3-10.2]	8.7 [7.3-10.5]	7.4 [6.1-8.7]	0.19
RV FAC (%)	35 (G1=26, G2=9)	46.4 [36.4-54.3]	42.3 [34.7-47.2]	57 [54.2-60.8]	0.001**
TAPSE (cm)*	34 (G1=25, G2=9)	2.1 ± 0.3	2.1 ± 0.3	2.3 ± 0.3	0.07
RV IVRT (cm)	20 (G1=13, G2=7)_	78 ± 27	75 ± 26	73 ± 29	0.41
Tei index (RV)	20 (G1=13, G2=7)	0.64 ± 0.14	0.65 ± 0.16	0.63 ± 0.11	0.75

Table 12. Baseline 2D, M-Mode, and Doppler echocardiography measurements

G1 representing patients with LVEF ≥ 53%. G2 representing patients with LVEF < 53%.
Data are presented as n (%) or mean ± SD or median [IQR].
*Inter-group differences for continuous variables measured using the Mann-Whitney U test or * student t test if data normally distributed*
*** p < 0.05*
LVIDd: left ventricular internal diameter at end-diastole, LVIDs: left ventricular internal diameter at end-systole, LV RWT: LV relative wall thickness, LVEDV: LV end-diastolic volume, LVESV: LV end-systolic volume, LVEF: LV ejection fraction, MV DecT: mitral valve deceleration time, IVRT: isovolumic relaxation time, RV EDA: right ventricular end-diastolic area, RV ESA: right ventricular end-systolic area, RV FAC: right ventricular fractional area change, TAPSE: tissue annular plane systolic excursion

In addition to the conventional echocardiography measures, the baseline LV strain and strain-rate (Table 13), RV strain and strain-rate (Table 14), left atrial strain and strain-rate (Table 15), and right atrial strain and strain-rate measures (Table 16) have been provided. These once again demonstrated similar baseline strain measures between G1 and G2 without any statistically significant difference between the two groups.

3.2.2 Baseline Strain Measures

Left ventricular strain and strain-rate

Variable	N	All patients	G1	G2	p value
GLS (%)*	41 (G1=31, G2=10)	-21.3 ± 2.5	-21.5 ± 2.6	-20.7 ± 1.8	0.37
MyoGLS (%)*	41 (G1=31, G2=10)	-18.5 ± 2.6	-18.7 ± 2.8	-17.8 ± 1.7	0.32
LV peak systolic longitudinal SR(1/s)	41 (G1=31, G2=10)	-1.1 [(-1.4) - (-1.01)]	-1.16 [(-1.4) - (-1)]	-1.1 [(-1.2) - (-1.01)]	0.26
LV end-systolic longitudinal SR (1/s)*	41 (G1=31, G2=10)	-0.04 ± 0.32	0.01 ± 0.32	-0.2 ± 0.36	0.09
GRS (%)*	41 (G1=31, G2=10)	35.9 ± 8.8	36.5 ± 9	34.2 ± 8.3	0.48
LV peak systolic radial SR (1/s)*	41 (G1=31, G2=10)	1.5 ± 0.34	1.5 ± 0.35	1.4 ± 0.29	0.23
LV end-systolic radial SR (1/s)	41 (G1=31, G2=10)	0.03 [(-0.2) - (0.2)]	0.03 [(-0.2) - (0.17)]	0.15 [(-0.1) - (0.40)]	0.27
LV GCS (%)	13 (G1=10, G2=3)	-30.7 ± 2.4	-30.7 ± 2.8	-30.6 ± 3.0	0.32
LV myoGCS (%)	13 (G1-10, G2=3)	-23.4 ± 3.1	-23.2 ± 3.2	-23.5 ± 2.8	0.38

Table 13. Baseline LV strain and strain-rate measures

G1: patients with LVEF ≥ 53%. G2: Group 2 representing patients with LVEF < 53%.
*Inter-group differences for continuous variables measured using the Mann-Whitney U test or * student t test if data normally distributed*
LV: left ventricle, GLS: global longitudinal strain, MyoGLS: LV myocardial strain, SR: strain-rate, GRS: global radial strain

Right ventricular strain and strain-rate

Variable	N	All patients	G1	G2	p value
RV GLS (%)*	34 (G1=25, G2=9)	-24.3 ± 3.2	-24 ± 2.7	-25.1 ± 4.4	0.38
RV myoGLS (%)*	34 (G1=25, G2=9)	-22.3 ± 3.2	-22 ± 2.5	-23.1 ± 4.7	0.35
RV peak systolic longitudinal SR (1/s)	34 (G1=25, G2=9)	-1.4 [(-1.6)-(-1.2)]	-1.4 [(-1.5)-(-1.2)]	-1.5 [(-2.0)-(-1.3)]	0.09
RV end-systolic longitudinal SR (1/s)*	32 (G1=24, G2=8)	-0.11 ± 0.44	-0.05 ± 0.43	-0.3 ± 0.45	0.49
RV FWS (%)*	34 (G1=25, G2=9)	-26.9 ± 4.2	-26.6 ± 4.1	-27.8 ± 4.5	0.48
RVFW peak systolic longitudinal SR (1/s)*	34 (G1=25, G2=9)	-1.69 ± 0.38	-1.6 ± 0.38	-1.8 ± 0.34	0.11
RVFW end-systolic longitudinal SR (1/s)	31 (G1=23, G2=8)	-1.0 [(-0.5)-(0.3)]	-0.1 [(-0.1)-(0.3)]	-0.5 [(-0.8)-(0.17)]	0.16

Table 14. Baseline RV strain and strain-rate measures

G1: patients with LVEF ≥ 53%. G2: Group 2 representing patients with LVEF < 53%.
*Inter-group differences for continuous variables measured using the Mann-Whitney U test or * student t test if data normally distributed*

RV: right ventricle, RV GLS: right ventricular global longitudinal strain; RV myoGLS: right ventricular myocardial strain, SR: strain-rate, RVFWS: right ventricular free wall strain

Left atrial strain and strain-rate

Variable	N	All patients	G1	G2	p value
LA 4Ch strain (%)*	32 (G1=23, G2=9)	31.8 ± 8.8	31.8 ± 9.6	31.8 ± 6.8	1
LASr 4Ch (%)*	32 (G1=23, G2=9)	39 ± 9.5	39.1 ± 10	38.7 ± 8.4	0.9
LAScd 4Ch (%)	29 (G1=21, G2=8)	-18.5 [(-27.3)-(-14.1)]	-18 [(-26.2)-(-14.1)]	-18.8 [(-29.9)-(-13.6)]	0.9
LASct 4Ch (%)*	29 (G1=21, G2=8)	-17.9 ± 7.7	-17.6 ±7.5	-18.8 ± 8.9	0.7
LA 4Ch peak systolic SR (1/s)	32 (G1=23, G2=9)	1.3 [1.1-1.7]	1.4 [1.1-1.8]	1.3 [1.0-1.6]	0.5
LA 4Ch early diastolic SR (1/s)*	25 (G1=18, G2=7)	-1.2 ± 0.48	-1.3 ± 0.5	-0.9 ± 0.3	0.06
LA 4Ch late diastolic SR (1/s)	25 (G1=18, G2=7)	-1.4 [(-1.8)-(-1.2)]	-1.5 [(-1.8)-(-1.2)]	-1.2 [(-1.6)-(1.2)]	0.3
LA 2Ch strain (%)*	22 (G1=16, G2=6)	29.1 ± 5.7	28.6 ± 5.9	30.3 ± 5.3	0.5
LASr 2Ch (%)*	22 (G1=16, G2=6)	36.7 ± 7.1	36.1 ± 7.2	38 ±7.3	0.6
LAScd 2Ch (%)	20 (G1=14, G2=6)	-14.6 [(-22.7)-(-11.7)]	-12.5 [(-18.8)-(-11.2)]	-21.5 [(-32.2)-(-12.7)]	0.1
LASct 2Ch (%)*	20 (G1=14, G2=6)	-18.5 ± 6.3	-19.5 ± 4.9	-16.1 ± 8.7	0.3
LA 2Ch peak systolic SR (1/s)	20 (G1=14, G2=6)	1.2 [1.0-1.7]	1.1 [1.0-1.4]	1.7 [1.3-2.1]	0.07
LA 2Ch early diastolic SR (1/s)*	15 (G1=11, G2=4)	-0.9 ± 0.3	-0.9 ± 0.3	-0.9 ± 0.3	0.9
LA 2Ch late diastolic SR (1/s)	16 (G1=12, G2=4)	-1.8 [(-2.2)-(-1.4)]	-1.8 [(-2.2)-(-1.4)]	-1.6 [(-2.3)-(-1.3)]	0.8
LA biplane strain (%)*	18 (G1=14, G2=4)	29.7 ± 5.5	29.1 ± 6.1	31.5 ± 2.5	0.4
LASr biplane (%)*	18 (G1=14, G2=4)	38.2 ± 7.9	37 ± 7.3	42.3 ± 9.5	0.2
LAcd biplane (%)	15 (G1=11, G2=4)	-15.8 [(-20.4)-(-12.4)]	-14.7 [(-20.4)-(-12)]	-18.6 [(-25.9)-(-13.7)]	0.3
LAct biplane (%)*	15 (G1=11, G2=4)	-18.1 ± 6.4	-18.1 ± 4.5	-17.9 ± 10.9	0.9

Table 15. Baseline left atrial strain measures

G1: patients with LVEF ≥ 53%. G2: Group 2 representing patients with LVEF < 53%.
*Inter-group differences for continuous variables measured using the Mann-Whitney U test or * student t test if data normally distributed*
LA: left atrial, 4Ch: 4chamber, LASr: left atrial strain during reservoir phase, LAScd: left atrial strain during conduit phase, LASct: left atrial strain during contraction strain, SR: strain-rate

Right atrial strain and strain-rate

Variable	N	All patients (n=45)	G1 (n=33)	G2 (n=12)	p value
RA strain (%)	29 (G1=20, G2=9)	30.1 ±8.3	31.3 ± 8.5	27.1 ± 7.1	0.2
RASr (%)	29 (G1=20, G2=9)	39.1 ± 9.0	40.1 ± 9.6	36.9 ± 7.4	0.4
RAScd (%)	26 (G1=19, G2=7)	-20.8 ± 8.4	-21.1 ± 9.2	-20.3 ± 6.1	0.8
RASct (%)	26 (G1=19, G2=7)	-18.4 ± 6.8	-18.7 ± 6.9	-17.6 ± 7.3	0.7
RA peak systolic SR (1/s)	27 (G1=19, G2=8)	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.6	0.4
RA early diastolic SR (1/s)	22 (G1=15, G2=7)	-0.9 ± 0.5	-0.9 ± 0.5	-0.9 ± 0.6	1.0
RA late diastolic SR (1/s)	21 (G1=15, G2=6)	-1.9 ± 0.7	-1.9 ± 0.6	-2.0 ± 0.7	0.6

Table 16. Baseline right atrial strain measures

G1: patients with LVEF ≥ 53%. G2: Group 2 representing patients with LVEF < 53%.
Inter-group differences for continuous variables measured using student t test
RA: right atrial, 4Ch: 4chamber, RASr: right atrial strain during reservoir phase, RAScd: right atrial strain during conduit phase, RASct: right atrial strain during contraction strain, SR: strain-rate

3.3 Changes in Echocardiographic Measures - All Patients

From T0 to T2, conventional and strain measures were analysed to determine which measure declined with, or preceded a decline in LVEF. These measures were initially assessed in all patients, followed by comparing these between patients in G1 and G2. The echocardiographic changes in all patients have been outlined in *Appendix 3*.

3.3.1 Conventional Echocardiographic Measures

With incremental doses of anthracyclines, in the left ventricle, a statistically significant increase in LVIDd, LVIDs, LVESV, LV mass (indexed to BSA) and IVRT, and a reduction in LV FS and LVEF between T0 and T2 were noted. Amongst these measures, LV FS and LVEF were the only two measures to show a statistically significant decline at T1 as well as T2 when compared to T0. However, despite these statistical changes the values for each of these measures were within the normal range at each time-point. Furthermore, there were no statistically significant changes in the LA volume (indexed to BSA), medial and lateral E', mean E/E' and TR velocity with time as highlighted in *Appendix 3*.

In the right ventricle, TAPSE was the only conventional echocardiographic measure to demonstrate a deterioration from T0 to T2 from a mean of 2.2 cm at T0 to a mean of 2.0 cm at T2 (p = 0.004) however, despite this reduction this was within the normal range considered for a normal TAPSE. No change in this measure was seen from T0 to T1. No significant change in the RA volumes were seen with time. The full results for the conventional echocardiography measures have been provided in *Appendix 3, Section 1.1.*

3.3.2 Strain Measures of Left and Right Sided Chambers

Given the lack of short axis apical views of the LV in the majority of patients in this study and poor-quality strain-rate curves, a conscious decision to exclude LV circumferential strain-rate measures, twist and torsion from the final analysis of this study was made. Only LV GCS and myoGCS results have been provided but these have to be interpreted with caution due to insufficient number of patients. Additionally, LV early and late diastolic strain-rate measures were also excluded from the final analysis of this study due to poor quality images when assessing the diastolic strain-rate curves.

3.3.2.1 Left Ventricular Strain Measures

A full table describing the changes in all LV strain and strain-rate measures have been provided in *Table 3, Appendix 3.* All patients demonstrated a decline in their GLS with time, with the mean GLS at T0 at -21.4%, at T1 -19.9% and T2 -17.9%. Despite a reduction in this measure, this was not statistically significant between T0 and T1 (mean change 1.59; p=0.18), but significant between T0 and T2 (mean change of 3.54 (16%); p=0.002). Figure 11 illustrates the changes in this measure at different time-points in all patients.

LV myoGLS showed a similar trend to GLS with a statistically significant decline between T0 and T2 (mean change 2.93; p=0.01) but no change between T0 and T1, or T1 and T2.

The LV peak systolic longitudinal strain-rate reduced with time and this decline was seen early in the treatment at T1 with a statistically significant reduction demonstrated between T0 and T1 (mean change 0.10; p=0.01), and between T0 and T2 (mean change 0.15; p<0.001).

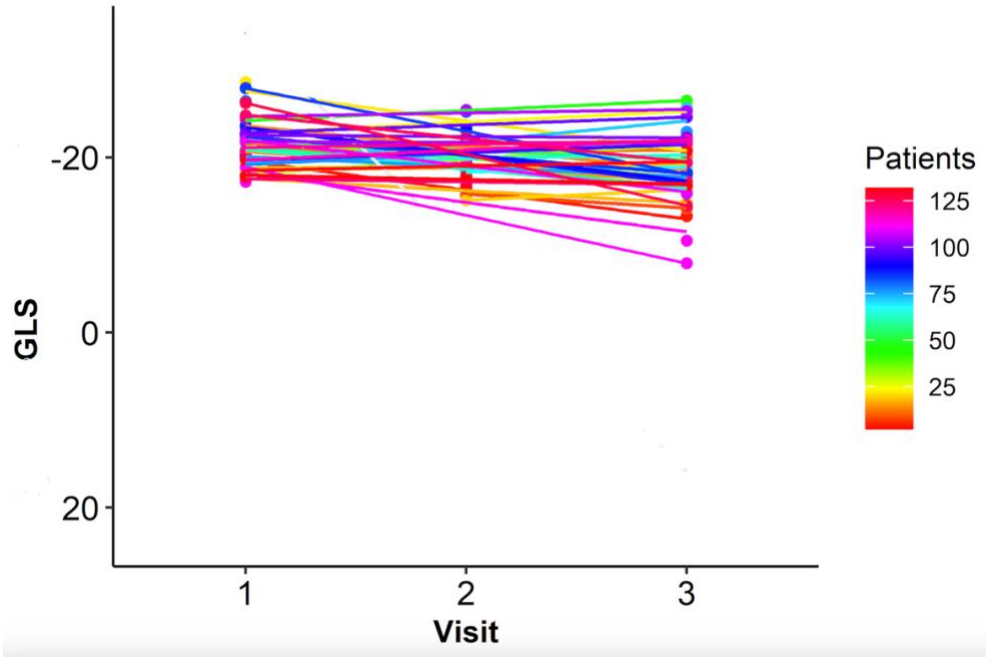


Figure 11. LV GLS in all patients at different time-points

LV GRS also showed a statistically significant deterioration reduced between T0 and T2 visits only (mean change -0.09, p=0.03) but despite this change the mean GRS at T2 was still within what is considered a normal range for GRS. The changes in LV GRS have been illustrated in Figure 12. A similar trend of decline was also seen in the LV peak systolic radial strain-rate (mean change -0.11; p=0.01).

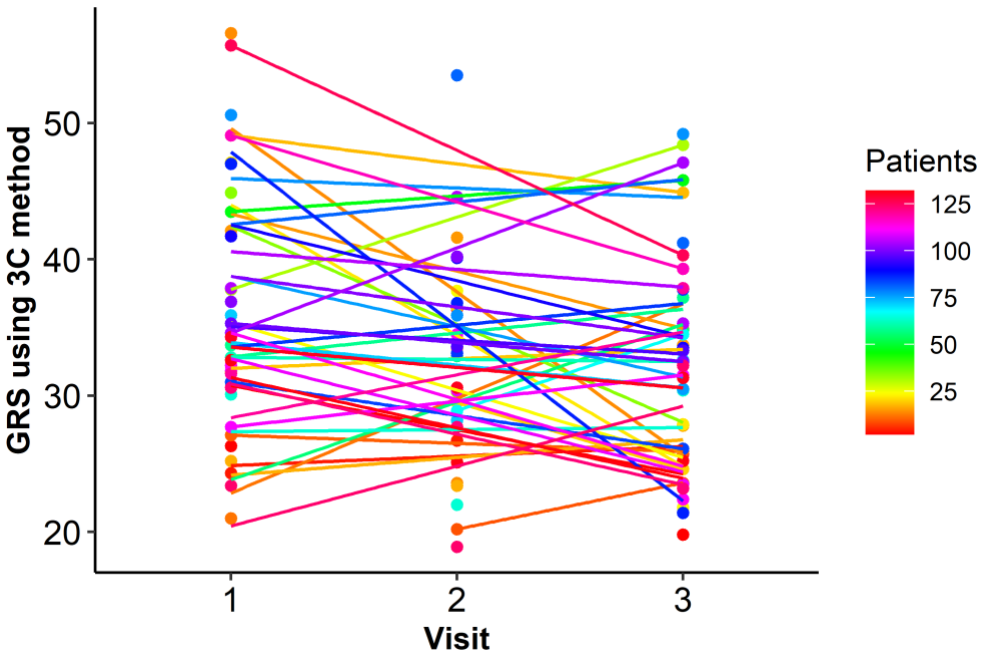


Figure 12. LV GRS change with time in all patients

LV GCS and myoGCS also showed a statistically significant decline in their measures with time however, as stated previously, extra caution has to be taken when interpreting these findings in view of the insufficient number of patients with adequate short axis images.

3.3.2.2 Right Ventricular Strain Measures

In the right ventricle, RV GLS and RV myoGLS showed a statistically significant decline in all patients with time which was evident as early as visit T1. Mean RV GLS at T0 was -24.3%, T1 -22.6% and T2 -20.9%. Between T0 and T1 the mean change in RV GLS was 1.61 with a p=0.01, and between T0 and T2 3.30 with a p<0.001 (Figure 13). RV peak systolic longitudinal strain-rate also showed a similar trend in decline at all visits, as highlighted in **Table 4, *Appendix 3*.**

Additionally, the RVFWS deteriorated with time with the mean RVFWS at T0 -26.9%, T1 -24.1% and T2 -22.9%. The mean change between T0 and T1 was 2.05, and T0 and T2 3.74 which were both statistically significant (p=0.03 and p<0.001, respectively). The RVFW peak systolic longitudinal strain-rate also followed the same pattern of decline as RVFWS with time which was statistically significant.

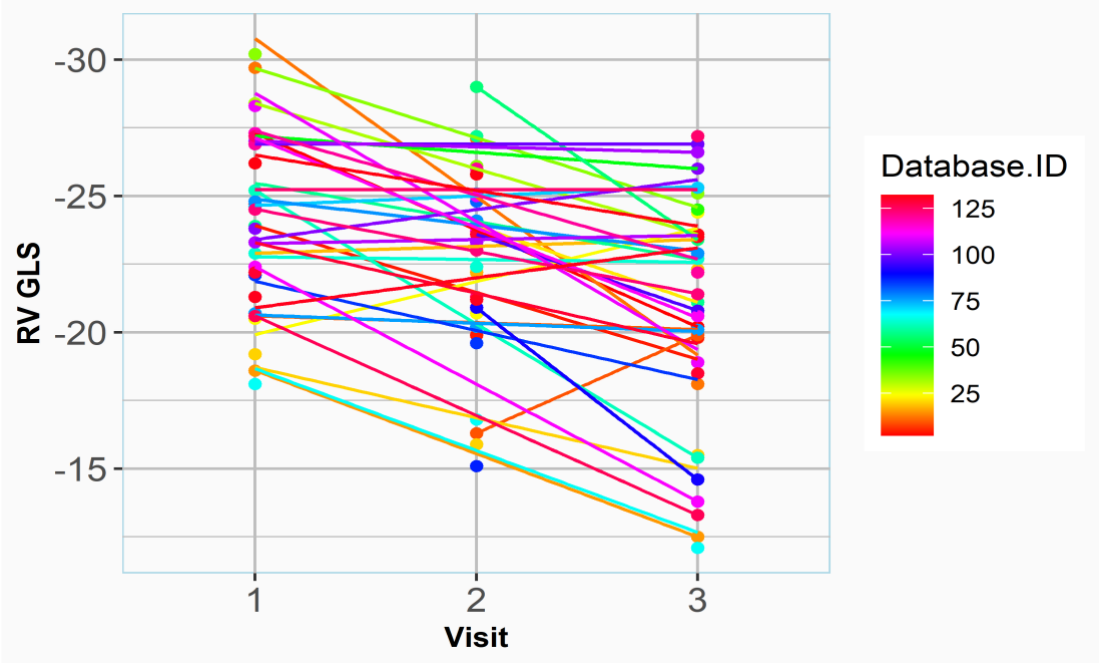


Figure 13. RV GLS in all patients at different time-points

3.3.2.3 Left Atrial Strain Measures

Left atrial strain measures have been provided in ***Table 5, Appendix 3.*** The LA GLS and LASr using the apical 4 chamber (Figure 14) and biplane views showed a decline with time which was statistically significant between T0 and T2. Additionally, the LAScd showed a statistically significant decline both between T0 and T1 (mean change 4.42; p=0.01) and T0 andT2 (mean change 3.60; p=0.04). This table also displays the changes in the LA strain-rate measures.

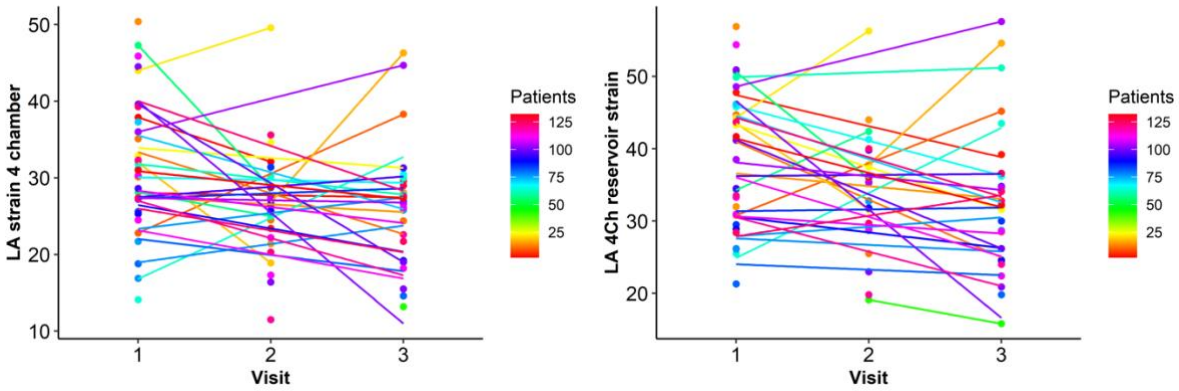


Figure 14. LA GLS and LASr change with time

Changes in LA GLS (using the apical 4Ch view) with time in all patients, demonstrated in the left image. Changes in LASr (using the apical 4Ch view) with time in all patients, demonstrated in the right image.

3.3.2.4 Right Atrial Strain Measures

In the right atrium, some changes were also noted in the strain measures which have been demonstrated in *Table 6, Appendix 3*. All patients showed a decline in their RA GLS with increasing dose of anthracyclines. The mean change in RA GLS between T0 and T1 was -0.11, and -0.13 between T0 and T2, which were both statistically significant (p=0.03 and p=0.008, respectively). This finding has been illustrated in (Figure 15). Furthermore, the RASr showed a reduction in its value from T0 to T2 with a mean RASr at T0 of 38.3% and at T2 34.3%, leading to a mean change of -3.71 which was again statistically significant. The RA peak systolic strain-rate showed a similar pattern of reduction to RA GLS at each timepoint.

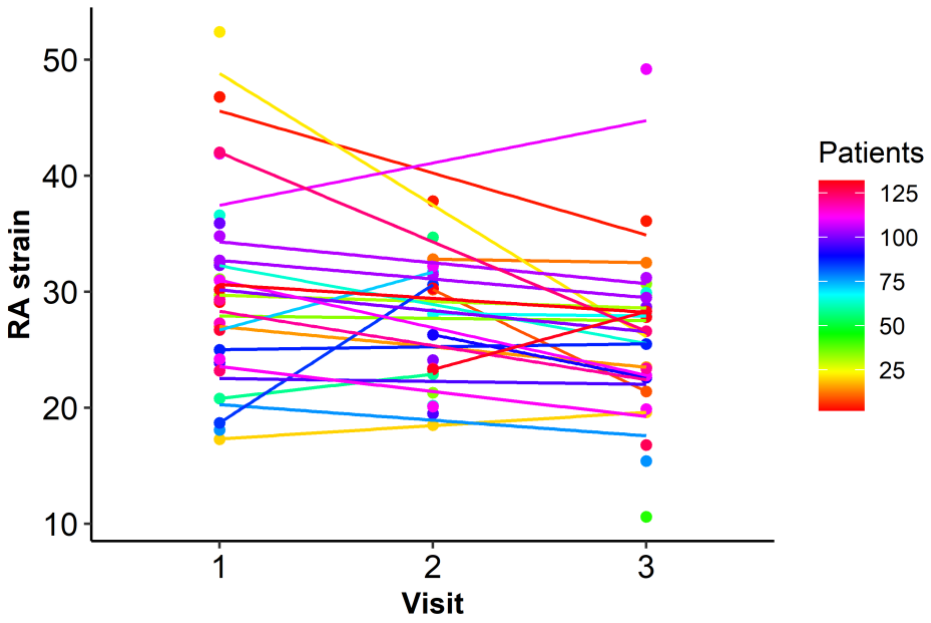


Figure 15. RA strain change in all patients

3.4 Changes in Echocardiography Measures - Between Groups

3.4.1 Conventional Echocardiography Measures

With incremental doses of anthracyclines an increase in the left ventricular dimensions in particular LVIDs was noted in patients in both G1 and G2. However, interestingly this increase was more statistically significant in G1 when compared to G2; LVIDs mean change of 0.09 between T0 and T1, p=0.04 and a mean change of 0.13 between T0 and T2, p=0.002. Additionally, LVEF deteriorated in both patient groups but despite the statistical decline from a LVEF of 67% at T0 to LVEF of 61% at T2 in G1, this remained within the normal range. However, the decline in LVEF in G2 was more significant with LVEF at T0 62% and at T2 50% (mean change of -0.09; p<0.001).

From a LV diastolic function point of view, some of the parameters such as the lateral and medial E' showed a statistically significant decline from baseline to T2 in patients in G2; lateral E' of 0.10m/s at T0 to 0.09m/s at T2 (mean change -0.22; p=0.02) and medial E' of 0.07m/s at T0 to 0.06m/s at T2 (mean change -0.23; p=0.04). However, other measures of LV diastolic function such as the LA volume indexed, average E/E' and TR velocity remained relatively unchanged. Other conventional echocardiographic measures of the LV have been described in detail in *Table 7, Appendix 3*.

In the assessment of the right sided chambers, the RV FAC reduced significantly from T0 to T2 in patients in G2. This change was seen as early as T1 with RV FAC at T0 54.9%, T1 44.2% and at T2 44.6%. The mean change between T0 and T1 was -0.22 with p=0.004 and between T0 and T2, -0.23 with p=0.003. Despite this reduction, RV FAC was still within the normal range (RV FAC >30%) at T2.[372] Interestingly, at baseline, patients in G2 had a higher RV FAC (54.9%) compared to patients in G1 (41.9%). In addition to RV FAC, TAPSE was another important measure of RV function seen to decline with time in patients in G2. At T0, this was 2.3cm which reduced to 1.8cm at T2 (mean change of -0.26 and p<0.001). This decline was not seen in patients in G1. Though, again, TAPSE was still within the normal range at T2.[372]

The indexed right atrial volume did not show any significant change between the two patient groups. Full results of the RV systolic and diastolic measures can be found in *Table 8, Appendix 3.*

3.4.2 Strain Measures of Left and Right Sided Chambers

The change in strain and strain-rate measures in both patient groups have been provided in detail in *Appendix 3.* For similar reasons as explained in section 3.3.2, LV GCS strain-rate measures, twist, torsion and the LV early and late diastolic strain-rate measures have not been provided in the final analysis of the results. LV GCS results have been provided on the limited number of patients where LV GCS was possible to measure as explained in section 3.3.2 Strain Measures of Left and Right Sided Chambers. Therefore, the results described for this measure on this section should be interpreted with caution.

3.4.2.1 Left Ventricular Strain Measures

In patients in G1 and G2, a significant deterioration in the LV GLS was noted between T0 to T2. However, the extent of this deterioration was more significant in the G2 group when compared to G1. In G1, the mean change of GLS between T0 and T2 was 3.25 with p=0.02 (15% change between the two visits). In G2, this change was 3.87 with P <0.001 (18% change). This finding was consistent with the definition of cardiotoxicity and correlated with a reduction in the LVEF. Interestingly, despite a reduction in GLS at T1, the mean change between T0 and T1 was not statistically significant (mean change 1.25; p=0.14) but significant between T1 and

T2 (mean change 2.61; p=0.002). LV peak systolic strain-rate also declined in both patient groups as outlined in Table 17.

In G2, LV GRS showed a more dramatic reduction with time compared to G1. The mean GRS at T0 was 34.8%, T1 32.2% and T2 28.3%. The mean change in GRS was only statistically significant between T0 and T2 with a mean change of -6.51 and p=0.03 which correlated with a drop in LVEF but this reduction was not statistically significant enough at T1 (mean change between T0 and T1 -2.52; p=0.38).

At baseline, the mean GCS was similar in both patient groups (-30.7% in G1 and -30.6% in G2). A more extensive decline in LV GCS was noted in G2 patients between T0 and T2 (-30.6% to -23.3%, respectively) compared to those in G1 (-30.7% to -26.7%, respectively). This was statistically significant (p=0.009). Despite a mean change of 3.12 between T0 and T1 in this patient group, this extent if decline did not reach statistical significance (p=0.16). A complete analysis of the LV strain measures have been provided in the table below (Table 17).

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Mean change (T1 from T0)	p value	Mean change (T2 from T1)	P value	Mean change (T2 from T0)	P value
GLS (%) G1	-21.6	-20.2	-18.4	1.439	0.37	1.81	0.26	3.25	0.02*
G2	-20.9	-19.3	-16.7	1.25	0.14	2.61	0.002*	3.87	<0.001*
MyoGLS (%) G1	-18.7	-17.7	-16.1	0.91	0.48	1.62	0.22	2.54	0.03*
G2	-18.0	-13.4	-14.1	4.67	0.12	-0.747	0.783	3.92	0.18
LV peak systolic longitudinal SR (1/s) G1	-1.22	-1.09	-1.07	0.12	0.02*	0.03	0.59	0.15	0.002*
G2	-1.05	-1.01	-0.89	0.04	0.40	0.12	0.01*	0.15	0.003*
LV end-systolic longitudinal SR (1/s) G1	-0.02	0.11	-0.06	0.08	0.29	-0.12	0.13	-0.03	0.6
G2	-0.10	-0.01	-0.06	0.09	0.20	-0.05	0.42	0.04	0.57
GRS (%) G1	36.2	31.9	34.0	-3.37	0.07	1.21	0.51	-2.16	0.17
G2	34.8	32.2	28.3	-2.52	0.38	-3.99	0.11	-6.51	0.03*
LV peak systolic	1.53	1.35	1.39	-0.14	0.07	-0.007	0.93	-0.14	0.03*

radial SR (1/s) G1									
G2	1.32	1.27	1.21	-0.05	0.67	-0.07	0.48	-0.12	0.29
LV end-systolic radial SR (1/s) G1	0.10	-0.19	0.13	-0.18	0.26	0.21	0.20	0.028	0.84
G2	0.08	-0.07	0.17	-0.14	0.48	0.23	0.21	0.09	0.65
GCS (%) G1	-30.7	-29.6	-26.7	0.88	0.66	3.03	0.16	3.9	0.06
G2	-30.6	-26.9	-23.3	3.12	0.16	3.64	0.12	6.76	0.009*
MyoGCS (%) G1	-23.2	-22.4	-20.4	0.49	0.78	2.23	0.25	2.72	0.13
G2	-23.5	-20.3	-18.9	2.69	0.19	1.4	0.48	4.09	0.06

Table 17. LV strain and strain-rate measures in both patient groups

White rows represent the changes in measures in G1 and grey rows representing the changes in G2.
**Statistically significant ($p < 0.05$)*

3.4.2.2 Right Ventricular Strain Measures

The detailed right ventricular strain and strain-rate measures have been provided in Table 18. RV GLS showed a statistically significant deterioration at T2 in both G1 and G2 ($p<0.001$). However, the extent of the decline in G2 was more pronounced when compared to G1 (mean change of 2.25 and a mean change of 6.28 between T0 and T2, in G1 and G2 respectively). This decline in RV GLS was seen early on in the treatment (at T1) which was only observed in G2, with a mean change of 2.87 between T0 and T1, $p = 0.02$. This reduction persisted to the end of treatment with a mean RV GLS of -18.8% and mean change of 3.41, $p = 003$ between T1 and T2. However, this early change was not seen in patients in G1. Furthermore, RV FWS reduced significantly in both groups at T2 with a more substantial decline seen in patients in G2. At T2, the mean RV FWS was -19.5% in G2 whilst this was -24.2% in G1 despite similar RV FWS measures at baseline. The changes in RV GLS and RV FWS have been shown in Figure 16.

The RV and RV FW peak systolic strain-rates deteriorated in both patient groups with a statistically significant early decline seen at T1.

A **B**

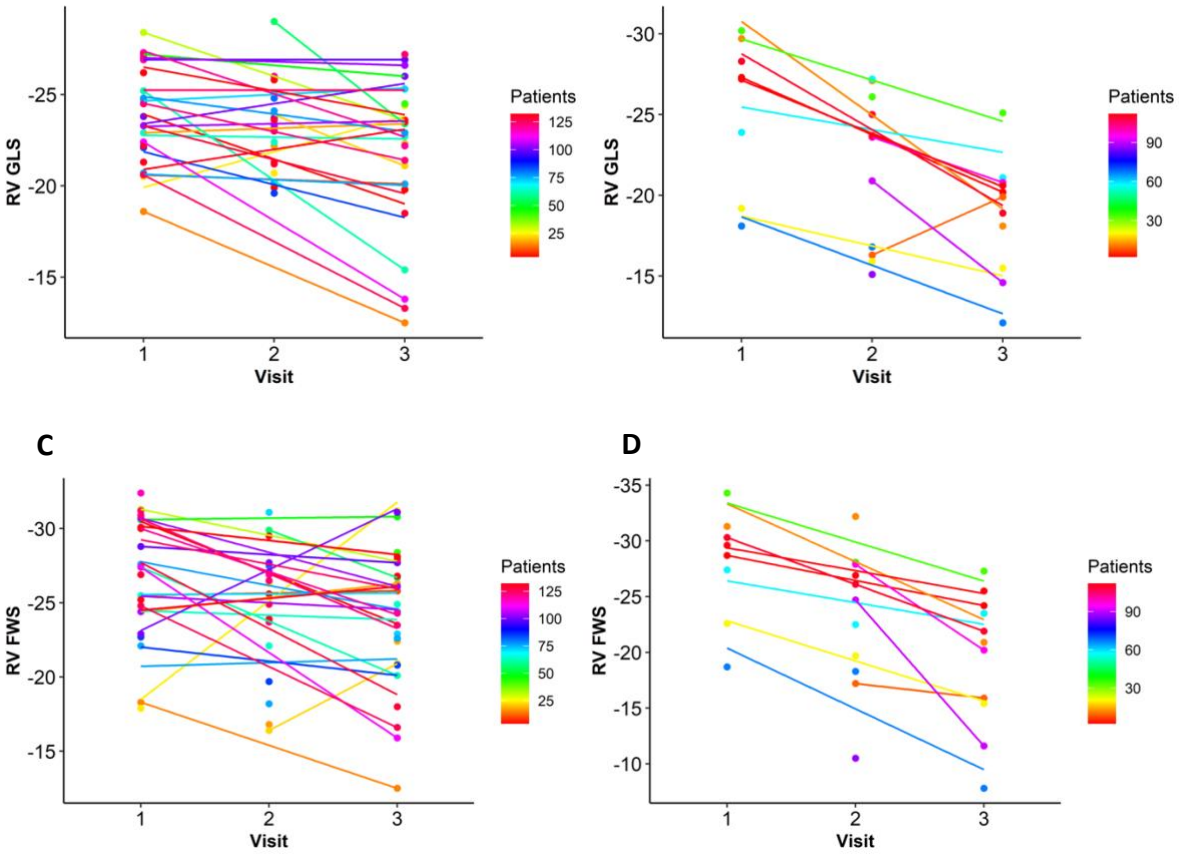


Figure 16. Change in RV GLS and RV FWS in both patient groups

A: Change in RV GLS in G1; B: change in RV GLS in G2, C: change in RV FWS in G1; D: RV FWS in G2

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Mean change (T1 from T0)	p value	Mean change (T2 from T1)	P value	Mean change (T2 from T0)	P value
RV GLS (%) G1	-23.9	-23.1	-21.8	1.17	0.10	1.07	0.12	2.25	<0.001*
G2	-25.5	-21.6	-18.8	2.87	0.02*	3.41	0.003*	6.28	<0.001*
RV myoGLS (%) G1	-21.9	-21.3	-19.9	0.99	0.18	1.18	0.10	2.18	0.001*
G2	-23.6	-20.1	-17.6	2.5	0.02*	3.09	0.003*	5.60	<0.001*
RV peak systolic longitudinal SR (1/s) G1	-1.4	-1.2	-1.1	0.17	0.03*	0.04	0.54	0.21	0.002*
G2	-1.55	-1.24	-1.01	0.30	0.02*	0.22	0.05	0.52	<0.001*
RV end-systolic longitudinal SR (1/s)	-0.08	0.04	-0.11	0.12	0.28	-0.16	0.14	-0.04	0.69

G1									
G2	-0.2	-0.06	-0.26	0.16	0.39	-0.19	0.27	-0.02	0.88
RV FWS (%) G1	-26.6	-24.7	-24.2	1.79	0.11	0.55	0.61	2.34	0.01*
G2	-27.8	-23.1	-19.5	2.66	0.07	4.90	0.001*	7.56	<0.001*
RVFW peak systolic longitudinal SR (1/s) G1	-1.6	-1.4	-1.3	0.22	0.02*	0.05	0.62	0.27	0.002*
G2	-1.78	-1.35	-1.2	0.42	0.003*	0.161	0.18	0.58	<0.001*
RVFW end-systolic SR (1/s) G1	-0.05	-0.005	-0.1	0.052	0.63	-0.09	0.37	-0.04	0.66
G2	-0.16	-0.02	-0.27	0.13	0.52	-0.24	0.21	-0.10	0.60

Table 18. Right ventricular strain and strain-rate measures in both patient groups

White rows represent the changes in measures in G1 and grey rows representing the changes in G2.
**Statistically significant ($p < 0.05$)*

3.4.2.3 Left Atrial Strain Measures

The changes in the LA strain and strain-rate measures with time have been illustrated in the table below (Table 19). LA biplane measurements should be interpreted with caution given that these measurements were possible in only a small number of patients as highlighted in Table 15. LA 4Ch and 2Ch strains showed a decline with time in both patient groups however, this change was more dramatic in patients in G2 though, this was only statistically significant for LA 4Ch strain measure and not LA 2Ch strain. LA 4Ch reservoir strain declined at each visit in both patient groups with the statistically significant reduction seen between T0 and T2. Interestingly, LA 4Ch and 2Ch conduit strains only showed a statistically significant decline in G2. There was no meaningful change in the LA 4Ch contractile strain but the 2Ch contractile strain showed a statistically significant increase from T0 to T1 in G2. Additionally, the LA biplane contractile strain showed a trend of increasing in its value with time in G2, despite this not reaching statistical significance.

Both LA 4Ch peak systolic and early diastolic strain-rates declined significantly in patients in G1 only but this change was not replicated in G2. Additionally, this change was not seen in LA 2Ch strain-rate measures. There was no other statistically significant change seen in the other strain-rate measures.

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
LA 4Ch strain (%) G1	32.5	26.9	27.0	-0.173	0.07	0.004	0.96	-0.16	0.06
G2	30.1	25.1	24.9	-0.18	0.005*	0.016	0.80	-0.17	0.01*
LASr 4Ch (%) G1	39.8	34.1	33.9	-0.093	0.16	-0.041	0.54	-0.13	0.04*
G2	36.1	35.2	29.2	-0.025	0.76	-0.181	0.04*	-0.20	0.03*
LAScd 4Ch (%) G1	-21.2	-18.4	-18.8	2.79	0.21	-0.67	0.76	2.12	0.33
G2	-21.8	-13.7	-13.6	8.06	0.007*	0.013	0.99	8.07	0.01*
LASct 4Ch (%) G1	-17.6	-16.1	-15.6	0.81	0.73	0.99	0.68	1.80	0.44
G2	-18.7	-21.5	-15.6	-2.78	0.39	4.70	0.19	1.92	0.58
LA 4Ch peak systolic SR (1/s) G1	1.5	1.4	1.2	-0.068	0.45	-0.185	0.06	-0.25	0.008*
G2	1.3	1.4	1.1	-0.02	0.90	-0.16	0.39	-0.18	0.34
LA 4Ch early diastolic SR (1/s) G1	-1.3	-1.1	-1.0	0.15	0.23	0.149	0.24	0.29	0.02*
G2	-0.9	-0.9	-0.9	0.05	0.77	0.02	0.89	0.08	0.67
LA 4Ch late diastolic SR (1/s) G1	-1.7	-1.7	-1.5	0.045	0.84	0.23	0.31	0.28	0.21
G2	-1.4	-1.8	-1.3	-0.38	0.15	0.46	0.09	0.07	0.77
LA 2Ch strain (%) G1	28.6	28.3	26.7	-0.05	0.54	-0.032	0.65	-0.088	0.31
G2	30.1	31.7	24.5	0.045	0.76	-0.25	0.08	-0.124	0.12
LASr 2Ch (%) G1	36.1	35.1	34.9	-0.061	0.36	0.008	0.90	-0.053	0.45
G2	38.0	41.2	32.1	0.09	0.44	-0.26	0.05	-0.167	0.13
LAScd 2Ch (%) G1	-15.1	-15.9	-17.6	1.34	0.52	-1.29	0.54	-2.63	0.24
G2	-22	-14.7	-12.2	7.96	0.06	3.64	0.32	11.6	0.009*
LASct 2Ch (%) G1	-19.5	-19.2	-17.7	0.44	0.81	1.47	0.46	1.92	0.36
G2	-16.0	-26.8	-19.8	-10.5	0.04*	5.64	0.24	-4.84	0.25
LA 2Ch peak systolic SR (1/s) G1	1.3	1.3	1.3	-0.03	0.80	-0.02	0.87	-0.05	0.69

G2	1.7	1.7	1.3	-0.43	0.84	-0.25	0.23	-0.29	0.14
LA 2Ch early diastolic SR (1/s) G1	-0.8	-1.1	-0.9	-0.26	0.16	0.187	0.31	-0.08	0.68
G2	-0.9	-0.8	-0.7	0.10	0.76	0.14	0.67	0.24	0.45
LA 2Ch late diastolic SR (1/s) G1	-1.8	-1.9	-1.7	-0.05	0.78	0.18	0.31	0.13	0.54
G2	-1.7	-2.1	-2.1	-0.27	0.51	-0.002	0.99	-0.27	0.45
LA biplane strain (%) G1	29.1	25.9	25.3	-0.127	0.09	-0.001	0.98	-0.128	0.09
G2	31.5	29.6	26.7	-0.067	0.62	-0.117	0.40	-0.184	0.17
LASr biplane (%) G1	36.7	33.4	31.7	-0.09	0.10	-0.033	0.55	-0.128	0.03*
G2	38.4	39.9	32.5	0.06	0.56	-0.212	0.09	-0.15	0.17
LAScd biplane (%) G1	-17.7	-17.8	-17.1	-0.12	0.95	0.46	0.80	0.34	0.86
G2	-19.4	-13.6	-13.4	3.54	0.28	2.83	0.37	6.37	0.08
LASct biplane (%) G1	-16.2	-17.3	-14.7	-1.04	0.58	2.57	0.18	1.52	0.44
G2	-17.9	-20.5	-19.1	3.54	0.28	6.4	0.08	1.45	0.82
LA biplane peak systolic SR (1/s) G1	1.3	1.3	1.2	-0.037	0.72	-0.127	0.22	-0.164	0.14
G2	1.8	1.2	1.1	-0.39	0.07	-0.036	0.85	-0.43	0.06
LA biplane early diastolic SR (1/s) G1	-1.1	-1.1	-0.9	-0.10	0.42	0.23	0.07	0.13	0.35
G2	-0.9	-1.1	-1.0	-0.32	0.29	0.22	0.07	-0.09	0.73
LA biplane late diastolic SR (1/s) G1	-1.7	-1.6	-1.5	-0.10	0.42	0.14	0.48	0.13	0.35
G2	-1.9	-1.9	-1.6	-0.02	0.93	0.28	0.30	0.30	0.30

Table 19. Changes in LA strain and strain-rate measures with time in G1 and G2

White rows represent the changes in measures in G1 and grey rows representing the changes in G2.
**Statistically significant ($p < 0.05$)*

3.4.2.4 Right Atrial Strain Measures

The full breakdown of the changes observed in the RA strain and strain-rate measures at different time-points in both G1 and G2, have been provided in Table 20. RA strain showed a deterioration in patients with time in patients in G1 which was statistically significant between T0 and T2. Interestingly, this change was not seen in G2 where RA strain remained largely unchanged between visits. Other parameters of the RA strain showed no significant change between visits in either group. This finding also applied to the RA strain-rate measures.

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
RA strain (%) G1	31.7	28.0	25.6	-0.117	0.12	-0.08	0.27	-0.202	0.005*
G2	26.7	25.1	27.3	-0.07	0.32	0.05	0.43	-0.02	0.76
RASr (%) G1	38.7	36.8	34.1	-0.073	0.33	-0.026	0.73	-0.099	0.16
G2	37.1	34.2	34.7	-0.113	0.16	0.01	0.85	-0.1	0.19
RAScd (%) G1	-21.6	-22.1	-17.9	-0.62	0.81	4.16	0.14	3.54	0.16
G2	-21.7	-14.6	-18.9	7.32	0.04	-4.54	0.16	2.78	0.41
RASct (%) G1	-17.9	-16.5	-17.8	1.8	0.48	-1.46	0.59	0.33	0.89
G2	-17.6	-19.5	-16.6	-1.76	0.48	2.86	0.22	1.10	0.66
RA peak systolic SR (1/s) G1	1.6	1.3	1.2	-0.125	0.26	-0.08	0.49	-0.205	0.06
G2	1.3	1.1	1.2	-0.19	0.27	0.12	0.49	-0.07	0.67
RA early diastolic SR (1/s) G1	-1.0	-1.2	-0.8	-0.211	0.15	0.37	0.02*	0.16	0.28
G2	-1.1	-0.8	-1.1	-0.21	0.25	-0.29	0.22	-0.12	0.47
RA late diastolic SR (1/s) G1	-1.9	-1.9	-1.8	0.025	0.91	0.071	0.77	0.09	0.69
G2	-1.9	-1.6	-1.6	0.34	0.18	0.03	0.86	0.37	0.16

Table 20. Changes in RA strain and strain-rate measures with time in G1 and G2

White rows represent the changes in measures in G1 and grey rows representing the changes in G2.
**Statistically significant ($p < 0.05$)*

3.5 Reliability of Different Strain Measures

3.5.1 Inter-observer variability

The full description of how inter-observer variability was assessed has been highlighted in section 2.1.6 Statistical analysis. Table 21 demonstrates the degree of consistency of the agreement between the two observers for LVEF and all strain measures. As clearly seen below, LA conduit strain, average LA strain, average LA reservoir and conduit strains were the only measures to show good level of agreement between the observers. The rest of measures had poor inter-observer agreement rates with the worst rate of agreement seen for RA strain; ICC -0.27 (CI -1.8-0.53). Interestingly, the level of agreement for LVEF and GLS measures were poor with ICC 0.60 (95% CI -0.11-0.86) for LVEF, ICC 0.61 (95% CI -0.24-0.73) for GLS (Autostrain) and ICC 0.60 (95% CI -0.28-0.84) for GLS using the 2D CPA method.

Variable	Intraclass Correlation*	95% Confidence Interval
LVEF	0.60	-0.11-0.86
GLS (AutoStrain)	0.61	-0.24-0.73
GLS (2D CPA)	0.60	-0.28-0.84
GRS	0.16	-0.23-0.57
RV GLS	0.24	-2.90-1.59
RV FWS	0.15	-1.86-1.58
LA 4Ch strain	0.57	-0.35-0.86
LA reservoir strain (4Ch)	0.61	-0.06-0.86
LA conduit strain (4Ch)	0.72	-0.03-0.92
LA contractile strain (4Ch)	0.42	-1.29-0.84
LA 2Ch strain	0.56	-0.92-0.91
LA reservoir strain (2Ch)	0.64	-1.24-0.93
LA conduit strain (2Ch)	0.75	-0.12-0.94
LA contractile strain (2Ch)	0.68	-0.23-0.93
Average LA strain	0.87	0.38-0.97
Average LA reservoir strain	0.80	-0.03-0.96
Average LA conduit strain	0.94	0.63-0.99
Average LA contractile strain	0.16	-5.95-0.84
RA strain	-0.27	-1.8-0.53
RA reservoir strain	0.11	-0.58-0.62
RA conduit strain	0.27	-0.37-0.72
RA contractile strain	0.29	-1.82-0.80

Table 21. Inter-observer variability

3.5.2 Intra-observer variability

Intra-observer variability assessment of different measures has been provided in the table below (Table 22). The description of how the level of agreement was measured has been explained in 2.1.6 Statistical analysis section. LVEF and GLS using 2D CPA showed an excellent intra-observer agreement level with both ICCs measured > 0.90. Despite a higher ICC in the RV GLS and RV FWS measures compared to the inter-observer variability data, the level of agreement still remained at a poor rate with ICC 0.64 (CI -0.24-0.77) for RV GLS and ICC 0.68 (CI -0.04-0.86) for RV FWS. The LA 4Ch, across all its strain measures showed a good level of consistency of agreement in its measures with ICC > 0.80 for all these variables. Interestingly and very differently to the inter-observer variability data, the RA strain measures showed a good level of agreement across all its strain variables with RA conduit strain demonstrating an excellent rate of agreement.

Variable	Intraclass Correlation*	95% Confidence Interval
LVEF	0.93	0.81-0.97
GLS (AutoStrain)	0.89	0.76-0.98
GLS (2D CPA)	0.97	0.91-0.99
GRS	0.45	-0.23-0.79
RV GLS	0.64	-0.24-0.77
RV FWS	0.68	-0.04-0.86
LA 4Ch strain	0.81	0.48-0.93
LA reservoir strain (4Ch)	0.85	0.59-0.94
LA conduit strain (4Ch)	0.95	0.84-0.98
LA contractile strain (4Ch)	0.85	0.56-0.95
LA 2Ch strain	0.70	0.44-0.89
LA reservoir strain (2Ch)	0.68	-0.23-0.72
LA conduit strain (2Ch)	0.71	-0.14-0.82
LA contractile strain (2Ch)	0.62	-0.12-0.77
Average LA strain	0.80	0.21-0.91
Average LA reservoir strain	0.78	0.11-0.83
Average LA conduit strain	0.71	0.20-0.88
Average LA contractile strain	0.68	-0.01-0.75
RA strain	0.89	0.59-0.96
RA reservoir strain	0.85	0.53-0.95
RA conduit strain	0.95	0.83-0.98
RA contractile strain	0.85	0.54-0.95

Table 22. Intra-observer variability

Chapter 4: Prospective Study Results

4.1 Study Population

From October 2018 to March 2020, a total number of 61 patients with a new diagnosis of lymphoma or breast cancer who fulfilled the eligibility criteria for the PROACT PLUS study (2.2.5.1 Inclusion criteria) were recruited after verbal and written consent was obtained. Due to the global SARS-CoV-2 pandemic, recruitment was halted in March 2020 with no further additional recruitment of patients planned given the uncertainty of the duration of the pandemic and the increased risk of infection with SARS-CoV-2 amongst this patient group. All patients were recruited at the South Tees NHS Foundation Trust. A unique study ID was allocated to each patient recruited into the study for anonymization purposes. Prior to commencing anthracycline based chemotherapy, all patients underwent an echocardiogram at baseline (V1) and 4-weeks after the final chemotherapy cycle (V2). Blood tests were taken at different time-points as highlighted in section 2.2.8.6 Blood sampling. During the study period, 6 patients passed away whilst undergoing chemotherapy treatment and 5 patients failed to attend their follow-up study investigations at V2 (Figure 17). An additional 2 patients passed away at 4 and 12 months respectively, post completion of their V2 study investigations.

For the purpose of this study, two patient groups **(G)** were defined (2.2.10 Statistical Analysis):
G1: patients with a preserved LVEF of ≥ 53% at V2
G2: patients with an absolute drop of > 10 percentage points in their LVEF to < 53% at V2

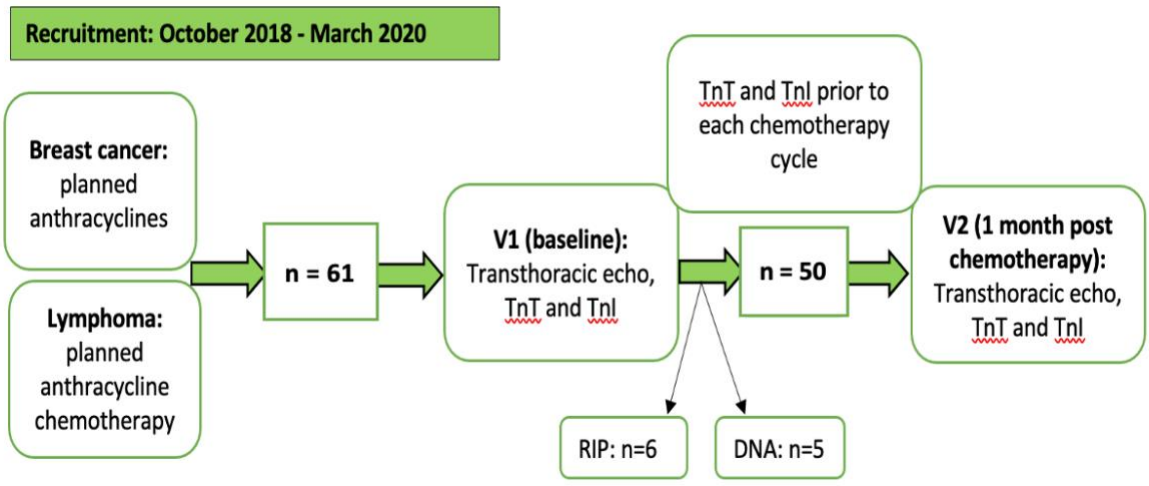


Figure 17. PROACT PLUS consort diagram

RIP: rest in peace; DNA: did not attend; TnT: troponin T; TnI: troponin I

Offline analysis of the echocardiograms was performed blindly at the Echocardiography Core laboratory. During the study period, a total number of 6 patients (10%) were noted to drop their LVEF by > 10 percentage points to < 53% from V1 to V2 with the remaining patients continuing to demonstrate a preserved LVEF > 53% at V2.

Full description of the statistical methods used for this chapter have been provided in section 2.2.10 Statistical Analysis.

4.2 Baseline Characteristics

The baseline characteristics of the patients can be visualized in Table 23. More detailed information regarding the underlying cancer diagnosis based on the histopathology results, the grade and stage of the malignancy and finally the surgical intervention undertaken has been described in *Appendix 7*.

For the purpose of this study, patients had been administered two different types of anthracyclines depending on their underlying cancer diagnosis, as highlighted in 2.2.5 Study Population. As different toxicity profile exists with the usage of each anthracycline, the total anthracycline dose for patients receiving epirubicin chemotherapy was converted into the cardiotoxicity equivalence dose of doxorubicin. As demonstrated in Table 23, patients in G2 received higher doses of anthracyclines (285.7 mg/m^2 [255.8-327 mg/m^2]) compared to patients in G1 (218.5 mg/m^2 [148.3-284.6 mg/m^2]) which was statistically significant ($p = 0.03$) however, this was mainly due to the presence of an outlier with 1 patient receiving 8 cycles of chemotherapy for their cancer treatment resulting in a total anthracycline dose of 413.8 mg/m^2 administered. All other baseline characteristics were similar between patient groups. 3 and 6 patients in G1 had a diagnosis of asthma and COPD respectively with 1 patient in G2 having a diagnosis of asthma only. However, these differences were not statistically significant. Interestingly, no patient was on an ACEi, ARB, betablocker or statin in patients in G2 however, a total of 24 patients in G1 were taking at least one of these medications. Despite these differences, this was not statistically significant.

In G1, 7 patients died during or after their chemotherapy treatment from cancer related complications with 1 patient passing away in G2. No patients passed away from cardiac related problems during the study period.

Variable	All patients (n=61)	G1 (n=55)	G2 (n=6)	p-value
Age	66 [52-74]	66 [51-74]	59 [48-72]	0.53
Female sex (%)	33 (54)	30 (55)	3(50)	1
Heart rate (bpm)	80 [70-88]	75 [70-85]	85 [69-103]	0.34
BSA (m^2)	1.88 ± 0.24	1.86 ± 0.23	2.02 ± 0.30	0.12
Systolic BP (mmHg)	136 ± 22	135 ± 22	143 ± 25	0.47
Diastolic BP (mmHg)	82 ± 11	81 ± 11	90 ± 8	0.07
Hb (g/L)	125 ± 18	126 ± 18	115 ± 17	0.14
Cr (umol/L)	73 ± 18	73 ± 18	73 ± 20	0.16
Caucasian (%)	61 (100)	55 (100)	6 (100)	1
Anthracycline dose (mg/m^2)	246.6 [149.6-285.7]	218.5 [148.3-284.6]	285.7 [255.8-327.0]	0.03*
Epirubicin chemotherapy (%)	10 (16.4)	10 (18.2)	0 (0)	0.57
Previous cancer diagnosis (%)	11 (18)	9 (16.4)	2 (33.3)	0.29
Previous anthracycline treatment (%)	1 (1.6)	1 (1.8)	0 (0)	1
IHD (%)	3 (4.9)	3 (5.5)	0 (0)	1
Previous LVSD (%)	1 (1.6)	1 (1.8)	0 (0)	1
Hypertension (%)	20 (32.8)	18 (32.7)	2 (33.3)	1
Diabetes (%) **Diet controlled (%)** **Tablet controlled (%)** **Insulin (%)** **Unknown (%)**	 2 (3.3) 4 (6.6) 1 (1.6) 1 (1.6)	 1 (1.8) 4 (7.3) 1 (1.8) 1 (1.8)	 1 (16.7) 0 (0) 0 (0) 0 (0)	0.38
Hypercholesterolaemia (%)	5 (8.2)	5 (9.1)	0 (0)	1
Smoking history (%) **Current smoker** **Ex-smoker** **Unknown**	 9 (14.8) 17 (27.9) 4 (6.6)	 7 (12.7) 16 (29.1) 4 (7.3)	 2 (33.3) 1 (16.7) 0 (0)	0.58
Asthma (%)	4 (6.6)	3 (5.5)	1 (16.7)	0.41
COPD (%)	6 (9.8)	6 (10.9)	0 (0)	1
CVA (%)	2 (3.3)	1 (1.8)	1 (16.7)	0.27
PVD (%)	0 (0)	0 (0)	0 (0)	1
ACEi (%)	5 (8.2)	5 (9.1)	0 (0)	1
ARB (%)	4 (6.6)	4 (7.3)	0 (0)	1
Betablocker (%)	8 (13.1)	8 (14.5)	0 (0)	0.66
CCB (%)	6 (9.8)	5 (9.1)	1 (16.7)	0.59
Statin (%)	7 (11.5)	7 (12.7)	0 (0)	1
Died (%)	8 (13.1)	7 (12.7)	1 (16.7)	1

Table 23. Baseline Characteristics

Inter-group differences for normally distributed continuous variables were measured using the student t test. The Mann-Whitney U test was used if data was not normally distributed.
Fisher's exact test was used for assessment of inter-group differences in the categorical data.
**Statistically significant ($p < 0.05$)*

4.2.1 Baseline Conventional Echocardiographic Measures

The baseline conventional echocardiographic data can be found in Table 24. There was a statistically significant difference in the mean MV DecT between G1 and G2 with $t(59) = 2.55$, $p = 0.01$. This was also the case with RV ESA, with patients in G1 having a higher mean RV ESA (9.6 ± 3.1) compared to those in G2 (8.3 ± 0.89) with $t(49) = 0.96$, $p = 0.03$. However, despite this difference, RV ESA was within the normal range in both groups and there was no statistically significant intergroup difference in RV FAC measure. The median RV free wall S' was also higher in patients in G2 compared to those in G1 which was statistically significant, $U = 196$, $z = 2.35$, $p = 0.01$ but again, this measure was within the normal range in both groups. Interestingly, TAPSE was similar in both groups. There was no statistically significant difference between the groups in the other conventional echocardiographic data.

	N in all patients (n in each group)	All patients	G1	G2	p value
Heart rhythm **Sinus (%)** **Atrial fibrillation** **Other**	61 (G1=55, G2=6)	59 (96.7) 1 (1.6) 1 (1.6)	53 (96) 1 (1.8) 1 (1.8)	6 (100) 0 (0) 0 (0)	1.00
LVIDd (cm)	60 (G1=54, G2=6)	4.5 ± 0.7	4.5 ± 0.7	4.6 ± 0.4	0.69
LVIDs (cm)	60 (G1=54, G2=6)	2.7 ± 0.7	2.7 ± 0.7	2.9 ± 0.4	0.55
Fractional shortening (%)	60 (G1=54, G2=6)	39 ± 11.3	40 ± 11.7	37 ± 6.1	0.61
LV mass index (mg/m^2)	60 (G1=54, G2=6)	78.9 ± 18.7	79.1 ± 19.3	76.3 ± 13.3	0.72
LV RWT (%)	59 (G1=54, G2=5)	0.42 ± 0.10	0.42 ± 0.1	0.41 ± 0.1	0.79
LA diameter (cm)	59 (G1=54, G2=5)	3.3 ± 0.6	3.4 ± 0.6	3.0 ± 0.7	0.16
LA volume biplane (ml/m^2)	42 (G1=39, G2=3)	21.1 [17.3-28.1]	21.5 [18.3-26.9]	16.8 [15.0-23.3]	0.32
LVEDV indexed (ml/m^2)	56 (G1=50, G2=6)	38.2 [34.0-46.5]	37.3 [33.3-47.3]	42.1 [38.1-48.9]	0.18
LVESV indexed (ml/m^2)	56 (G1=50, G2=6)	14.4 [11.6-18.9]	14.0 [11.2-19.0]	18.2 [14.4-19.8]	0.17
LVEF (%)	61 (G1=55, G2=6)	60 [57-66]	60 [56-66]	60 [59-62]	0.64
MV E (m/s)	61 (G1=55, G2=6)	0.67 [0.59-0.77]	0.67 [0.59-0.77]	0.67 [0.52-0.88]	0.89
MV E/A	59 (G1=53, G2=6)	0.78 [0.69-0.99]	0.77 [0.68-1.15]	0.82 [0.73-0.91]	0.93
MV DecT (cm)	61 (G1=56, G2=6)	186 ± 57	192 ± 56	132 ±35	0.01*
Lateral E/E'	60 (G1=54, G2=6)	7.3 [5.8-9.4]	7.3 [6.3-9.6]	6.4 [5.0-8.5]	0.23
Medial E/E'	58 (G1=52, G2=6)	9.6 [7.9-11.6]	9.9 [8.2-11.8]	7.5 [5.7-9.7]	0.03*
Mean E/E'	58 (G1=52, G2=6)	8.9 [7.2-10.1]	8.9 [7.6-10.1]	6.8 [5.4-9.1]	0.05

TR maxPG (mmHg)	57 (G1=51, G2=6)	17 [0-24]	16 [0-24]	23 [14-25]	0.15
IVRT (cm)	50 (G1=45, G2=5)	91 ± 22	90 ± 22	97 ± 23	0.53
Tei Index (LV)	50 (G1=45, G2=5)	0.53 ± 0.13	0.52 ± 0.13	0.61 ± 0.10	0.13
RA volume indexed (ml/m^2)	46 (G1=42, G2=4)	16.6 [13.8-20.4]	16.1 [12.3-20.4]	12.3 [9.8-17.1]	0.13
RV basal-wall diameter (cm)	51 (G1=47, G2=4)	3.8 ± 0.6	3.8 ± 0.6	3.5 ± 0.5	0.34
RV mid-wall diameter (cm)*	51 (G1=47, G2=4)	2.9 ± 0.59	3.0 ± 0.6	2.8 ± 0.4	0.50
RV free wall S' (m/s)	47 (G1=41, G2=6)	0.13 [0.11-0.15]	0.13 [0.11-0.15]	0.16 [0.13-0.19]	0.01*
RV EDA (cm^2)	51 (G1=46, G2=5)	16.0 [13.8-20.4]	16.3 [13.7-20.8]	15.1 [14.4-16.2]	0.54
RV ESA (cm^2)	51 (G1=46, G2=5)	9.5 ± 3.0	9.6 ± 3.1	8.3 ± 0.89	0.03*
RV FAC (%)	51 (G1=46, G2=5)	45.2 ± 7.3	45.1 ± 7.4	45.7 ± 6.2	0.87
TAPSE (cm)*	56 (G1=50, G2=6)	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.5	0.94
RV IVRT (cm)	44 (G1=39, G2=5)	76 [57-92]	77 [56-91]	68 [47-107]	0.88
Tei index (RV)	44 (G1=39, G2=5)	0.56 ± 0.17	0.56 ± 0.17	0.59 ± 0.16	0.65

Table 24. Baseline conventional echocardiographic measures

Inter-group differences for normally distributed continuous variables were measured using the student t test. The Mann-Whitney U test was used if data was not normally distributed.
**Statistically significant ($p < 0.05$)*

4.2.2 Baseline Strain Measures

4.2.2.1 Left ventricular strain and strain-rate

LV strain and strain-rate measures at baseline, for all patients and both patient groups, has been provided in Table 25. Apart from LV GRS, there was no statistically significant difference in any other of the strain and strain-rate measures of the left ventricle between G1 and G2. However, the mean LV GRS was significantly higher in G1 (37.4 ± 7.2 %) compared to G2 (30.2 ± 7.1); $t(51) = 2.29$, $p = 0.02$. Due to limited number of cases with the presence of all three parasternal short axis views, LV GRS was measured using the three apical left ventricular views instead as explained in detail in 2.5.1.3.2 2D strain analysis. Additionally, as LV GCS, torsion and twist along with the LV circumferential strain-rates are all reliant on the presence of all 3 short axis apical views, the results provided have to be interpreted with caution due to the insufficient number of cases with all three images. Interestingly, LV GLS, GRS and GCS

were also higher in patients in G1 compared to those in G2 but this was only statistically significant for the GRS value and not GLS or GCS.

4.2.2.2 Right ventricular strain and strain-rate

As per Table 26, there were some statistically significant baseline differences in the RV strain and strain-rate measures between G1 and G2. Patients in G1 had a higher baseline RV GLS and myoGLS compared to those in G2. These changes correlated well with the conventional RV S' measure demonstrated earlier in this chapter. Despite the statistically significant difference between the groups, RV GLS was still within the considered normal range in both of these groups. Although the number of patients with underlying respiratory disease was higher in G1, this did not appear to have a significant effect on the RV GLS and myoGLS measures in these patients. The mean RV FWS was also higher in patients in G1 compared to those in G2 with a mean difference of -3.67 (95% CI -8.22-0.87), t (49) = -1.62 but this did not reach statistical significance (p = 0.11).

Variable	N	All patients	G1	G2	p value
GLS (%)	53 (G1=47, G2=6)	-20.6 ± 2.6	-20.7 ± 2.6	-19.3 ± 2.5	0.18
MyoGLS (%)	53 (G1=47, G2=6)	-17.8 ± 2.5	-17.9 ± 2.5	-16.8 ± 2.4	0.33
LV peak systolic longitudinal SR(1/s)	53 (G1=47, G2=6)	-1.07 ± 0.16	-1.08 ± 0.17	-1.0 ± 0.13	0.24
LV end-systolic longitudinal SR (1/s)	53 (G1=47, G2=6)	-0.25 ± 0.34	-0.25 ± 0.32	-0.21 ± 0.52	0.80
LV early diastolic longitudinal SR (1/s)	47 (G1=42, G2=5)	0.99 ± 0.30	0.98 ± 0.30	1.1 ± 0.27	0.41
LV late diastolic longitudinal SR (1/s)	42 (G1=37, G2=5)	0.80 ± 0.20	0.79 ± 0.19	0.86 ± 0.32	0.51
GRS (%)	53 (G1=47, G2=6)	36.6 ± 7.57	37.4 ± 7.2	30.2 ± 7.1	0.02*
LV peak systolic radial SR (1/s)	53 (G1=47, G2=6)	1.45 ± 0.26	1.46 ± 0.27	1.39 ± 0.24	0.56
LV end-systolic radial SR (1/s)	53 (G1=47, G2=6)	0.35 ± 0.45	0.36 ± 0.42	0.29 ± 0.70	0.71
LV early diastolic radial SR (1/s)	51 (G1=45, G2=6)	-1.34 ± 0.43	-1.37 ± 0.43	-1.19 ± 0.37	0.36
LV late diastolic radial SR (1/s)	43 (G1=38, G2=5)	-1.03 ± 0.32	-0.82 ± 0.30	-0.87 ± 0.45	0.71
GCS (%)	20 (G1=18, G2=2)	-31.2 ± 4.47	-31.4 ± 4.68	-29.4 ± 0.85	0.55
MyoGCS (%)	20 (G1=18, G2=2)	-22.3 ± 3.43	-22.5 ± 3.53	-20.1 ± 1.20	0.36

LV peak systolic circumferential SR (1/s)	19 (G1=17, G2=2)	-1.73 ± 0.36	-1.74 ± 0.37	-1.58 ± 0.45	0.56
LV end-systolic circumferential SR (1/s)	20 (G1=18, G2=2)	-0.15 ± 0.69	-0.15 ± 0.73	-0.20 ± 0.09	0.92
LV early diastolic circumferential SR (1/s)	16 (G1=14, G2=2)	1.61 ± 0.48	1.62 ± 0.45	1.49 ± 0.90	0.72
LV late diastolic circumferential SR (1/s)	12 (G1=12, G2=2)	0.86 ± 0.41	0.93 ± 0.34	0.44 ± 0.72	0.12
LV twist (degrees)	20 (G1=18, G2=2)	15.1 ± 8.3	15.3 ± 8.6	13.5 ± 7.1	0.77
LV torsion (degrees/cm)	20 (G1=18, G2=2)	2.06 ± 1.13	2.08 ± 1.16	1.89 ± 1.12	0.82

Table 25. Baseline LV strain and strain-rate measures

Inter-group differences for normally distributed continuous variables were measured using the student t test.
**Statistically significant (p < 0.05*

Variable	N	All patients	G1	G2	p value
RV GLS (%)	51 (G1=47, G2=4)	-25.1 ± 3.8	-25.5 ± 3.5	-20.1 ± 3.5	0.005*
RV myoGLS (%)	51 (G1=47, G2=4)	-22.9 ± 3.7	-23.5 ± 3.3	-17.4 ± 3.7	0.001*
RV peak systolic longitudinal SR (1/s)	50 (G1=47, G2=3)	-1.29 ± 0.20	-1.30 ± 0.19	-1.10 ± 0.26	0.10
RV end-systolic longitudinal SR (1/s)	51 (G1=47, G2=4)	-0.11 ± 0.33	-0.07 ± 0.31	-0.52 ± 0.42	0.01*
RV early diastolic SR (1/s)	47 (G1=44, G2=3)	1.08 ± 0.35	1.11 ± 0.33	0.66 ± 0.30	0.03*
RV late diastolic SR (1/s)	44 (G1=41, G2=3)	0.92 [0.62-1.2]	0.95 [0.60-1.2]	0.90 [0.80-1.06]	0.86
RV FWS (%)	51 (G1=47, G2=4)	-28.9 ± 4.4	-29.2 ± 4.3	-25.5 ± 3.6	0.11
RVFW peak systolic longitudinal SR (1/s)	50 (G1=47, G2=3)	-1.50 [-1.70- (-1.40)]	-1.50 [-1.70-(-1.40)]	-1.50 [-1.80-(-1.35)]	0.96
RVFW end-systolic longitudinal SR (1/s)	51 (G1=47, G2=4)	-0.12 ± 0.40	-0.08 ± 0.37	-0.60 ± 0.47	0.01*
RVFW early diastolic longitudinal SR (1/s)	48 (G1=45, G2=3)	1.23 ± 0.39	1.25 ± 0.39	0.93 ± 0.42	0.17
RVFW late diastolic longitudinal SR (1/s)	48 (G1=45, G2=3)	1.09 ± 0.47	1.08 ± 0.48	1.21 ± 0.11	0.67

Table 26. Baseline RV strain and strain-rate measures

Inter-group differences for normally distributed continuous variables were measured using the student t test. The Mann-Whitney U test was used if data was not normally distributed.
**Statistically significant (p < 0.05)*

4.2.2.3 Left atrial strain and strain-rate

Table 27 demonstrates all the baseline mean left atrial strain and strain-rate measures in all patients and also both patient groups. Due to insufficient sample size in G2 for the measurement of baseline biplane left atrial strain and strain-rate measures, the decision was made to exclude these measures from further inter-group analysis in the study. However, the baseline measures for G1 have been provided in the current table.

As it is clearly highlighted in Table 27, no statistically significant difference was seen in any of the LA strain and strain-rate measures between the groups at baseline.

Variable	N	All patients	G1	G2	p value
LA 4Ch strain (%)	42 (G1=39, G2=3)	27.0 ± 9.1	27.1 ± 9.3	25.6 ± 6.1	0.78
LASr 4Ch (%)	42 (G1=39, G2=3)	34.8 ± 10.3	35.1 ± 10.6	30.2 ± 3.4	0.43
LAScd 4Ch (%)	42 (G1=39, G2=3)	-17.7 ± 8.0	-18.0 ± 8.1	-13.6 ± 5.1	0.35
LASct 4Ch (%)	42 (G1=39, G2=3)	-17.0 ± 6.5	-17.1 ± 6.6	-16.6 ± 6.5	0.90
LA 4Ch peak systolic SR (1/s)	42 (G1=39, G2=3)	1.19 ± 0.42	1.21 ± 0.42	0.93 ± 0.25	0.26
LA 4Ch early diastolic SR (1/s)	40 (G1=37, G2=3)	-1.10 [-1.77-(-0.8)]	-1.1 [-1.80-(-0.80)]	-0.80 [-0.85-(-0.7)]	0.15
LA 4Ch late diastolic SR (1/s)	41 (G1=38, G2=3)	-1.71 ± 0.64	-1.71 ± 0.63	-1.67 ± 0.74	0.90
LA 2Ch strain (%)	37 (G1=34, G2=3)	26.7 ± 8.2	26.7 ± 8.5	25.8 ± 2.9	0.85
LASr 2Ch (%)	37 (G1=34, G2=3)	36.2 ± 11.4	36.1 ± 11.7	37.1 ± 8.7	0.88
LAScd 2Ch (%)	37 (G1=34, G2=3)	-15.5 ± 6.8	-15.4 ± 6.9	-16.6 ± 5.0	0.77
LASct 2Ch (%)	37 (G1=34, G2=3)	-20.7 ± 7.7	-20.7 ± 7.9	-20.5 ± 4.6	0.96
LA 2Ch peak systolic SR (1/s)	37 (G1=34, G2=3)	1.13 ± 0.40	1.14 ± 0.42	1.00 ± 0.17	0.55
LA 2Ch early diastolic SR (1/s)*	33 (G1=31, G2=2)	-0.80 [-1.3-(-0.65)]	-0.80 [-1.30-(-0.70)]	-0.62 [-0.9-(-0.35)]	0.37
LA 2Ch late diastolic SR (1/s)	35 (G1=33, G2=2)	-2.00 ± 0.78	-2.02 ± 0.79	-1.65 ± 0.49	0.51
LA biplane strain (%)	32 (G1=31, G2=1)	26.8 ± 7.4	26.8 ± 7.5	NA	NA
LASr biplane (%)	32 (G1=31, G2=1)	35.1 ± 9.8	35.2 ± 9.8	NA	NA
LAcd biplane (%)	32 (G1=31, G2=1)	-16.3 ± 6.1	-16.3 ± 6.2	NA	NA
LAct biplane (%)	32 (G1=31, G2=1)	-18.7 ± 5.9	-18.9 ± 6.0	NA	NA
LA biplane peak systolic SR (1/s)	32 (G1=31, G2=1)	1.18 ± 0.35	1.19 ± 0.35	NA	NA
LA biplane early diastolic SR (1/s)	29 (G1=28, G2=1)	-0.95 [-1.30-(-0.82)]	-0.98 [-1.30-(-0.82)]	NA	NA

LA biplane late diastolic SR (1/s)	31 (G1=30, G2=1)	-1.85 ± 0.64	-1.87 ± 0.64	NA	NA

Table 27. Baseline left atrial strain and strain-rate measures

Inter-group differences for normally distributed continuous variables were measured using the student t test. The Mann-Whitney U test was used if data was not normally distributed.
NA - Not Analysed due to insufficient sample size

4.2.2.4 Right atrial strain and strain-rate

The baseline RA strain and strain-rate measures have been provided in the table below. Due to insufficient sample size in G2, the baseline mean RA strain-rate measures were not possible to analyse in G2. Given this issue, the RA strain-rate measures were omitted from the subsequent analyses in the study.

As highlighted below, the RA contractile strain was higher in G2 than in G1 with a mean difference of 12.2 (95% CI 3.3-21.0), t(35) = 2.80; p = 0.008. This higher value of the RA contractile strain in G2 correlated well with a lower RV GLS in this patient group as shown earlier in this chapter. This could potentially be explained by the close mechanical relationship between the RA and RV, and the importance of RA in providing a compensatory mechanism for a lower RV GLS. No other statistically significant inter-group difference was seen in the RA strain measures at baseline.

Variable	N	All patients	G1	G2	p value
RA strain (%)	37 (G1=35, G2=2)	34.3 ± 9.0	34.1 ± 9.2	37.3 ± 5.2	0.63
RASr (%)	37 (G1=35, G2=2)	40.3 ± 9.1	39.9 ± 9.2	46.9 ± 3.2	0.29
RAScd (%)	37 (G1=35, G2=2)	-23.3 ± 7.3	-23.5 ± 7.2	-18.3 ±7.3	0.33
RASct (%)	37 (G1=35, G2=2)	-17.0 ± 6.5	-16.3 ± 6.0	-28.5 ± 4.0	0.008*
RA peak systolic SR (1/s)	34 (G1=33, G2=1)	1.6 [1.00-1.60]	1.5 [1.05-1.65]	NA	NA
RA early diastolic SR (1/s)	33 (G1=32, G2=1)	-1.17 ± 0.39	-1.18 ± 0.40	NA	NA
RA late diastolic SR (1/s)	33 (G1=32, G2=1)	-1.62 ± 0.57	-1.60 ± 0.58	NA	NA

Table 28. Baseline right atrial strain and strain-rate measures

Inter-group differences for normally distributed continuous variables were measured using the student t test. The Mann-Whitney U test was used if data was not normally distributed.
NA - Not Analysed due to insufficient sample size
**Statistically significant (p < 0.05)*

4.3 Changes in Echocardiographic Measures in All Patients

4.3.1 Conventional echocardiographic measures

Changes in the conventional echocardiographic measures between V1 and V2 have been provided in section 2 of *Appendix 7.* Full description of the statistical analysis used for the purpose of this section has been provided in 2.2.10 Statistical Analysis.

After completion of anthracycline chemotherapy, it is clearly seen that the LV end systolic diameter and volumes increased at V2 in all patients. This increase corresponded with a decline in the LVEF. Although these changes were statistically significant, they were all within the dedicated normal ranges for their measures. Nevertheless, these changes highlight the effect of anthracyclines on different LV measures in particularly the ones related to LV systolic function. Interestingly, no significant change was observed in the RV conventional measures with time.

4.3.2 Left ventricular strain and strain-rate measures

Section 3.1 of *Appendix 7* contains the data for changes in the LV strain and strain-rate measures with time in all patients. Both LV GLS and GCS showed a significant decline in their measures at V2. However, the decline in LV GLS was < 10% from V1 and V2 and despite this statistically significant change, this did not meet the definition for cardiotoxicity. LV myoGLS and myoGCS additionally declined with time correlating with a decline in LV GLS and GCS, respectively. GRS did not show any major change with completion of chemotherapy treatment.

On reviewing the strain-rate measures, early diastolic strain-rate was the only measure that showed a deterioration with greater effect in all three longitudinal, radial and circumferential domains. No other significant change was noted in the other strain-rate measures.

4.3.3 Right ventricular strain and strain-rate measures

RV strain and strain-rate measures can be found in the first table in section 3.2 of *Appendix 7.* Similar to the LV strain measures, RV GLS and RV FWS showed a major decline at V2 when compared to V1, with a mean change of 2.73; $p < 0.001$ and a mean change of 2.41; $p = 0.01$,

respectively. However, the values at V2 were once again within the normal range. Figure 18 demonstrates the change in RV GLS between visits. Interestingly, RV and RVFW early diastolic strain-rates also showed a decline similar to the LV early diastolic strain-rate as mentioned above which correlated with a drop in the RV GLS and RV FWS. No other change in the RV strain-rate measures were observed.

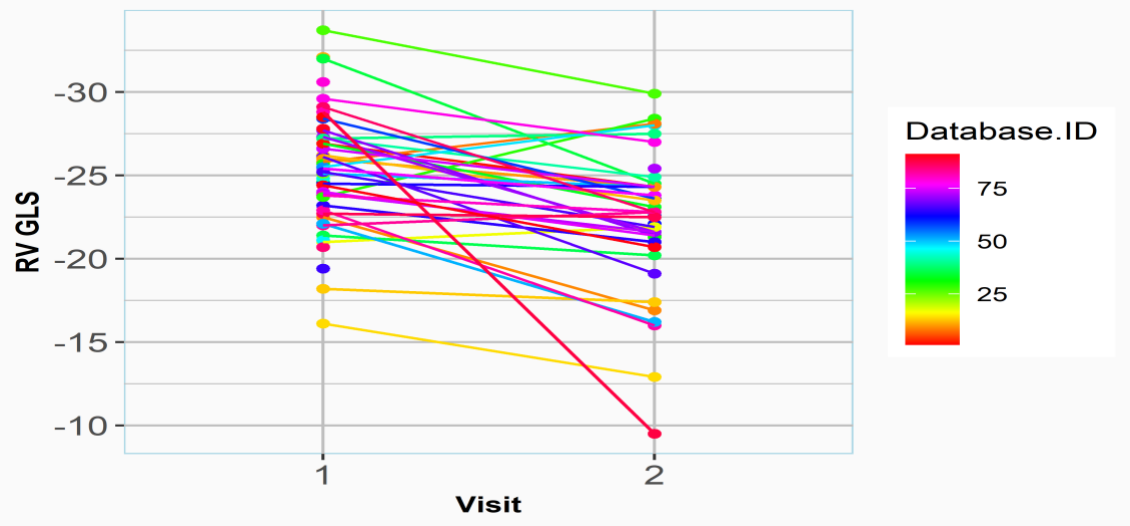


Figure 18. Change in RV GLS between visits in all patients

4.3.4 Left atrial strain and strain-rate measures

The full results for LA strain and strain-rate measures have been provided in section 3.3 of *Appendix 7*. The mean LA 4Ch conduit strain showed a worsening in its strain value at V2 with a mean change of 2.90; p=0.04 (Figure 19). Furthermore, LA 4Ch and biplane early diastolic strain-rates showed a decline after the completion of chemotherapy treatment, mean change of 0.38; p = 0.001 and mean change of 0.18; p = 0.04 respectively.

Apart from these measures, no other significant changes were visualised in the other LA strain and strain-rate parameters.

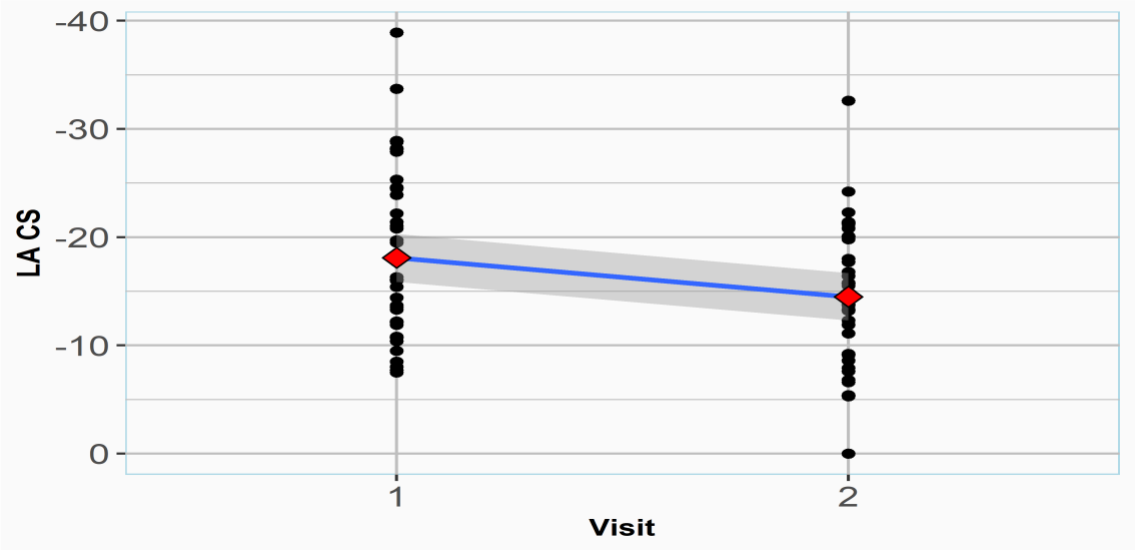


Figure 19. LA conduit strain in all patients

4.3.5 Right atrial strain and strain-rate measures

In section 3.4 of *Appendix 7*, the full results for all the RA strain and strain-rate measures are available. The RA GLS showed a decline at V2 in line with a drop in the RV GLS, with a mean change of -4.25 between the visits; p = 0.02 (Figure 20). Also, the RA early diastolic strain-rate showed a deterioration with time between V1 and V2, with a mean change of 0.18; p = 0.02. No other significant change in the RA strain and strain-rate parameters were observed.

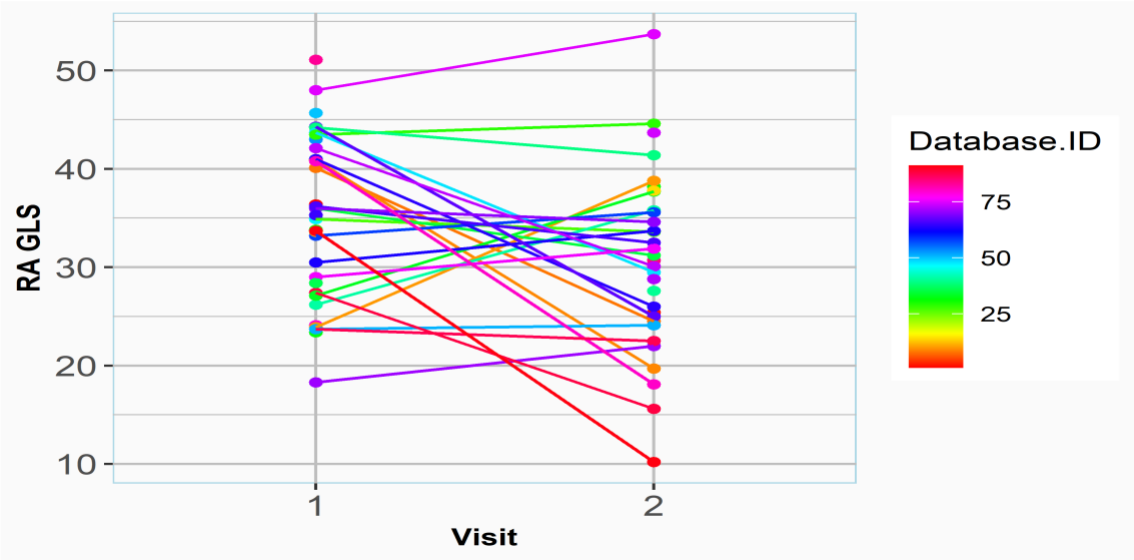


Figure 20. Change in RA GLS in all patients

4.4 Changes in Echocardiography Measures Between Groups

4.4.1 Conventional echocardiography measures

The changes in the conventional echocardiography parameters in both G1 and G2 at the two different time-points have been provided in the table below (Table 29). At V2, the mean LVIDs showed a statistically significant increase in its dimension in patients in G1 however, this was still within the normal range for LVIDs. Interestingly, this change was not seen in patients in G2 despite a greater mean change between the visits and a higher mean LVIDs at V2. This can partially be explained by the smaller number of patients in G2 compared to G1. Additionally, LVEF showed a statistically significant deterioration in both patient groups although, the mean change in G1 was 2 percentage points, with LVEF value at V2 still within the normal range. However, the mean change in LVEF in patients in G2 was greater at 13 percentage points with a LVEF below the normal range at V2. There was no real change in the LV diastolic parameters between visits in both groups.

Furthermore, in G2, the mean LA volumes increased at V2 in patients, however, this did not reach statistical significance

On assessment of the right sided chambers, there was an increase in the mean RV EDA at V2 in patients in G2, with a mean change of 2.64; $p = 0.01$. No change was seen in this measure, in patients in G1. Despite the increase in RV EDA in G2, there was no statistically significant decline in RV FAC. RV S' and TAPSE remained largely unchanged between visits in both patient groups. Additionally, the mean RA volumes did not show any major change in their mean values between V1 and V2.

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
BSA (m^2)	1.87	1.88	-0.01	0.18	2.02	2.00	-0.02	0.36
LVIDd (cm)	4.5	4.5	-0.006	0.90	4.6	4.7	0.15	0.62
LVIDs (cm)	2.7	2.9	0.20	0.04*	2.9	3.5	0.63	0.12
Fractional shortening (%)	39.7	35.2	-4.3	0.03*	37.2	29.4	-7.8	0.09
LV mass index (mg/m^2)	79.1	82.8	4.06	0.07	76.3	87.3	11.02	0.16
LV RWT (%)	0.42	0.49	0.06	0.06	0.42	0.51	0.53	0.09
LA diameter (cm)	3.4	3.6	0.2	0.78	3.0	3.5	0.05	0.73
LA volume biplane (ml/m^2)	24.4	21.3	-1.07	0.27	19.9	25.6	5.00	0.19
LVEDV indexed (ml/m^2)	40.4	41.3	0.04	0.96	44.2	52.9	8.70	0.06
LVESV indexed (ml/m^2)	15.4	16.9	1.33	0.05	17.7	28.2	10.6	0.002*
LVEF (%)	62	60	-2.07	0.01*	60	47	-13	<0.01*
MV E (m/s)	0.70	0.61	-0.09	0.003*	0.69	0.55	-0.14	0.18
MV E/A	0.93	0.82	-0.11	0.01*	0.80	0.66	-0.13	0.15
MV DecT (cm)	192	192	0.10	0.99	132	191	59	0.07
Lateral E/E’	7.9	7.4	-0.42	0.26	6.7	7.3	0.70	0.49
Medial E/E’	9.9	9.6	-0.38	0.38	7.6	9.3	1.7	0.15
Mean E/E’	8.9	8.5	-0.46	0.20	7.1	7.9	0.87	0.44
TR maxPG (mmHg)	14.6	13.2	0.04	0.98	19.7	17.8	-1.83	0.37
IVRT (cm)	90	83	-5.07	0.22	97	86	-8.38	0.56
Tei Index (LV)	0.52	0.53	0.01	0.72	0.61	0.63	0.029	0.47
RA volume indexed (ml/m^2)	18.4	16.2	-1.81	0.18	13.1	14.3	0.46	0.66
RV basal-wall diameter (cm)	3.8	3.5	0.31	0.23	3.5	3.4	0.12	0.71
RV mid-wall diameter (cm)	3.0	3.2	0.20	0.18	2.8	3.0	0.18	0.75
RV free wall S’ (m/s)	0.13	0.13	0.002	0.67	0.16	0.13	-0.02	0.20
RV EDA (cm^2)	17.6	17.0	-0.74	0.37	15.2	17.9	2.64	0.01*
RV ESA (cm^2)	9.6	9.7	-0.04	0.93	8.3	10.7	2.4	0.07
RV FAC (%)	45.1	43.0	-2.06	0.23	45.7	40.7	-5.02	0.37
TAPSE (cm)*	2.2	2.1	-0.01	0.80	2.2	2.3	0.13	0.61
RV IVRT (cm)	80	79	-1.12	0.84	75	59	-8.59	0.38
Tei index (RV)	0.56	0.59	0.03	0.31	0.59	0.58	-0.02	0.62

Table 29. The changes in conventional echocardiography measures at two different time-points in G1 and G2

**Statistically significant ($p < 0.05$)*

4.4.2 Left ventricular strain and strain-rate measures

Table 30 highlights the changes in all LV strain and strain-rate parameters in detail between V1 and V2, for both patient groups. A significant deterioration in the LV GLS and myoGLS was seen at V2 from V1, in both G1 and G2. Although this reduction was statistically

significant in both these groups, closer inspection of the results revealed a greater deterioration in these measures in G2 in comparison with G1 (mean change of 4.00 percentage points in GLS in G2 compared to a mean change of 0.9 percentage points in G1). The mean LV GCS and myoGCS showed a decline at V2 in both groups with a larger degree of drop in the parameters observed in G2, however this did not reach statistical significance in either group. LV GRS did not show any major change between the two time-points. Surprisingly, both the mean LV twist and torsion increased at V2 in patients in G2 but no change was noted in G1.

Moreover, the mean LV peak systolic longitudinal strain-rate declined in G2 only ($p = 0.006$). LV early diastolic longitudinal strain-rates deteriorated in G1 and G2, but once again the degree of this reduction was greater in patients in G2. Apart from a reduction in the LV early diastolic radial strain-rate in G1, no other significant change was detected in the other strain and strain-rate parameters.

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
GLS (%)	-20.7	-19.9	0.90	0.01*	-19.3	-15.3	4.00	0.004*
MyoGLS (%)	-17.9	-16.9	0.96	0.01*	-16.8	-12.8	3.98	0.001*
LV peak systolic longitudinal SR (1/s)	-1.08	-1.06	0.01	0.62	-1.00	-0.78	0.22	0.006*
LV end-systolic longitudinal SR (1/s)	-0.25	-0.25	0	0.99	-0.22	-0.40	-0.18	0.35
LV early diastolic longitudinal SR (1/s)	0.98	0.85	-0.11	0.01*	1.10	0.67	-0.42	0.02*
LV late diastolic longitudinal SR (1/s)	0.79	0.82	0.03	0.49	0.86	0.72	-0.21	0.10
GRS (%)	37.4	35.7	-1.78	0.18	30.2	32.5	2.33	0.61
LV peak systolic radial SR (1/s)	1.46	1.43	-0.02	0.56	1.39	1.27	-0.13	0.22
LV end-systolic radial SR (1/s)	0.36	0.35	-0.005	0.95	0.29	0.56	0.27	0.31
LV early diastolic radial SR (1/s)	-1.37	-1.19	0.15	0.009*	-1.19	-1.07	0.12	0.41
LV late diastolic radial SR (1/s)	-0.82	-0.89	-0.06	0.26	-0.87	-0.80	0.18	0.09
GCS (%)	-31.4	-28.8	2.36	0.07	-29.4	-22.6	5.3	0.72
MyoGCS (%)	-22.5	-19.9	2.5	0.05	-20.1	-16.1	4	0.28
LV peak systolic circumferential SR (1/s)	-1.74	-1.66	-0.06	0.55	-1.58	-1.15	0.43	0.37
LV end-systolic circumferential SR (1/s)	-0.15	-0.20	-0.06	0.60	-0.20	-0.11	0.17	< 0.01*
LV early diastolic circumferential SR (1/s)	1.63	1.33	-0.29	0.04*	1.49	1.10	-0.39	0.65
LV late diastolic circumferential SR (1/s)	0.93	0.96	0.05	0.66	0.44	0.73	0.29	0.64
LV twist (degrees)	15.3	13.5	-2.52	0.33	13.5	17	1.3	<0.01*
LV torsion (degrees/cm)	2.08	1.89	-0.33	0.35	1.88	2.34	0.23	<0.01*

Table 30. Change in LV strain and strain-rate between V1 and V2 in both groups (G)

**Statistically significant ($p < 0.05$)*

4.4.3 Right ventricular strain and strain-rate measures

On assessment of the RV, the RV GLS and myoGLS worsened at V2 in patients in G1 only. Although these measures also showed a trend towards declining in patients in G2, this was not statistically significant. Interestingly, RV FWS did not alter in G1 (mean change 1.17, p = 0.46) despite a drop in the RV GLS and myoGLS, but this measure did deteriorate significantly in G2 with a mean RV FWS of -25.5% at V1 to a mean of -22.7% at V2; mean change of 4.9, p = 0.03 (Figure 21). However, RVFW early diastolic longitudinal strain-rate did deteriorate in patients in G1 only. The full results of the other RV strain and strain-rate measures can be found in Table 31.

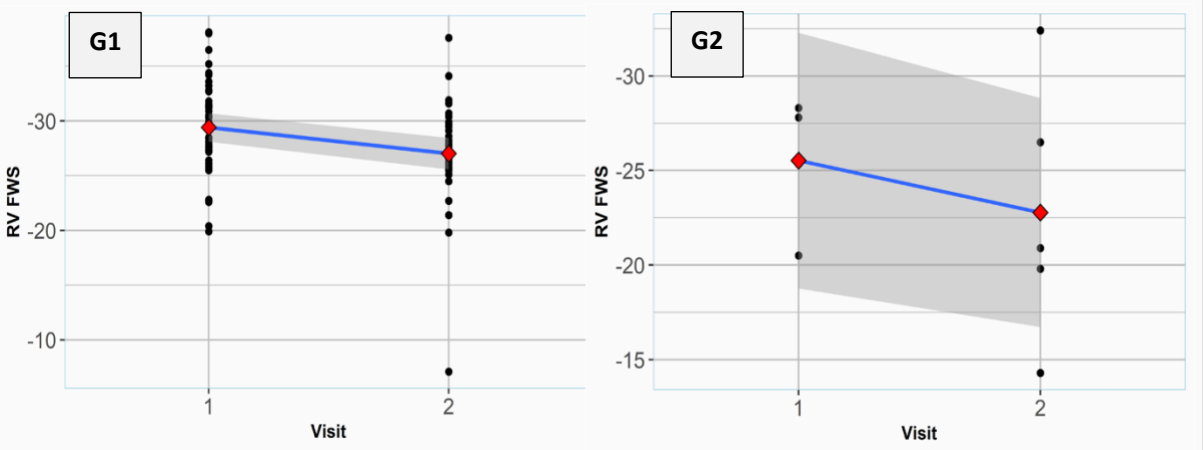


Figure 21. RV FWS in G1 and G2 between visits

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
RV GLS (%)	-25.8	-22.9	2.89	<0.01*	-20.1	-19.0	3.03	0.11
RV myoGLS (%)	-22.9	-21.1	2.36	0.006*	-17.4	-18.8	-1.40	0.64
RV peak systolic longitudinal SR (1/s)	-1.30	-1.30	0.01	0.79	-1.1	-1.04	0.06	0.72
RV end-systolic longitudinal SR (1/s)	-0.06	-0.03	0.01	0.81	-0.52	-0.24	0.29	0.11
RV early diastolic longitudinal SR (1/s)	1.11	0.93	-0.18	0.02*	0.67	0.71	-0.10	0.47
RV late diastolic longitudinal SR (1/s)	0.96	0.94	-0.03	0.76	0.94	1.15	0.35	0.43
RV FWS (%)	-28.2	-27.0	1.17	0.46	-25.5	-22.7	4.9	0.03*
RVFW peak systolic longitudinal SR (1/s)	-1.56	-1.61	-0.05	0.54	-1.60	-1.34	0.26	0.46
RVFW end-systolic longitudinal SR (1/s)	-0.06	-0.05	0.005	0.95	-0.60	-0.32	0.28	0.16
RVFW early diastolic longitudinal SR (1/s)	1.25	1.08	-0.17	0.03*	0.93	0.98	-0.09	0.23
RVFW late diastolic longitudinal SR (1/s)	1.06	1.14	0.07	0.51	1.21	1.29	0.20	0.70

Table 31. Changes in RV strain and strain-rate measures between visits in G1 and G2

**Statistically significant ($p < 0.05$)*

4.4.4 Left atrial strain and strain-rate measures

The full results for the changes in the LA strain and strain-rate parameters in both patient groups can be found in Table 32. The LA 4Ch conduit strain showed a deterioration in G1 with a mean LAScd 4Ch of -18% at V1 to a mean of -14.8% at V2; mean change of 3.22, p = 0.04. However, this did not change in G2. No other LA strain or strain-rate measure demonstrated a significant change, post completion of chemotherapy.

4.4.5 Right atrial strain and strain-rate measures

Similar to the LA strain measures, the RA conduit strain declined in patients in G1 with a mean RAScd of -24.1% at V1 to a mean RAScd of -20.4% at V2; mean change 3.79, p = 0.03. These results were not replicated in patients in G2 with no major change in the RAScd observed in this patient group. However, in patients in G2 a reduction in the RA reservoir and contractile strains were seen after the completion of their chemotherapy treatment with the mean RASr of 46.9% at V1 to a mean of 36.6% at V2; mean change -10.3, p = 0.04 and the mean RASct -

28.5% at V1 to a mean of -14.1%; mean change 14.4, p = 0.01. The results for the other RA strain parameters can be found in Table 33.

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
LA 4Ch strain (%)	27.7	25.6	-1.93	0.14	25.6	29.3	3.79	0.48
LASr 4Ch (%)	35.1	32.9	-2.47	0.12	30.2	35.8	4.5	0.38
LAScd 4Ch (%)	-18.0	-14.8	3.22	0.04*	-13.6	-15.1	2.61	0.19
LASct 4Ch (%)	-17.1	-18.1	-0.79	0.51	-16.6	-20.6	-4.92	0.39
LA 4Ch peak systolic SR (1/s)	1.21	1.13	-0.09	0.27	0.93	1.18	0.33	0.30
LA 4Ch early diastolic SR (1/s)	-1.27	-0.89	0.38	0.002*	-0.77	-0.60	0.17	0.42
LA 4Ch late diastolic SR (1/s)	-1.71	-1.80	-0.08	0.47	-1.67	-2.10	-0.31	0.43
LA 2Ch strain (%)	26.8	26.3	-0.52	0.73	25.8	25.7	-0.51	0.78
LASr 2Ch (%)	36.1	36.2	-0.05	0.97	27.1	33.8	-1.38	0.62
LAScd 2Ch (%)	-15.4	-15.3	0.35	0.76	-16.6	-14.5	-0.12	0.97
LASct 2Ch (%)	-20.7	-20.9	-0.23	0.87	-20.5	-19.3	1.25	0.75
LA 2Ch peak systolic SR (1/s)	1.14	1.11	-0.03	0.69	1.00	1.06	0.06	0.82
LA 2Ch early diastolic SR (1/s)	-1.01	-0.95	0.11	0.20	-0.62	-0.40	0.10	0.61
LA 2Ch late diastolic SR (1/s)	-2.02	-2.18	-0.15	0.38	-1.65	-2.27	-0.66	0.24

Table 32. Change of LA strain and strain-rate measures in both patient groups

**Statistically significant (p < 0.05*

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
RA strain (%)	34.8	30.7	-4.12	0.05	37.3	31.5	-5.8	0.35
RASr (%)	40.7	37.1	-3.67	0.06	46.9	36.6	-10.3	0.04*
RAScd (%)	-24.1	-20.4	3.79	0.03*	-18.3	-22.5	-4.18	0.51
RASct (%)	-16.5	-16.7	-0.18	0.90	-28.5	-14.1	14.4	0.01*

Table 33. Change in RA strain and strain-rate measures in both patient groups

**Statistically significant (p < 0.05*

4.5 Changes in Heart Rate and Blood Pressure

The baseline heart rate and blood pressure values have been provided earlier in Table 23. Despite a higher baseline median heart rate and a mean systolic and diastolic blood pressures in G2 compared to G1, there was no statistically significant difference between the groups.

Additionally, at V2, no change was observed in any of these measures in all patients (

Table 34) however, in G1, the mean heart rate was lower at 72 bpm with a mean change of 4.70, p =0.02 making this change statistically significant. The systolic and diastolic blood pressures remained unchanged in G1. Despite a lower heart rate and systolic and diastolic blood pressures post completion of chemotherapy in patients in G2, these did not reach statistical significance (Table 35).

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
HR (bpm)	79	82	3.29	0.10
Systolic BP (mmHg)	136	132	-3.57	0.31
Diastolic BP (mmHg)	82	81	-0.12	0.95

Table 34. Changes in heart rate and blood pressure in all patients

Variable	G1				G2			
	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
HR (bpm)	78	72	4.79	0.02*	85	76	-8.33	0.25
Systolic BP (mmHg)	135	132	-2.60	0.45	143	131	-11.65	0.53
Diastolic BP (mmHg)	81	81	0.47	0.85	90	84	-4.14	0.29

Table 35. Changes in heart rate and blood pressure in both groups

4.6 Changes in Troponin Levels

For the purpose of this thesis, hs-cTnT results have only been provided. The hs-cTnI results could not be included in this thesis due to time constraints imposed by the MD duration, the impact of the SARS-CoV-2 19 pandemic, and the logistical issues surrounding the timely sending of blood samples to the Queen's Medical Research Institute at the University of Edinburgh.

Table 36 and Table 37 have been provided to illustrate the changes in the hs-cTnT during chemotherapy treatment. The 1-month post chemotherapy hs-cTnT visit has been labelled as cycle 9 in the tables.

4.6.1 Changes in hs-cTnT in all patients

During the study period, only 1 patient underwent an 8-cycle chemotherapy treatment plan for their underlying cancer diagnosis. The remaining majority of patients had an average of 6 cycles of chemotherapy. In addition, hs-cTnT levels were measured in only 24 patients at the 1-month post-chemotherapy visit due to the SARS-CoV-2 pandemic, which prevented patients at high risk of contracting the virus from attending their hospital appointments. This inadvertently led to insufficient results for analysis. Additionally, 6 patients passed away during their treatment and some lacked adequate venous access for blood sampling purposes which were also contributory factors to the inadequate hs-cTnT samples at the final visit.

The results of hs-cTnT during the different chemotherapy cycles and the mean change of hs-cTnT at different cycles from the baseline results for all patients have been provided in Table 36. As it can be observed from this table, there was an incremental increase in the hs-cTnT levels with increasing chemotherapy cycles. However, this increase only became statistically significant from cycle 3 onwards and continued all the way to the 1-month post chemotherapy visit (cycle 9) with the highest mean change seen between this visit and the baseline hs-cTnT; mean change of 24.09, $p < 0.01$

Cycles	Mean hs-cTnT	Change from baseline	p value
Baseline	11.3 ± 12.4	N/A	N/A
2	12.1 ± 8.0	2.27	0.31
3	16.6 ± 16.1	5.38	0.01*
4	21.4 ± 23.7	9.81	<0.01*
5	28.5 ± 22.5	15.56	<0.01*
6	34.4 ± 21.3	21.98	<0.01*
7	90	78.54	<0.01*
8	112	100.54	<0.01*
9**	34.9 ± 25.4	24.09	<0.01*

Table 36. hs-cTnT changes at different cycles - all patients

**Statistically significant (p < 0.05)*
*** Cycle 9 is equivalent to the 1-month post chemotherapy sample*

4.6.2 Changes in hs-cTnT in G1 and G2

The hs-cTnT levels in both patient groups have been highlighted in Table 37. The mean baseline hs-cTnT levels were similar in both patient groups. A total of 61 patients had a baseline hs-cTnT level checked and in only n=14 (n=13 in G1 and n=1 in G2) of these, the hs-cTnT was negative with the remaining of the patients having raised baseline troponins. A total of n=24 patients had their one-month post chemotherapy blood test (n=20 in G1 and n=4 in G2). In both G1 and G2, hs-cTnT increased at each cycle with levels persistently showing an increase at 4- weeks post chemotherapy visit (cycle 9). However, the rate of increase in the hs-cTnT levels was more rapid in G1 with statistically significant rises seen as early as cycle 3; mean change of 5.52 from baseline, p=0.02. In G2, hs-cTnT only began to show a significant increase at cycle 6 with a mean change of 21.17 from baseline, p<0.01. Only one patient in G2 had additional chemotherapy cycles (cycles 7 and 8) with none in G1. Interestingly, the mean hs- cTnT at cycle9 was higher in G2 (45.7 ± 24.0) compared to G1 (33.4 ± 25.8) with a higher mean change seen in the hs-cTnT levels when compared to baseline; mean change 33.21 and 22.75 in G2 and G1, respectively.

Cycles	Mean hs-cTnT in G1 (n=20)	Change from baseline	p value	Mean hs-cTnT in G2 (n=4)	Change from baseline	p value
Baseline	11.3 ± 12.6	NA	NA	11.5 ± 11.0	NA	NA
2	12.0 ± 7.63	2.14	0.38	13.0 ± 11.9	3.27	0.55
3	16.8 ± 16.5	5.52	0.02*	13.0 ± 10.3	4.07	0.49
4	21.9 ± 25.3	10.38	<0.01*	18.0 ± 11.4	6.50	0.22
5	29.7 ± 23.9	16.59	<0.01*	21.8 ± 11.5	10.33	0.05
6	34.7 ± 22.0	22.13	<0.01*	32.7 ± 19.1	21.17	<0.01*
7	NA	NA	NA	90	77.05	<0.01*
8	NA	NA	NA	112	99.05	<0.01
9	33.4 ± 25.8	22.75	<0.01*	45.7 ± 24.0	33.21	<0.01*

Table 37. hs-cTnT changes in both patient groups

4.7 Changes in Other Blood Results

The mean Hb and Cr results at both visits in all patients and those in G1 and G2 have been illustrated in the table below. No significant change was observed in any of these measures during the study period. The mean Hb was lower in G2 compared to those in G1 with a mean Hb of 115 g/l in G2 and a mean of 126 g/l in G1 however despite this difference, this was not statistically significant (p = 0.14).

Variable	All				G1				G2			
	Mean at V2	Mean at V2	Mean change from V1	P value	Mean at V1	Mean at V2	Mean change from V1	p value	Mean at V1	Mean at V2	Mean change from V1	P value
Hb (g/l)	125	124	-1.54	0.55	126	125	-1.95	0.48	115	118	2.66	0.64
Cr (umol/l)	73	75	2.05	0.67	73	70	-2.12	0.20	73	102	29.83	0.43

Table 38. Hb and Cr results

4.8 Reliability of Different Strain Measures

In order to determine the reproducibility and reliability of the LVEF and different strain measures, inter- and intra-observer variability was assessed as described in 2.2.10 Statistical Analysis section.

4.8.1 Inter-observer variability

The full detailed result for assessment of inter-observer variability can be found in Table 39. For measurements of LV, there was a good agreement between the two observers on the reported and re-evaluated LVEF; ICC 0.74 (95% CI 0.18-0.92). This was even higher on assessment of LV GLS with an ICC 0.85 (95% CI 0.53-0.95) when using the semi-automated AutoSTRAIN© method and 0.84 (95% CI 0.33-0.95) when using 2D CPA. The agreement was moderate with GRS and poor in GCS as highlighted in the table below.

In assessing the reproducibility of the RV measures, there was a good agreement between observers for both measures of RV GLS and RV FWS, ICC 0.81 (95% CI 0.42-0.93) and ICC 0.86 (0.57-0.95), respectively.

The inter-observer agreement for LA 4Ch strain measures was poor with only good agreement observed in the LA 4Ch conduit strain only; ICC 0.76 (95% CI 0.30-0.92). Good agreement was seen in all LA 2Ch strain measures.

There was very poor agreement in the RA strain measures with ICC 0.06 (95% CI -2.21-0.70) in the RA strain, ICC 0.09 (95% CI -1.94-0.71) in the RA reservoir strain, ICC 0.49 (95% CI -0.29-0.82) RA conduit strain and ICC 0.36 (95% CI -0.94-0.79).

Variable	Intraclass Correlation*	95% Confidence Interval
LVEF	0.74	0.18-0.92
GLS (AutoStrain)	0.85	0.53-0.95
GLS (2D CPA)	0.84	0.33-0.95
GRS	0.62	-0.18-0.90
GCS	0.41	-0.2-0.83
RV GLS	0.81	0.42-0.93
RV FWS	0.86	0.57-0.95
LA 4Ch strain	0.48	-0.40-0.82
LA reservoir strain (4Ch)	0.34	-1.2-0.79
LA conduit strain (4Ch)	0.76	0.30-0.92
LA contractile strain (4Ch)	0.59	-0.29-0.87
LA 2Ch strain	0.73	0.02-0.9
LA reservoir strain (2Ch)	0.78	0.21-0.94
LA conduit strain (2Ch)	0.90	0.65-0.97
LA contractile strain (2Ch)	0.82	0.32-0.95
RA strain	0.06	-2.21-0.70
RA reservoir strain	0.09	-1.94-0.71
RA conduit strain	0.49	-0.29-0.82
RA contractile strain	0.36	-0.94-0.79

Table 39. Inter-observer variability

** Type A interclass correlation coefficients using an absolute agreement definition.*

4.8.2 Intra-observer variability

The intra-observer variability of LVEF and the strain measures were also assessed for reproducibility purposes and can be found in Table 40. There was good agreement for both LVEF and GLS using 2D CPA however, this was excellent for GLS when using the semi-automated AutoSTRAIN© method with ICC 0.93 (95% CI 0.81-0.98). The intra-observer reproducibility of GRS was moderate and poor for GCS with ICC 0.62 (95% CI -0.18-0.90) and ICC 0.41 (95% CI -0.2-0.83), respectively.

Both RV GLS and RVFWS had good intra-observer variability with ICC exceeding >0.80 for both measures as seen in the table below.

Similar to the results in section 4.8.1 Inter-observer variability, LA 4Ch strain measures also had poor agreement rates between their measures however, this was good for LA 2Ch strain ICC 0.83 (95% CI 0.14-0.96), LA 2Ch reservoir strain ICC 0.84 (95% CI 0.48-0.97), LA 2Ch conduit strain ICC 0.90 (95% CI 0.65-0.95) and LA 2Ch contractile strain ICC 0.83 (95% CI 0.34-0.95). The reproducibility of the RA strain measures was poor.

Variable	Intraclass Correlation*	95% Confidence Interval
LVEF	0.84	0.47-0.95
GLS (AutoStrain)	0.93	0.81-0.98
GLS (2D CPA)	0.88	0.53-0.96
GRS	0.54	-0.13-0.88
GCS	0.73	-0.16-0.97
RV GLS	0.85	0.66-0.98
RV FWS	0.86	0.56-0.98
LA 4Ch strain	0.32	-1.33-0.78
LA reservoir strain (4Ch)	0.58	-0.22-0.86
LA conduit strain (4Ch)	0.59	-0.21-0.87
LA contractile strain (4Ch)	0.61	-0.17-0.87
LA 2Ch strain	0.83	0.14-0.96
LA reservoir strain (2Ch)	0.84	0.48-0.95
LA conduit strain (2Ch)	0.90	0.65-0.97
LA contractile strain (2Ch)	0.83	0.34-0.95
RA strain	0.52	-0.004-0.82
RA reservoir strain	0.57	-1.53-0.85
RA conduit strain	0.58	-0.11-0.86
RA contractile strain	0.62	0.14-0.86

Table 40. Intra-observer variability

** Type A interclass correlation coefficients using an absolute agreement definition.*

Chapter 5: Discussion

5.1 Key Findings

In our research, the effects of anthracycline chemotherapy treatment on a total number of 106 patients with breast cancer or lymphoma across two distinct studies by means of 2D transthoracic echocardiography and measurement of cardiac troponins, were demonstrated. Both investigations involved patients of a similar age range, and the rate at which cardiotoxicity developed was comparable to that of prior studies [36, 373, 374] but exceeded the expected incidence for the dose of anthracyclines that was administered.[24, 375]During our study, no patient died of cardiovascular complications of cancer treatment.

5.1.1 Echocardiography in cardiotoxicity assessment

To the best of our knowledge, this study is the first to use both conventional and more novel echocardiographic techniques to examine the impact of anthracyclines on each of the four cardiac chambers. The key discovery of this thesis is that anthracycline chemotherapy effect is a global phenomenon that affects the entire heart, as opposed to just the left ventricle, where the majority of previous research have mostly concentrated their attention. However, despite changes observed in all chambers, the LV and RV measures were the ones to show consistent findings in both the retrospective and prospective studies of this thesis. Measurements obtained via STE appeared to be more consistent, representing the true changes seen in the LV and RV.

5.1.1.1 Conventional echocardiography measures

Using conventional echocardiography measures, LVEF was the only measure to show a consistent statistically significant decline with time in both studies. However, despite a reduction in this measure in both patient groups, LVEF remained within the normal range at the final visit in patients in G1 making the drop in this group clinically non-significant. LVEF continues to be routinely used for surveillance of patients undergoing chemotherapy treatment. It is widely acknowledged that a meaningful decline in the LVEF to below the "normal value" must be observed in order to make a diagnosis of CTRCD.[376] However, the complexity of the issue arises in the inconsistencies and challenges observed across different guidelines and studies in defining cardiotoxicity using LVEF as a marker, with different threshold changes in

LVEF and normal LVEF values used.[14, 24, 113, 290, 291, 377, 378] In this thesis, a reduction of >10 percentage points in the LVEF to <53% was considered as evidence of AIC to replicate the same threshold criteria that was set by the guidelines and studies at the time of undertaking this research.[3, 14] More recently, new threshold criteria have once again been introduced for the purpose of cardiotoxicity definition.[378, 379] Albeit these revised standards, it is generally recognised that LVEF is highly dependent on loading conditions including blood pressure, heart rate, volume status, fever and anaemia. However, in this thesis, no statistically or clinically significant variation in the patients' baseline or between-visit blood pressure, heart rate, or haemoglobin was observed, to explain the changes in LVEF throughout treatment, especially in those in G2. It is also noteworthy that LVEF is associated with a poor level of reproducibility amongst different readers. The level of agreement between two independent readers in this thesis showed an ICC of 0.60 (95% CI -0.11-0.86) and 0.74. (95% CI 0.18-0.92) in the retrospective and prospective studies, respectively. This was similar to the findings of a study published in the American Society of Echocardiography where the authors evaluated the reproducibility of different echocardiographic measures including LVEF in patients with breast cancer treated with doxorubicin +/- trastuzumab across two academic echocardiography core laboratories.[380] In this study, the agreement level which was determined by the proportion of all pairwise comparisons between readers (coverage probability [CP], showed a CP of 0.67 for LVEF, (a CP $\geq$ 0.80 considered acceptable) suggesting poor inter-laboratory reproducibility. Notwithstanding these well-recognised flaws, LVEF still remains as one of the chief echocardiographic measures in the definition of cardiotoxicity and surveillance of patients across all guidelines.[60, 378, 379]

On assessment of LV diastolic function, no change in the diastolic measures were detected in the retrospective study, through the course of chemotherapy treatment. However, in the PROACT PLUS study, the LA volume was seen to increase with time in patients in G2 with a reduction in MV E/A. Despite these changes, neither of these measures reached statistical significance. Some studies have revealed that in patients treated with anthracyclines and trastuzumab, baseline LV diastolic function could potentially be associated with a small risk of future LV systolic impairment, though the evidence for this has been somewhat inconsistent, similar to the findings of this thesis.[280, 381] This confirms the unreliability and inconsistency of these measures which limits their utility in the assessment of chemotherapy induced cardiotoxicity.

Conventional measures of RV function were also found to be inconsistent and lacked sufficient sensitivity in reflecting the true changes in the RV. For example, a statistically significant decline in RV FAC and TAPSE were seen with anthracycline treatment in the retrospective study though these values were still within their normal range at the final visit. However, these findings were not reproducible in the PROACT PLUS study where no statistically or clinically significant decline in these measures were detected. This reflects the same findings as the previously published scarce and conflicting data on the assessment of RV function in chemotherapy induced cardiotoxicity using conventional echocardiography measures.[210, 269, 382, 383] As seen with the PROACT PLUS study, in a study of 42 patients with breast cancer undergoing chemotherapy, Lang et al did not find any significant deterioration in TAPSE, RV FAC or RV S' with chemotherapy treatment.[383] However, Tanindi et al found a significant deterioration in RV FAC in patients with breast cancer who received anthracycline based chemotherapy demonstrating similar findings to the retrospective study of this thesis.[211]

Finally, no valuable information was concluded through the measurement of LA and RA conventional measures. Studies on assessment of LA/RA volumes in the setting of cardiotoxicity have been scarce with LA volumes being predominantly assessed in the context of diastolic function. A previous retrospective study assessing RA area in 49 patients with breast cancer undergoing anthracycline chemotherapy, found a statistically significant increase in the RA area post chemotherapy however, despite this increase, RA area was still within the normal range.[269] Therefore, measures of LA and RA dimension and volumes alone do not provide any valuable information regarding the effects of chemotherapy on the heart.

5.1.1.2 LV strain measures

As opposed to earlier published results where the peak systolic GLS was evaluated, this thesis was the first study to investigate end-systolic GLS by manually measuring the aortic valve closure time. This was in line with the recommendations by the ASE and EACVI speckle tracking task force.[153, 251] In both studies of this thesis, LV GLS showed a deterioration during treatment in all patients however, the significance of the decline was more pronounced in patients who exhibited a reduction in their LVEF to < 53% at the final visit. A relative decrease of >15% was seen in LV GLS in patients in G2 in keeping with the diagnosis of AIC, as also seen in previous studies[3, 4, 35, 115, 174]. Interestingly, a reduction in GLS was not seen to precede

a decline in LVEF in the retrospective study and GLS was found to only deteriorate significantly at the same visit where a significant LVEF reduction was observed. This was contrary to previous studies however, similar to the findings published by Narayan et. al where the authors did not find GLS to be an early predictor of CTRCD in patients undergoing anthracycline +/- trastuzumab chemotherapy.[125] Additionally, as seen with previous studies[384, 385], in this thesis, a reduction in GLS did not indicate LV systolic dysfunction in all patients.

Although several studies have proposed that GLS is a more sensitive surrogate marker of early cardiotoxicity compared to LVEF, [2, 119, 170] and a decline in GLS can predict the development of clinical cardiotoxicity in patients undergoing chemotherapy, it is yet not confirmed whether a GLS based approach in monitoring patients undergoing chemotherapy is superior to an LVEF based strategy. The SUCCOUR clinical trial was the first ever study to address this very important question.[386] This multicenter prospective clinical trial randomly allocated patients undergoing chemotherapy treatment, to either a GLS-guided surveillance strategy versus a LVEF-guided one. The study failed to meet its primary endpoint which was the between-group change in LVEF during the study period. More importantly, no difference in the proportion of patients with LVEF < 55% was seen at 1-year.[387] An objective evaluation of these results suggests that the GLS assessment of cardiotoxicity lacks efficacy. Remarkably, there was no difference in GLS at one year between the two groups (1.5% vs. 1.4%) despite the expectation that this would be the case with a GLS-initiated intervention strategy.[343, 388] Furthermore, a recent meta-analysis evaluating the utility of GLS in cardio-oncology which demonstrated the presence of marked heterogeneity in the methodology of the studies, the presence of publication bias, and limited data on incremental value of GLS highlights the need for larger clinical trials in the use of GLS.[175] This may explain why in the latest European Society of Cardiology guidelines on cardio-oncology,[379] GLS has not been incorporated as a sole measurement for defining moderate to high-risk asymptomatic cancer therapy-related cardiovascular toxicity, and both measures of LVEF and GLS have been highlighted as essential measurements for reaching this definition.

In this thesis, the evaluation of GRS was conducted using a different methodology than is typically outlined in most research.[118, 125, 126, 171, 181] To the best of our knowledge, our study was the only one to use this method to investigate GRS using this method. This was done in order to determine whether this approach offered better consistency of results as compared to

the previous widely employed trio of short -axis LV views. Despite the adoption of this method, the results were unsatisfactory as they failed to demonstrate consistent outcomes throughout the two studies. Although, a statistically significant reduction in GRS was observed in the retrospective anaylsis in patients in G2, the PROACT PLUS study did not discover the same findings, and GRS was not seen to reduce significantly in patients who had developed LV systolic dysfunction. Due to this, employing GRS, regardless of the method used to detect it, does not seem to be helpful for assessing cardiotoxicity, which is why its measurement has not been included in the most recent guidelines for cardio-oncology.[379]

In our research, the insufficient number of cases made it impossible to draw any definitive or significant conclusions from the analysis of GCS, twist, or torsion. However, based on the information that was gathered, GCS consistently decreased with increasing anthracycline dosage in both studies, and the severity of this drop was more clinically meaningful in individuals who had developed LV systolic dysfunction at the final visit. This decline in this group of patients was statistically significant between T0 and T2 in the retrospective research. Although this measure showed a relative decrease of >10% between T0 and T1, it did not reach statistical significance. This result was consistent with research by Narayan et al.[125] In their prospective study, 2D echocardiography was used to analyse 135 breast cancer patients who were undergoing anthracycline +/- trastuzumab treatment at baseline and at various intervals. The authors concluded that GCS had the strongest predictive ability for CTRCD (AUC: 0.655; 95% CI: 051-0.767). Although the most recent guidelines acknowledge the value of GCS and its potential to identify individuals at risk of CTRCD, they do not advocate its routine use in clinical practice due to the lack of sufficient data supporting its usefulness in this context.[379] Given the consistent decline that we observed in this measure across both our studies, we strongly believe that its utility should not be overlooked and though still in the exploratory stage, it should be incorporated into further research and clinical trials in cardio-oncology.

In our prospective study, twist and torsion significantly increased in contrast to the results reported by Motoki et al.[203] Motoki et al. discovered a significant early decline in twist and torsion one month after starting chemotherapy treatment in their study of 25 patients undergoing low-dose anthracycline (100mg/m2) chemotherapy treatment. As previously stated, it is difficult to draw any firm conclusions on torsional deformation measures in our research due to the lack of sufficient data for these parameters. In this thesis, a higher mean anthracycline dose was administered particularly to those in G2, with the mean anthracycline

doses exceeding >250mg/m^2. Whether twist and torsion genuinely increase as a compensatory mechanism for the loss of the longitudinal fibres and reduction in GLS in patients with a reduction in LVEF, is unknown. Further research is required to determine the utility of these measures in surveillance of patients undergoing chemotherapy treatment.

5.1.1.3 LV strain-rate measures

Although, strain-rate has a strong correlation with contractility[3, 389, 390] and its use has been explored in various studies,[171, 172, 180] we were unable to obtain reliable results in our investigation of this measure. Our study was the first to incorporate the assessment of end-systolic strain-rate in addition to measures of systolic and diastolic strain-rate parameters during the surveillance of patients undergoing chemotherapy treatment. However, no major change in the longitudinal or radial end-systolic strain rates were observed in our research. Though, in the PROACT PLUS study, a substantial reduction in the circumferential end-systolic strain-rate was observed in patients in G2, it is challenging to give these findings any clinical significance due to the lack of sufficient number of cases.

Despite a reduction in LV peak systolic longitudinal strain-rate in patients with LV impairment, in the PROACT PLUS study, these results were contradictory in the retrospective study which could be argued by the better image quality that was available in our prospective study. Our results from the prospective study were consistent with the findings published by Negishi et al.[171] In their study of 81 patients with breast cancer undergoing anthracycline +/- trastuzumab treatment, the authors found a significant reduction in the LV peak systolic longitudinal strain-rate in those who had developed cardiotoxicity at 12-months. Additionally, the authors concluded that a change in this measure at 6-months was a predictor of future development of >10% decrease in LVEF at 12-months. Recently, the Copenhagen City Heart Study demonstrated a significant correlation between peak systolic strain rate and the development of heart failure in the general population after monitoring 4013 participants for 5.4 years.[391] In their study, an optimal cutoff value of peak systolic strain-rate of <1.028 1/s was associated with a 4-fold increased risk of heart failure.

In addition to the peak systolic longitudinal strain-rate, the early diastolic longitudinal/radial and circumferential strain-rates further demonstrated a statistically significant decline at the

final visit in the PROACT PLUS study, but these measures only reached statistical significance in patients in G1. Although the early diastolic radial and circumferential strain rates similarly declined throughout time, these values did not reach statistical significance. In the study by Negishi et al, the authors discovered that subsequent LV systolic impairment may also be predicted by changes in the early diastolic longitudinal strain-rate at 6-months.[171] Although diastolic assessment is important and thought to precede LV systolic dysfunction and diastolic strain-rate is believed to have a strong correlation with LV relaxation,[392] we were unable to replicate the same results in our research study, highlighting that there are still limitations to the evaluation of diastolic function. Despite the fact that diastolic strain-rate is thought to be less load-dependent, it is important to be aware that low frame rates and signal noise might restrict strain-rate readings, leading to loss of information and therefore complicating analysis.[393] Before these measures make their way into routine clinical practice, there is need for a more advanced technology to eliminate the associated restrictions and further larger studies to demonstrate consistent results.

5.1.1.4 RV strain and strain-rate measures

At the time of the write-up of our research protocols, we were surprised by the limited number of studies that had incorporated RV assessment in their investigation of CTRCD.[210, 211, 269] It is well known from histological biopsies that anthracyclines exert their effects beyond the left ventricle.[89, 90] Our study, to the best of our knowledge, was the first to use STE to examine the complete RV systolic and diastolic parameters. The influence of chemotherapy on the RV diastolic strain-rate parameters had not been investigated in any prior investigations.

In our retrospective study, we discovered a significant reduction in RV GLS and RV FWS in both patient groups with patients with LV systolic impairment seeing a more drastic decline. Interestingly, these changes were noticeable as early as T1 with changes of >10% seen between T0 and T1, in patients in G2. However, this decline did not reach statistical significance for RV FWS. Although patients in the PROACT PLUS study also experienced a reduction in RV GLS, this was only statistically significant for patients in G1. However, this study showed a reduction in RV FWS in patients in G2 only. Although, minor inconsistencies in the RV GLS findings were identified across our studies, this was not the case for RV FWS indicating that this may be a preferable parameter for right ventricular monitoring. Our findings were consistent with the results of Boczar et al and other research studies that have since been

published.[181, 269, 394, 395]. In their retrospective study of 49 patients with breast cancer undergoing anthracycline-based chemotherapy treatment, Boczar et al found RV FWS to decline from -16.2% at baseline to -13.8% at the follow-up visit (p=0.04) suggesting that anthracycline chemotherapy exerts its adverse effects on the right ventricle.

Interestingly, in a recent study evaluating patients undergoing trastuzumab treatment for their breast cancer, the authors found a reduction in RV GLS 6-months post treatment and therefore concluded that a relative reduction of -14.8% was predictive of cardiotoxicity development.[373] Fascinatingly, in our retrospective study the extent of the reduction in RV GLS was >15%, from T0 to T1, in patients in G2 with this being less in patient in G1 consistent with this study's findings.

Given the prior favourable histology findings supporting the overall effect of anthracyclines, the outcomes of our investigation and subsequent studies are not surprising. Owing to its thin wall structure, the RV is susceptible to cardiotoxicity, and therefore, RV GLS and mostly RV FWS appear to be robust measures in identifying these early changes when compared to conventional echocardiography measures. Therefore, it is logical the RV is no longer the neglected chamber and the most recent cardio-oncology guidelines now include RV assessment along with measurement of RV FWS in the monitoring of patients receiving chemotherapy.[379] However, its prognostic impact in the outcome and survival of patients with cancer is not yet known highlighting the need for larger studies and clinical trials.

RV peak systolic longitudinal strain-rate is believed to have a significant correlation with RV contractility. In one study, Jamal et al.[396] compared echocardiographic strain-rate imaging results to sonomicrometry and demonstrated the feasibility of the echo technique for quantifying changes in RV contractile function in an ingenious animal experiment. During our investigation of the strain-rate measures, we found a reduction in RV and RV FW peak systolic longitudinal strain-rates in both patient groups with this decline seen as early as T1, in the retrospective study. Although these findings were not replicated in the PROACT PLUS study, a reduction was still observed in these measures in patients with LV systolic impairment, which was not statistically significant.

Furthermore, recent results from a pilot study investigating 40 patients with breast cancer in the Israel Cardio-Oncology Registry (ICOR) who had undergone anthracycline-based

chemotherapy, found a relative reduction of ≥ 10% in the RV GLS and RV FWS peak systolic strain-rate measures post treatment which was similar to our findings.[394]

On assessment of the RV diastolic function using STE, we also found RV and RVFW early diastolic strain-rate measures, which are markers of RV relaxation, to significantly decline with time But, given this finding was seen in patients in G1 only, it is difficult to interpret the significance of these results. As seen with the LV, RV diastolic impairment should also predate RV systolic dysfunction and coexist in cases where RV systolic impairment has been established. However, in G2, despite a reduction in RV FWS, no change in the diastolic measures were witnessed. This could be related to the restrictions that exist with strain-rate measurement as previously discussed or could suggest lack of sensitivity of RV diastolic strain-rate parameters in identifying any diastolic changes.

5.1.1.5 LA strain and strain-rate measures

This research was the first to our knowledge, to use 2D STE to examine the impact of anthracycline treatment on LA function. A few recent studies evaluating the utility of the LA strain in cardiotoxicity were published at the time this thesis was written; however, the majority of these studies were retrospective in nature, increasing the risk of bias in their findings.[262, 397-399] Interestingly, our results from our retrospective study suggested significant reductions in measures of LA 4Ch and LAScd 4Ch strains (in G2 only), and LASr 4Ch strain with anthracycline chemotherapy treatment. Considering the close interaction between LA and LV mechanics, it seemed plausible to believe that anthracycline chemotherapy-effect extends beyond the left ventricle affecting the left atrium, and that STE appears to be a robust method to evaluate these changes. However, these findings were not reciprocated in the PROACT PLUS study with only the LAScd 4Ch strain demonstrating a substantial decline with time, seen in patients in G1 only. The only consistent results between the two studies were the reduction of the LA 4Ch early-diastolic strain-rate measures but oddly this significant reduction was only observed in patients in G1 with conflicting results in those in G2.

In a recent retrospective study of 91 patients with breast cancer treated with chemotherapy, LA strain parameters were assessed at three different time-points. The authors of this study identified that all three components of LA strain were affected by chemotherapy with a

significant decline seen in LASr, LAScd and LASct parameters ($p<0.01$).[397] However, in an earlier study by Timoteo which investigated a higher number of patients (n=100) with breast cancer in the span of 1 year, no statistically significant change in LA strain parameters were identified.[262] Another study by Laufer-Perl and colleagues discovered that 50% of patients treated with anthracyclines exhibited a 10% relative reduction in LA reservoir strain and/or a reduction to a value < 35%.[399]

Due to the close interplay between the two chambers, it is well-known that LA function plays an essential role in LV diastolic function. Owing to the limitations of angle-dependency of tissue Doppler imaging, STE was developed to tackle this limitation for LA function assessment.

Despite the advantages of STE imaging, our studies failed to demonstrate consistent results during chemotherapy treatment. One important reason could be the variability in the methodologies used for LA strain measurement across studies. Other potential explanations could be related to a more posterior location of the LA, making it more challenging to trace the thin-walled LA structure. Additionally, dependency of LA strain measurement on good-quality not-foreshortened images with the need for lower heart rates to enable better tracking of the LA wall could be a further explanation for these findings. There is hope that with more advanced machine learning techniques, we will be able to overcome some of the current limitations that exist with LA strain measurement increasing the chances of more robust and consistent results becoming available in the future. Currently, there is need for larger studies and trials for LA strain assessment before its measurement is implemented into clinical practice.

5.1.1.6 RA strain and strain-rate measures

As part of this thesis, we felt it prudent to assess the effects of anthracycline chemotherapy on the right atrial function. To the best of our knowledge, our study was the first to assess right atrial strain in this context. No previous or subsequent studies have since being published to evaluate right atrial function in the setting of chemotherapy related cardiotoxicity. Most studies on right atrial strain have focused on the evaluation of pulmonary hypertension.[400, 401]

Our research demonstrated interesting findings in regards to the effects of chemotherapy on the RA with more consistent results seen across both studies. RA GLS was noted to significantly reduce in all patients, in both studies, in line with a reduction in the RV GLS and RV FWS, highlighting the close relationship that exists between the two chambers. A deterioration in the RASr and peak systolic strain-rate, in the retrospective study, and early diastolic strain-rate in the PROACT PLUS study were also seen but these were not reproducible across both studies. Additionally, in the PROACT PLUS study, the RASr and RASct declined in line with the reduction in RVFWS, in G2, though, these findings were not present in the retrospective study. While, some of these findings were not replicated across both studies, a reduction in RA GLS appeared to be a consistent finding suggesting a role in its measurement in monitoring of patients in chemotherapy. Given the better quality images we had for the PROACT PLUS study, it is possible that the changes observed in the RASr and RASct in correlation with a decrease in RVFWS represent a true finding; however, it is hard to ignore the reproducibility data we obtained from RA strain measurements with these highlighting poor levels of inter and intra-observer agreement. This could be related to the use of LA strain tracking software for the analysis of the RA strain due to the absence of dedicated software for RA strain measurement which may have affected the results. Additionally, lack of operator experience in RA strain assessment could be another explanation for this. RA strain measurements are still in the very early stages of research, and larger studies with longer follow-up studies will be required to assess their utility in monitoring patients undergoing chemotherapy prior to their implementation in clinical practice. Although LA strain has become more of a focus of research interest in recent years and has been discussed in various studies for the assessment of heart failure with preserved ejection fraction, it is somewhat surprising that RA strain has not received the same level of attention, especially given its less posterior position in the chest for echocardiography purposes, which should somewhat reduce the limitations associated with LA strain measurement.

5.1.2 Troponin measurement in cardiotoxicity assessment

It is widely accepted that measurement of hs-cTnT or hs-cTnI have a superior advantage in detecting early cardiac damage compared to the use of normal troponins and their use can be beneficial in the context of cardiotoxicity surveillance due to their ability to uncover cardiomyocyte injury prior to the development of LV dysfunction.[402] Several studies have

evaluated cardiac troponin measurement in the field of cardio-oncology in an effort to identify a simple, useful, and cost-effective method for detecting early CTRCD to aid the management of patients undergoing cancer treatment. Using high sensitivity assays, some of these have demonstrated a positive correlation between elevated troponin levels during or after chemotherapy and the subsequent development of LV systolic dysfunction. [64, 170, 316] In a study by Cardinale et al, the authors revealed that in patients treated with high dose anthracycline chemotherapy, a negative troponin during and at 1-month post chemotherapy can effectively exclude significant cardiotoxicity.[333] Consequently, one of the goals of our PROACT PLUS study was to also measure this cardiac biomarker in order to assess its usefulness in detecting cardiotoxicity in conjunction with more advanced echocardiographic parameters.

During our study, we found an incremental increase in hs-cTnT release with increasing doses of anthracyclines, in all patients, reaching a maximum peak at 1-month after completion of chemotherapy. However, contrary to the findings of other studies,[170, 302, 316, 402] our subgroup analysis did not demonstrate any correlation between hs-cTnT release and subsequent LV systolic dysfunction as hs-cTnT was noted to increase in both G1 and G2 with an earlier increase observed in patients in G1. It is important to note, however, that due to the SARS-CoV-2 pandemic, more than half of our patients were unable to have their post-chemotherapy hs-cTnT blood test due to their high-risk clinical condition during this time period. If more data had been available, our results may have revealed more interesting findings. Nevertheless, our results were similar to those published by Diaz-Anton et al, who evaluated biomarker alterations in patients undergoing anthracycline and trastuzumab chemotherapy using the same assay (Elecsys hs-cTnT Roche) as our research team.[374] In their study of 72-patients with breast cancer undergoing chemotherapy treatment, the authors did not find any significant difference between the hs-cTnT levels in patients with or without cardiotoxicity, demonstrating a lack of ability for hs-cTnT in predicting subsequent cardiotoxicity. In another prospective study investigating the utilization of hs-cTnT using the Elecsys assay (Roche), in patients with non-Hodgkin's lymphoma receiving anthracycline-based chemotherapy treatment, no correlation between hs-cTnT and LVEF was observed.[403]

In light of multiple studies demonstrating a correlation between troponin and the development of cardiotoxicity by Cardinale et al,[69, 316, 333, 337] the ICOS-ONE (Prevention of anthracycline-induced cardiotoxicity) randomised clinical trial was conducted to assess the role of troponins in this setting further.[343] In their study, two therapeutic strategies for guiding cardioprotective

treatment in 273 patients receiving anthracycline-based chemotherapy were compared, one of which involved troponin-guided therapy. Cardinale et al. found that the incidence of troponin elevation was 23% in patients receiving concomitant enalapril treatment with their anthracycline chemotherapy, compared to 26% in those receiving enalapril when troponin levels were elevated ($p = 0.50$), indicating that there was no difference between the two strategies for the prevention of cardiotoxicity. However, one of the drawbacks of the study was the use of various troponin subunits and reagents among patients, which may have affected the results due to the variability in threshold values, resulting in comparability issues.

Although hs-cTnI analysis was not included in this study, it is likely that similar results to that of the findings of hs-cTnT would have been observed as both enzymes are largely cardiac specific and are commonly regarded as interchangeable. As it can be demonstrated from our results and various studies, cardiac troponins have not yet fully established their place in cardio-oncology. Although their use is complementary to imaging techniques and have been proposed in the latest guidelines,[379] larger studies with longer follow-up periods are required to assess their utility in clinical practice further. Currently the findings on these cardiac biomarkers are inconsistent across studies which can largely be attributed to the significant heterogeneity observed in patient characteristics, the types of troponins used, and the variability in the sensitivity of the assays employed. Additionally, there is possibility that high sensitivity cardiac troponins may not be as useful in the setting of CTRCD monitoring. We do know that a small fraction of cardiac troponins exist in the cytosolic pool; this has been estimated as 6-8% for cTnT and 3.5% for cTnI.[404] The remaining vast majority of troponins lie within the myofibrils' contractile apparatus.[405] Although high sensitivity cardiac troponins are advantageous in the setting of ischaemia due to their ability to detect early release of the cytosolic troponin, it is questionable whether this benefit extends to the detection of CTRCD, as a small level of cardiac troponins can be detected in the setting of other non-cardiac conditions, such as inflammation, and do not necessarily indicate cell damage. As cancer is an inflammatory process and hs-cTnT is considered slightly less specific than hs-cTnI,[406, 407] this may be a potential explanation as to why all of our patients exhibited elevated troponin levels during treatment.

With newly emerging clinical trials such as our own PROACT clinical trial[408] and CARDIAC-CARE,[409] it is hoped that the role of cardiac troponins in the field of cardio-oncology will be finally determined.

5.2 Clinical Relevance

In this study, we demonstrated the global effects of anthracyclines on all cardiac chambers by using 2D conventional echocardiography, STE and cardiac biomarkers. Using echocardiography, we identified that LVEF continues to play a major role in the diagnosis of cardiotoxicity, and strain measures such as GLS and possible GCS add complementary value to LVEF in the assessment of cardiotoxicity. However, despite numerous studies proposing GLS as an early surrogate marker of subsequent LV systolic dysfunction, our study failed to show the same results. Additionally, the SUCCOUR trial published after we concluded this research, failed to demonstrate a benefit in a GLS-based approach in the surveillance and management of patients undergoing chemotherapy when compared to an LVEF-based approach.[343] This trial suggests, in contrary to what was once believed, GLS may not have a major role in the surveillance of patients undergoing cancer treatment. Nevertheless, its use has been incorporated into the recent cardio-oncology guidelines and its use currently still recommended in conjunction with LVEF assessment.[378, 379] Emerging studies with longer follow up periods may provide more useful information about the prognostic implication of this measure in this field and whether treating patients based on this measure to prevent future LV systolic impairment is beneficial.[408, 409]

Furthermore, we found that RV GLS and more specifically RV FWS deteriorated with anthracycline chemotherapy and these measures preceded a decline in LVEF which could potentially make these a more sensitive marker in identifying patients at risk of cardiotoxicity. Although measuring RV FWS has also been recommended in the recent guidelines,[379] the clinical relevance of any change remains unknown. Our findings in combination with other recent studies will inform future clinical trials. This will determine the significance of this measure in the clinical setting and its sensitivity and consistency in predicting future LV systolic dysfunction when compared to GLS.

Changes in LA and RA strain parameters were also seen in our study which highlighted how anthracycline effect is not confined to the left ventricle. With future advancements in technology, the role of these measures may become increasingly significant in the field of cardio-oncology. In diastolic function assessment, LASr has gained interest where a value of <18% is considered useful in predicting LV filling pressures better than the conventional 2D LA volume assessment.[410] Therefore, some studies have recommended the routine use of LA

reservoir strain in diastolic function assessment to aid diagnosis when the use of conventional measures provide conflicting results.[411, 412] Despite finding changes in LA strain measures in our research, the small sample size of our studies and the dependence of diastolic measures on age and sex, did not allow us to draw any definitive conclusions. Nevertheless, this highlighted that changes do occur and larger studies and clinical trials may yet find the utility of these measures in diastolic function assessment and prediction of atrial arrhythmias.

Furthermore, despite the recommendation for cardiac troponin monitoring in the latest clinical guidelines,[379] our research and previous studies suggest conflicting findings. Therefore, larger studies with longer follow up periods are required using modern troponin assays. Until then, these biomarkers will continue to have a complementary role alongside imaging techniques in the field of cardio-oncology.

Although our research has advanced knowledge of the cardiotoxic effects of anthracycline chemotherapy, it is not yet clear what role these measures will play in clinical practice. It is therefore crucial to stress that early introduction of these measures into clinical practice and placing too much emphasis on certain measures (such as GLS) can pose a risk on cancer patients undergoing treatment. This can lead to unnecessary life-saving cancer treatment interruption in favour of initiation of new cardiac-directed medications which can result in unwanted side-effects. It is important to keep in mind that many of the new measures developed as a result of technological advancement are still in the exploratory phase. Therefore, strain and strain-rate imaging should continue to serve as research tools until their predictive significance on the outcome and survival of cancer patients is confirmed in larger trials and their unique value at the intersection of imaging and clinical care is completely established.

Currently the PROACT clinical trial which is a multicenter, prospective, randomized, open-label, blind end-point trial has completed recruitment and will report on the role of enalapril in preventing cardiotoxicity in patients with breast cancer and lymphoma undergoing anthracycline-based chemotherapy treatment.[408] In this trial, cardiac troponin T release at any time during treatment and 1-month post last dose of chemotherapy will be assessed in addition to assessment of cardiac function using LVEF and GLS. With our findings of our current observational research we will incorporate RV GLS, RV FWS and LA strain measures, into this trial. Through doing so, we will explore further the potential usefulness of these measures

and assess their utility in a larger setting and explore whether they could potentially be useful in clinical practice.

5.3 Study Limitations

This thesis, was a single-center study with relatively small number of patients. Unfortunately, the unprecedented challenges posed by the SARS-CoV-2 pandemic had a major effect on study recruitment, especially in the PROACT PLUS study. The original plan to recruit more patients (n=85) in a multicenter setting was affected and this option was removed by the pandemic. In March 2020, we had no choice but to suspend further recruitment. This was based on a UK wide government recommendation on shielding and public health priorities. Additionally, the travel restrictions and quarantine guidelines on our patients due to their high-risk clinical status posed further obstacles to our patient numbers and the timely collection of all the data required for this research project. Due to the time constraints of the MD and the occurrence of the SARS-CoV-2 pandemic, the follow-up period was limited to 1-month post chemotherapy treatment. Unfortunately, the 12-months follow-up results were not within the scope of this MD thesis.

In light of the exploratory nature of this research, the studies in this thesis were not statistically powered to detect a change in various measures with anthracycline chemotherapy. A further limitation, was that in addition to assessing the changes in different echocardiographic parameters in all patients, the retrospective division of patients into two different sub-groups based on the LV function assessment at the end of the treatment had the potential to increase the risk of bias in our interpretation of the results; however, this was the only way to understand the changes in novel echocardiographic measures associated with definite cardiotoxicity.

One of the other important key limitations in this study was the insufficient number of apical short axis views that were taken during the study as this was not routinely taken in the Echocardiography department as part of clinical care and was therefore commonly forgotten during this research period. This therefore precluded any conclusive results to be produced for measures such as LV GCS, torsion, twist and their corresponding strain-rate measures.

Furthermore, this study's findings were only representative of the cardiac changes seen in Caucasians who were treated with anthracyclines. Although we sought to include patients from under-represented groups, we were unable to recruit any participants from different racial and

ethnic backgrounds. As a result, our capacity to draw broad generalisations about the impact of anthracyclines on the population at large may be constrained.

Finally, in our center, GLS was a routinely used measure in the assessment of cardio-oncology patients. However, there was limited experience in the utilisation of other strain measures by our highly experienced physiologists. Given that STE imaging requires extensive knowledge and experience in the use of the technique on a regular basis, this could explain the poor inter-observer variability that was found for most of our strain measures and therefore, these results have to be interpreted with caution.

5.4 Future Perspectives

5.4.1 Use of Artificial Intelligence (AI)

At the time this study was conducted, only GLS was semi-automated with all other measurements performed manually, increasing the potential for human error. With advancing technology, and the increasing popularity of machine learning and AI, there is hope that in the near future better techniques may become available to improve the accuracy and reproducibility of the current measures. Due to the increased risk of cardiovascular complications associated with anthracycline exposure, early recognition of cardiotoxicity has been the focus of most studies, including ours. In the recent years, the use of computer algorithms to stimulate human intelligence has propelled AI advancements. The continuous machine learning through the availability of longitudinal clinical data with the addition of cardiovascular imaging and ECGs, provides hope that better and more efficient ways in predicting and detecting cardiac dysfunction will become available.[413-415] Additionally, this may help develop efficient surveillance algorithms for patients deemed at a high risk of developing cardiotoxicity.

5.4.2 Use of Biomarkers

Finding new biomarkers to aid in the early detection of cardiotoxicity in individuals thought to be at high risk of developing LV dysfunction, has been a persistent focus in the field of cardio-oncology in the recent years. Some of these biomarkers such as fatty acid binding protein

(FABP) and glycogen phosphorylase isoenzyme BB (GPBB) have been identified and studied in this context showing promising results.[416, 417]A number of other novel protein biomarkers, such as TGF-ß1, ST-2, MPO, GDF-15, PIGF and many others have additionally been explored suggesting a potential role for these markers in cardiotoxicity assessment.[354, 418-422] However, due to the limited amount of research in this field, they have not yet made their way into clinical practice. Nonetheless, this underlines the recent push to uncover sensitive markers of cardiotoxicity. With deployment of emerging biomarkers in large-scale research, it is hoped that additional information regarding the prediction capabilities of these markers in detecting cardiotoxicity will become available, allowing for their future introduction into clinical practice.

5.4.3 Role of Genomics

Although age and preexisting cardiovascular diseases are established risk factors for cardiac toxicity, there is still considerable inter-individual variability in the onset of this effect. While some people can safely take high doses of chemotherapy, others may experience detrimental effects beginning at much lower levels. Therefore, a drive to identify possible genetic variants associated with developing cardiotoxicity has been the focus of recent research. Several single nucleotide polymorphisms (SNPs) of interest have been identified through genome wide association studies however, due to the small number of studies these have not yet become clinically applicable.[423-425] Nonetheless, some of these studies have yielded interesting results, which may pave the path and direct future research into better comprehending the genetic overlap with environmental influences, and help define a personalized genetic approach in identifying those patients susceptible to cardiotoxicity.[424, 426]

In the PROACT PLUS study, DNA samples were also collected to develop a future study for the purpose of exploring potential gene variants that could increase the risk of CTRCD development. In conclusion, it is hoped that, in the near future, the means of identifying cardiotoxicity will be vastly improved owing to genomic testing, advanced imaging techniques, and the availability of novel and specific biomarkers.

5.4.4 Treatment of Cardiotoxicity

A number of studies have evaluated the use of ACEi, ARB and betablockade treatment in the management of patients with CTRCD[63, 68, 343, 427] due to their proven benefit in cardiovascular medicine and treatment of patients with heart failure.[428-431] In addition to promoting ventricular recovery through their direct influence on adrenergic and neuro-endocrine pathways, some studies have further proven the ability of these agents in reducing ROS formation responsible for cardiotoxicity.[73, 75, 77, 432] In patients exhibiting LV systolic dysfunction secondary to anthracyclines, early commencement of neurohormonal antagonists have proven beneficial in improving cardiovascular outcomes.[24, 36, 333] Interestingly, our prospective study demonstrated similar findings despite not reaching statistical significance. A trend towards maintaining normal LV function was observed in those patients who were either on ACEi, ARB or betablocker treatment at baseline and during treatment suggesting similar findings to previous studies.

In addition to these agents, some studies have also investigated the role of MRAs' potential usefulness in this context, but research has been insufficient making definitive statement on their application difficult.[116]

Despite the recognised advantage in early detection and treatment of cardiotoxicity, preventative/early treatment in patients undergoing cancer therapy remains controversial with the current guidelines acknowledging the use of ACEis/ARBs and betablockers in early treatment of CTRCD but recommending their use based on level IIa evidence only which highlights the need for more studies.[379] The PROACT and CARDIAC-CARE clinical trials are both upcoming multicentre studies which will provide exciting information regarding the role of primary prevention in the setting of chemotherapy treatment which will undoubtedly shape future guidelines and gain more understanding into the use of these medications in the field of cardio-oncology.[408, 409] Furthermore, PRADA II clinical trial will also assess the use of sacubitril/valsartan in the management of patients receiving treatment for their cancer.[433]

5.5 Conclusions

This research was the first study to evaluate the effects of anthracyclines on all four cardiac chambers using 2D conventional echocardiography, STE and troponin assessment. This

demonstrated anthracycline-effect to be a global phenomenon and not confined to the LV, as once believed. LVEF continues to play a major role in the definition of cardiotoxicity, with LV GLS adding complementary value during treatment surveillance. Furthermore, LV GCS could have a potential role in the assessment of cardiotoxicity however, no firm conclusion can be drawn from our results.

Additionally, changes in RV FWS demonstrated interesting findings in our research and suggest that this measure could serve as a more advantageous and sensitive surrogate marker than LV GLS, in predicting future LV systolic dysfunction. Before its use becomes established in clinical practice, larger clinical studies should assess its utility.

Despite alterations in the LA and RA strain measures, these did not appear to be consistent across both studies which could potentially be explained by the image quality across both studies. At present neither of these strain measures have a role in cardio-oncology surveillance however, with advancement of technology and improvement in tracking software there may be hope that more consistent results could be produced. Therefore, these measures should continue to be explored with a focus of their role assessed in predicting LV diastolic dysfunction, atrial arrhythmias and pulmonary hypertension in the setting of cancer treatment.

Finally, measurement of hs-cTnT did not add any additional value to surveillance of patients undergoing chemotherapy treatment within this study which contrasts with the current orthodoxy, but may be supported by the CARDIAC-CARE findings. Additionally, despite not reaching statistical significance due to the low numbers of patients in this study, a greater use in ACEi in the cohort of patients with preserved LV function was observed at baseline and during treatment. This may suggest that ACEi could have a role in preventing LV dysfunction in patients undergoing anthracycline chemotherapy. The PROACT clinical trial will provide further insight into these unknowns.

To conclude, comprehensive assessment of all cardiac chambers during chemotherapy is feasible. At present, these should remain in the research domain and not detract from cancer care. In the PROACT trial echo sub-study, the utilisation of these measures will provide more insight into the clinical utility of parameters.

6. Publications and Poster Presentations

S Vahabi, E Kharati-Koopaei, M Norouzi, J Maddox, A Humphreys, H Hancock, A Zaman, D Austin, Right ventricular mechanics in anthracycline chemotherapy: insights into the PROACT PLUS study, *European Heart Journal - Cardiovascular Imaging*, Volume 23, Issue Supplement_1, February 2022, jeab289.418, https://doi.org/10.1093/ehjci/jeab289.418

S Vahabi, E Kharati-Koopaei, M Norouzi, J Maddox, A Humphreys, H Hancock, A Zaman, D Austin, Anthracycline chemotherapy and its effects on left ventricular mechanics: insights into the PROACT PLUS study, *European Heart Journal - Cardiovascular Imaging*, Volume 23, Issue Supplement_1, February 2022, jeab289.026, https://doi.org/10.1093/ehjci/jeab289.026

S Vahabi, E Kharati-Koopaei, M Norouzi, J Maddox, A Humphreys, H Hancock, A Zaman, D Austin, Atrial mechanics in anthracycline chemotherapy: insights into a prospective study, *European Heart Journal - Cardiovascular Imaging*, Volume 23, Issue Supplement_1, February 2022, jeab289.027, https://doi.org/10.1093/ehjci/jeab289.027

S Vahabi, E Kharati-Koopaei, M Stewart, H Hancock, M Norouzi, J Maddox, D Austin, The effects of doxorubicin on left and right atrial mechanics in patients with lymphoma, *European Heart Journal*, Volume 41, Issue Supplement_2, November 2020, ehaa946.3284, https://doi.org/10.1093/ehjci/ehaa946.3284

S Vahabi, M Stewart, A Kasim, H Hancock, M Norouzi, J Maddox, D Austin, P1383 The effects of doxorubicin on left and right ventricular strain in patients with lymphoma: insights from a retrospective study, *European Heart Journal - Cardiovascular Imaging*, Volume 21, Issue Supplement_1, January 2020, jez319.816, https://doi.org/10.1093/ehjci/jez319.816

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8. Appendices

8.1 Appendix 1 - Study Protocol 1

Detection of Anthracycline Induced Cardiotoxicity in Lymphoma

PROTOCOL

Full Title:	Detection of early anthracycline induced cardiotoxicity using speckle tracking echocardiography in patients with lymphoma: a retrospective cohort study
Short Title:	Detection of anthracycline induced cardiotoxicity in Lymphoma
Lay Title:	Can we detect early chemotherapy-related heart damage in patients with lymphoma?
Registry Identifiers:	IRAS ID 251233 ISRCTN84544539
Protocol version:	1.0, dated 18TH of July 2018
Funder:	South Tees Hospital NHS Foundation Trust
Chief Investigator:	Dr Sharareh Vahabi Clinical Research Fellow The James Cook University Hospital South Tees Hospitals NHS Foundation Trust, Marton Road, Middlesbrough, TS4 3BW Tel: 01642 850850
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1. Protocol Signatures

1.1 Authorisation Signatories:

Signature Date:

Dr Sharareh Vahabi, Chief Investigator

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Dr David Austin, Clinical Supervisor

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Dr Mike Stewart, Clinical Supervisor

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Dr Jamie Maddox, Consultant Haematologist

2. Background

Lymphoma is characterised by an abnormal growth in the lymphatic system and is considered the fifth most common cancer in Europe.[434, 435] In the late 1960s, owing to their excellent anti-tumour activities, anthracyclines were acknowledged as the cornerstone in the management of lymphomas and leukaemias.[13, 14, 434, 435] To date, their clinical use has revolutionised the outcome for patients, leading to improved cancer survival and prognosis.[436]

Despite their high efficacy in the management of malignancies, anthracyclines have been widely associated with dose-dependent toxicity affecting the cardiovascular system; a limiting factor in their use.[434-436] Cardiomyocyte apoptosis secondary to oxidative stress and mitochondrial dysfunction are thought to be the major mechanistic causes of anthracycline-induced cardiotoxicity,[13, 118, 119] with the clinical course ranging from transient asymptomatic left ventricular dysfunction to the development of chronic heart failure and even cardiovascular death.[30, 121, 434] The incidence of anthracycline related left ventricular dysfunction has been reported to range from 1-48% depending on the type and dose of anthracycline used.[24] Therefore, better means of detecting early anthracycline induced cardiotoxicity is required to facilitate diagnosis, and the development of strategies for early prevention or treatment for those patients affected.

Current guidelines have recommended the use of left ventricular ejection fraction (LVEF) monitoring using transthoracic echocardiography, in patients treated with cytotoxic drugs.[2, 4, 14, 24] However it is well established that the use of LVEF has major limitations with the temporal variability of this measurement being ~10%. This factor is considered a particular concern given the definition of cardiotoxicity relies on a decline in LVEF by 5-10%.[14, 35, 122] Furthermore, due to the sensitivity of this measurement to physiological factors which can affect the loading conditions of the heart, the true underlying left ventricular contractility can be masked.[14, 120]Most importantly, when a true reduction in LVEF is seen, cardiotoxicity is already established reducing the chance of full recovery, and opportunity for early intervention.[122-126] Given these downfalls of the measurement and the late manifestation of LVEF reduction in the pathophysiology of cardiotoxicity, better means of detection are required.

In the past couple of decades, Speckle Tracking Echocardiography (STE) has been established as a valid measure, and proposed as more objective when compared to the traditional methods of quantifying cardiac function.[114, 117, 127] STE has been widely applied in the assessment of different cardiovascular conditions and its use has gained increasing recognition in the field of

cardio-oncology owing to its potential to detect subclinical cardiac dysfunction before changes in LVEF are established.[13, 114]

Global longitudinal strain (GLS), which assesses the shortening and lengthening of the left ventricle along its' long axis using STE, has been shown to precede and therefore predict subsequent declines in the LVEF. So far, it has been the most studied and validated alternative measurement to LVEF, with an altered GLS considered a robust predictor of later development of cardiotoxicity.[13, 35, 115, 118, 119, 122, 162] Although GLS measurement is recommended in clinical practice, other domains of STE imaging are less validated.[13] Furthermore the effects of chemotherapy on the other chambers of the heart are not well established.

Table 1: Common chemotherapy regimens used in the treatment of lymphoma

Regimen	Type of Cancer	Description	No. of cycles	No. anthracycline cycles	Dose of anthracycline	Total dose of anthracycline
R-CHOP	Advanced Non-Hodgkin's Lymphoma and Hodgkin's lymphoma (nodular lymphocyte type)	Rituximab 375mg/m2, Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (for 1 to 5 days)	6	6	50mg/m2	300mg/m2
CHOP	Advanced Non-Hodgkin's Lymphoma	Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (for 1-5 days)	6	6	50mg/m2	300mg/m2
ABVD	Advanced Hodgkin's Lymphoma	**Adriamycin (doxorubicin)** 25mg/m2, Bleomycin 10,000 IU/m2, Vinblastine 6mg/m2, Dacarabazine 375/m2	6*	12*	25mg/m2	300mg/m2
Escalated BEACOPP	Advanced Hodgkin's Lymphoma (based on PET scan results)	Bleomycin 10,000 IU/m2, Etoposide 200mg/m2, **Adriamycin (doxorubicin)** 35mg/m2, Cyclophosphamide 1250mg/m2, Oncovin (vincristine) 1.4mg/m2 (max 2mg), Procarbazine 100mg/m2, Prednisolone 40mg/m2, Filgrastim 300mcg	4-6**	4-6**	35mg/m2	140mg/m2-210mg/m2

*Doxorubicin 25mg/m2 given 2 weekly x 12 doses (6 cycles of treatment – each cycle is a 4 week block)
**Esc BEACOPP either given from the outset due to high clinical risk (x6 Esc BEACOPP) or given due to a poor PET/CT after two cycles of ABVD (x2 ABVD, x4 Esc BEACOPPP)

Table 1 reveals the different chemotherapy regimens used in the UK, for the treatment of lymphoma. In the mid-1980s, CHOP (cyclophosphamide, doxorubicin, vincrisitine, and prednisolone) chemotherapy became the standard treatment for aggressive lymphomas.[435] However after demonstration of the superiority of the addition of R (rituximab) to CHOP, R-

CHOP has now been considered as the gold standard first line therapy for the treatment of aggressive Non-Hodgkin's lymphoma.[435, 437] Other anthracycline containing regimens are used in the treatment of other types of lymphoma which have been highlighted in table 1.

Given the high efficiency of anthracyclines in the treatment of lymphoma and other haematological and solid organ tumours, their use will continue to form an important part of treatment pathways.[437] Therefore, ability to detect cardiotoxicity secondary to these agents sooner can help identify those patients at risk and allow early instigation of treatment for those affected.

Assessment of anthracycline induced cardiotoxicity using STE has not been widely studied in patients with a diagnosis of lymphoma. Most studies to date have focused on LVEF measurement[434, 438-440] with only a minority of these using additional STE measurements such as GLS, GRS, GCS, twist and RV strain.[203, 206, 441-443] Given the small number of patients examined in these limited studies, results obtained have been somewhat conflicting and contradictory. Furthermore, no studies to date have assessed atrial strain in this cohort of patients.

3. Justification:

Owing to their excellent anti-tumour properties and improvement in cancer survival, anthracyclines will continue to play an important role in the treatment of cancer. However cardiotoxicity secondary to these agents remains an important issue in cancer survivorship. Hence, early detection of anthracycline induced cardiotoxicity is crucial to allow early appropriate measures to be taken for the management of those patients affected. A number of studies have explored if better measures of cardiotoxicity exist, and although the findings have been promising, further research is required in this field. Furthermore some advanced echocardiographic measurements have not been systematically assessed in the field of haematology with limiting and conflicting evidence for other measurements.

Therefore the "Detection of anthracycline induced cardiotoxicity in lymphoma" study has been designed to explore whether using advanced speckle tracking echocardiography can help detect early anthracycline induced cardiotoxicity in patients with lymphoma. This will allow the assessment of reliability and reproducibility of the findings, which can help inform the design of further prospective research studies in this field.

4. Aims and Objectives:

4.1 *Aims*

- To evaluate the role of advanced speckle tracking echocardiography in the early detection of anthracycline induced cardiotoxicity in patients treated with lymphoma
- To evaluate which patient specific factors increase the risk of developing anthracycline induced cardiotoxicity

4.2 *Objectives*

- To collect detailed information on a cohort of patients who have received anthracycline chemotherapy for the treatment of their lymphoma between January 2015 to January 2018
- To assess cardiac function in detail by speckle tracking echocardiography measuring LV GLS, global circumferential strain (GCS) and global radial strain (GRS), LV twist and torsion, right ventricular free wall strain RV FWLS, left and right atrial strains, and strain rates.
- To determine which measure, or combination of measurements, are most sensitive and specific for early cardiac damage, in the subset of patients whose LVEF, measured by Simpson's biplane method, has declined by >10% after anthracycline treatment and whether this was evident at an earlier time point.
- To assess which single or combination of measurements are better at detecting subclinical LV systolic dysfunction when compared to GLS
- To assess if any routinely available clinical or demographic factors are associated with echo changes following anthracycline chemotherapy

5. Protocol:

5.1 Design

- Retrospective
- Observational

5.2 Study period

- January 2015 to January 2018

5.3 Study population

Inclusion criteria

- Patients with a new diagnosis of histopathologically confirmed lymphoma between January 2015 to January 2018
- Patients who have received anthracycline based chemotherapy for the treatment of their lymphoma

Exclusion criteria

- No adequate echocardiographic imaging on PACS database
- Explicit dissent and unwillingness to participate in research detailed in the medical notes

6. Outcome Measures:

In addition to the full description of the population in the study, the following outcome will be measured:

- Cardiac function assessed by echocardiogram including measurement of LVEF, and measurement of all the novel echocardiographic strain parameters (ie. GLS, GRS, GCS, torsion and twist, RVFWL, left and right atrial strain and strain rates) on already performed echo scans done prior to chemotherapy, mid-treatment and post chemotherapy.

7. Research Setting:

This research study will be conducted at South Tees NHS Foundation Trust. The site is fully accommodated with research nurse support, and facilities to help with the conduction of the study. All echocardiograms that have been carried out as part of standard care will be re-analysed by a British Society of Echocardiography (BSE) accredited advanced imaging trainee, Dr Sharareh Vahabi. No additional patient imaging specifically for this study will be required.

15% of these scans will be checked by an Imaging Consultant Cardiologist or BSE accredited echocardiographer for validation.

8. Research Procedures:

The haematology team at James Cook University Hospital will identify those patients with a diagnosis of lymphoma, who have received anthracycline based chemotherapy between January 2015 to January 2018, through a computerised search of their haematology database. A list of patients deemed suitable for the study will then be forwarded to the Clinical Research Fellow, Dr Sharareh Vahabi, containing only patient hospital numbers.

8.1 Demographic Information

The following demographic data will be obtained and recorded from patients' medical records:

- Age
- Gender
- Ethnicity

8.2 Medical History

Information regarding patients' full medical history including a list of medication will be obtained from the medical records and recorded in a password protected excel sheet.

8.3 Echocardiograms

As part of standard care, patients will have undergone baseline, mid-treatment and post chemotherapy echocardiograms. These will be reviewed and post processing of images performed, will enable measurements using speckle tracking echocardiography. These measurements will be performed at The James Cook University Hospital (JCUH) echocardiography core laboratory. No hospital visits or investigations will be required for the purpose of this study.

9. JCUH Echo Core Laboratory:

One of the potential drawbacks of GLS, and other measures in STE, is inter-vendor variability. For the purpose of the "Detection of Anthracycline Induced Cardiotoxicity in Lymphoma" study, all measurements will be performed on the TOMTEC^{TM} work station at JCUH core echocardiography lab by a BSE accredited advanced imaging trainee, Dr Sharareh Vahabi.

TOMTEC^{TM} software allows for all the strain measurements mentioned to be performed in an offline fashion, and reduces the inter-vendor variability dramatically.

10. Data Collection

Data collection and analysis will be carried out by Dr Sharareh Vahabi who is bound by the rules of strict confidentiality. Each patient hospital number will be allocated a unique study ID which will be stored in a password protected excel sheet. Research data including the patient study ID, age, gender and ethnicity (but no specific patient identifiers) will be entered into a separate password protected excel sheet accessible by delegated members of the research team. Both sheets will be stored at The James Cook University Hospital.

Any data passed onto the statistical team for advice will not identify any individual patients and will be handled in a confidential manner. Results obtained from the data analysis will be presented in an aggregated manner.

Research data will be stored confidentially for a period of 15 years after the end of the registry, after which these will be securely and confidentially destroyed.

11. Statistical Analysis

Data cleaning and analysis will be provided by Dr Sharareh Vahabi. Guidance if required, will be obtained from the statistical team at Durham University. A full statistical analysis plan will be developed during data collection, but will include assessment of inter and intra-observer variability, description of changes in echo parameters with confidence intervals, and linear regression analysis for factors affecting changes in echo findings.

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

8.2 Appendix 2 - Ethical Approval 1

Dr Sharareh Vahabi
South Tees NHS Foundation Trust
Cardiology Department
The James Cook University Hospital
Marton Road, Middlesbrough
TS4 3BW

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

06 November 2018

Dear Dr Vahabi

HRA and Health and Care

Study title:	**Detection of early anthracycline induced cardiotoxicity using speckle tracking echocardiography in patients with lymphoma: a retrospective cohort study**
IRAS project ID:	**251233**
REC reference:	**18/SS/0139**
Sponsor:	**South Tees NHS Foundation Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales? You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

This is a single site study sponsored by the site. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your nonNHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including: Registration of research

- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?

You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Sharareh Vahabi
Tel: 01642 850 850
Email: sharareh.vahabi@nhs.net

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **251233**. Please quote this on all correspondence.

Yours sincerely

Michael Higgs
Assessor

Copy to: Mr Joe Millar, South Tees NHS Foundation Trust (Sponsor and NHS R&D office) **List of Documents**

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	Version	Date
Covering letter on headed paper		23 August 2018
IRAS Application Form [IRAS_Form_05102018]		05 October 2018
Other [Confirmation of local CG approval]		31 October 2018
Other [Evidence of indemnity for non-NHS collaborators]		01 August 2018
Research protocol or project proposal	1.0	18 July 2018
Response to Additional Conditions Met		31 October 2018
Summary CV for Chief Investigator (CI) [Dr Vahabi]		
Summary CV for supervisor (student research) [Dr Austin]		
Summary CV for supervisor (student research) [Dr Stewart]		
Summary CV for supervisor (student research) [Prof Hnacock]		

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/ consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	This is a single site study sponsored by the site. Study specific agreements are not expected to be used.
4.2	Insurance/ indemnity arrangements assessed	Yes	No comments
4.3	Financial arrangements assessed	Yes	No application for external funding has been made.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	Only members of the care team will be involved in accessing personal data, including any processing for the purpose of pseudonymisation.
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments

6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is a single participating NHS organisation which is also the sponsor. If this study is subsequently extended to other NHS organisation(s) in England or Wales, an amendment should be submitted, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England or Wales. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Principal Investigator should be in place for all participating NHS organisations in England and Wales. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken

All research activity at participating NHS organisations in England and Wales will be conducted by members of the local care team for the relevant patient population. Therefore, access arrangements and pre-engagement checks are not expected to be relevant for this study.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

8.3 Appendix 3 - Chapter 3 results

1. Changes in echocardiographic measures in all patients

1.1 Conventional Echocardiography Measures

Table 1. Changes in LV systolic and diastolic measures

Variable	All patient								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
LVIDd (cm)	4.57	4.63	4.75	-0.014	0.84	0.038	0.02*	0.18	0.008*
LVIDs (cm)	2.61	2.86	3.02	0.07	0.07	0.06	0.11	0.13	<0.001*
FS (%)	42.7	38.3	37.3	-3.764	0.04*	-0.048	0.33	5.35	0.001*
LV mass indexed (g/m²)	84.9	87.4	90.5	0.016	0.63	0.049	0.14	0.06	*0.03
LV RWT (%)	0.42	0.43	0.42	0.03	0.31	-0.03	0.24	-0.005	0.86
LA diameter (cm)	3.4	3.4	3.5	-0.058	0.39	0.036	0.09	0.073	0.23
LA volume indexed (ml/m²)	23.6	23.4	22.1	-0.005	0.92	-0.05	0.30	-0.05	0.25
LVEDV indexed (ml/m²)	42.2	39.4	43.2	-3.523	0.006*	0.1	0.001*	0.816	0.47
LVESV indexed (ml/m²)	14.5	15.4	18.4	0.02	0.56	0.171	<0.001*	0.19	<0.001*
LVEF (%)	65.4	60.2	58.1	-0.08	*0.001	-0.049	0.03*	-0.13	<0.001*
MV E (m/s)	0.68	0.61	0.65	-0.10	0.03*	0.05	0.30	-0.05	0.2
MV DecT (ms)	163	144	148	-20.08	0.06	0.032	0.66	-14.6	0.13
MV E/A	0.83	0.77	0.80	-0.09	0.03*	0.048	0.28	0.048	0.24
Lateral E/E’	6.7	6.3	7.01	-0.07	0.01*	0.082	0.17	-0.05	0.35
Medial E/E’	10.2	8.9	10.1	-0.15	0.02*	0.121	0.06	-0.04	0.56
Mean E/E’	7.9	7.5	8.5	-0.046	0.44	0.111	0.06	0.06	0.23
IVRT (cm)	84.9	93.3	94.8	6.875	0.18	0.069	0.54	9.89	0.03*
TEI index	0.54	0.54	0.59	0.008	0.89	0.073	0.23	0.08	0.12

Table 2. Changes in RV systolic and diastolic measures

Variable	All patient								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
RA volume indexed (ml/m^2)	17.6	17.3	20.64	0.026	0.72	0.105	0.17	0.13	0.07
RVD1 (cm)	3.5	3.7	3.7	0.132	0.22	-0.012	0.71	0.099	0.33
RVD2 (cm)	2.9	3.0	3.0	0.082	0.42	-0.016	0.64	0.05	0.59
RV S' (m/s)	0.12	0.13	0.12	0.103	0.15	-0.145	0.05	-0.042	0.49
RV EDA (cm^2)	15.9	16.0	15.6	0.047	0.25	-0.064	0.12	-0.017	0.67
RV ESA (cm^2)	8.7	9.3	9.3	0.107	0.04*	-0.048	0.36	0.06	0.23
RV FAC	44.9	42.8	41.4	-2.558	0.17	-0.038	0.44	-3.572	0.05
TAPSE (cm)	2.2	2.1	2.0	-0.05	0.21	-0.054	0.12	-0.1	0.003*
RV TEI index	0.64	0.62	0.63	-0.02	0.74	0.015	0.84	-0.01	0.88
RV IVRT (ms)	78.5	73.6	76.8	-6.88	0.42	0.07	0.54	-3.18	0.96

**Statistically significant ($p < 0.05$)*

1.2 Strain Measures

1.2.1 Left and Right Ventricular Strain

Table 3. Changes in LV strain and strain-rate measures

Variable	All patient								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
GLS (%)	-21.4	-19.9	-17.9	1.59	0.18	1.95	0.09	3.54	0.002*
MyoGLS (%)	-18.5	-16.1	-15.6	2.34	0.06	0.59	0.62	2.93	0.01*
LV peak systolic longitudinal SR(1/s)	-1.18	-1.06	-1.03	0.10	0.01*	0.06	0.16	0.15	<0.001*
LV end-systolic longitudinal SR (1/s)	-0.04	0.07	-0.06	0.078	0.19	-0.09	0.10	-0.017	0.75
GRS (%)	35.9	32.02	32.4	-0.08	0.06	-0.006	0.88	-0.09	0.03*
LV peak systolic radial SR (1/s)	1.49	1.39	1.18	-0.09	0.06	-0.02	0.65	-0.11	0.01*
LV end-systolic radial SR (1/s)	0.10	-0.15	0.14	-0.178	0.17	0.22	0.08	0.044	0.707
GCS (%)	-30.7	-28.6	-25.4	1.60	0.27	3.31	*0.03	4.09	0.001*
MyoGCS (%)	-23.4	-21.7	-19.9	1.26	0.33	1.89	0.16	3.15	0.02*

**Statistically significant ($p < 0.05$)*

Table 4. Changes in RV strain and strain-rate measures

Variable	All patient								
	Mean at T0	**Mean at T1**	**Mean at T2**	**Change from T1 to T0**	**p value**	**Change from T2 to T1**	**P value**	**Change from T2 to T0**	**P value**
RV GLS (%)	-24.3	-22.6	-20.9	1.617	0.01*	1.68	0.007	3.304	<0.001*
RV myoGLS (%)	-22.3	-20.9	-19.3	1.478	0.02*	1.57	*0.01	3.049	<0.001*
RV peak systolic longitudinal SR (1/s)	-1.44	-1.23	-1.14	0.195	0.004*	0.12	0.11	0.297	<0.001*
RV end-systolic longitudinal SR (1/s)	-0.11	0.01	-0.16	0.116	0.23	-0.16	0.09	-0.044	0.62
RV FWS (%)	-26.9	-24.1	-22.9	2.058	0.03*	1.69	0.07	3.748	<0.001*
RVFW peak systolic longitudinal SR (1/s)	-1.69	-1.39	-1.33	0.279	$8x10^{-4}$*	1	0.29	0.355	<0.001*
RVFW end-systolic radial SR (1/s)	-0.08	-0.01	-0.15	0.069	0.47	-0.13	0.15	-0.065	0.47

**Statistically significant ($p < 0.05$)*

1.2.2 Left and Right Atrial Strain

Table 5. Changes in left atrial strain and strain-rate measures

Variable	All patient								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
LA 4Ch strain (%)	31.8	26.4	26.6	-0.17	0.01	0.008	0.91	-0.16	0.02*
LASr 4Ch (%)	38.9	34.5	32.9	-3.106	0.09	-1.82	0.34	-4.928	0.01*
LAScd 4Ch (%)	-21.3	-16.9	-17.6	4.421	0.01*	-0.81	0.65	3.605	0.04*
LASct 4Ch (%)	-17.9	-17.8	-15.6	-0.421	0.82	2.23	0.26	1.802	0.35
LA 4Ch peak systolic SR (1/s)	1.45	1.38	1.18	-0.05	0.67	-0.18	0.03*	-0.23	*0.004
LA 4Ch early diastolic SR (1/s)	-1.21	-1.12	-0.99	0.123	0.24	0.11	0.30	0.234	0.03*
LA 4Ch late diastolic SR (1/s)	-1.62	-1.70	-1.44	-0.056	0.75	0.29	0.12	0.231	0.20
LA 2Ch strain (%)	28.9	29.0	25.9	-0.03	0.68	-0.09	0.21	-0.12	0.10
LASr 2Ch (%)	36.6	36.4	33.9	-0.828	0.69	-2.4	0.24	-3.238	0.12
LAScd 2Ch (%)	-17.1	-15.7	-15.8	1.375	0.50	0.14	0.95	1.511	0.47
LASct 2Ch (%)*	-18.5	-20.7	-18.4	-2.142	0.28	2.2	0.27	0.101	0.96
LA 2Ch peak systolic SR (1/s)	1.40	1.38	1.27	-0.05	0.66	-0.07	0.51	-0.12	0.28
LA 2Ch early diastolic SR (1/s)	-0.89	-1.06	-0.86	-0.185	-0.27	0.21	0.17	0.032	0.85
LA 2Ch late diastolic SR (1/s)	-1.79	-1.94	-1.83	-0.096	0.60	0.19	0.50	0.022	0.90
LA biplane strain (%)	29.7	26.6	25.7	-3.093	0.08	-0.84	0.63	-3.93	0.02*
LASr biplane (%)	37.1	34.6	31.9	-2.26	0.18	-2.2	0.19	-4.465	0.01*
LAcd biplane (%)	-18.1	-17.1	-16.1	0.926	0.58	0.95	0.56	1.881	0.28

LA biplane peak systolic SR (1/s)	1.43	1.28	1.2	-0.12	0.30	-0.14	0.24	-0.26	*0.04
LA early diastolic SR (1/s)	-1.04	-1.1	-0.96	-0.96	-0.13	0.21	0.06	0.08	0.47
LA late diastolic SR (1/s)	-1.7	-1.7	-1.5	0.13	0.44	0.17	0.32	0.30	0.10

**Statistically significant (p < 0.05)*

Table 6. Changes in right atrial strain measures

Variable	**All patient**								
	Mean at T0	**Mean at T1**	**Mean at T2**	**Change from T1 to T0**	**p value**	**Change from T2 to T1**	**P value**	**Change from T2 to T0**	**P value**
RA strain (%)	30.3	26.8	26.2	-0.11	0.03*	-0.02	0.66	-0.13	0.008*
RASr (%)	38.3	35.8	34.3	-2.853	0.14	-0.85	0.64	-3.712	0.04*
RAScd (%)	-21.6	-18.8	-18.3	2.8	0.19	0.36	0.87	3.158	0.13
RASct (%)	-17.9	-17.8	-17.3	0.299	0.87	0.40	0.83	0.704	0.70
RA peak systolic SR (1/s)	1.51	1.24	1.22	-0.26	0.01*	-0.01	0.86	-0.28	0.008*
RA early diastolic SR (1/s)	-1.03	-1.05	-0.95	-0.023	0.86	0.09	0.49	0.076	0.59
RA late diastolic SR (1/s)	-1.93	-1.80	-1.71	0.137	0.42	0.06	0.69	0.205	0.26

**Statistically significant (p < 0.05)*

2. Changes in Echocardiographic measures between groups

2.1 Conventional Echocardiography Measures

Table 7. Changes in LV systolic and diastolic measures

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
LVIDd (cm) G1	4.5	4.6	4.7	0.003	0.89	0.032	0.11	0.035	0.05
G2	4.8	4.8	4.9	-0.009	0.76	0.053	0.10	0.043	0.16
LVIDs (cm) G1	2.5	2.8	2.9	0.09	0.04*	0.036	0.43	0.13	0.002*
G2	2.9	3.0	3.4	0.01	0.86	0.129	0.11	0.142	0.07
FS (%) G1	44.1	38.6	39.1	-4.86	0.02*	-0.13	0.95	-5	0.009*
G2	38.4	37.6	32.3	-0.012	0.89	-0.161	0.095	-0.173	0.06
LV mass indexed (g/m²) G1	82.7	84.3	89.9	0.01	0.76	6.4	0.06	0.07	0.03*
G2	91.8	94.4	92.1	0.022	0.75	0.02	0.77	0.042	0.53
LV RWT (%) G1	0.43	0.43	0.43	0.009	0.81	0.002	0.89	0.005	0.88
G2	0.40	0.42	0.39	0.086	0.20	-0.116	0.08	-0.03	0.64
LA diameter (cm) G1	3.4	3.3	3.4	-0.019	0.45	0.133	0.10	0.017	0.43
G2	3.5	3.6	3.6	0.001	0.98	0.038	0.34	0.039	0.32
LA volume (ml/m²) G1	23.6	23.5	22.0	-0.003	0.95	-1.54	0.22	-0.07	0.15
G2	23.7	23.4	22.4	-0.01	0.92	0.019	0.85	0.009	0.93
LVEDV indexed (ml/m²) G1	40.6	37.2	41.8	-0.09	0.01*	4.67	0.002*	0.01	0.70
G2	47.1	43.2	47.1	-0.094	0.10	0.084	0.12	-0.01	0.85
LVESV (ml/m²) G1	13.4	13.6	16.5	0.02	0.72	2.64	0.01*	0.17	0.003*
G2	18	18.4	23.5	0.038	0.53	0.24	<0.001*	0.28	<0.001*
LVEF (%) G1	66.6	61.6	61.1	-0.07	0.008*	-0.53	0.74	-0.09	<0.001*
G2	62.1	57.6	49.7	-0.077	0.02*	-0.146	<0.001*	-0.222	<0.001*
MV E (m/s)	0.67	0.62	0.66	-0.09	0.15	0.05	0.25	-0.018	0.74

G1									
G2	0.71	0.59	0.61	-0.145	0.05	-0.009	0.89	-0.154	0.03*
MV DecT (ms) G1	171	158	159	-18.4	0.18	7.25	0.60	-11.1	0.35
G2	138	118	115	-20.5	0.22	-2.8	0.86	-23.4	0.17
MV E/A G1	0.85	0.81	0.83	-0.07	0.23	0.04	0.37	-0.03	0.55
G2	0.78	0.69	0.73	-0.13	0.06	0.04	0.55	-0.09	0.18
Lateral E/E' G1	6.7	6.3	6.9	-0.06	0.44	0.67	0.23	0.009	0.89
G2	6.8	6.3	7.0	-0.056	0.55	0.114	0.20	0.057	0.54
Medial E/E' G1	10.1	8.3	9.7	-0.21	0.01*	1.61	0.03*	-0.06	0.39
G2	10.4	9.8	10.4	-0.055	0.61	0.103	0.28	0.048	0.67
Mean E/E' G1	7.9	7.3	8.5	-0.06	0.40	1.13	0.06	0.05	0.43
G2	8.0	7.8	8.6	-0.006	0.94	0.108	0.21	0.102	0.26
IVRT (cm) G1	88.8	98.2	97.0	0.10	0.10	-0.37	0.94	0.08	0.15
G2	74.8	72.2	87.7	-5.11	0.69	16.4	0.24	11.3	0.30
TEI index G1	0.54	0.55	0.58	0.007	0.91	0.02	0.57	0.03	0.63
G2	0.50	0.49	0.61	-0.006	0.96	0.223	0.12	0.217	0.08

**Statistically significant ($p < 0.05$)*

Table 8. Changes in RV systolic and diastolic measures

Variable	Mean changes in variables between visits								
	Mean at T0	Mean at T1	Mean at T2	Change from T1 to T0	p value	Change from T2 to T1	P value	Change from T2 to T0	P value
RA volume indexed (ml/m²) G1	18.1	18.7	22.0	0.09	0.36	0.058	0.58	0.151	0.13
G2	16.4	14.3	17.2	-0.109	0.16	0.202	*0.02	0.093	0.23
RVD1 (cm) G1	3.5	3.7	3.7	0.068	0.11	-0.009	0.83	0.059	0.12
G2	3.7	3.6	3.6	-0.01	0.73	-0.027	0.49	-0.04	0.32
RVD2 (cm) G1	2.9	3.0	3.0	0.07	0.12	-0.038	0.42	0.033	0.40
G2	3.1	3.0	3.1	-0.03	0.56	0.01	0.84	-0.02	0.70
RV S' (m/s) G1	0.12	0.14	0.12	0.115	0.22	-0.128	0.17	-0.013	0.86
G2	0.12	0.13	0.10	0.06	0.50	-0.193	0.07	-0.13	0.18
RV EDA (cm²) G1	15.5	16.5	15.8	0.109	0.02*	-0.113	0.035	-0.004	0.93
G2	17.2	15.2	15.1	-0.079	0.30	-0.009	0.89	-0.088	0.26
RV ESA (cm²) G1	9.0	9.8	9.7	0.113	0.06	-0.099	0.13	0.015	0.79
G2	7.7	8.6	8.2	0.135	0.21	0.006	0.95	0.141	0.20
RV FAC (%) G1	41.9	41.8	39.9	0.032	0.60	-0.099	0.13	-0.029	0.61
G2	54.9	44.2	44.6	-0.222	0.004*	-0.012	0.83	-0.234	0.003*
TAPSE (cm) G1	2.2	2.1	2.1	-0.012	0.77	-0.019	0.63	-0.031	0.37
G2	2.3	2.1	1.8	-0.12	0.06	-0.139	0.02*	-0.259	<0.001*
RV TEI index G1	0.63	0.57	0.63	-0.063	0.49	0.087	0.34	0.023	0.76
G2	0.65	0.70	0.62	0.07	0.56	-0.147	0.28	-0.072	0.55
RV IVRT (ms) G1	74	68	75	-0.074	0.57	0.136	0.29	0.063	0.56
G2	88	86	81	-0.147	0.49	-0.101	0.64	-0.247	0.25

**Statistically significant ($p < 0.05$)*

8.4 Appendix 4 - Study Protocol 2

PROACT PLUS REGISTRY & ECHOCARDIOGRAPHY SUB-STUDY PROTOCOL

South Tees Hospitals NHS
NHS Foundation Trust

Newcastle University

PROTOCOL

Full Title:	PROACT PLUS Registry and Echocardiographic substudy: An observational, prospective, cohort study assessing the use of novel echocardiographic tools and measurement of troponin to help detect early signs of cardiotoxicity in patients treated for breast cancer and lymphoma
Short Title:	PROACT PLUS Registry and Echo Sub-study
Lay Title:	PROACT PLUS Registry and Echo Sub-study: Can we detect early chemotherapy-related heart damage in patients with breast cancer and lymphoma?
Registry Identifiers:	ClinTrials.gov: ISRCTN11676341 IRAS ID: 245613
Protocol version:	2.0, dated 10th of August 2018
Funder:	South Tees Hospital NHS Foundation Trust
Chief Investigator:	Dr Sharareh Vahabi Clinical Research Fellow The James Cook University Hospital South Tees Hospitals NHS Foundation Trust, Marton Road, Middlesbrough, TS4 3BW Tel: 01642 850850
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Protocol Authors:	Vahabi S, Hancock H, Humphreys A, Graham J, Stewart M, Austin D

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1. PROTOCOL SIGNATURES

1.1 Authorisation Signatories:

Signature Date:
Dr Sharareh Vahabi, Chief Investigator

Signature Date:
Dr David Austin, Clinical Supervisor

Signature Date:
Dr Mike Stewart, Clinical Supervisor

Signature Date:
Professor Helen Hancock, Academic Supervisor

1.2 Principal Investigator Signature

By signing this protocol page, I confirm I have read and agree to:
Conduct the trial in accordance with the protocol, and the principles of GCP and the appropriate regulations
Personally conduct and supervise the registry and echo sub-study and ensure that all colleagues assisting with the trial are appropriately delegated and are informed about their obligations
Ensure that the requirements with regard to obtaining informed consent are adhered to without exception
Report all AEs and SAEs that occur during the registry period, in accordance with the protocol
Principal Investigator's Name:

Principal Investigator's Signature: Date:

2. INTRODUCTION

2.1 Synopsis

This protocol describes the **PROACT PLUS registry and echocardiographic sub-study**, which are complimentary projects related to the NIHR funded PROACT clinical trial (see section 3.1 and PROACT protocol). This document describes the study processes for the **PROACT PLUS registry**, which will mirror the assessments performed in the PROACT clinical trial (see section 6.0). The document also describes the additional novel echocardiographic measures that will be undertaken beyond those being performed as part of the PROACT clinical trial. This is the **PROACT echocardiographic sub-study**. The additional echo measures will be undertaken on echo studies that have been collected both as part of the PROACT clinical trial and PROACT PLUS registry. This protocol describes both aspects and will ultimately allow comparison between the PROACT trial and PROACT PLUS registry populations to meet the study objectives (see section 5.0).

2.2 Background

Cancer is considered the leading cause of the death worldwide. Lymphoma, characterised by an abnormal growth of the lymphatic system, and breast cancer are both frequent forms of cancer affecting individuals across the world. [1-3] For the past number of decades, chemotherapy has been the mainstay treatment for different types of cancer leading to improvement in cancer prognosis and survival. Owing to their excellent anti-tumour properties, anthracyclines have been acknowledged as the cornerstone in the management of a wide range of malignancies, in particular breast cancer and lymphoma. Anthracyclines were first discovered in the 1950s after extraction of daunorubicin from the soil bacterium, Streptomyces peucetius. It was not until the 1960s when these agents were found to be remarkably effective in the treatment of leukaemias and lymphomas.[4, 5] Their substantial influence on the outcome of patients with cancer has been evident throughout the past 15 years.[5-8]

As with all cytotoxic chemotherapy, benefits come at the cost of adverse effects. Anthracyclines are associated with cardiotoxicity which is the limiting factor in their use. Cardiomyocyte apoptosis secondary to oxidative stress and mitochondrial dysfunction are thought to be the major mechanistic causes of anthracycline-induced cardiotoxicity;[4, 10, 11] a continuous phenomenon manifesting itself as left ventricular systolic dysfunction and later heart failure.[12, 13] Although the adverse effects of these agents are more pronounced with higher cumulative doses, histopathological changes have been evident in the endomyocardial biopsies of patients receiving lower doses, suggesting there is no safe dose.[4, 14-17] Cardiac dysfunction can be prognostically important in its own right, but can also limit the use of other anti-cancer chemotherapies such as Herceptin.[9] Therefore early detection of subclinical myocardial dysfunction is crucial in the better understanding of anthracycline-induced cardiotoxicity and the development of strategies for prevention or early treatment.

2.3 Echocardiography and cardiotoxicity

Detection of anthracycline-induced cardiotoxicity has been dependant on serial cardiac imaging to identify a reduction in the left ventricular ejection fraction (LVEF). Several consensus statements and guidelines focusing on cardio-oncology have recommended the use of transthoracic echocardiography surveillance, owing to its wide availability, cost effectiveness and evidence base.[16] Conventionally, a reduction in LVEF of ≥5% to <55% with symptoms of heart failure[3, 7]or an asymptomatic drop in the LVEF of ≥10% and to below the normal range (<53%) has been regarded as echocardiographic evidence of cardiotoxicity.[1, 8] This measurement has to be confirmed by repeat echocardiography after a few weeks of the initial scan, before a decision on chemotherapy is made, such as stopping a cardiotoxic agent or starting treatment for LV dysfunction.[1, 8]

However, it is well established that use of LVEF has major limitations in this setting.[1, 3, 9-12] For example, the technique is subject to a moderate level of inter- and intra-observer variability[5, 7, 13-16] and the temporal variability of this measurement has also been found to be ~10%. These factors are of particular concern given the definition of cardiotoxicity relies on a decline in LVEF by 5-10%.[5, 17, 18] Furthermore, LVEF measurement is sensitive to physiological factors creating variability in the loading conditions of the heart, masking the true underlying contractility of the left ventricle.[5, 19] Crucially, when a true reduction in LVEF is seen, cardiotoxicity is already established, the chance of full recovery is low and the opportunity for early intervention has already been missed.[18, 20-23] Clearly with this degree of variation in measurement, and the late manifestation of LVEF reduction in the pathophysiology of cardiotoxicity, better methods of detection are required.[11]

2.3.1 Myocardial Deformation

A number of studies conducted in cardio-oncology have focused on novel echocardiographic-derived measures of myocardial mechanics, namely strain and strain-rate, providing an insight into more accurate measurements of cardiac function.[12, 22, 24] "Strain" denotes deformation which measures local shortening and thickening of the myocardium during stress at end-systole compared to its original length in a relaxed state at end-diastole.[25] Due to the fractional change in the myocardial length, this is expressed as a percentage which can be negative or positive indicating shortening or lengthening, respectively.[1, 11, 12, 25-27] Strain measurements focus on myocardial velocity, displacement and deformation to quantify regional and global systolic and diastolic function.[12]
"Strain rate" is the speed at which deformation occurs with respect to time and has a unit of 1/s.[1, 11, 12, 26-30]

Tissue Doppler Imaging (TDI), and more recently Speckle Tracking Echocardiography (STE), have been established as valid measures of strain and are proposed as more objective, when compared to traditional methods, in quantifying cardiac function.[9, 24, 25] However, TDI has several major limitations in this context, in particular the dependency on the Doppler angle of incidence; only velocities parallel to the ultrasound beam can be measured with this technique. TDI has therefore been superseded by STE, allowing a more comprehensive assessment of myocardial deformation, independent of the Doppler angle.[1, 9, 27, 28]

Since its introduction, STE (also known as two-dimensional strain analysis (2DS)) has been widely applied in the assessment of different cardiovascular conditions.[4] However in the recent years, STE has gained increasing recognition in the field of cardio-oncology owing to its potential to detect subclinical cardiac dysfunction before changes in LVEF are established.[21] "Speckles" are natural acoustic markers formed by the grey scale ultrasound interference patterns within the myocardial tissue.[12, 25, 27, 29, 30] The movement of these speckles, identified in discrete sections of the myocardium, can be followed ("tracked") frame by frame throughout the cardiac cycle enabling the differentiation between active thickening and passive wall motion.[12, 28, 31] Using this method, STE has the ability to track speckles in two dimensions, along the direction of the wall rather than along the ultrasound beam.[1, 21, 25, 28, 31, 32]

Other advantages of STE, when compared to TDI, include better spatial resolution, less sensitivity to signal noise and the use of lower frame rate when acquiring the images.[26] STE is semi-automated, and as a consequence has better measurement reproducibility. It is quick to perform, user friendly with straightforward data processing.[26] However, STE requires high resolution image quality for accurate measurements and this can be a limitation in some patients.[9, 26] Nevertheless, a number of validation studies have proven the consistency of STE when compared to other modalities with reasonable intra- and inter-observer variability (<8% and <6% respectively).[4, 9, 21]

2.3.2 Types of Strain

2.3.2.1 Global Longitudinal Strain

Given the different orientation of the myocardial fibres and the complex multi-dimensional deformation that the left ventricle (LV) undergoes during the cardiac cycle, three principle types of LV strain are described by STE: longitudinal, radial and circumferential.[3] Longitudinal strain represents the shortening of the LV along its long axis, radial strain denotes the thickening of the LV wall along its radius and circumferential strain relates to the reduction in the LV cavity circumference during the cardiac cycle. Beyond these linear deformation measurements, peak systolic LV torsion by STE, is a further measurement that focuses on the myocardial rotational deformation owing to the helical orientation of the myocardial fibres.[21, 32-34]

Abnormalities in strain and strain-rate, have shown an association with chronic heart failure prognosis.[10, 11, 27, 35] This finding is particularly strongly observed for global longitudinal strain (GLS), which is a combined measure of LV regional longitudinal strains.[17, 36] In relation to chemotherapy induced cardiotoxicity, GLS has shown to precede and therefore predict subsequent declines in the LVEF and hence has been the most studied and validated measurement so far.[4, 8, 10, 11, 17, 18, 36] An altered GLS is an independent and robust predictor of later cardiotoxicity, with a 10-20% reduction observed amongst studies during treatment.[17, 18, 21] A relative decrease of >15% in GLS has been identified as evidence of anthracycline-induced cardiotoxicity.[3, 8, 17, 21, 27, 37]

Although GLS is recommended in clinical practice,[6, 27] the other domains of strain imaging are less well validated.[4] Furthermore, the effects of chemotherapy on other chambers of the heart (such as RV and LA) are not well established.

2.3.2.2 Radial and Circumferential Strain

In the context of chemotherapy induced cardiotoxicity, a number of studies have assessed the application of global radial (GRS) and global circumferential (GCS) strain in addition to GLS.[4, 10, 11, 18, 23, 34, 38] Of these, some identified that global radial strain could be seen to reduce after 1 week to 3 months of administration of anthracyclines and could potentially be considered a robust parameter in detecting early myocardial damage during chemotherapy.[23, 39-42] However, other studies have been conflicting, failing to prove such findings and have demonstrated GRS to not be predictive of future toxicity.[11, 19, 34, 43] This has also been the case for GCS, with studies revealing contradictory results.[4, 10, 11, 22, 34, 38, 43] These results have been attributed to a lower reproducibility of these measurements,[4, 17, 18] and therefore the use of these parameters in routine clinical practice has not yet been validated.

2.3.2.3 Torsion and Twist

Since the early 1990s, a number of studies assessed "torsion" using tagged magnetic resonance imaging (tMRI).[32, 44-49] However due to the high cost and complex data analysis processing of tMRI, this technique has not gained widespread use.[32] However, with the recent development of STE, torsion has once again gained interest. Torsion has been widely used in the assessment of different cardiac conditions.[19, 34, 50, 51]

During systole, owing to the helical myocardial fibre architecture, the base of the LV rotates in a clockwise pattern, with the apex demonstrating a counter-clockwise rotation.[32-34, 50, 52] "Twist" is the absolute angle difference between LV base and apex, measured in degrees.[12, 50] "Untwist" is the reverse of this phenomenon, during diastole.[50] As a consequence, a torsional deformation is created leading to a dynamic interaction between the opposing epicardial and endocardial myocardial fibre helices.[19, 33, 52] LV torsion is expressed in degrees/radians per centimetre, and is measured by dividing the twist angle by the distance between the cross-sectional planes of the LV base and apex.[50, 52]

In patients with cardiomyopathy, abnormalities in the twist and untwist measurements have been clearly seen providing evidence that these measurements can deliver mechanistic information in the assessment of myocardial diseases.[50, 51]

In relation to chemotherapy induced cardiotoxicty one study[34] demonstrated that with cumulative doses of anthracyclines, torsional deterioration can be seen;[48] thus torsion may be a useful parameter in early detection of anthracycline induced subclinical LV dysfunction.[19, 34] The addition of GLS to LV twist has also been shown to improve the prediction of cardiotoxicity in one other study.[19]

2.3.2.4 Right ventricular (RV) function and RV strain

In the 1970s, anthracycline induced cardiotoxicity was diagnosed by means of obtaining endomyocardial biopsies from left and right ventricles of patients who had been receiving these agents.[53, 54] Histological analysis of these biopsies proved that anthracyclines can affect

both ventricles.[53, 54] Given the invasive nature of endomyocardial biopsy, non invasive methods for diagnosing and monitoring the effects of the anthracyclines have been preferred in the modern era. Most studies of anthracycline cardiotoxicity have focussed on the left ventricle with only limited number of these assessing the right ventricle,[18, 37, 55-58] hence sometimes termed the "forgotten chamber".[59, 60] The relative lack of study could be due to the crescentic anatomic and morphological structure of the RV, which complicates its full assessment by conventional echocardiography.[37] However, a number of studies have being able to detect changes in the RV in the context of anthracycline treatment.[37, 61, 62] More recently, RV free wall longitudinal strain (RVFWLS) has demonstrated prognostic value in some cardiovascular conditions and is emerging as a tool for the detection of subclinical myocardial dysfunction.[18, 43, 63-67] Therefore the full assessment of the right ventricle and the use of RVFWLS has been recommended, in an updated American and European guidelines on the chamber quantification.[18, 24]

2.3.2.5 Left Atrial Strain

The left atrium plays an important role in the cardiovascular function and contributes 20-30% to the total LV stroke volume, or even higher in the setting of LV dysfunction.[68, 69] It is now well established that enlargement of the left atrium, is an independent predictor of adverse cardiovascular outcomes.[68, 70-74] More recently, LA strain and function has been studied predominantly in the setting of valvular heart disease and in prediction of atrial arrhythmias.[75, 76] However, data on the measurement of these parameters in the context of chemotherapy has been scarce. In one study,[77] which looked at LA function in breast cancer patients after the administration of anthracyclines, the intra- and inter mechanical delays, which are well known to be electrophysiological features of the atrium prone to atrial fibrillation, were found to be prolonged. This could potentially put these patients at risk of atrial arrhythmias and hence increasing their morbidity and mortality.[77] Furthermore, preliminary data has suggested that 2D STE measurement of peak LA strain, may be a method for measuring instantaneous LA pressure which could potentially be useful.[27] Given the lack of data in this field, measuring left atrial function and strain could provide some insight into the effect of anthracyclines on this cardiac chamber, which plays a fundamental role in maintaining cardiac function.[77]

2.3.2.6 Right Atrial Strain

The right atrium has also been relatively neglected in the assessment of cardiac function. It was only after 1979, when Bloomer et al was the first to have measured its dimensions, when this chamber gained some interest.[78] However, even then its purpose was mainly studied in mass lesions or electrophysiological assessments. The role this chamber played in the right heart systolic and diastolic function was not fully explored.[78] Nevertheless, recently, there have been some studies that have assessed the right atrium, its function and strain, mainly in relation to the evaluation of patients with pulmonary arterial hypertension (PAH).[79, 80] Despite the sparse literature available, the results from these studies have demonstrated the usefulness of right atrial function and strain measurements and how these could potentially add valuable information in the assessment of right heart function and be predictive of clinical outcomes in PAH.[35, 80-83] Furthermore, right atrial strain

measurement as an adjunct to simultaneous strain assessment of the other chambers could provide new insight into inter-chamber relationships.[35]
Right atrial function/strain has not yet been studied in the context of chemotherapy induced cardiotoxicity. Given the evidence that anthracyclines could affect the right heart function in addition to the left ventricle, the use of this measurement could add supplementary information about the changes the right heart undergoes during treatment with these agents.

2.4Troponin and Cardiotoxicity

The use of biochemical markers for the detection of possible myocardial damage was initially introduced in the early 1950s by Karmen et al, when an increase in the levels of serum glutamate oxaloacetate transaminase (now aspartate transaminase) in those patients presenting with acute myocardial infarction was noted.[84] This finding led to more stimulating attempts to aid identify better markers of myocardial damage (e.g lactate dehydrogenase, creatinine kinase and their isoenzymes). However due to the lack of specificity and sensitivity of these biomarkers, it was not until the 1980s when the attention of the researchers shifted towards the myofibrillar proteins of the myocardium named cardiac troponins.[85]

Troponins, protein complexes involved in the modulation of contraction and relaxation of striated muscle, consist of three different subunits: troponin I, T and C (cTnI, cTnT, cTnC).[85-88] Amongst these, cTnI and cTnT are considered the most sensitive and specific biomarkers for detecting cardiac damage.[85-88] Their clinical utility has been well established in the evaluation of patients with suspected myocardial infarction and they are now considered "gold standard" for the biochemical diagnosis of myocardial necrosis in acute coronary syndromes.[87, 89-94]

The role of troponin in identifying cardiac damage, and therefore potentially predicting subsequent chemotherapy-induced cardiotoxicity has been extensively investigated.[87, 88, 95-99] Seino et al. were the first to report cTnT as a biomarker of doxorubicin cardiotoxicity in spontaneously hypertensive rats in the early 1990s.[85] Since then a number of studies have demonstrated that in patients with raised troponin levels during high dose chemotherapy, a higher risk of cardiotoxicity exists.[85, 88, 100] Thus the troponin biomarkers may have a role in potentially risk stratifying those who could be at future risk of developing cardiotoxicity.(87, 88) Troponin may have a greater utility in exclusion of cardiotoxicity, as a negative troponin during and a 1 month after anthracycline chemotherapy essentially excludes significant cardiotoxicity; the negative predictive value of undetectable troponin was found to be 99% by Cardinale et al.[87, 88]

3. PROACT Clinical Trial

Current literature suggests higher cumulative doses of anthracyclines can augment the risk of cardiotoxicity.[6, 101, 102] In one study doses ≥ 250mg/m2 of epirubicin was found to cause cardiotoxicity as early as the third cycle of chemotherapy.[101]

At The James Cook University Hospital (JCUH) we are currently conducting the PROACT clinical trial (PRevention Of Anthracycline Cardiovascular Toxicity in patients treated for breast cancer). PROACT is an NIHR-funded, multicentre, phase 3, randomised, open label, blinded end-point study that aims to assess the effectiveness of enalapril in preventing cardiotoxicity in patients with newly diagnosed breast cancer requiring anthracycline based chemotherapy. One hundred and seventy patients, in whom high-dose anthracycline chemotherapy (≥300mg/m2) (table 1) is planned, will be randomised to either usual care plus enalapril or usual care. The primary end point is the presence of detectable troponin T (14ng/L or greater), measured at the end of each chemotherapy cycle (in total six time points). As part of the assessment of key secondary endpoints in the trial, transthoracic echocardiography will be performed at two time points using a standardised British Society of Echocardiography (BSE) template: prior to commencing chemotherapy, and 1 month after the completion of anthracycline chemotherapy. The clinical trial includes an additional optional consent to allow participants to be invited for further echocardiography follow up. Trial echocardiographic secondary endpoints will focus on established measures of LVEF and global longitudinal strain (GLS).

Regimen	Type of Cancer	PROACT clinical trial eligibility	Description	No. of cycles	No. anthracycline cycles	Dose of anthracycline	Total dose of anthracycline	Cardio-toxic equivalent dose for doxorubicin***
EC 90	Breast	**Yes**	**Epirubicin** 90mg/m2, Cyclophosphamide 600mg/m2- 6 cycles	6	6	90mg/m2	540mg/m2	378mg/m2
FEC 75	Breast	**Yes**	Fluorouracil 600mg/m2, **Epirubicin** 75mg/m2, Cyclophosphamide 600mg/m2- 6 cycles	6	6	75mg/m2	450mg/m2	315mg/m2
FEC-T	Breast	**No**	Fluorouracil 500mg/m2, **Epirubicin** 100mg/m2, Cyclophosphamide 500mg/m2, Taxane (docetaxel) 100mg/m2-6 cycle	6	3	100mg/m2	300mg/m2	210mg/m2
R-CHOP	Advanced Non-Hodgkin's Lymphoma and Hodgkin's lymphoma (nodular lymphocyte type)	**No**	Rituximab 375mg/m2, Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (for 5 days)	6	6	50mg/m2	300mg/m2	300mg/m2
CHOP	Advanced Non-Hodgkin's Lymphoma	**No**	Cyclophosphamide 750mg/m2, **Doxorubicin** 50mg/m2, Vincristine 1.4mg/m2 (max 2mg), Prednisolone 40mg/m2 (5 days)	6	6	50mg/m2	300mg/m2	300mg/m2
ABVD	Advanced Hodgkin's Lymphoma	**No**	**Adriamycin (doxorubicin)** 25mg/m2, Bleomycin 10,000 IU/m2, Vinblastine 6mg/m2, Dacarabazine 375mg/m2	6*	12*	25mg/m2	300mg/m2	300mg/m2
Escalated BEACOPP	Advanced Hodgkin's Lymphoma (based on PET scan results)	**No**	Bleomycin 10,000 IU/m2, Etoposide 200mg/m2, **Adriamycin (doxorubicin)** 35mg/m2, Cyclophosphamide 1250mg/m2, Oncovin (vincristine) 1.4mg/m2 (max 2mg), Procarbazine 100mg/m2, Prednisolone 40mg/m2, Filgrastim 300mcg	4-6**	4-6**	35mg/m2	140mg/m2-210mg/m2	140mg/m2-210mg/m2

Table 1. Most commonly used anthracycline based chemotherapy regimens in breast cancer and lymphoma treatment in North East England

*Doxorubicin 25mg/m2 given 2 weekly x 12 doses (6 cycles of treatment – each cycle is a 4 week block)

**Esc BEACOPP either given from the outset due to high clinical risk (x6 Esc BEACOPP) or given due to a poor PET/CT after two cycles of ABVD (x2 ABVD, x4 Esc BEACOPPP)

***Anthracycline toxicity equivalence ratio for assessment of cardiotoxicity: relative cardiotoxicity of doxorubicin rapid infusion = 1 and epirubicin= 0.7 as per the latest ESC position paper on cancer treatments and cardiovascular toxicity

4. PROACT PLUS REGISTRY & ECHO SUBSTUDY

4.1 Rationale

As with all clinical trials, PROACT has established inclusion and exclusion criteria that will result in a specially selected population to answer the trial question (Table 2). To further supplement the findings of the PROACT trial, we propose to conduct a parallel prospective observational cohort study. Called the "**PROACT PLUS Registry**", this will allow assessment of the effects of anthracyclines on the hearts of those patients who fall outwith the eligibility criteria for the PROACT trial. The registry will also include patients with a diagnosis of lymphoma who are due to receive anthracycline based chemotherapy as part of their treatment, to increase the cohort of patients studied.

There are limited information of the effects of lower dose anthracyclines regimens and the utility of newer echocardiographic methods.[38] The PROACT PLUS registry will mirror the assessments from the main trial, and will also include the novel echocardiographic measures proposed in the echo substudy. There are three major patient groups who will not be represented in the clinical trial, that are of particular interest:

1)Patients who are due to receive a qualifying regimen, but who meet one or more exclusion criteria

A retrospective study conducted at JCUH identified the total number of patients newly diagnosed with breast cancer who receive a qualifying high dose anthracycline regimen (EC90 – six cycles epirubicin 90mg and cyclophosphamide). In a 6 month period (01/05/17-31/10/17), 26 patients received EC90. Eighteen patients would have been considered eligible for the PROACT trial. Of the eight patients ineligible, six were due to existing treatment with an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB), with the remaining two having uncontrolled hypertension and a diagnosis of possible metastatic breast cancer.

2) Patients who are due to receive a lower dose anthracycline regimen

Feasibility work, prior to application for trial funding, identified that around half of patients with breast cancer receive FEC-T regimen (see table 1). The dose of anthracycline given in JCUH is 100mgx3 of epirubicin (total 300mg/m2). It is thought that cardiotoxicity in this patient group is lower, hence the patients were not included in the specific trial population. However, as discussed above there is no "safe" anthracycline dose although detailed prospective and contemporary data are limited, and the utility of novel echocardiographic parameters in identifying patients at risk is unknown.

3) Herceptin and 1 year echo follow up

The clinical trial is designed to isolate the question of anthracycline toxicity. Final troponin and echocardiography is performed four weeks after the final epirubicin dose. It is known that LV function can change up to one year, and in some cases beyond. A significant and important subgroup (around 20% of the total cases with breast cancer) will go on to receive Herceptin for 12 months, which exerts a distinct cardiotoxic effect.[103, 104] To preserve the integrity of

randomisation beyond the initial end-point, the PROACT trial is stratified by Herceptin status. Patients in this group routinely undergo echocardiographic monitoring. The trial and registry will collect information on Herceptin monitoring and will plan a 12 month echo in trial and registry patients.

4) Patients with Lymphoma

In addition to the breast cancer population, we plan to include patients with a new diagnosis of lymphoma into the PROACT PLUS registry. Anthracyclines are commonly used in the treatment of lymphoma and cardiotoxicity has been evident in this subgroup of cancer patients. A local audit at JCUH revealed that out of 46 patients with a diagnosis of lymphoma requiring anthracyclines as part of treatment, 3 patients developed moderate to severe LV systolic dysfunction at the end of the treatment. However only 66% of the total patients had undergone a mid- and end of chemotherapy echocardiogram.

The clinical trial focuses on patients with breast cancer only, however we would like to include patients with a diagnosis of lymphoma (i.e. Hodgkin's or non-Hodgkin's lymphoma) into the registry to increase our understanding of cardiotoxicity in other cancer populations.

Table 2. PROACT clinical trial eligibility criteria

Inclusion criteria	Exclusion criteria
1. Histopathologically* confirmed breast carcinoma who have received surgery for the their breast carcinoma	1. Positive baseline cardiac troponin T
2. Planned to receive 6 cycle of adjuvant epirubicin based chemotherapy >300mg/m2	2. Known contraindication to ACE inhibitor
3. Written informed consent	3. Are taking or previously intolerant to ACE inhibitors
	4. Already on agents acting on the renin-angiotensinaldosterone system (e.g. ARBs, Aliskiren, MRA, entresto.)
	5. LVEF <50%
	6. Estimated GFR <30mL/min/1.73m2 at baseline
	7. Symptomatic hypotension, or systolic BP <100mmHg
	8. Poorly controlled hypertension (BP >160/100mmHg or ambulatory BP of 150/95mmHg)
	9. Previous myocardial infarction
	10. Previous metastatic breast cancer
	11. Previous exposure to anthracycline chemotherapy
	12. Patients pregnant or breast feeding
	13. For patients of child bearing age refusing to use contraception throughout the trial
	14. Previous Herceptin treatment or planned Herceptin treatment within 4 weeks following anthracycline chemotherapy
	15. Treatment of other invasive cancer in the past 5 years*
	16. Symptomatic or severe asymptomatic previous radiation induced heart injury*
	17. Participation in other interventional medicinal trials in the past 6 months
	18. Judgement by the investigator that the patient should not participate in the trial

***** Amendments of the PROACT clinical trial currently pending ethical approval

5. PROACT PLUS REGISTRY AND ECHO SUB-STUDY AIMS & OBJECTIVES

5.1 Aims and Objectives

5.1.1 Aims

To collect data for a prospective cohort of patients (PROACT PLUS Registry) who are not eligible for the PROACT trial

To collect data for a prospective cohort of patients with a new diagnosis of lymphoma

To generate a combined prospective cohort of patients from the PROACT trial and PROACT PLUS registry

5.1.2 Objectives

To assess troponin T and troponin I release in the PROACT PLUS registry patients, during, one month and twelve months after chemotherapy, and to compare the registry group with the patients enrolled in the trial (treatment and control)

To assess cardiac function by echocardiogram, using echocardiographic measures such as GLS, radial and circumferential strain, torsion, right ventricular free wall strain, left atrial and right atrial strain and strain rates.

To determine which measure, or combination of measures, are most sensitive and specific for early cardiac damage caused by anthracyclines

To model the factors that are associated with cardiac dysfunction following anthracycline chemotherapy

To assess the effect of randomised therapy (enalapril or control) on novel echocardiographic measurements within the PROACT clinical trial population

5.1.3 Main Research Questions

What are the inter and intra observer variability of LVEF, GLS and the novel strain parameters measured within PROACT trial and registry?

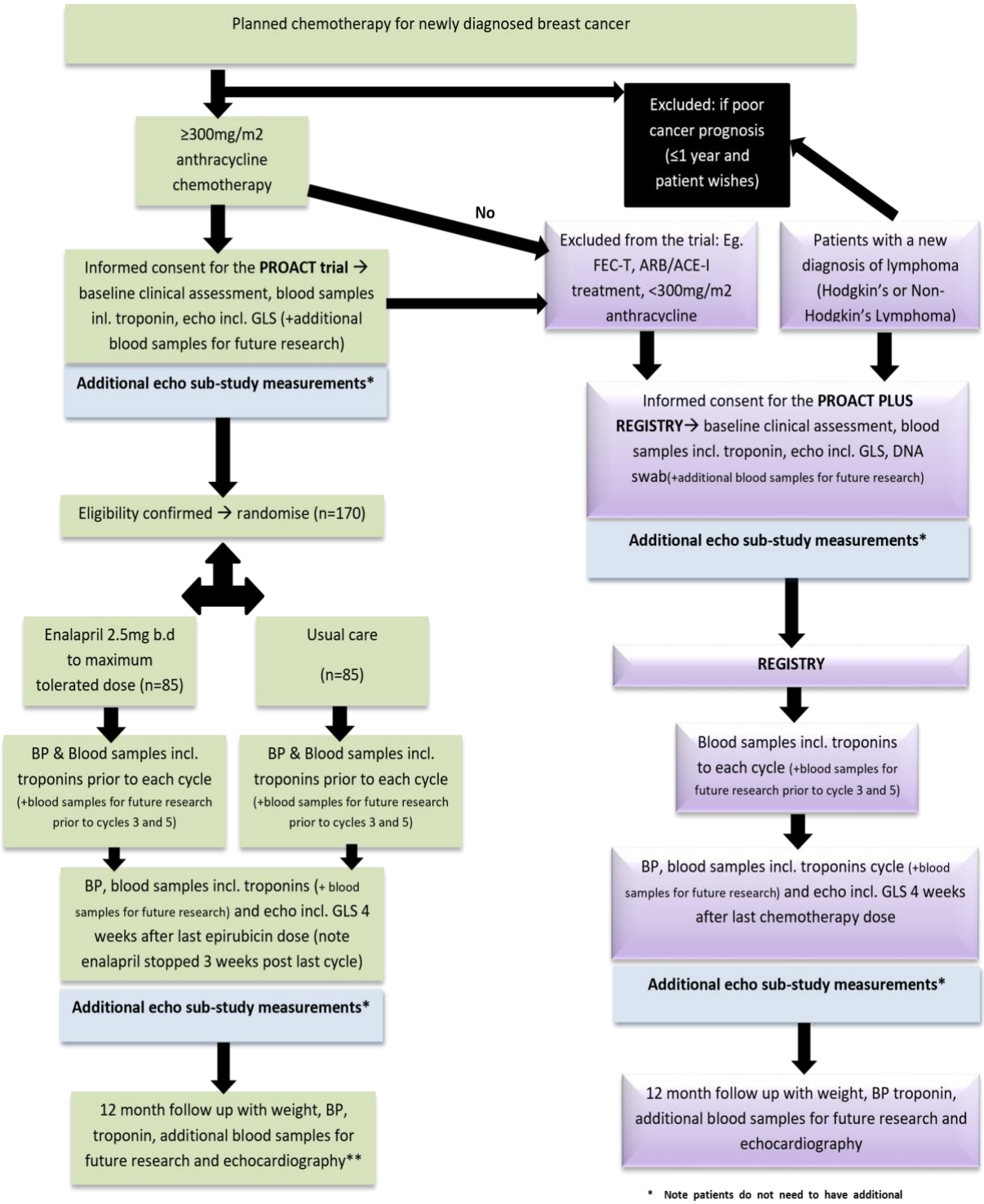
What are the rate of troponin T and I release within the PROACT registry, and how does this compare to the clinical trial population (ACEI and control)?

What are the changes from baseline for LVEF, GLS and the novel LV strain parameters measured within PROACT trial and registry?

- To explore if there are any measurable changes in RV function and strain within the PROACT trial and PROACT PLUS registry groups, and does randomised therapy affect these parameters?

- To assess what happens to the LA function/strain parameters in PROACT trial and registry? Does this precede any change in the LV diastolic function and is there an influence from ACEI?
- Does RA strain change with chemotherapy, how is this related to RV parameters and is there an influence from ACEI?
- To determine whether any factors (clinical, medical therapy, biochemical, echocardiographic) predict change in GLS and LVEF in the PROACT trial and registry following chemotherapy and at twelve months?
- To explore whether novel echocardiographic measures better predict later cardiotoxicity as measured by LVEF and GLS? Within the PROACT trial, how does ACEI therapy effect novel LV strain parameters?

6. PROACT PLUS REGISTRY AND ECHO SUB-STUDY DESIGN



* Note patients do not need to have additional scans - extra measurements wiill be done on the scans already taken

20

**Separate application will be put in for 12 months follow up and investigations for PROACT trial

7. OUTCOMES MEASURES IN THE PROACT PLUS REGISTRY AND ECHOCARDIOGRAPHIC SUB-STUDY

In addition to the full description of the population in the registry, the following outcomes will be measured which will mirror the same schedule as the PROACT trial enabling direct comparison:
The presence (≥14ng/L) or absence of cardiac troponin T (<14ng/L) release at any time during anthracycline treatment, 4 weeks and 12 months after the last dose of anthracycline.
Cardiac troponin I release during, 4 weeks and 12 months after the last dose of anthracyclines;
Cardiac function assessed by echocardiogram, including GLS, measurement of LVEF, and measurement of all the novel echocardiographic strain parameters (ie. GRS, GCS, torsion and twist, RVFW, left and right atrial strain), at baseline, 4 weeks following completion of chemotherapy and at 12 months

8. RESEARCH SETTING

Recruitment for the Registry will occur at South Tees NHS Foundation Trust and participating NHS trusts and will be for a total of 18 months. These sites will be able to fully accommodate the needs of the registry including research nurse support, facilities for the registry investigations and assessments, and British Society of Echocardiography (BSE) accredited advance imaging trainee, echocardiographers or consultant cardiologist to carry out scans in accordance with the PROACT echo protocol.

9. REGISTRY POPULATION

Adult patients with a new diagnosis of breast cancer due to receive anthracycline based treatment who do NOT meet the eligibility criteria for the PROACT trial will be included in this study.

9.1 Inclusion Criteria

Adult patients with a new diagnosis of histopathologically confirmed breast carcinoma

Adult patients with a new diagnosis of histopathologically confirmed lymphoma (Hodgkin's and Non-Hodgkin's Lymphoma)

Age ≥ 18

Planned to receive anthracycline based treatment (adjuvant or neo-adjuvant) – any dose • Written informed consent

9.2 Exclusion criteria

Meets eligibility criteria for the PROACT trial*

Known metastatic cancer

Poor cancer prognosis of ≤ 1 year

*patients who are otherwise eligible for the PROACT trial will not be eligible to participate in the PROACT registry

10. SCREENING, RECRUITMENT AND CONSENT

10.1 Identification

Patients likely to fulfil the PROACT PLUS Registry inclusion/exclusion criteria will be identified by the clinical teams prior to commencing their chemotherapy and approached for inclusion in the registry.

10.2 Recruitment and consent

Once patients have been identified as likely eligible candidates for the registry, they will be invited to participate. Potential participants will be provided a patient information sheet outlining the main principles of the registry. The time between giving the information and taking consent may be on the same day. All steps will be taken to ensure that patients are afforded a reasonable time to consider enrolment into the registry, to ask questions, and have all their queries answered prior to consent.
If patient is happy to participate, written informed consent will be obtained by a delegated member of the research team. The Consent Form will be retained in the Study File, with a copy filed in the clinical notes and one given to the patient. Additional consent will be requested for DNA swab and storage of additional blood samples for translational research. Beyond the final echocardiogram, we will seek consent to contact registry patients for further follow up including additional echocardiography. We will also seek consent for the use of and storage of personal data for a total period of 15 years.

11. REGISTRY PROCEDURES

11.1 Demographic Information

The following demographic data will be obtained and recorded from patients' medical records at baseline:

Month and year of birth

Gender

Ethnic group

11.2 Medical History

Information regarding patients' full medical history including a list of baseline medication will be obtained from the medical notes or patients at the time of consent and recorded in the CRF.

11.3 Physical Examination

11.3.1 Height and Weight

Information regarding patients' height and weight which will have been taken by their oncology/haematology team as part of standard care will be obtained from the medical notes at baseline and recorded in the CRF. The weight will be re-measured 12 months after chemotherapy at the time of echocardiography.

11.3.2 Heart Rate and Blood pressure

Heart rate and blood pressure measurements will be obtained from patients' medical notes at baseline and documented in the CRF. If this has not been done, a heart rate and blood pressure measurement will be taken at the time of consent to avoid unnecessary hospital admissions. This will be repeated 4 weeks, and 12 months after the last dose of anthracycline treatment and at the time of echocardiography.

11.4 JCUH Echo core laboratory

Studies that use echocardiography not blinded to treatment assignment are prone to observer bias. For the PROACT clinical trial we have established an echo core laboratory to facilitate blinded echo review. The blinded end-point design is a strength of the PROACT study. The echo core lab will be utilised for the PROACT PLUS Registry and echocardiographic sub-study. Echocardiography will be performed by local teams according to the protocol, and then images transferred to the echocardiographic core lab without personal identifiers. These will be analysed by Dr Sharareh Vahabi, Chief Investigator of the study, alongside PROACT trial analyses. We plan to perform inter- and intra- observer variability measures as directed by the statistical analysis plan for the trial.

One of the potential drawbacks of GLS, and other measures of strain, is inter-vendor variability. For the purposes of the PROACT clinical trial, PROACT PLUS Registry and the echo sub-study, all measurements will be performed on the TOMTEC^{TM} work station at JCUH core echocardiography lab by a BSE accredited advanced imaging trainee. The basic TOMTEC package and autostrain function (which measures GLS and strain rate) are already available in the department for the purposes of the PROACT clinical trial. We wish to add a further licence to TOMTEC software to allow the additional measurements in the PROACT echo sub-study. This funding application includes the additional licencing and training costs that will be incurred to allow these additional analyses.

11.5 Registry Blood Sampling

11.5.1 Troponin T and I

Patients who consent to the PROACT PLUS registry will have a blood sample taken to assess the troponin T and I, taken to coincide with blood samples for routine care. Up to 5mL of blood will be taken in a serum-separation tube (SST) for troponin T and I. The sample collected will be sent for immediate local processing. Two aliquots of serum will be stored at -80°C for subsequent troponin T and I analysis. The sample will be subsequently sent for central analysis at Newcastle upon Tyne NHS Foundation Trust Laboratories, who routinely measure high sensitivity (HS) troponin T for clinical use. During the course of chemotherapy, up to 5mL of blood will be collected in a SST, in addition to the patient's standard care blood tests to check troponin T and I. These will be collected within 72 hours prior to the intended start of chemotherapy before cycle 2, and subsequent chemotherapy cycles. Again, these will be collected at the same time as patient's routine pre-chemotherapy blood tests to avoid additional hospital admissions. These will be processed immediately after collection and stored.

Four weeks after the last dose of chemotherapy (anthracycline), a blood test of up to 5mL will be collected in a SST for troponin T and I . The results will be processed as described above. This will be an additional visit to the hospital for the patient, that we would plan to coincide with the post chemotherapy echocardiogram. Troponin T and I will be batch tested during and at the end of the registry. The clinical team and patient will be blinded to the troponin results. However, any abnormality in the echocardiography findings during the collection period will be relayed to the patient's treating clinical team for further action.

At least 12 months after the final dose of chemotherapy, patients will be invited to attend a final clinic visit where a blood test of up to 5mL will be collected in a SST for troponin T and I and processed and stored by means described earlier. All effort will be made to plan this appointment at the same time as the 12 months echocardiogram.

The principal investigator at the participating hospital will keep full traceability of the samples collected whilst in storage at the site until shipment and keep records of shipping for each sample. The receiver will acknowledge receipt of each sample and keep full traceability of the samples during storage and use until samples used or disposed of.

The results of the patient's blood tests taken as part of standard care will be recorded in the CRF at baseline. 4 weeks after last dose of chemotherapy the patients' latest FBC result taken as part of standard care will also be recorded in CRF.

11.5.2 Additional blood sampling for further research

If consent is given, additional blood samples will be collected for further research purposes at the beginning of the study, before cycle 3, before cycle 5, 4 weeks and 12 months after chemotherapy. This will be up to 5mL in one SST and up to 5mL in one EDTA. These will be processed immediately and stored at -80°C at the participating NHS site until analysis or for a

maximum period of 15 years following the patient's last visit for the registry. After this period all blood samples will be destroyed.
As with the troponin samples, the principal investigator at the study site will keep full traceability of collected samples while in storage at the site until shipment or disposal, and keep records of shipping for each sample. Once again, the receiver will acknowledge receipt of each sample and keep full traceability of the samples whilst in storage, during use and until disposed of.

11.6 DNA sampling

If the patient has given additional consent a buccal swab will be taken for DNA analysis purposes. A single buccal swab will be collected at baseline ideally prior to the initial chemotherapy cycle. This will be done at the same time as collection of the standard care blood tests to avoid additional hospital admissions. The samples will be processed into lysis buffer and stored at -20°C for up to one month. The samples will then be transported to the Newcastle University where DNA will be extracted. The DNA will then be stored at -80°C until analysis for a maximum of 15 years following the patient's final visit for the registry after which time they will be destroyed.

Table 3. Summary of registry procedures

	Baseline (prior to commencement of chemotherapy)	Prior to each cycle of chemotherapy	4 weeks after final dose of anthracyclines	12 months after final dose of anthracyclines
Demographics	X			
Cancer history*	X			
Medical history*	X			
Medication history*	X			
Eligibility check	X			
Physical assessment (height and weight)*	X			X*
Blood pressure and heart rate*	X		X	X
Troponin T and I	X	X	X	X
Additional blood samples for future research	X	X**	X	X
Other blood tests as per usual care (FBC, U&Es)***	X		X	
Buccal swabs for future research	X			
Echocardiogram (with advanced echo measurements)	X		X	X

* Cancer history, medical history, medication, physical assessment, blood pressure and heart rate will be obtained from patient's medical notes where possible. Patients may be asked some questions about their medical history at time of consent. Note: HR and BP will be taken 4 weeks and 12 months after chemotherapy and weight will be taken 12 months after chemotherapy.

** Additional blood samples taken prior to cycles 3 and 5

*** Other blood tests' (FBC, U&Es) results taken as part of standard care prior to chemotherapy will be obtained from patient's medical records at baseline. 4 weeks after last dose of chemotherapy a FBC result taken as part of standard care will be recorded aswell (note that this blood sample may have been taken any time before the last dose of chemotherapy).

12. DATA COLLECTION

For PROACT echo sub-study patients, an additional data entry page will be created in the PROACT trial CRF to capture the novel echo parameters. For the registry, we will design a specific database that will capture the key data, mapped to the clinical trial to allow easy comparison.

13. STATISTICAL ANALYSIS

The initial collection period for the registry will be for 1 year. The aim is to recruit as many patients as possible during the one year period, aiming for a total number of 85 patients. Once all the data collection is finalised, data cleaning and analysis will be carried out primarily by the research staff located at JCUH. Every effort will be made to retain and include all patients that have been recruited into the registry.

Prior to undertaking any analysis for the registry and echo substudy, a full statistical analysis plan will be developed. As part of analysis for the echo substudy, and assessing the effect of different types of strain measurements on prediction of future cardiotoxicity, linear regression analysis will be used. For the purpose of the registry, the primary analysis of the presence or absence of troponin will be assessed using Fisher's exact test. Inter- and intraobserver variabilities of 15% of the echocardiographic assessments that have occurred in both the trial and registry population will be measured using the Bland-Altman analysis.

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8.5 Appendix 5 - PROACT PLUS Study Forms

NHS Recruiting Centre logo

and relevant contact details to be entered

Participant Information Sheet

PROACT PLUS REGISTRY

Title of Project: PROACT PLUS REGISTRY**:** Can we detect early chemotherapy-related heart damage in patients with breast cancer and lymphoma?

Lead Investigators: Dr Sharareh Vahabi, Clinical Research Fellow, The James Cook University Hospital, Middlesbrough
Dr David Austin, Consultant Cardiologist**,** The James Cook University Hospital, Middlesbrough

Principal Investigator: *insert details of NHS recruiting centre Principal Investigator*

You are being invited to take part in research study called the PROACT PLUS registry. Before you decide whether you want to take part, it is important that you understand why the research is being done and what being a registry participant (taking part) will involve. Please take the time to decide if you wish to take part. Taking part is entirely your choice.

The following information sheet provides details about the research, and aims to answer any questions that you may have about what being a registry participant will involve. Please ask us if there is anything that is not clear. Our full contact details can be found at the end of this information sheet.

It is important that you take time to read the following information carefully.

Why have I been invited to take part?

You have been invited to take part because you have recently been diagnosed with either lymphoma or breast cancer and are planned to receive chemotherapy as part of your cancer treatment. We are inviting patients who require this treatment to participate in this registry.

What is the purpose of this Registry?

Many patients with cancer receive anti-cancer (chemotherapy) drugs as part of their cancer treatment. One of the most commonly-used chemotherapy drugs are called anthracyclines, which are extremely effective at treating cancer. Unfortunately, as with all chemotherapy drugs, anthracyclines have side-

effects. One of these side-effects is heart damage. Although heart damage is caused at the time of treatment, symptoms may only occur many years later. It is important to know that heart damage only occurs in a minority of patients. However, when it does occur, it can affect the length and quality of people's lives. A year after chemotherapy, one in ten patients will have heart damage and be unaware of it; up to one in twenty patients may go on to develop heart failure in the future.

Currently the detection of adverse effects of chemotherapy on the heart relies on ultrasound scanning called echocardiography (or echo for short). However, the usual measurements used in echo to detect heart damage are not very good. These measurements can only identify heart damage when this is already established. Waiting until heart damage is established means a reduced chance of heart recovery. Furthermore, in the UK, not all patients having anthracyclines are routinely monitored with echo.

In light of these issues, we have designed the **PROACT PLUS registry** to monitor the heart function of patients receiving anthracyclines, with the aim of finding better ways of detecting heart damage earlier. The PROACT PLUS registry explores the potential use of new echo measurements and heart specific blood tests (called troponins) to be able to answer this question. We do not know whether these measurements will be better than what is currently used, however this study will be able to help us answer this question.

The PROACT PLUS registry is an observational study and will **not** involve administering any study specific medication. Patients will receive their planned chemotherapy as usual and will receive additional cardiac monitoring by means of echo scans. Information from the PROACT PLUS registry will be combined with another study called PROACT, to help us answer the study questions. Patients cannot be in both studies, and your treating team will know which study is appropriate in your case.

What will happen to me if I decide to take part?

Participation in the PROACT PLUS registry is entirely your choice and if you decide not to take part, your care will not be affected in any way. However, if you wish that you would like to participate, we will ask you to formally become a registry participant (a volunteer who is officially taking part in the registry).

To become a registry participant we will:

1. Provide you with any further information that you would like about the registry.
2. Answer any questions that you may have about the registry.
3. Ask you to sign a consent form.

Taking part in this study may involve up to three extra visits to the hospital along with your usual appointments

Before chemotherapy:

As part of standard care, you will be invited to attend a pre-chemotherapy outpatient clinic visit with your oncologist or haematologist depending what type of cancer you have (breast cancer or lymphoma). At this visit after discussing treatment options for your cancer the PROACT PLUS registry will be introduced to you. Once you are happy with the information provided and have decided that you would like to participate in the registry with all your relevant questions answered, you will be asked to sign a consent form. At this appointment we will obtain information about your medical history, any medication you are taking, your height, weight, heart rate and blood pressure measurements often using your medical notes. Where adequate information is not available we may ask you further questions about your health. Measurements of your height, weight, heart rate and blood pressure may also be taken if these are not available from your notes. We will then take a small amount of blood (5 mL, which is approximately 1 teaspoon) to measure a substance in your blood (called troponin). The troponin test is very sensitive. It may tell us if there are subtle signs of heart cell injury. The results of the troponin tests will not be available to either you or your doctors. This is called blinding; at present doctors do not know how to respond to a “positive” blood test in this setting. The research team will eventually know the results, but may not have analysed the blood sample until after your chemotherapy has finished. If you have provided additional consent, two further 5mL blood samples will be taken for further research. Additionally, we will collect results of your standard care chemotherapy blood tests from your medical records.

A swab will be taken from the inside of your mouth for DNA testing. This will be stored and used for additional research purposes, but only if you agree and consent to this.

We will then arrange a detailed echocardiogram before your chemotherapy starts. Echocardiograms are not performed on all patients having chemotherapy, so this may be an additional check in your case. Echocardiograms are the usual method a doctor would use to assess heart function. The results of your heart scan will be available to your doctors and GP during the study. Having an echocardiogram may mean another trip to hospital if it can’t be arranged on the same day as your standard care appointments.

During chemotherapy:

During your chemotherapy you will visit the hospital a number of times as part of your routine care and see your caring clinical team. You will have a blood sample (5mL, 1 teaspoon) taken for troponin measurements before each of your chemotherapy treatments. Wherever possible, this blood sample will be taken at the same time as your routine blood tests. If you have provided additional consent, two further 5mL blood samples will be taken before cycle 3 and cycle 5 of your chemotherapy treatment.

After chemotherapy:

Four weeks after your last dose of chemotherapy we will ask you to attend hospital for a follow-up echocardiogram. You will be asked to provide a blood sample for troponin measurements (5 mL, 1 teaspoon). If you have provided additional consent, two further 5mL blood samples will be taken after cycle 3 and cycle 5 of your chemotherapy treatment. You will also have your blood pressure checked at this visit.

At least 12 months after your last dose of anthracycline chemotherapy, we will ask you to attend hospital for a final follow-up echocardiogram. You will also have your blood pressure and weight checked at this visit. We will ask you to provide a final blood sample for troponin measurement (5mL, 1 teaspoon) and two further 5mL blood samples for future research. All efforts will be made to take these samples at the same time as your echo visit. Some patients will receive cardiac monitoring as part of routine care, for example if Herceptin treatment is planned for patients with breast cancer. The research team intend to collect information and/or analyse any subsequent routine heart monitoring, but no additional study visits beyond those described are planned.

In summary, study investigations that will be performed:

	Before chemotherapy	During chemotherapy (prior to each chemotherapy cycle)	4 weeks after last chemotherapy treatment	12 months after the last chemotherapy treatment
Blood pressure and heart rate	✓*		✓	✓
Weight and Height	✓*			✓
Blood samples to measure troponin (up to 5mL, a teaspoon)	✓	✓	✓	✓
Additional blood samples for future research	✓	✓**	✓	✓
Swabs for DNA	✓			
Heart scan	✓		✓	✓

*Blood pressure and heart rate will be taken 4 weeks and 12 months after the last chemotherapy dose. The baseline blood pressure and heart rate will be obtained from the medical notes. Weight will only be taken 12 months after the last dose of chemotherapy with baseline weight taken from the medical notes.

**before cycles 3 and 5

Do I have to take part?

No, you do not have to take part. You are under no obligation and taking part in the registry is entirely voluntary.

Will it affect my future medical care if I decide not to take part? What happens if I change my mind?

No, deciding not to take part will not affect your future medical care. Taking part in the registry is entirely your choice. If you decide to take part you may withdraw at any time. If you decide to withdraw from the registry, the data and blood samples for troponin measurements collected up to that point will be kept and analysed. You will be asked about whether further information may be collected from your medical notes. Your decision to withdraw will not affect your standard care in any way.

Do I need to tell my GP?

You do not need to contact your GP. With your permission, if you become a registry participant we will send your GP a letter informing them that you are taking part. We may also contact your GP if we need any information about you during the registry that is not available from your medical notes at the hospital.

Are there any risks to my health?

We know that there are side-effects from chemotherapy and your oncology team will talk to you about these and give you information on what to expect. They will also give you some medications to help you deal with side-effects as part of routine care. The additional risks from taking part in the study are:

Risks associated with taking blood

Risks associated with drawing blood from your arm include pain, bleeding, bruising, light-headedness, and, on very rare occasions, infection.

Risks associated with having a heart scan (echo)

When you have an echo, the instrument will be held firmly to your chest and this pressure can be uncomfortable.

Will I get paid for taking part in the Registry?

No, taking part in the registry is a voluntary process and you will not be paid for your time. However, you may need to attend hospital more times than you would normally to have heart scans and we can reimburse any travel costs for these visits that you have kindly made.

What will happen to the results of the Registry?

We expect to publish the results in a medical journal and discuss the results at medical conferences but no reference will be made at any point to any individual patient. The results will also be used for the purposes of a research degree (Dr Vahabi).

Will my taking part in the registry be kept confidential?

All the members of the research team who have been delegated to the registry will be bound by rule of strict confidentiality. Therefore, all the information collected about you during the course of the research registry will be kept confidential. Your own GP and any other doctor who is currently treating you will be notified of your participation in the registry.

The registry involves gathering information from different places. To keep your data confidential you will be allocated a unique study ID. Only your month and year of birth/age, gender, and your ethnicity will leave the NHS Trust.

Additionally we may need to transfer your data in an anonymised format to other NHS providers, Newcastle and/or Durham Universities where some of the research will be carried out. When we publish the results of this registry or any research related to this registry, we will not use any information which could identify individuals.

What will happen to the data collected?

The research team for this registry includes:

- Oncologists, Haematologists and Cardiologists at your hospital;
- A group of experts at The James Cook University Hospital in Middlesbrough who will review your heart scans;
- Researchers from Newcastle and Durham Universities.

South Tees NHS Foundation Trust is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. South Tees NHS Foundation Trust will keep identifiable information about you for 5 years after the study has finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. Data leaving the Trust for research purposes will have all patient identifiable data removed except age, month and year of birth, gender, ethnicity and registry number (unique study ID).

Your local NHS hospital will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from South Tees NHS Foundation Trust may look at your medical and research records to check the accuracy of the research study. Your local NHS hospital will pass these details to South Tees NHS Foundation Trust along with the information collected from you and your medical records. The only people in South Tees NHS Foundation Trust who will have access to information that identifies you will be people who need to contact you to obtain other relevant information if not already provided for research purposes, or audit the data collection process, or for invitation for participation in future research.

The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

Note that your data will be stored securely at the NHS trust before being destroyed. Following the end of the registry, the team will keep the copies of your heart scans and, along with other data collected within the registry, may use these data for further research.

Research data containing your month and year of birth/age, gender, ethnicity and unique study ID will be stored for a period of 15 years after the end of the registry. It will be confidentially and securely destroyed after this point. We seek consent for the use and storage of your personal data for this period of time.

You can find out more about how we use your information by contacting:

1. Mr Joe Millar at joe.millar@nhs.net or
2. Dr Sharareh Vahabi at sharareh.vahabi@nhs.net or
3. Dr David Austin at david.austin@nhs.net

Who has reviewed the Registry?

The registry has been reviewed by an NHS Research Ethics Committee. The Committee needs to be satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits, and that you have been given sufficient information on which to make an informed decision to take part or not. The Nottingham 1 Research Ethics Committee has reviewed this registry and agreed that it is ethical to proceed.

Who is organising and funding the research?

The registry is the responsibility of the South Tees Hospitals NHS Foundation Trust. It is funded by South Tees Cardiothoracic Research and Development Fund.

What if something goes wrong?

If you are harmed by taking part in this research registry, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this registry, the normal National Health Service complaints mechanisms are available to you.

The hospital has a *Patient Advice and Liaison Service (PALS)/PALS equivalent*. If you wish to speak to them they can be contacted at:

insert contact details for PALS or equivalent for the participating NHS recruiting centre

Contact for further information

For further information regarding the registry, to tell us that you would like to withdraw from the registry or if you wish to discuss any matters related to the registry with a member of the research team, please contact us as detailed below:

Enter full address of recruiting NHS Trust

Enter contact details of PI at the recruiting NHS Trust

Enter contact details of the research nurse team at the recruiting NHS Trust

You can also contact the Research and Development Department at the Trust for general advice using the following details:

Enter contact details of the R&D department at the recruiting NHS Trust

PROACT PLUS Registry consent form

NHS Recruiting Centre logo

and relevant contact details to be entered

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: PROACT PLUS REGISTRY**:** Can we detect early chemotherapy-related heart damage in patients with breast cancer and lymphoma?

Name of Researcher: *Dr Sharareh Vahabi*

Please initial each of the boxes to confirm your agreement

1. I confirm that I have read, or had read to me, and understand the information sheet dated 14/08/2018 (version 4.0) for the PROACT PLUS REGISTRY. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time from all, or any part, of the registry without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to my GP being informed of my participation in this registry, and contacted as required during the registry. ☐
4. I understand that relevant sections of my medical notes and data collected during the registry may be looked at by individuals from the research team. I give permission for these individuals to have access to my records. ☐
5. I understand that information about me that is relevant to this registry, including my month and year of birth, gender and ethnicity, will leave the Trust. I understand that the research team at South Tees Hospitals NHS Foundation Trust will not publish any information that identifies me. ☐
6. I understand that my data will be stored securely and managed confidentially as part of this registry. I understand that the research team may keep this information for up to 15 years following the end of the registry before confidentially destroying it. ☐
7. I understand that anonymised copies of my heart scans will be used for research purposes and presentations at different research meetings/conferences. I agree that this can happen. ☐
8. I agree to take part in the above study. ☐

9. I agree that the team can collect extra blood samples for future research, and that they can link this information to the data collected on me within the registry (Please initial your choice into the Yes or No boxes. If you initial No, you can still participate in the registry).

Yes ☐ No ☐

10. I agree that the team can collect a DNA swab from inside my mouth for future cardiac research, and that they can link this information to the data collected on me within the registry. (Please initial your choice into the Yes or No boxes. If you initial No, you can still participate in the registry).

Yes ☐ No ☐

11. I agree that following the end of the registry, the research team can contact me again in the future to follow up my progress and to invite me to have further heart scans. (Please initial your choice into the Yes or No boxes. If you initial No, you can still participate in the registry).

Yes ☐ No ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

GP information letter

Date: *NHS Recruiting Centre logo*

and relevant contact details to be entered

Insert patient's name and details

Dear Dr *insert patient's named GP,*

Re: PROACT PLUS REGISTRY: Can we detect early chemotherapy-related heart damage in patients with breast cancer and lymphoma?

We would like to inform you that *[insert patient name and date of birth],* who is registered with your practice, has agreed to take part in the PROACT PLUS registry.
This registry is an observational cohort study that is assessing patients undergoing chemotherapy for breast cancer or lymphoma. The study will prospectively follow patients before, during and after anthracycline chemotherapy measuring cardiac function with echocardiography, and the cardiac biomarker troponin. Standard echo findings will be available, but troponin samples are blinded to patient and treating clinicians. No study drugs are given in this registry.
The study is funded by the South Tees Cardiothoracic Research and Development fund and sponsored by South Tees Hospitals NHS Foundation Trust with academic partners at Durham University and Newcastle University. PROACT PLUS registry has been approved by Nottingham 1 Research Ethics Committee.
If you would like further information about this study, please contact me, [insert local Principal Investigator details], at [insert NHS recruiting centre name], on[insert telephone number], or the Research Nurse [insert details for site research nurse].

Yours sincerely,
Insert name of PI at recruiting site

Detecting Early Chemotherapy Related Cardiotoxicity in Breast Cancer and Lymphoma Patients: PROACT PLUS

LABORATORY MANUAL

V 2.0, 14th of August 2018

ISRCTN11676341

IRAS ID: 245613

Contents

1. Introduction

This is the laboratory manual for the PROACT PLUS registry. Herein are described the biological samples collected from the patients in the study, including descriptions of the processing, shipping, analysis and results.

2. Contacts

a. Central laboratory

The central laboratory for this study will be Newcastle Laboratories:

Core Biochemistry
Dept of Blood Sciences
Royal Victoria Infirmary
Newcastle upon Tyne Hospitals NHS Foundation Trust
NE14LP

Tel: 0191 2824305 /2824041 for sample/results queries

For general queries:
Laboratory Medicine Business Unit:
email address: tnu-tr.NewcastleLaboratories@nhs.net
Tel: 0191 2231135 (Option 1) monitored during office hours (Monday – Friday, 8.30am – 17.00)

b. Lead research centre/CI/lead research nurse

James Cook University Hospital
Marton Road
Middlesbrough
TS4 3BW
Chief Investigator: Sharareh Vahabi
Lead Research Nurse: Laura Thompson
Email: sharareh.vahabi@nhs.net or laura.thompson@nhs.net
Tel: 01642 850850

3. Schedule of Assessments

	Baseline (prior to commencement of chemotherapy)	Prior to each cycle of chemotherapy	4 weeks after final dose of anthracyclines	12 months after final dose of anthracyclines
Demographics	X			
Cancer history	X			
Medical history	X			
Medication history	X			
Eligibility check	X			
Physical assessment (height and weight)	X			X
Blood pressure and heart rate	X		X	X
Troponin T	X	X	X	X
Troponin I	X	X	X	X
Additional blood samples for future research*	X	X*	X	X
FBC**	X		X	
U+Es**	X			
Buccal swabs for future research	X			
Echocardiogram (with advanced echo measurements)	X		X	X

*Additional blood samples taken prior to cycle 3 and 5

**FBC and U+Es results taken as part of standard care prior to chemotherapy will be obtained from patients' medical records at baseline. FBC result taken after chemotherapy as part of standard care will be recorded 4 weeks after last dose of chemotherapy

4. Consent

Written informed consent will be sought for all biological sampling. Consent for the main part of the registry will be sought for blood sampling (troponin (T and I)).
Patients must give separate additional consent for the blood sampling for future analysis. This additional consent also applies to the request for the buccal swab. A patient may still take part in the registry without providing consent to the additional bloods or to the buccal swab.

5. Samples

The samples to be obtained from the patients are detailed in this section. Table 1 and 2 provides a full summary of the samples requested, the timepoints, the processing, shipping, analysis, reporting and labelling. Figure 1 shows the recommended order of blood draw where more than one type of blood collection tube is used.

a. Troponin T and I (baseline, pre each chemotherapy cycle, 4 weeks post anthracyclines and 12 months post anthracyclines)

The test requires blood samples to be processed to extract the serum which is then analysed. Samples will be processed, and then sent to a central laboratory (Newcastle Laboratories) for analysis. Samples will be labelled using registry specific labels
The assessment of troponin T is the primary endpoint of this study and troponin I assessments are a secondary outcome. There are eight timepoints where blood samples should be taken for these assessments. Blood collection ***must*** occur prior to the chemotherapy dose for each cycle and may be taken up to 72 hours before (chemotherapy) dosing. If the sample is found to be haemolysed, a repeat draw should be requested immediately.

No special processing is required for the serum. Standard BD Vacutainer instructions are: BD Vacutainer® SST™ Serum Separation Tubes should be inverted five times, allowed 30 minutes clotting time, and centrifuged for 10 minutes at 1000-1300 RCF (g) in a swing bucket centrifuge.) Once separated, the serum should be pipetted into 2 appropriately labelled aliquots, one for troponin T and one for troponin I.

For all troponin T serum samples, freeze and store at -20 °C and ship on dry-ice on a monthly frequency or upon request.

All serum aliquots for troponin I analysis should be frozen and stored at -20 °C and shipped on dry-ice upon request. Analysis will occur in batches and data will be supplied directly from the central laboratory to the James Cook University Hospital.

b. Future bloods (baseline, pre cycle 3, pre cycle 5, 4weeks post anthracyclines and 12months post anthracyclines)

Future bloods will be collected, processed, labelled and transported to Newcastle Laboratories for future analysis.

i. serum

Where patients have provided additional consent, up to 5 mL of blood should be collected in an SST tube at the 4 time points described above. No special processing is required for the serum. Standard BD Vacutainer instructions are: BD Vacutainer® SST™ Serum Separation Tubes should be inverted five times, allowed 30 minutes clotting time, and centrifuged for 10 minutes at 1000-1300 RCF (g) in a swing bucket centrifuge.) Once separated, the serum should be pipetted into up to 4 appropriately labelled aliquots, frozen and stored at -20 °C until shipment to Newcastle Laboratories.

ii. plasma

Where patients have provided additional consent, up to 5 mL of blood should be collected in an EDTA tube at the 4 time points described above. No special processing is required for the plasma. Standard BD Vacutainer instructions are: invert the collection tube 8-10x, centrifuge for 10 mins at ≤1300 RCF (g). Once separated, pipette the plasma should be pipetted into up to 4 appropriately labelled aliquots, frozen and stored at -20 °C until shipment to Newcastle Laboratories.

c. Buccal swab (baseline)

Where patients have provided additional consent, a buccal swab should be collected at baseline. This should be posted immediately in the envelopes provided to:

Dr Jason Gill
PROACT PLUS Registry
Northern Institute for Cancer Research (NICR)
Paul O'Gorman Building
Medical School
Newcastle University
Framlington Place
Newcastle upon Tyne
NE2 4HH

TABLE 1: MANDATORY SAMPLING

VISIT	SAMPLE	COLLECTION AND SAMPLING TUBES	LOCAL PROCESSING	SHIPPING	CENTRAL LAB ANALYSIS AND RESULTS	EXAMPLE LABELS
1. **Baseline** 2. **Cycle 2 – Cycle 6 (pre-dose)** 3. **4 weeks post anthracycline** 4. **12 months post anthracycline**	**Troponin T**	 1x(up to) 5mL SST 	• Invert 5x, allow 30mins to clot, centrifuge for 10 mins at 1000-1300 RCF (g) in a swing bucket centrifuge • Aliquot into 2x vials and label for troponin T and I: **Troponin T –** freeze at -20 °C and store until shipment notification **Troponin I –** freeze at -20°C and store until shipment notification	On request/monthly to Newcastle Laboratories On dry ice	-Stored at -80°C -Batch analysis -Results to be supplied to database by central lab	PROACT PLUS registry ID:-------- DOB --/----- (mm/yyyy) Date collected: --/--/--(dd/mm/yy) Time collected --:--(hh:mm 24 hr clock) Visit: Baseline, cycle no - , 4 weeks post chemo –, 12 months post chemo Sample type: Troponin T
1. **Baseline** 2. **Cycle 2- Cycle 6 (pre-dose)** 3. **4 week post anthracycline** 4. **12 months post anthracycline**	**Troponin I**			On request/monthly to Newcastle Laboratories On dry ice	-Stored at -80°C -Batch analysis -Results to be supplied to database by central lab	PROACT PLUS registry ID:-------- DOB --/----- (mm/yyyy) Date collected: --/--/--(dd/mm/yy) Time collected --:--(hh:mm 24 hr clock) Visit: Baseline, cycle no - , 4 weeks post chemo –, 12 months post chemo- Sample type: Troponin I

TABLE 2: SAMPLES TO BE COLLECTED WITH ADDITIONAL CONSENT

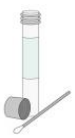
VISIT	SAMPLE	COLLECTION AND SAMPLING TUBES	LOCAL PROCESSING	SHIPPING	CENTRAL LAB ANALYSIS AND RESULTS	EXAMPLE LABELS
1. **Baseline** 2. **Cycle 3 and Cycle 5 (pre-dose)** 3. **4 weeks post anthracycline** 4. **12 months post anthracycline**	**Future research bloods (additional consent required)**	 1x(up to) 5mL SST   1x(up to) 5mL EDTA 	• Invert 5x, allow 30mins to clot, centrifuge for 10 mins at 1000-1300 RCF (g) in a swing bucket centrifuge • Pipette serum into up to 4x vials, label and freeze at -20°C and store until shipment notification • Invert 8-10x, centrifuge for 10 mins at ≤1300 RCF (g) • Pipette plasma into up to 4x vials, label and freeze at -20°C and store until shipment notification	On request/monthly to Newcastle Laboratories On dry ice	-Stored at -80°C -Post study analysis	PROACT PLUS registry ID:-------- DOB --/----- (mm/yyyy) Date collected: --/--/--(dd/mm/yy) Time collected --:--(hh:mm 24 hr clock) Visit: Baseline, cycle 3, cycle 5, 4 weeks post chemo, 12 months post chemo Sample type: Future blood serum 1, 2, 3, 4, 5 PROACT PLUS registry ID:-------- DOB --/----- (mm/yyyy) Date collected: --/--/--(dd/mm/yy) Time collected --:--(hh:mm 24 hr clock) Visit: Baseline, cycle 3, cycle 5, 4 weeks post chemo, 12 months post chemo Sample type: Future blood serum 1, 2, 3, 4, 5
• **Baseline**	**Buccal swab**	 1xswab	Immediate shipment	Send immediately to Newcastle University in the envelopes provided	-Stored at -80°C -Post study analysis	PROACT PLUS registry ID:-------- DOB --/----- (mm/yyyy) Date collected: --/--/--(dd/mm/yy) Time collected --:--(hh:mm 24 hr clock) Visit: Baseline Sample type: BUCCAL SWAB

Figure 22: Recommended order of blood draw

BD Vacutainer®

BD Diagnostics - Preanalytical Systems

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Tube Guide & Recommended Order of Draw*

* Clinical and Laboratory Standards Institute (Formerly NCCLS) Guidelines H3-A6, 6th Edition

Primary Care Guide - 06.14

Blood samples should be taken in the following order:

Cap Colour	Cat. No.	Additive	Determinations	Special Instructions
	Cat. No. 363095 Draw Volume 2.7ml	Sodium Citrate	All Coagulation Studies (INR, APTR, Thrombophilia*). *4 Blue Tubes required for Thrombophilia.	Clean puncture and accurate volume are ESSENTIAL. Mix 3-4 Times
	Cat. No. 367954 Draw Volume 5ml	SST™ II	All Routine Biochemistry Tests except where otherwise stated. All Microbiology, Immunology and Virology. Serum B12 and Folate.	For Vitamins A and E protect from light. Mix 5-6 Times
	Cat. No. Draw Volume 6ml	Trace Element	Manganese, Cobalt and Chromium.	Discard first drawn blood if no previous sample taken. Mix 8-10 Times
	Cat. No. 367885 Draw Volume 6ml	Lithium Heparin	Carboxyhaemoglobin.	Mix 8-10 Times
	Cat. No. 367839 Draw Volume 4ml	EDTA	FBC, ESR, Glandular Fever, Haemoglobinopathy. HbA1c, Lead, PTH.	Mix 8-10 Times
	Cat. No. 367941 Draw Volume 6ml	Cross Match	Blood Group, Antibody Screen, Cross Match.	Mix 8-10 Times
	Cat. No. 367934 Draw Volume 2ml	Fluoride Oxalate	Glucose.	Mix 8-10 Times

IMPORTANT MIXING GUIDELINES Mix 8-10 Times

All BD Vacutainer® tubes require immediate mixing following collection. Insufficient mixing can result in inaccurate test results and the need to re-draw. Correct mixing technique is to invert each tube by the recommended number of times shown on the right hand side of the table.

BD

BD Diagnostics - Preanalytical Systems
The Danby Building
Edmund Halley Road
Oxford Science Park
Oxford OX4 4DQ
Tel: 01865 781603
Fax: 01865 781528

6. Example Request Form for Troponin Measurement

Research Sample Request Form

N.B Please make sure all hand writing on the form is in block capitals and legible

PROACT PLUS Registry

PATIENT STUDY ID (in format Z123, where Z denotes the site)	VISIT NUMBER (circle as appropriate)	MONTH AND YEAR OF BIRTH (mm/yyyy)	GENDER (circle as appropriate)
____	Baseline Cycle 2, 3, 4, 5, 6 4 weeks post chemotherapy 12 months post chemotherapy	__/____	**F or M**
Date sample taken: __/__/____ (dd/mm/yyy)	Time sample taken: __:__ (hh:mm 24hr clock)		
Site:	**Research nurse investigator:**		

SAMPLES TO BE TAKEN

Mandatory:

1x (up to) **5mL SST (gold top) tube** **☐(tick box)**

If additional consent has been given:

1x (up to) **5mL SST (gold top) tube** **☐(tick box)**

1x (up to) **5mL EDTA (purple top) tube** **☐(tick box)**

SPECIMEN REQUIREMENTS	INVESTIGATIONS REQUIRED	
LOCAL PROCESSING LAB	RVI LAB (Central lab) PLEASE INPUT & PROCESS (Apex Codes)	CENTRAL LAB USE (Barcodes)
MANDATORY SAMPLES, PROCESS AS FOLLOWS		
Gold SST tube (1x 5mL)  **Invert 5x, allow 30mins to clot, centrifuge for 10mins at 1000-1300 RCF (g) in a swing bucket centrifuge and divide into two 1mL cryovials.**	**Troponin T** – 1mL cryovial **and** **Troponin I** – 1mL cryovial (use label provided) Freeze at -20°C until next available transport to Newcastle	1 x 1mL cryovial for Troponin T **and** **1 x 1mL cryovial for Troponin I** **FOR -80°C STORAGE UNTIL BATCH ANALYSIS**	

LOCAL LAB: Photocopy this form and keep for records

7. Example Request Form for Bloods for Future Research

Research Sample Request Form

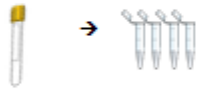
N.B Please make sure all hand writing on the form is in block capitals and legible

PROACT PLUS Registry

PATIENT STUDY ID (in format Z123, where Z denotes the site)	VISIT NUMBER (circle as appropriate)	MONTH AND YEAR OF BIRTH (mm/yyyy)	GENDER (circle as appropriate)
____	Baseline Cycle 3, 5 4 weeks post chemotherapy 12 months post chemotherapy	__/____	F or M
Site:		Research nurse investigator:	

SPECIMEN REQUIREMENTS	INVESTIGATION REQUIREMENTS	
LOCAL PROCESSING LAB	RVI LAB (Central lab) PLEASE INPUT & PROCESS (Apex Codes)	CENTRAL LAB USE (Barcodes)

IF ADDITIONAL CONSENT HAS BEEN GIVEN, PROCESS SAMPLES AS FOLLOWS:

Gold SST tube (1x 5mL)  Invert 5x, allow 30mins to clot, centrifuge for 10mins at 1000-1300 RCF(g) in a swing bucket centrifuge. Spin and divide into four 0.5mL serum cryovials.	STORE AT -20°C AND TRANSPORT WHEN REQUESTED USE LABELS PROVIDED No. of vials collected: __	Receipt up to 4 vials FOR -80°C STORAGE No. of vials received: __	
EDTA tube (1x5mL)  Invert 8-10x, centrifuge for 10mins ≤1300 RCF (g). Spin and divide into four 0.5mL plasma cryovials.	STORE AT -20°C AND TRANSPORT WHEN REQUESTED USE LABELS PROVIDED No. of vials collected: __	Receipt up to 4 vials FOR -80°C STORAGE No. of vials received: __	

NEWCASTLE LAB STAFF NOTES:
Book in APEX location as: NLSTUDY
Enter into Study code field and Visit No into reasons for request field – against all specimen numbers used

LOCAL LAB: Photocopy this form and keep for site records

8. Storage and Shipping Logs

Figure 2: Example sample log for Troponin T

PATIENT STUDY ID (e.g. Z001)	MONTH/YEAR OF BIRTH (mm/yyyy)	SAMPLE ID (optional use)	SAMPLE TYPE	VISIT (baseline, cycle2,3,4,5,6,7,8, 4 weeks post chemo, 12 months post chemo)	DATE SAMPLE COLLECTED (dd/mm/yyyy)	TIME SAMPLE COLLECTED (hh:mm, 24hr clock)	LOCAL STORAGE LOCATION-BOX POSITION(optional use)	DATE SHIPPED
			Troponin T					
			Troponin T					

Figure 3: Example sample log for Troponin I

PATIENT STUDY ID (e.g. Z001)	MONTH/YEAR OF BIRTH (mm/yyyy)	SAMPLE ID (optional use)	SAMPLE TYPE	VISIT (baseline, cycle2,3,4,5,6,7,8, 4 weeks post chemo, 12 months post chemo)	DATE SAMPLE COLLECTED (dd/mm/yyyy)	TIME SAMPLE COLLECTED (hh:mm, 24hr clock)	LOCAL STORAGE LOCATION-BOX POSITION(optional use)	DATE SHIPPED
			Troponin I					
			Troponin I					

Figure 4: Example sample log for future research blood samples – plasma aliquots

PATIENT STUDY ID (e.g. Z001)	MONTH/YEAR OF BIRTH (mm/yyyy)	SAMPLE ID (optional use)	SAMPLE TYPE	VISIT (baseline, cycle2,3,4,5,6,7,8, 4 weeks post chemo, 12 months post chemo)	DATE SAMPLE COLLECTED (dd/mm/yyyy)	TIME SAMPLE COLLECTED (hh:mm, 24hr clock)	LOCAL STORAGE LOCATION-BOX POSITION(optional use)	DATE SHIPPED
			Future bloods plasma					

Figure 5: Example sample log for future research blood samples – serum aliquots

PATIENT STUDY ID (e.g. Z001)	MONTH/YEAR OF BIRTH (mm/yyyy)	SAMPLE ID (optional use)	SAMPLE TYPE	VISIT (baseline, cycle2,3,4,5,6,7,8, 4 weeks post chemo, 12 months post chemo)	DATE SAMPLE COLLECTED (dd/mm/yyyy)	TIME SAMPLE COLLECTED (hh:mm, 24hr clock)	LOCAL STORAGE LOCATION-BOX POSITION(optional use)	DATE SHIPPED
			Future bloods serum					

9. Shipping and couriers

a. Courier details (for each site)

A hopper service exists between Newcastle Laboratories (based at the Royal Victoria Infirmary) and North and South Tees Hospitals. This is a daily service which should be utilised for all samples which need to be shipped to Newcastle Laboratories for longer-term storage before analysis (all troponin T, all troponin I and all serum and plasma samples collected for future research).

Samples collected from within Newcastle Hospitals should follow the routine in-hospital transport system for clinical specimens (porters/messengers/air tube system)

b. Packaging

Samples collected in this study can be classified under the risk category UN3373 (diagnostic specimens). The policies associated with the transport of goods as defined within this category must be adhered to, including the use of appropriate UN3373 'triple pack' containers, such as 'bio-bottles' and clearly UN3373 labelled.

c. Shipping manifests

All samples must be clearly labelled and accompanied by sample request forms. Where batched samples are sent, an aggregated sample log (forming the shipping manifest) should also be sent.

d. Receipt of samples and reconciliation

Once samples are received at Newcastle Laboratories, they will be checked and entered into the local laboratory information management system (LIMS).

The samples will be reconciled against the shipping manifests and a full list of samples received will be returned to the sender. Where discrepancies are noted both the research site sending the sample(s) and the Trials Unit will be alerted.

10. Analysis and Results

All other samples collected for the study will be batch-analysed. Where the results form part of the primary and secondary objectives, they will be either entered into the CRF by the central laboratory or provided to the Research Team at James Cook University Hospital in a quality-checked spreadsheet format. Results from future research will not form part of the main dataset for the study.

11. Lab accreditations/quality statements

Quality statements and laboratory accreditation details can be found on the following website: https://www.newcastlelaboratories.com/quality/quality-statement/

Research Sample Request Form

N.B Please make sure all hand writing on the form is in block capitals and legible

PROACT PLUS Registry

PATIENT STUDY ID (in format Z123, where Z denotes the site)	VISIT NUMBER (circle as appropriate)	MONTH AND YEAR OF BIRTH (mm/yyyy)	GENDER (circle as appropriate)
____	Baseline Cycle 2, 3, 4, 5, 6 4 weeks post chemotherapy 12 months post chemotherapy	__/____	**F or M**
Date sample taken: __/__/____ (dd/mm/yyy)	Time sample taken: __:__ (hh:mm 24hr clock)		
Site:	**Research nurse investigator:**		

SAMPLES TO BE TAKEN

Mandatory:

1x (up to) **5mL SST (gold top) tube** **□(tick box)**

If additional consent has been given:

1x (up to) **5mL SST (gold top) tube** **□(tick box)**

1x (up to) **5mL EDTA (purple top) tube** **□(tick box)**

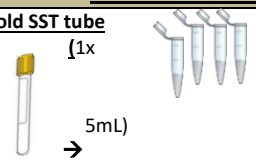
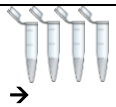
SPECIMEN REQUIREMENTS	INVESTIGATIONS REQUIRED	
LOCAL PROCESSING LAB	RVI LAB (Central lab) PLEASE INPUT & PROCESS (Apex Codes)	CENTRAL LAB USE (Barcodes)
MANDATORY SAMPLES, PROCESS AS FOLLOWS		
 **Gold SST tube** (1x 5mL) →  Invert 5x, allow 30mins to clot, centrifuge for 10mins at 1000-1300 RCF (g) in a swing bucket centrifuge and divide into two 1mL cryovials.	**Troponin T** – 1mL cryovial **and** **Troponin I** – 1mL cryovial (use label provided) Freeze at -20°C until next available transport to Newcastle	1 x 1mL cryovial for Troponin T **and** **1 x 1mL cryovial for Troponin I** **FOR -80°C STORAGE UNTIL BATCH ANALYSIS**	

LOCAL LAB: Photocopy this form and keep for records

N.B Please make sure all hand writing on the form is in block capitals and legible

PROACT PLUS Registry

PATIENT STUDY ID (in format Z123, where Z denotes the site)	VISIT NUMBER (circle as appropriate)	MONTH AND YEAR OF BIRTH (mm/yyyy)	GENDER (circle as appropriate)
____	Baseline Cycle 3, 5		

	4 weeks post chemotherapy 12 months post chemotherapy	__/____	**F or M**
Site:		**Research nurse investigator:**	
SPECIMEN REQUIREMENTS		**INVESTIGATION REQUIREMENTS**	
LOCAL PROCESSING LAB		RVI LAB (Central lab) PLEASE INPUT & PROCESS (Apex Codes)	CENTRAL LAB USE (Barcodes)
IF <u>ADDITIONAL</u> CONSENT HAS BEEN GIVEN, PROCESS SAMPLES AS FOLLOWS:			
<u>Gold SST tube</u> (1x 5mL) →  Invert 5x, allow 30mins to clot, centrifuge for 10mins at 1000-1300 RCF (g) in a swing bucket centrifuge. Spin and divide into four <u>0.5mL serum</u> cryovials.	STORE AT -20°C AND TRANSPORT WHEN REQUESTED USE LABELS PROVIDED No. of vials collected: __	Receipt up to 4 vials **FOR -80°C STORAGE** No. of vials received: __	
<u>EDTA tube</u> (1x5mL) →  Invert 8-10x, centrifuge for 10mins ≤1300 RCF (g). Spin and divide into four <u>0.5mL plasma</u> cryovials.	STORE AT -20°C AND TRANSPORT WHEN REQUESTED USE LABELS PROVIDED No. of vials collected: __	Receipt up to 4 vials **FOR -80°C STORAGE** No. of vials received: __	

NEWCASTLE LAB STAFF NOTES:
Book in APEX location as: NLSTUDY
Enter into Study code field and Visit No into reasons for request field – against all specimen numbers used

LOCAL LAB: Photocopy this form and keep for site records

PROACT PLUS STUDY ECHO PROTOCOL

STUDY DETAILS

AUTHORS

DATE

ECHOCARDIOGRAPHY IMAGE ACQUISITION PROTOCOL

GENERAL INSTRUCTIONS

STAGE OF TREATMENT:-

PATIENT MEASUREMENTS:-

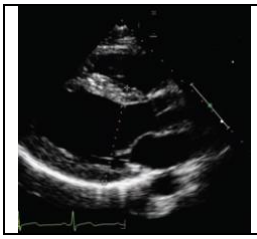
Height cm

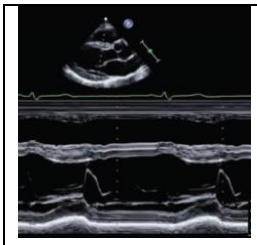
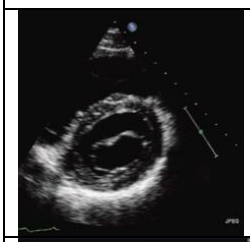
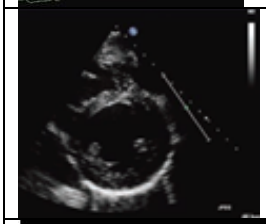
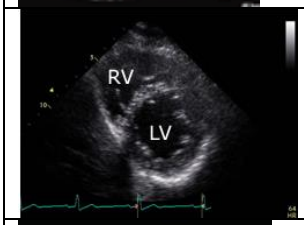
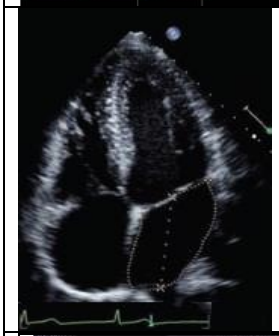
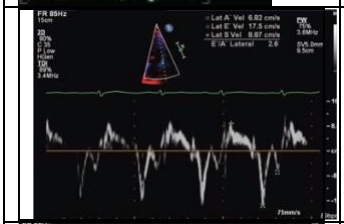
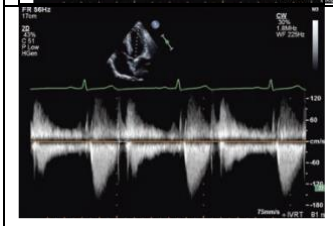
Weight kg

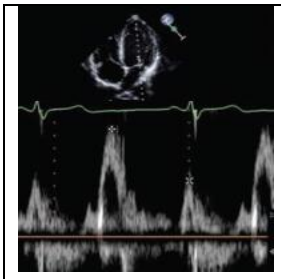
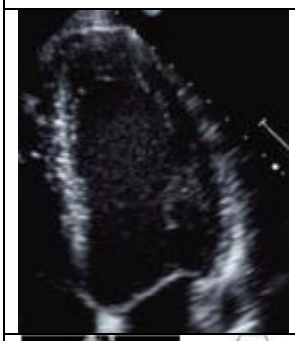
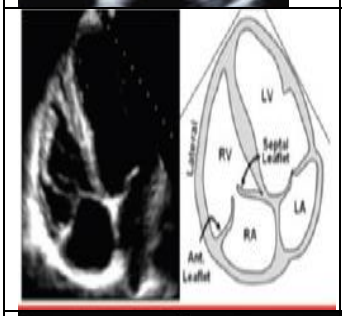
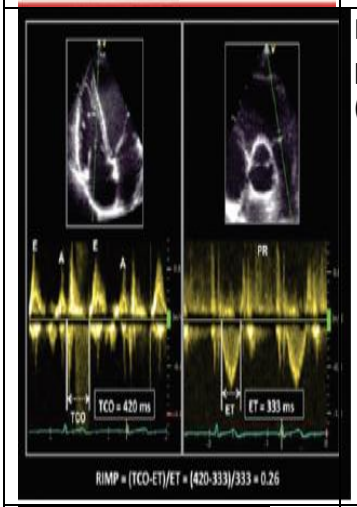
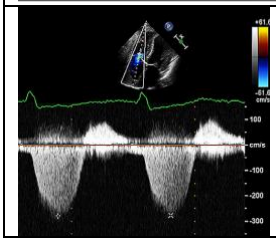
Blood Pressure / mmHg

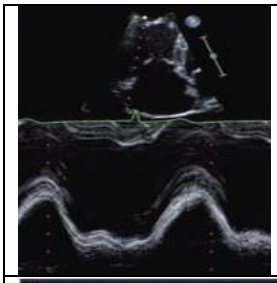
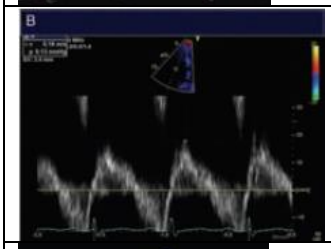
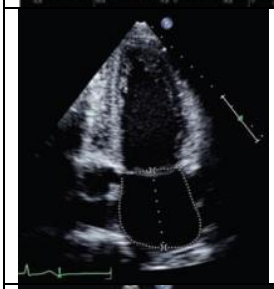
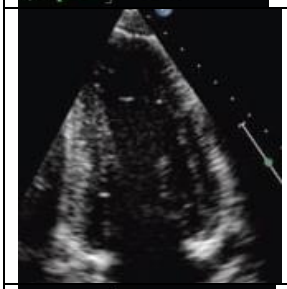
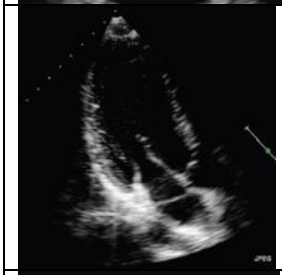
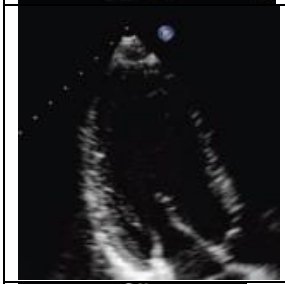
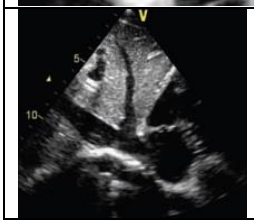
Heart Rate bpm sinus/AF/other

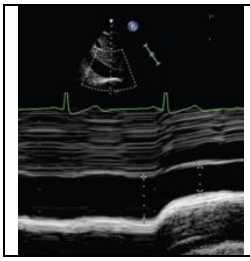
- An ECG should be attached throughout the scan
- No patient identifiable details should be on screen other than the unique study identification number
- A minimum of three cardiac cycles should be stored for each image obtained if the patient is in Sinus rhythm
- A minimum of five cardiac cycles should be stored for each image obtained if the patient is in AF or has frequent ectopy/ pacing
- A full standard template in line with BSE recommendations should be obtained for each study
- Particular attention should be paid to the following views which are essential to meet study criteria;-

		2D Parasternal Long Axis to demonstrate chamber sizes, MV structure and motion and LV function	

	Plax M-mode Just distal to MV tips LV end-diastolic and end- systolic dimensions	
	PSax basal view	
	PSax papillary level	
	Psax apical view	
	Apical 4 chamber view for chamber sizes and LA volume measurement	
	TDI at base of lateral and septal walls for E’ and S-wave velocity measurements.	
	CW Doppler between Aortic and Mitral valve for IVRT measurement	

	PW Doppler at MV tips for E and A wave velocity and E decel time	
	LV focus during held respiration for GLS measurement and Simpson's biplane EF% assessment	
	RV focused view Achieved with medial or lateral transducer orientation	
	Pulsed Doppler through TV tips and pulsed Doppler through pulmonary valve in short axis view (to measure pulsed Doppler RIMP)	
	Colour and CW Doppler for TR Vmax measurement (RVsp estimate)	

	M-mode at TV lateral annulus for TAPSE measurement	
	TDI at base of RV free wall (TV annulus)	
	Apical 2 –chamber for LA volume measurement	
	LV focus during held respiration for GLS measurement and Simpson's biplane EF% assessment	
	Apical 3 chamber view for LV function assessment	
	LV focus during held respiration for GLS measurement and Simpson's biplane EF% assessment	
	Subcostal image to show IVC size	

		M-mode subcostal view with’ sniff’ to assess IVC compliance	

8.6 Appendix 6 - Ethical Approval 2

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Sharareh Vahabi
Clinical Research Fellow
South Tees NHS Foundation Trust (The James Cook University Hospital)
Marton Road
Middlesbrough
TS4 3BW

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

14 August 2018

Dear Dr Vahabi

HRA and Health and Care

Study title:	**PROACT PLUS Registry and Echocardiography sub-study: An observational, prospective, cohort study assessing the use of novel echocardiographic tools and troponin to detect early signs of cardiotoxicity in patients treated for breast cancer and lymphoma**
IRAS project ID:	**245613**
REC reference:	**18/EM/0177**
Sponsor	**South Tees NHS Foundation Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales? You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your nonNHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including: Registration of research

- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?

You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Dr Sharareh Vahabi

Tel: 01642 850850

Email: sharareh.vahabi@nhs.net

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **245613**. Please quote this on all correspondence.

Yours sincerely

Simon Connolly

Senior Assessor

Email: hra.approval@nhs.net

Copy to: Mr Joe Millar, South Tees NHS Foundation Trust

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Covering letter on headed paper [PROACT PLUS cover letter]		03 May 2018
GP/consultant information sheets or letters [PROACT PLUS registry GP letter]	1.0	25 April 2018
HRA Schedule of Events	1	29 May 2018
HRA Statement of Activities	1	29 May 2018
Instructions for use of medical device [PROACT PLUS Echo template]	1.0	05 March 2018
IRAS Application Form [IRAS_Form_09052018]		09 May 2018
Letter from funder		
Letters of invitation to participant	2.0	03 July 2018
Other [PROACT clinical trial protocol]	1.3	27 September 2017
Participant consent form	1.0	03 March 2018
Participant consent form [PROACT clinical trial consent form]	1.2	24 July 2017
Participant information sheet (PIS)	3	13 August 2018
Research protocol or project proposal [PROACT PLUS Registry and Echo sub-study protocol]	1.0	09 March 2018
Response to Request for Further Information		
Summary CV for Chief Investigator (CI) [Sharareh Vahabi CV]		25 April 2018
Summary CV for student [Sharareh's CV]		24 April 2018
Summary CV for supervisor (student research) [Supervisor 3 - Helen Hancock CV]		
Summary CV for supervisor (student research) [Supervisor 1 Dr Austin CV]		01 December 2015
Summary CV for supervisor (student research) [Supervisor 2 Dr Stewart CV]		02 April 2018

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments

1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	Minor amendment made to document subsequent to REC Favourable Opinion.
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	A Statement of Activities and Schedule of Events have been provided for use with participating NHS organisations in England. Exchange of the SoA will confirm capacity and capability of an NHS organisation to host the research.
4.2	Insurance/indemnity arrangements assessed	Yes	No comments
4.3	Financial arrangements assessed	Yes	External funding bids are in progress. If successful, funds will be used to run the study at additional NHS organisations, where funds will be made available as described in the Statement of Activities.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
Section	**Assessment Criteria**	**Compliant with Standards**	**Comments**
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments

6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.

There is a single type of participating NHS organisation in England and Wales, i.e. the research activity at all sites shall be the same.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS or on the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net, or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A Principal Investigator should be in place at each participating NHS organisation in England and Wales. Suitable individuals have been identified for the sites listed in Part C of the IRAS form. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken

As a non-commercial study undertaken by local staff, it is unlikely that letters of access or honorary research contracts will be applicable, except where local network staff employed by another Trust or researchers employed by a University are involved (and then it is likely that arrangements are already in place). Where arrangements are not already in place, such researchers undertaking any of the research activities listed in A18 or A19 of the IRAS form (except for administration of questionnaires), would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. These would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed) and should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance. For research team members only administering questionnaires, a Letter of Access based on standard DBS checks and occupational health clearance would be appropriate.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.

The applicant has indicated that they intend to apply for inclusion on the NIHR CRN Portfolio.

8.7 Appendix 7 - Chapter 4 results

1. Baseline characteristics - cancer information

Variable	All patients (n=61)	G1 (n=56)	G2 (n=6)	p-value
Cancer diagnosis (%)				
DLBCL	42 (68.9)	38 (69.1)	4 (66.7)	
B Cell NHL	3 (4.9)	3 (5.5)	0 (0)	
Classical HL	4 (6.6)	3 (5.5)	1 (16.7)	
HL (nodule lymphocyte)	1 (1.6)	1 (1.8)	0 (0)	
T cell lymphoma	1 (1.6)	0 (0)	1 (16.7)	0.22
Breast - ductal	9 (14.8)	9 (16.4)	0 (0)	
Breast - lobular	1 (1.6)	1 (1.8	0 (0)	
Breast - mixed	0 (0)	0 (0)	0 (0)	
Breast - mucinous	0 (0)	0 (0)	0 (0)	
Breast - metaplastic	0 (0)	0 (0)	0 (0)	
Breast- medullary	0 (0)	0 (0)	0 (0)	
Regional lymph node involvement (%)				
N0	1 (1.6)	1 (1.8)	0 (0)	
N1	6 (9.8)	6 (10.9)	0 (0)	
N1 mi	1 (1.6)	1 (1.8)	0 (0)	1.00
N2	1 (1.6)	1 (1.8)	0 (0)	
N3	1 (1.6)	1 (1.8)	0 (0)	
NA	51 (83.6)	45 (81.8)	6 (100)	
Metastases (%)				
M0	10 (16.4)	10 (18.2)	0 (0)	
M1	0 (0)	0 (0)	0 (0)	0.57
NA	51 (83.6)	45 (81.8)	6 (100)	
Cancer stage (%)				
I	9 (14.8)	8 (1.8)	1 (16.7)	
II	5 (8.2)	5 (9.1)	0 (0)	
III	10 (16.4)	9 (16.4)	1 (16.7)	
IV	27 (44.3)	23 (41,8)	4 (66.7)	
T0	0 (0)	0 (0)	0 (0)	1.00
T1	2 (3.3)	2 (3.6)	0 (0)	
T2	4 (6.6)	4 (7.3)	0 (0)	
T3	4 (6.6)	4 (7.3)	0 (0)	
T4	0 (0)	0 (0)	0 (0)	
Cancer grade				
1	0 (0)	0 (0)	0 (0)	
2	5 (8.2)	5 (9.1)	0 (0)	
3	5 (8.2)	5 (9.1)	0 (0)	1.00
GX	0 (0)	0 (0)	0 (0)	
NA	51 (83.6)	45 (81.8)	6 (100)	
Chemotherapy treatment (%)				
R CHOP (x6 cycles)	43 (70.5)	39 (70.9)	4 (66.7)	
R CHOP (x3 CHOP, x3 CEOP)	2 (3.3)	2 (3.6)	0 (0)	0.11
CHOP	1 (1.6)	0 (0)	1 (16.7)	
ABVD	5 (8.2)	4 (7.3)	1 (16.7)	
FEC-T	10 (16.4)	10 (18.2)	0 (0)	
FEC 75	0 (0)	0 (0)	0 (0)	
Cancer surgery (%)				
Lumpectomy/WLE	2 (3.3)	2 (3.6)	0 (0)	
Mastectomy	7 (11.5)	7 (12.7)	0 (0)	1.00
Other	2 (3.3)	2 (3.6)	0 (0)	

2. Changes in conventional echocardiographic measures - all patients

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
BSA (m^2)	1.89	1.90	-0.01	0.10
LVIDd (cm)	4.5	4.5	0.01	0.80
LVIDs (cm)	2.7	3.0	0.26	0.009*
Fractional shortening (%)	39	35	-4.8	0.01*
LV mass index (mg/m^2)	78.9	83.3	4.8	0.02*
LV RWT (%)	0.42	0.44	0.02	0.06
LA diameter (cm)	3.3	3.5	0.18	0.25
LA volume biplane (ml/m^2)	24.1	21.9	-0.53	0.58
LVEDV indexed (ml/m^2)	40.8	42.7	1.23	0.26
LVESV indexed (ml/m^2)	15.6	18.4	2.55	0.001*
LVEF (%)	62	58	-3.4	<0.01*
MV E (m/s)	0.70	0.59	-0.09	0.001*
MV E/A	0.91	0.80	-0.11	0.005*
MV DecT (cm)	186	192	6.3	0.51
Lateral E/E'	7.8	7.4	-0.3	0.39
Medial E/E'	9.7	9.6	-0.14	0.72
Mean E/E'	8.8	8.5	-0.30	0.37
TR maxPG (mmHg)	15.1	13.8	-0.06	0.97
IVRT (cm)	91	84	-5.29	0.17
Tei Index (LV)	0.53	0.54	0.01	0.58
RA volume indexed (ml/m^2)	17.9	15.9	-1.54	0.20
RV basal-wall diameter (cm)	3.8	3.8	0.08	0.93
RV mid-wall diameter (cm)	2.9	3.1	0.3	0.35
RV free wall S' (m/s)	0.14	0.13	-0.001	0.79
RV EDA (cm^2)	17.4	17.1	-0.35	0.64
RV ESA (cm^2)	9.5	9.8	0.26	0.56
RV FAC (%)	45.2	42.7	-2.39	0.14
TAPSE (cm)*	2.2	2.2	0.006	0.92
RV IVRT (cm)	79	77	-2.40	0.64
Tei index (RV)	0.56	0.59	0.02	0.37

3. Changes in strain and strain-rate measures - all patients

3.1 Changes in LV strain and strain-rate measures

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
GLS (%)	-20.6	-19.3	1.3	<0.01*
MyoGLS (%)	-17.8	-16.4	1.3	<0.01*
LV peak systolic longitudinal SR (1/s)	-1.07	-1.04	0.03	0.25
LV end-systolic longitudinal SR (1/s)	-0.25	-0.26	-0.02	0.74
LV early diastolic longitudinal SR (1/s)	0.99	0.83	-0.15	<0.001*
LV late diastolic longitudinal SR (1/s)	0.80	0.82	0.01	0.72
GRS (%)	36.6	35.3	-1.39	0.30
LV peak systolic radial SR (1/s)	1.45	1.42	-0.03	0.52
LV end-systolic radial SR (1/s)	0.35	0.38	0.03	0.70
LV early diastolic radial SR (1/s)	-1.34	-1.18	0.15	0.003*
LV late diastolic radial SR (1/s)	-0.82	-0.87	-0.03	0.51
GCS (%)	-31.2	-28.2	2.62	0.03*
MyoGCS (%)	-22.3	-19.5	2.54	0.03*
LV peak systolic circumferential SR (1/s)	-1.73	-1.61	-0.07	0.47
LV end-systolic circumferential SR (1/s)	-0.15	-0.19	-0.03	0.69
LV early diastolic circumferential SR (1/s)	1.61	1.31	-0.30	0.04*
LV late diastolic circumferential SR (1/s)	0.86	0.93	0.07	0.50
LV twist (degrees)	15.1	13.9	-2.04	0.39
LV torsion (degrees/cm)	2.06	1.93	-0.26	0.41

3.2 Changes in RV strain and strain-rate measures

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
RV GLS (%)	-25.1	-22.5	2.73	<0.001*
RV myoGLS (%)	-22.2	-20.9	2.26	0.004*
RV peak systolic longitudinal SR (1/s)	-1.28	-1.20	0.08	0.31
RV end-systolic longitudinal SR (1/s)	-0.11	-0.07	0.02	0.67
RV early diastolic longitudinal SR (1/s)	1.08	0.91	-0.16	0.01*
RV late diastolic longitudinal SR (1/s)	0.96	0.96	-0.01	0.89
RV FWS (%)	-28.9	-26.6	2.41	0.01*
RVFW peak systolic longitudinal SR (1/s)	-1.57	-1.59	-0.02	0.76
RVFW end-systolic longitudinal SR (1/s)	-0.12	-0.09	0.01	0.81
RVFW early diastolic longitudinal SR (1/s)	1.23	1.07	-0.15	0.02*
RVFW late diastolic longitudinal SR (1/s)	1.07	1.15	0.08	0.45

3.3 Changes in left atrial strain and strain-rate measures

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
LA 4Ch strain (%)	27.0	26.1	-1.32	0.30
LASr 4Ch (%)	34.8	33.3	-1.79	0.23
LAScd 4Ch (%)	-17.7	-14.8	2.90	0.04*
LASct 4Ch (%)	-17.0	-18.5	-1.18	0.31
LA 4Ch peak systolic SR (1/s)	1.19	1.14	-0.05	0.48
LA 4Ch early diastolic SR (1/s)	-1.24	-0.85	0.38	0.001*
LA 4Ch late diastolic SR (1/s)	-1.71	-1.84	-0.11	0.29
LA 2Ch strain (%)	26.7	26.2	-0.53	0.70
LASr 2Ch (%)	36.2	35.9	-0.34	0.84
LAScd 2Ch (%)	-15.5	-15.2	0.34	0.75
LASct 2Ch (%)	-20.7	-20.7	0.01	0.99
LA 2Ch peak systolic SR (1/s)	1.13	1.11	-0.02	0.72
LA 2Ch early diastolic SR (1/s)	-0.98	-0.87	0.13	0.11
LA 2Ch late diastolic SR (1/s)	-2.00	-2.19	-0.18	0.24
LA biplane strain (%)	26.8	25.6	-1.3	0.30
LASr biplane (%)	35.1	34.5	-0.79	0.65
LAcd biplane (%)	-16.3	-15.3	1.10	0.37
LAct biplane (%)	-18.7	-19.2	-0.20	0.84
LA biplane peak systolic SR (1/s)	1.18	1.07	-0.11	0.11
LA biplane early diastolic SR (1/s)	-1.12	-0.91	0.18	0.04*
LA biplane late diastolic SR (1/s)	-1.85	-1.92	-0.06	0.65

3.4 Changes in right atrial strain and strain-rate measures

Variable	Mean at V1	Mean at V2	Mean change from V1	p value
RA strain (%)	34.3	30.8	-4.25	0.02*
RASr (%)	40.3	37.1	-3.50	0.08
RAScd (%)	-23.3	-20.6	2.78	0.11
RASct (%)	-17.0	-16.5	0.47	0.74
RA peak systolic SR (1/s)	1.37	1.25	-0.10	0.23
RA early diastolic SR (1/s)	-1.17	-0.99	0.18	0.02*
RA late diastolic SR (1/s)	-1.62	-1.70	-0.08	0.60

8.8 Appendix 8 - CRF

PROACT PLUS – Registry

Table of Contents

Baseline Assessments

Baseline eligibility		
1.	Subject ID	
2.	Date of Birth	☐ ☐ / ☐ ☐ ☐ ☐
3.	Date of eligibility check	☐ ☐/☐ ☐/☐ ☐ ☐ ☐
4.	Name of person checking eligibility	
5.	Version of protocol	☐.☐
6.	Does the subject meet all inclusion criteria	Yes ☐ No ☐
7.	Does the subject meet any of the exclusion criteria	Yes ☐ No ☐
8.	Confirm the patient is eligible	Confirm ☐

Inclusion Criteria		
1.	Adult patients with a new diagnosis of histopathologically confirmed breast carcinoma	Yes ☐ No ☐
2.	Adult patients with a new diagnosis of histopathologically confirmed lymphoma (Hodgkin's or Non-Hodgkin's lymphoma)	Yes ☐ No ☐
3.	Age ≥ 18	Yes ☐ No ☐
4.	Planned to receive anthracycline based treatment (adjuvant or neo-adjuvant) – any dose	Yes ☐ No ☐
5.	Written informed consent	Yes ☐ No ☐

Exclusion Criteria		
1.	Meets eligibility criteria for the PROACT trial* ** patients who are otherwise eligible for the PROACT trial will not be eligible to participate in the PROACT registry*	Yes ☐ No ☐
2.	Known metastatic cancer	Yes ☐ No ☐
3.	Poor cancer prognosis ≤ 1 year	Yes ☐ No ☐

Baseline Informed Consent		
1.	Confirm the patient consented to participate in the trial	Yes ☐ No ☐
2.	Date of written consent	☐ ☐/☐ ☐/☐ ☐ ☐ ☐
3.	Name of person taking consent	
4.	Version of PIS	☐.☐
5.	Version of consent form	☐.☐
6.	Did the patient consent to have data stored for up to 15 years	Yes ☐ No ☐
7.	Did the patient consent to having a swab taken	Yes ☐ No ☐
8.	Did the patient consent to having blood taken for future research	Yes ☐ No ☐
9.	Did the patient agree to long term follow-up	Yes ☐ No ☐

**A full medical history will be recorded for each patient at baseline and will include details of all clinically significant cardiovascular medical conditions and a full cancer history (from patients' medical notes). Review of other hospital notes (and GP notes) may be required to complete the medical history.*

Baseline Medical History		
1.	Atrial fibrillation or flutter (known history)	Yes ☐ No ☐
2.	Coronary heart disease (clinical diagnosis of angina, angiographically documented CAD, previous coronary angioplasty or coronary artery bypass grafting)	Yes ☐ No ☐ If 'yes' Describe ----------
3.	Previous MI	Yes ☐ No ☐
4.	Hypertension	Yes ☐ No ☐
5.	Hyperlipidaemia (>5mmol/l total cholesterol/treatment with lipid lowering medication)	Yes ☐ No ☐
6.	Diabetes mellitus (known history)	Yes ☐ No ☐ 1. Diet controlled ☐ 2. Tablet controlled ☐ 3. Insulin ☐
7.	Known LV impairment (EF<50%)	Yes ☐ No ☐
8.	Previous stroke or TIA	Yes ☐ No ☐
9.	Smoking	Current ☐ Ex ☐ Never☐
10	Chronic Obstructive Pulmonary Disease (COPD) (known history)	Yes ☐ No ☐
11	Asthma (Known history)	Yes ☐ No ☐
12	Peripheral Arterial Disease (Symptomatic PVD or previous peripheral angioplasty or bypass operation)	Yes ☐ No ☐
13	Any previous cancer diagnosis	Yes ☐ No ☐
14	Any previous anthracycline treatment	Yes ☐ No ☐
15	Any previous Herceptin treatment	Yes ☐ No ☐
16	Any previous radiotherapy (mediastinal/thoracic)	Yes ☐ No ☐
Cancer History		
1.	Type of cancer	Breast ☐ Hodgkin's lymphoma ☐

		Non-Hodgkin's lymphoma ☐
2.	Date of cancer diagnosis	☐ ☐/☐ ☐/☐ ☐ ☐ ☐
3.	How was the diagnosis of cancer made	☐Fine needle aspiration cytology ☐Core needle biopsy ☐Excision biopsy ☐Incisional biopsy ☐Laparascopic ☐Other
4.	Previous chest radiotherapy	☐Left side ☐Right side ☐Both sides ☐None
5.	Date of index surgery	☐ ☐/☐ ☐/☐ ☐ ☐ ☐ ☐N/A
6.	If breast cancer, types of surgery	☐ Lumpectomy/wide local excision ☐ Mastectomy ☐Radical mastectomy ☐Other, specify
7.	Types of axillary surgery	☐Axillary clearance ☐Sentinel lymph node biopsy
Current Breast Cancer Details		
1.	If breast cancer: histological subtype (s)	☐Ductal ☐Lobular ☐Mixed ☐Mucinous ☐Papillary ☐Metaplastic ☐Medullary ☐Other, specify
2.	T (size of original tumour)	☐T0 ☐TX ☐Tis ☐T1 ☐T2 ☐T3 ☐T4
3.	N (regional lymph nodes involved)	☐ N0 ☐NX ☐N1

			☐N1 mi ☐N2 ☐N3
4.	M (metastasis)		☐M0 ☐M1
5.	Grade		☐ 1 ☐2 ☐3 ☐GX
6.	Receptor details	Estrogen: Progesterone: HER2: (autopopulates from randomisation	☐ Positive (≥3) ☐Negative (≤2) ☐Positive (≥3) ☐Negative (≤2 ☐Not done ☐Positive ☐Negative
Current Lymphoma Details			
1.	If Hodgkin's lymphoma - histological type (s):		☐Classical Hodgkin's lymphoma ☐Nodular lymphocyte-predominant Hodgkin's lymphoma ☐other, specify
2.	If Non-Hodgkin's lymphoma – histological type (s):		***B cell non-Hodgkin's Lymphoma*** ☐Diffuse large B cell lymphoma ☐Burkitt lymphoma ☐Mantle cell lymphoma ☐Follicular lymphoma ☐Small lymphocytic lymphoma ☐Lymphoplasmacytic lymphoma ☐Marginal zone Non-Hodgkin's Lymphoma ***T cell non-Hodgkin's Lymphoma*** ☐T cell non-Hodgkin's lymphoma
3.	Lymphoma staging		☐Stage 1 ☐Stage 2 ☐Stage 3

		☐Stage 4

Concomitant medications - baseline assessment (repeat section editable by sites)

Concomitant medications		
1.	Generic medication name	
2.	Dose	
3.	Frequency	☐OD ☐BD ☐TDS ☐QDS ☐PRN ☐other
4.	Units	☐g ☐ mg ☐µg ☐ml ☐unit ☐other
5.	Medication started more than 14 days prior to consent	☐Yes ☐No
6.	Start date (if <14 days)	☐☐/☐☐/☐☐☐☐
7.	End date (inclusive)	☐☐/☐☐/☐☐☐☐
8.	End date not applicable	☐Lifelong ☐Ongoing at end of study ☐Unknown

Baseline-Physical assessment		
1.	Date of assessment	☐☐/☐☐/☐☐☐☐
2.	Height	☐☐☐cm
3.	Weight	☐☐☐kg
4.	Heart rate	☐☐☐bpm
5.	BP systolic	☐☐☐mmHg
6.	BP diastolic	☐☐☐mmHg

Baseline-Demographics		
1.	Gender at birth	☐Male ☐Female
2.	Ethnic group	White: ☐British ☐Irish ☐Other Asian: ☐Chinese ☐Bangladeshi ☐Indian ☐Pakistani ☐Other Black: ☐African ☐Caribbean ☐Other

		Mixed: □White/Asian □White/African □White/Caribbean □Other Other: □ Specify_____

Baseline-ECOG-Performance status	
1.	☐0 (Fully active, able to carry on all pre-disease performance without restriction) ☐1. (Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work) ☐2. (Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours) ☐3. (Capable of only limited self-care, confined to bed or chair more than 50% of waking hours) ☐4. (Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair) ☐5. (Dead)

Baseline-NYHA class		
1.	Date of assessment	☐☐/☐☐/☐☐☐☐
2.	☐I (No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea ☐II (Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnoea ☐III (Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnoea ☐IV (Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases)	

Baseline-Blood parameters		
U & Es		
1.	Date of U & Es	☐☐/☐☐/☐☐☐☐
2.	Sodium	☐☐☐mmol/l
3.	Potassium	☐.☐mmol/l
4.	Creatinine	☐☐☐µmol/l
5.	Urea	☐☐.☐mmol/l
Estimated glomerular filtration rate (eGFR)		
6.	eGFR greater than 60 ml/min/1.7m2	☐Yes☐No☐
7.	If ‘No’ eGFR result	☐☐ml/min/1.7m
FBC		
8.	Date of FBC	☐☐/☐☐/☐☐☐☐
9.	Hb	☐☐☐g/L
Troponin		
10	Date of troponin sample	☐☐/☐☐/☐☐☐☐

11	Confirm troponin T sample was not haemolysed	☐Confirm
12	Confirm troponin I sample was not haemolysed	☐Confirm

Baseline-Further research samples			
1.	Did the patient have a mouth swab taken		☐Yes☐No
2.	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm
3.	Did the patient have blood for further research taken		☐Yes☐No
4.	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm

Echocardiograms

Echocardiograms Baseline-Echocardiogram-TTE		
1.	Date of TTE	☐☐/☐☐/☐☐☐☐
2.	Assessment of study quality	☐Good ☐Adequate ☐Poor
3.	Rhythm	☐Sinus ☐Atrial fibrillation ☐Atrial flutter ☐Other
4.	Left atrial volume (indexed)	☐☐☐mL/m2
5.	Left ventricular end systolic dimensions	☐☐.☐cm
6.	Left ventricular end diastolic dimensions	☐☐.☐cm
7.	Left ventricular fractional shortening (%)	☐☐
8.	Left ventricular end systolic volume (indexed)	☐☐☐ml /m2
9.	Left ventricular end diastolic volume (indexed)	☐☐☐ ml/m2
10	Left ventricular ejection fraction	☐☐%
11	If LVEF could not be measured, a visual assessment confirmed the LVEF as:	☐>55% ☐50-55% ☐45 - 49% ☐40 -44% ☐35 - 39% ☐<35%
12	LV mass (indexed)	☐☐☐g/m2
13	TAPSE (Tricuspid annular plane systolic excursion)	☐☐.☐cm
14	Tricuspid regurgitation peak systolic pressure	☐☐mmHg
15	Estimated right atrial pressure	☐0-5mmHg ☐5-10mmHg ☐10-15mmHg

		□15-20mmHg □>20mmHg
16	RV fractional change (FAC) (%)	□□
17	RV free wall S’	□.□□ m/s
18	E:	□.□□m/s
19	A:	□.□□m/s
20	IVRT:	□□□ms
21	MV deceleration time	□□□ms
22	e’ (lateral)	□.□□m/s
23	a’ (lateral)	□.□□m/s
24	s’ wave (lateral)	□.□□m/s
25	e’ (septal)	□.□□m/s
26	a’ (septal)	□.□□m/s
27	s’ wave (septal)	□.□□m/s
28	Peak global longitudinal strain (GLS) (%)	-□□.□
29	Longitudinal strain rate (1/sec)	-□.□□
30	Global radial strain (GRS) (%)	+□□.□
31	Radial strain rate (1/sec)	+□.□□
32	Global circumferential strain (GCS) (%)	-□□.□
33	Circumferential strain rate (1/sec)	-□.□□
34	Apical rotation (°)	+□□.□
35	Basal rotation (°)	-□□.□
36	LV twist (°)	□□.□
37	LV length end-diastole	□.□cm
38	LV torsion (°/cm)	□□.□
39	Right ventricular free wall strain (RVFWS) (%)	-□□.□
40	Right ventricular free wall strain rate (1/s)	-□.□□
41	LA GLS (%)	+□□.□
42	LA strain rate (1/sec)	+□.□□
43	RA GLS (%)	+□□.□
44	RA strain rate (1/sec)	+□.□□

4 Weeks Post Chemotherapy Echocardiogram-TTE		
1.	Date of TTE	□□/□□/□□□□
2.	Assessment of study quality	□Good □Adequate □Poor

3.	Rhythm	□Sinus □Atrial fibrillation □Atrial flutter □Other
4.	Left atrial volume (indexed)	□□□mL/m2
5.	Left ventricular end systolic dimensions	□□.□cm
6.	Left ventricular end diastolic dimensions	□□.□cm
7.	Left ventricular fractional shortening (%)	□□
8.	Left ventricular end systolic volume (indexed)	□□□ml /m2
9.	Left ventricular end diastolic volume (indexed)	□□□ ml/m2
10	Left ventricular ejection fraction	□□%
11	If LVEF could not be measured, a visual assessment confirmed the LVEF as:	□>55% □50-55% □45 - 49% □40 -44% □35 - 39% □<35%
12	LV mass (indexed)	□□□g/m2
13	TAPSE (Tricuspid annular plane systolic excursion)	□□.□cm
14	Tricuspid regurgitation peak systolic pressure	□□mmHg
15	Estimated right atrial pressure	□0-5mmHg □5-10mmHg □10-15mmHg □15-20mmHg □>20mmHg
16	RV fractional change (FAC) (%)	□□
17	RV free wall S’	□.□□ m/s
18	E:	□.□□m/s
19	A:	□.□□m/s
20	IVRT:	□□□ms
21	MV deceleration time	□□□ms
22	e’ (lateral)	□.□□m/s
23	a’ (lateral)	□.□□m/s
24	s’ wave (lateral)	□.□□m/s
25	e’ (septal)	□.□□m/s
26	a’ (septal)	□.□□m/s
27	s’ wave (septal)	□.□□m/s
28	Peak global longitudinal strain (GLS) (%)	-□□.□
29	Longitudinal strain rate (1/sec)	-□.□□
30	Global radial strain (GRS) (%)	+□□.□
31	Radial strain rate (1/sec)	+□.□□

32	Global circumferential strain (GCS) (%)	-□□.□
33	Circumferential strain rate (1/sec)	-□.□□
34	Apical rotation (°)	+□□.□
35	Basal rotation (°)	-□□.□
36	LV twist (°)	□□.□
37	LV length end-diastole	□.□cm
38	LV torsion (°/cm)	□□.□
39	Right ventricular free wall strain (RVFWS) (%)	-□□.□
40	Right ventricular free wall strain rate (1/s)	-□.□
41	LA GLS (%)	+□□.□
42	LA strain rate (1/sec)	+□.□□
43	RA GLS (%)	+□□.□
44	RA strain rate (1/sec)	+□.□□

12 Months Post Chemotherapy Echocardiogram-TTE		
1.	Date of TTE	□□/□□/□□□□
2.	Assessment of study quality	□Good □Adequate □Poor
3.	Rhythm	□Sinus □Atrial fibrillation □Atrial flutter □Other
4.	Left atrial volume (indexed)	□□□mL/m2
5.	Left ventricular end systolic dimensions	□□.□cm
6.	Left ventricular end diastolic dimensions	□□.□cm
7.	Left ventricular fractional shortening (%)	□□
8.	Left ventricular end systolic volume (indexed)	□□□ml /m2
9.	Left ventricular end diastolic volume (indexed)	□□□ ml/m2
10	Left ventricular ejection fraction	□□%
11	If LVEF could not be measured, a visual assessment confirmed the LVEF as:	□>55% □50-55% □45 - 49% □40 -44% □35 - 39% □<35%
12	LV mass (indexed)	□□□g/m2
13	TAPSE (Tricuspid annular plane systolic excursion)	□□.□cm
14	Tricuspid regurgitation peak systolic pressure	□□mmHg

15	Estimated right atrial pressure	□0-5mmHg □5-10mmHg □10-15mmHg □15-20mmHg □>20mmHg
16	RV fractional change (FAC) (%)	□□
17	RV free wall S’	□.□□ m/s
18	E:	□.□□m/s
19	A:	□.□□m/s
20	IVRT:	□□□ms
21	MV deceleration time	□□□ms
22	e’ (lateral)	□.□□m/s
23	a’ (lateral)	□.□□m/s
24	s’ wave (lateral)	□.□□m/s
25	e’ (septal)	□.□□m/s
26	a’ (septal)	□.□□m/s
27	s’ wave (septal)	□.□□m/s
28	Peak global longitudinal strain (GLS) (%)	-□□.□
29	Longitudinal strain rate (1/sec)	-□.□□
30	Global radial strain (GRS) (%)	+□□.□
31	Radial strain rate (1/sec)	+□.□□
32	Global circumferential strain (GCS) (%)	-□□.□
33	Circumferential strain rate (1/sec)	-□.□□
34	Apical rotation (°)	+□□.□
35	Basal rotation (°)	-□□.□
36	LV twist (°)	□□.□
37	LV length end-diastole	□.□cm
38	LV torsion (°/cm)	□□.□
39	Right ventricular free wall strain (RVFWS) (%)	-□□.□
40	Right ventricular free wall strain rate (1/s)	-□.□
41	LA GLS (%)	+□□.□
42	LA strain rate (1/sec)	+□.□□
43	RA GLS (%)	+□□.□
44	RA strain rate (1/sec)	+□.□□

Chemotherapy Cycle 1

Chemotherapy dose 1 details			
1.	Did the patient receive any of their chemotherapy dose 1		☐Yes ☐No
2.	If No, reason patient did not receive any dose 1 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide Procarbazine Filgrastim	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mcg ☐N/A

Chemotherapy Cycle 2

Troponin		
1.	Troponin sample taken (non haemolysed)	☐Yes☐No
2.	If no, reason why	
3.	Date of troponin sample	☐☐/☐☐/☐☐☐☐
4.	Time of troponin sample	☐☐:☐☐hh:mm

Chemotherapy dose 2 details			
1.	Did the patient receive any of their chemotherapy dose 2		☐Yes ☐No
2.	If No, reason patient did not receive any dose 2 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A

		Procarbazine Filgrastim	□□□mg/m2 □N/A □□□mcg □N/A

Chemotherapy Cycle 3

Troponin		
1.	Troponin sample taken (non haemolysed)	☐Yes☐No
2.	If no, reason why	
3.	Date of troponin sample	☐☐/☐☐/☐☐☐☐
4.	Time of troponin sample	☐☐:☐☐hh:mm

Further research			
1.	Was a further research sample taken		☐Yes☐N
2.	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm

Chemotherapy dose 3 details			
1.	Did the patient receive any of their chemotherapy dose 3		☐Yes ☐No
2.	If No, reason patient did not receive any dose 3 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A

		Procarbazine Filgrastim	□□□mg/m2 □N/A □□□mcg □N/A

Chemotherapy Cycle 4

Troponin		
1.	Troponin sample taken (non haemolysed)	☐Yes☐No
2.	If no, reason why	
3.	Date of troponin sample	☐☐/☐☐/☐☐☐☐
4.	Time of troponin sample	☐☐:☐☐hh:mm

Chemotherapy dose 4 details			
1.	Did the patient receive any of their chemotherapy dose 4		☐Yes ☐No
2.	If No, reason patient did not receive any dose 4 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide Procarbazine Filgrastim	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mcg ☐N/A

Chemotherapy Cycle 5

Troponin		
1.	Troponin sample taken (non haemolysed)	☐Yes☐No
2.	If no, reason why	
3.	Date of troponin sample	☐☐/☐☐/☐☐☐☐
4.	Time of troponin sample	☐☐:☐☐hh:mm

Further research			
1.	Was a further research sample taken		☐Yes☐N
2.	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm

Chemotherapy dose 5 details			
1.	Did the patient receive any of their chemotherapy dose 5		☐Yes ☐No
2.	If No, reason patient did not receive any dose 5 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A

		Procarbazine Filgrastim	□□□mg/m2 □N/A □□□mcg □N/A

Chemotherapy Cycle 6

Troponin		
1.	Troponin sample taken (non haemolysed)	☐Yes☐No
2.	If no, reason why	
3.	Date of troponin sample	☐☐/☐☐/☐☐☐☐
4.	Time of troponin sample	☐☐:☐☐hh:mm

Chemotherapy dose 6 details			
1.	Did the patient receive any of their chemotherapy dose 6		☐Yes ☐No
2.	If No, reason patient did not receive any dose 6 chemotherapy		☐Withdrawal ☐Other, please specify
3.	Date of chemotherapy		☐☐/☐☐/☐☐☐☐
4.	Drug regimen		☐FEC-T ☐FEC 75 ☐EC90 ☐R CHOP ☐CHOP ☐ABVD ☐Escalated BEACOPP
5.	Did the patient receive the full chemotherapy dose per protocol		☐Yes☐No
6.	If reduced, actual dose given	Fluorouracil Epirubicin Cyclophosphamide Rituximab Cyclophosphamide Doxorubicin Vincristine Prednisolone Bleomycin Vinblastine Dacarbazine Etoposide Procarbazine Filgrastim	☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐mg/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐.☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐☐☐IU/m2 ☐N/A ☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mg/m2 ☐N/A ☐☐☐mcg ☐N/A

4 weeks post chemotherapy visit

4 weeks post chemotherapy visit (same time as echo)		
1.	Date of study visit	☐☐/☐☐/☐☐☐☐
2.	Heart rate	☐☐☐bpm
3.	Systolic BP	☐☐☐mmHg
4.	Diastolic BP	☐☐☐mmHg

Concomitant medications - 4 weeks post chemotherapy assessment (repeat section editable by sites)

Concomitant medications		
1.	Change in medication since baseline	☐Yes ☐No
2.	Medication stopped	Name
3.	Medication started since last assessment	
4.	Dose	-----
4.	Frequency	☐OD ☐BD ☐TDS ☐QDS ☐PRN ☐other
5.	Units	☐g ☐ g ☐µg ☐ml ☐unit ☐other
6.	Start date (if <14 days)	☐☐/☐☐/☐☐☐☐
7.	End date (inclusive)	☐☐/☐☐/☐☐☐☐
8.	End date not applicable	☐Lifelong ☐Ongoing at end of study ☐Unknown

FBC			
1.	Date of FBC		☐☐/☐☐/☐☐☐☐
2.	Hb		☐☐☐g/L
Troponin			
7.	Troponin sample taken (non haemolysed)		☐Yes☐No
8.	If no, reason why		
9.	Date of troponin sample		☐☐/☐☐/☐☐☐☐
10	Time of troponin sample		☐☐:☐☐hh:mm
Further research			
11	Was a further research sample taken		☐Yes☐No
12	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm

Planned further treatment			
1.	Is trastuzumab (Herceptin®) treatment planned		☐Yes ☐No
2.	Is radiation therapy planned (thoracic/mediastinal)		☐Yes ☐No
3.	Is endocrine treatment planned		☐Yes ☐No
4.	If yes,	Tamoxifen Aromatase inhibitor LHRH +Tamoxifen or aromatase inhibitor	☐Yes ☐No ☐Yes ☐No ☐Yes ☐No
5.	Is further surgery planned		☐Yes ☐No
6.	Is further chemotherapy planned		☐Yes ☐No

12 months post chemotherapy visit

12months post chemotherapy visit (same time as echo)		
1.	Date of study visit	☐☐/☐☐/☐☐☐☐
2.	Heart rate	☐☐☐bpm
3.	Systolic BP	☐☐☐mmHg
4.	Diastolic BP	☐☐☐mmHg

Concomitant medications - 12 months post chemotherapy assessment (repeat section editable by sites)

Concomitant medications		
1.	Change in medication since baseline	☐Yes ☐No
2.	Medication stopped	Name
3.	Medication started since last assessment	
4.	Dose	
4.	Frequency	☐OD ☐BD ☐TDS ☐QDS ☐PRN ☐other
5.	Units	☐g ☐ g ☐µg ☐ml ☐unit ☐other
6.	Start date (if <14 days)	☐☐/☐☐/☐☐☐☐
7.	End date (inclusive)	☐☐/☐☐/☐☐☐☐
8.	End date not applicable	☐Lifelong ☐Ongoing at end of study ☐Unknown
12 Months Follow Up - Cardiac History		
1.	Any shortness of breath on exertion since the 4 weeks post chemotherapy appointment	☐Yes ☐No
2.	Any leg oedema since the 4 weeks post chemotherapy appointment	☐Yes ☐No
3.	Has there been a BNP checked since the 4 weeks post chemotherapy appointment	☐Yes ☐No

4.	If yes, was this >100ng/L	☐Yes ☐No
5.	Any diagnosis of heart failure since the 4 weeks post chemotherapy appointment	☐Yes ☐No
5a	If yes, was there a drop in EF to below 50%	☐Yes ☐No
5b	If yes, was there a drop in the GLS by 15%	☐Yes ☐No
6.	Any hospital admissions with heart failure since the 4 weeks post chemotherapy appointment	☐Yes ☐No
7.	Any intravenous diuretic use since the 4 weeks post chemotherapy appointment	☐Yes ☐No
8.	Any admissions with MI since the 4 weeks post chemotherapy appointment	☐Yes ☐No
9.	Any additional cancer treatment commenced since the 4 weeks post chemotherapy appointment	☐Yes ☐No

Troponin			
7.	Troponin sample taken (non haemolysed)		☐Yes☐No
8.	If no, reason why		
9.	Date of troponin sample		☐☐/☐☐/☐☐☐☐
10	Time of troponin sample		☐☐:☐☐hh:mm
Further research			
11	Was a further research sample taken		☐Yes☐No
12	If yes:	Date: Time:	☐☐/☐☐/☐☐☐☐ ☐☐:☐☐hh:mm

Troponin T

Baseline Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 2 (Day 1) Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 3 (Day 1) Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 4 (Day 1) Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 5 (Day 1) Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 6 (Day 1) Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

4 weeks post chemotherapy Troponin T		
1.	Date of sample	□□/□□/□□□□

2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

12 months post chemotherapy Troponin T		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Troponin I

Baseline Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 2 (Day 1) Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 3 (Day 1) Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 4 (Day 1) Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Cycle 5 (Day 1) Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No

4.	If no, troponin T result	□□□□□ng/l

Cycle 6 (Day 1) Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

4 weeks post chemotherapy Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

12 months post chemotherapy Troponin I		
1.	Date of sample	□□/□□/□□□□
2.	Time of sample	□□:□□
3.	Troponin T result less than 5ng/l	□Yes□No
4.	If no, troponin T result	□□□□□ng/l

Study completion/withdrawal

Study completion/withdrawal		
1.	Did the participant complete the trial	☐Completed ☐Withdrew ☐Died
2.	Date of completion/withdrawal/death	☐☐/☐☐/☐☐☐☐
3.	If withdrew, reason	☐Withdrawal of consent ☐No longer eligible ☐Disease progression ☐Lost to follow up ☐Pregnancy ☐AE/SAE, specify ☐Other, specify
4.	If patient withdrew, did they agree for further information to be collected from medical notes	☐Yes ☐No
5.	If patient withdrew, did they agree for DNA from buccal swab to be used	☐Yes ☐No
6.	If patient withdrew, did they agree for sera/plasma to be used	☐Yes ☐No
7.	If patient withdrew, did they agree for heart scans and data to be kept for 15 years	☐Yes ☐No
8.	If patient withdrew, did they agree to be approached for future research	☐Yes ☐No
9.	If patient died, cause of death	