Remote Ischaemic Conditioning as a cardioprotective mechanism against anthracycline induced cardiac injury and multimodality monitoring of patients receiving anthracycline chemotherapy

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Signed Declaration

‘I, Michael Mallouppas confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

Abstract

Introduction: Remote ischaemic conditioning was investigated as a cardioprotective strategy against anthracycline cardiotoxicity in the clinical setting. Identifying ways to best monitor these was also assessed.

Methodology: A randomised double-blind controlled trial of remote ischaemic conditioning vs sham was performed in patients receiving anthracyclines. Conditioning was delivered with 4 cycles of 5-minute inflations/deflations prior to each chemotherapy cycle. High sensitivity troponin T was analysed at baseline, during chemotherapy and at follow-up. Echocardiography was performed at baseline and at follow-up. Arrhythmia assessment was performed during chemotherapy. Clinical events were documented at each visit. A cohort of patients had rapid sequence cardiac MRI and cardiac myosin binding protein C. The main outcome was change in troponin T between the two groups. Secondary outcomes included LV function, clinical events and arrhythmia outcomes. The relationship between peak troponin and secondary outcomes was assessed in a prospective observational manner.

Results: Thirty-seven patients were randomly allocated to the remote ischaemic conditioning (n=19) or sham group (n=18). After excluding withdrawals, 16 patients in each group were included in the intention to treat analysis. Troponin T significantly increases during chemotherapy and peaks 1 month after. Regression analysis shows no difference in troponins between the groups during chemotherapy (mean difference 1.59ng/L, p=0.245) or follow-up (mean difference 0.62ng/L, p=0.744). Clinical events were similar. There was a significant trend towards more admissions with sepsis/neutropenia in the RIC group. There is a moderate correlation between peak troponin and total cumulative dose received (r=0.422, p=0.016) but no correlation with other outcomes. cMyC has a 4-fold median peak increase compared to 2-fold for TnT (p<0.001). Rapid sequence CMR is feasible (mean scanning time 14 minutes) and acceptable (85% acceptance rate).

Conclusions: Remote ischaemic conditioning does not reduce anthracycline cardiotoxicity as assessed with troponin T despite a significant rise in troponin from chemotherapy.

Impact Statement

What we know so far, is that anthracycline induced cardiomyopathy still affects a significant amount of cancer patients as anthracyclines are still widely used chemotherapy drugs. Currently there are limited cardioprotective strategies. Remote ischaemic conditioning has been shown to be cardioprotective against anthracyclines in pre-clinical studies. What this study adds is that this is the first proof of concept study to test remote ischaemic conditioning as a cardioprotective mechanism against anthracycline cardiotoxicity in humans. It further provides a detailed cardiac phenotyping of patients receiving anthracyclines to be used as a template for future studies. The study had a neutral result but it falls short in that the number of patients was low and should thus be used only as a hypothesis generating exercise.

Inside academia on a local level, as cardiovascular scientists little expertise on how to perform research in cancer patients. This thesis should allow the design of further studies investigating cardioprotection from chemotherapy induced cardiotoxicity using insight from the methodology and results. Based on the thesis results, an incremental increase in troponin T concentration is noted during chemotherapy. If troponin is to be used as a monitoring tool, a useful timeline would be to perform at baseline, mid-point and end of chemotherapy as well as at one month post chemotherapy when it tends to peak. Immediately post-chemotherapy blood tests for troponin do not seem to offer any additional benefit and in fact may be a barrier to recruitment. A baseline assessment of cardiac function and an assessment within one month from end of treatment which would coincide with the highest rise in troponin, as well as at 6- and 12-months post chemotherapy are likely to be the most useful timepoints. In addition, the dynamic trends of troponin T concentrations at each cycle of chemotherapy and follow-up using troponin

as a continuous variable has not been previously shown in such details which would be of benefit to the wider research community to understand how biomarker concentrations fluctuate during chemotherapy if we are to use biomarkers in the future to try and predict risk of cardiotoxicity, particularly as we know the risk is greater in the first year post chemotherapy.

Secondly, one of the strengths of this thesis is the detailed reporting of serious adverse events. The methodology and accurate recording of this is important for any future clinical research to ensure there is no impact on clinical care.

Outside academia, the main benefit from this thesis is in clinical service. With the recent development of the cardio-oncology service at UCLH, the findings of this thesis, will hopefully provide a benchmark of a detailed cardiac phenotyping using biomarkers, imaging and arrhythmia assessment of patients who often receive high dose anthracyclines such as the sarcoma and lymphoma patients for which UCLH is a tertiary referral centre. Furthermore, using the results of this thesis and already existing evidence, will allow local guidelines and protocols to be developed for monitoring of patients receiving anthracycline chemotherapy.

Table of Contents

Abbreviations

2D	Two dimensional
3D	Three dimensional
AAR	Area at risk
ABVD	doxorubicin, bleomycin, vinblastine, dacarbazine
ACE-I	Angiotensin Converting Enzyme Inhibitor
AF	Atrial Fibrillation
ALL	Acute Lymphoblastic Leukaemia
Ang 1-7	Angiotensin 1-7
ANP	Atrial Natriuretic peptide
ARB	Angiotensin Receptor Blocker
ASCO	American Society of Clinical Oncology
ASE	American Society of Echocardiography
AT1	Angiotensin II Type 1a
AT1 KO	Angiotensin II Type 1a receptor knock out
ATP	Adenosine Triphosphate
AUC	Area under the curve
AV	Atrio-ventricular
BB	Beta blocker
BNP	Brain natriuretic peptide
BP	Blood Pressure
CAD	Coronary Artery Disease
CCB	Calcium Channel Blocker
CCSS	Childhood Cancer Survivor Study
CECCY	Carvedilol Effect in Chemotherapy-induced CardiotoxicitY trial
CHF	Congestive Heart Failure

CHOEP	Cyclophosphamide – Doxorubicin – Vincristine – Etoposide – Prednisolone
CHOP	cyclophosphamide, doxorubicin, vincristine, prednisolone
CK	Creatine Kinase
CK MB	Creatine kinase myocardial band
CMR	Cardiac magnetic resonance imaging
cMyC	Cardiac myosin binding protein C
CONDI-2/ERIC PPCI	Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction trial
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic Obstructive Pulmonary Disease
CsA	Cyclosporin A
CT	Computed Tomography
CTRCD	Cancer therapeutic related cardiac dysfunction
D-Cis	Doxorubicin – Cisplatin
D-Ifos	Doxorubicin – Ifosfamide
DNA	Deoxoribonucleic acid
D-Ola	Doxorubicin – Olaratumab
Dox	Doxorubicin
EACVI	European Association of Cardiovascular Imaging
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ECV	Extracellular volume
EDTA	ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EHRA	European Heart Rhythm Association

ERIC-ONC	Effect of Remote Ischaemic Conditioning in Oncology patients trial
ESC	European Society of Cardiology
ESMO	European Society of Medical Oncology
Fe	Iron
FEC DT	Fluorouracil-Epirubicin-Cyclophosphamide-Docetaxel-Trastuzumab
FEC-PC	Fluorouracil-Epirubicin-Cyclophosphamide-Paclitaxel-Carboplatin
GLIDES	Guidelines Into Decision Support
GLS	Global Longitudinal Strain
H_2O_2	Hydrogen peroxide
HCM	Hypertrophic cardiomyopathy
HER-2	Human epidermal growth factor receptor 2
HF	Heart Failure
HFrEF	Heart Failure with reduced Ejection Fraction
Hsp 20	Heat shock protein 20
ICOS-ONE	International Cardio-Oncology Society-ONE trial
IL	Interleukin
IL6	Interleukin 6
IQR	Interquartile range
IRI	Ischaemic Reperfusion Injury
IVA-Dox	Ifosfamide – Vincristine – Dactinomycin – Doxorubicin
IVSd	Interventricular Diastolic Septal diameter
JAKs	Janus Kinases
LA	Left atrium
LAD	Left Anterior Descending Artery
LDH	Lactate dehydrogenase
LGE	Late gadolinium enhancement

LoD	Limit of detection
LoQ	Limit of quantification
LPS	Lipopolysaccharide
LV	Left ventricle
LVEDd	Left Ventricular End Diastolic Diameter
LVEDs	Left Ventricular End Systolic Diameter
LVEDV	Left Ventricular End Diastolic Volume
LVEF	Left Ventricular Ejection Fraction
LVESV	Left Ventricular End Systolic Volume
LVPWd	Left ventricular posterior wall diastolic diameter
MACCE	Major adverse cardiovascular and cancer events
MAP	Methotrexate – Doxorubicin – Cisplatin
Mdivi-1	mitochondrial division inhibitor 1
MI	Myocardial Infarction
MPTP	mitochondrial permeability transition pore
MRA	Mineralocorticoid Receptor Antagonist
MRI	Magnetic Resonance Imaging
mRNA	Messenger ribonucleic acid
MUGA	Multi-gated acquisition scan
NADH	Nicotinamide adenine dinucleotide hydrogen
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric Oxide
NO-cGMP-PKG	Nitric-Oxide cyclic guanosine 3′,5′-monophosphate Protein Kinase G
NT-pro-BNP	N-terminal-pro-brain-natriuretic-peptide
NYHA	New York Heart Association

OH	hydroxyl
OVERCOME	Prevention of left Ventricular dysfunction with Enalapril and Carvedilol in patients submitted to intensive Chemotherapy for the treatment of Malignant hEmopathies trial
PCI	Percutaneous Coronary Intervention
PICC	Peripherally inserted central venous catheter
PPAR	Proliferator activated receptors
PPCI	Primary Percutaneous Coronary Intervention
PPI	Protein Pump Inhibitor
PRADA	Prevention of cardiac dysfunction during adjuvant breast cancer therapy trial
RAS	Renin-angiotensin system
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone
RCHOP-Mtx	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone, methotrexate
RIC	Remote Ischaemic Conditioning
RISK	Reperfusion Injury Salvage Kinase
rRIC	Repeated remote ischaemic conditioning
RV	Right ventricle
SAFE	Survivor Activator Factor Enhancement
SD	Standard deviation
SST	Serum separating tube
STAT 3	Signal Transducer and Activator of Transcription 3
STEMI	ST elevation myocardial infarction
STI	Speckle Tracking Imaging
SVE	Supraventricular Ectopic beat

SVT	Supraventricular tachycardia
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler Imaging
TIMI	Thrombolysis in Myocardial Infarction
TNF	Tumour Necrosis Factor
TnI	Troponin I
TnT	Troponin T
TTN	Titin
UCL	University College London
UCLH	University College London Hospital
VE	Ventricular Ectopic beat
VI-Dox	Vincristine – Ifosfamide – Doxorubicin
VT	Ventricular tachycardia
WHO	World Health Organisation
τ_{ic}	Intracellular lifetime of water

Acknowledgements

The MD Thesis presented below is in part based on patients and date collected from the ERIC-ONC study (Effect of Remote Ischaemic Conditioning in Oncology Patients). This was a randomised controlled study of remote ischaemic conditioning vs sham which was already in progress when I started my MD. The design and methodology therefore of the comparison between the remote ischaemic conditioning and sham groups of the thesis was in part based on the already existing design of the ERIC-ONC study. When I started my MD, I successfully submitted major amendments to the ethical committee to include cardiac MRI, cardiac myosin binding protein C as well as to expand the referral of patients to include lymphoma patients. In addition, the data I present are based on the patients that I recruited into the ERIC-ONC study and not of any patients previously recruited into the study. Furthermore, the statistical analysis and results presentation was performed by myself and were independent of any study analysis. A preliminary mid-point interim analysis of the entire ERIC-ONC study showed similar findings to current thesis findings.

Dedicated to Rachel and Yiannis, for their patience

Chapter 1 Anthracyclines and Anthracycline induced cardiomyopathy

1.1 History of Anthracyclines

Anthracyclines, with daunorubicin being the first to be developed, are antibiotics that were first isolated from streptomyces peucetius in the 1960s(1) in Milan, Italy. They were soon realised to have anti-tumour properties; observed following such effects seen on tumours in mice(2). Not long after, the beneficial anti-tumour effects were also noted in children with leukaemia(3,4) as well as other types of tumours(5). Adriamycin (today's doxorubicin and named after the Adriatic sea whose panoramic view could be enjoyed from where the soil that the s.peucetius was isolated from(6)), was developed soon after, with a chemical structure similar to that of daunorubicin but for the substitution of a hydrogen atom with a hydroxyl group. It too, was found to be effective against childhood leukaemia and lymphomas in particular, as well as solid tumours(7,8). However, even early on from their discovery, their detrimental effect on the heart was noted(9,10). Over the following decades, more than 2000 anthracycline analogues have been developed, all in an attempt to primarily minimize the cardiac side effects(11) . However only a minority have made it into the clinical arena and the prototypes daunorubicin and doxorubicin are still widely used. Epirubicin, idarubicin and mitoxantrone are other examples of anthracyclines that are in use in clinical practice particularly in breast and gastric cancer and haematological cancers(12–14). However, the potential to cause cardiac injury (short or long term) has not been completely diminished.

1.2 Anthracycline use in cancer patients

Currently, anthracyclines still play an important role in the treatment of patients with a variety of cancers. For example in haematological malignancies and in particular in one of the most common types of Non-Hodgkin's Lymphoma, diffuse large cell lymphoma, doxorubicin as part of the CHOP regime has been standard of care for more than 30 years

with early studies suggestive of complete remission rates of up to 70%(15) and overall survival rates at 3 years of 50%(16). More recently, addition of rituximab, a monoclonal antibody against CD-20, has also been shown to improve response rate(17) and thus the R-CHOP regime is now standard of care in most patients with diffuse Non-Hodgkin's Lymphoma. Similarly, in classical Hodgkin's Lymphoma, the commonest form of Hodgkin's Lymphoma, doxorubicin as part of the ABVD regime is now considered standard of care for limited and intermediate-stage disease and is a treatment option for advanced-stage disease(18).

Bone sarcomas are another cancer where anthracyclines have been found to be beneficial. In osteosarcoma, seen most commonly in adolescents, doxorubicin in combination with either cisplatin or ifosfamide and/or high dose methotrexate increases disease free survival from 10-20% to more than 60% in the non-metastatic setting(19) and is currently first line treatment. Similarly in Ewing's sarcoma, commonly seen in children and adolescents, doxorubicin usually forms part of a six-drug systemic chemotherapy regime that includes cyclophosphamide, ifosfamide, vincristine, dactinomycin and etoposide which, when added to radiotherapy and surgery, improves 5-year survival from less than 10% to 60-75% in localized disease and 20-40% in the metastatic setting(19). In chondrosarcoma, the most common bone cancer of adulthood, response to chemotherapy is more variable with anthracycline based regimes potentially of some benefit in certain types of chondrosarcoma(20).

In soft tissue sarcoma, the situation is less clear at least in localized disease where surgery and radiotherapy seem to have a more important role, though anthracycline based chemotherapy (usually combined with ifosfamide) remains an option in certain

patients(21). In metastatic soft tissue sarcoma however, anthracycline chemotherapy is first line, usually as single agent doxorubicin or combined with ifosfamide(21).

In early breast cancer, anthracycline/taxane combination regimes are now the standard of care for the majority of cases both in the neo- and adjuvant setting (Level of evidence IA)(22) and in meta-analyses have been found to reduce mortality by one third(23,24) regardless of anti-HER2 or hormonal therapy. Similarly in advanced breast cancer, anthracyclines or taxanes as single agents are the preferred choice in the absence of any contraindications (Level of evidence IA)(25).

1.3 Mechanisms of action of anthracyclines in cancer (cytotoxicity)

Anthracyclines have a core four-ring structure that is linked to an amino acid, daunosamine(26).They are administered intravenously, have a long plasma half-life of more than 24 hours, are rapidly absorbed by tissues and in general, apart from idarubicin, do not cross the blood-brain barrier. They are primarily excreted by the biliary system with some renal excretion(27). Their mechanism of action is multifactorial, complex, confusing and ultimately not entirely known.

When human chromosomes are treated with anthracyclines, they can be seen as a fluorescent well defined band(28) thought to represent the formation of a tight bond with nuclear double-stranded DNA. This tight bond is responsible for their toxicity(26). Once bound to DNA, through the process of intercalation (i.e. the insertion of molecules between the planar DNA bases) they cause local uncoiling of the double-stranded helical structure(26). This intercalation interferes with the action of topoisomerase II. Topoisomerase II is an enzyme involved in DNA transcription and replication by transiently uncoiling DNA strands. Leukaemia cell lines that are resistant to Adriamycin have

significantly reduced topoisomerase II catalytic activity compared to Adriamycin-sensitive cell lines(29), suggesting strong evidence of a link between anthracycline cytotoxicity and topoisomerase II activity. There are two types of topoisomerase II enzymes, IIA and IIB. Tumour cells express large amounts of topoisomerase IIA whereas cardiac cells express IIB. Anthracyclines affect both topoisomerase IIA and IIB(30).

The generation of reactive oxygen species is another popular theory of how anthracyclines cause cytotoxicity primarily through their interaction with cellular iron (Fe) metabolism. Doxorubicin readily binds to iron to form complexes which cause DNA damage by reducing Fe(III) to Fe(II) and reacting with hydrogen peroxide (H_2O_2) to form hydroxyl (OH) reactive oxygen species(26). Furthermore, doxorubicin affects the release of iron from ferritin, the cellular iron storage protein(31,32), which impacts on iron dependent cellular functions. In addition, anthracyclines have the ability to produce free radicals independent of their interaction with iron. Their structure allows them to act as electron acceptors that interact with various enzymes (cytochrome P450 reductase, NADH dehydrogenase, xanthine oxidase) to form semiquinone structures that can cause direct DNA injury or interact further with oxygen to form further oxygen free radicals that themselves cause DNA injury(33). These reactive oxygen species have been proposed to also cause cytotoxicity via the process of lipid peroxidation of the cell membrane(11,31)(a process by which free radicals attack lipids and ultimately lead to cell death(34)) though with conflicting evidence. The general criticism of both the free radical and the lipid peroxidation theory of anthracycline cytotoxicity is that many of the mechanistic studies have used drug concentrations that were not clinically relevant(11,33).

When a chemical substance forms a covalent bond to DNA, DNA adducts are formed(35). Similarly, when a substance forms covalent bonds between two DNA nucleotides, DNA

cross-linking is thought to occur(36). Adriamycin has been found to form DNA adducts that prevent DNA transcription(37) and their cytotoxic activity has been found to be strongly correlated to the amount of inter-strand DNA crosslinking(38). These, and other, experiments therefore suggest DNA adduction and cross-linking as other potential mechanisms of action for anthracycline cytotoxicity(33). Furthermore, most of the anthracyclines have also been found to inhibit the action of DNA helicases, enzymes that are essential in preparing the DNA strands for replication and transcription by unzipping them(39) thus offering a further potential mechanism of action.

There is increasing evidence that anthracyclines may in part be causing cytotoxicity by inducing apoptosis. Apoptosis, also known as programmed cell death, is an important mechanism by which many chemotherapeutic agents cause cancer death(40) and anthracyclines, at least in certain concentrations, seem to induce apoptotic cell death of cancer cells(41,42) with some evidence that this is undertaken via the p53 apoptosis-related gene family(11).

1.4 Anthracyclines and the heart

1.4.1 Prevalence of anthracycline induced cardiomyopathy

As alluded to above, early on in the discovery and use of anthracyclines in patients, their toxic effect on the heart was recognised(43) and its link to cumulative dose received, acknowledged(44). Early retrospective reports suggested an incidence of congestive heart failure of 3% at 400mg/m^2, 7% at 550mg/m^2 and 18% at 700mg/m^2 for doxorubicin(45) and 1.5% at 600mg/m^2 and 12% at 1000mg/m^2 for daunorubicin(10) in both adults and children. Speyer et al, when analysing the placebo arm of their breast cancer cardioprotection study, found clinical congestive heart failure (i.e. New York Heart Association (NYHA) II or more) in 27% of adult women with a median cumulative

doxorubicin dose of 440mg/m^2 and a median decrease in LVEF of 4% between 275-399 mg/m^2 of doxorubicin and 15% between 400-499 mg/m^2 (46). There was a 70% chance of remaining free of clinical CHF for cumulative doses up to 500 mg/m^2 and a 50% chance of remaining free of a decrease in LVEF for the same cumulative dose(46). However, even though this was a prospective analysis, the number of patients was small (n=74). Therefore, Swain et al used the placebo arms of 3 randomised controlled trials investigating dexrazoxane, as a cardioprotective agent, to assess the incidence of anthracycline-induced cardiotoxicity with a combined number of 630 patients (all adults)(47). A total of 32 patients (5.1%) developed congestive cardiac failure. As expected, the incidence increased according to the dose given with 5% developing heart failure after a cumulative dose of 400 mg/m^2, 16% at 500 mg/m^2, 26% at 550 mg/m^2 and 28% at 700mg/m^2 suggesting that cardiotoxicity was commoner than early reports suggested. Furthermore, the analysis confirmed previous suspicions that other factors increase the risk, particularly older age, with patients >65 having at least a 2-fold increase in risk compared to younger patients. Therefore, a prevalence of clinical heart failure of around 5% when using cumulative doses of up to 400mg/m^2 is commonly quoted in expert documents(48).

However, with improvements in particularly echocardiographic analysis of cardiac function, it is now increasingly recognised that subclinical asymptomatic cardiotoxicity is more common. This was initially noted in studies of children who survived cancer with subclinical echocardiographic indices of cardiotoxicity in up to 57% of cases(49) in one study and 23% in another study, of which only 4% developed symptomatic heart failure(50). Furthermore, subclinical cardiac abnormalities such as LV fractional shortening and changes in isovolumetric relaxation time, were noted in up to a third of patients treated for childhood

ALL receiving low dose anthracyclines(51) suggesting that there is no truly safe anthracycline dose.

More recently, in a large prospective study of 2625 adult patients receiving an anthracycline based chemotherapy regime for a variety of cancers, cardiotoxicity as defined by a reduction in LVEF by more than 10% from baseline and to less than 50%, occurred in 9% of patients, of which 81% were in NYHA I or II and 19% in NYHA III or IV after a median follow up of 5.2 years(52).

As already eluded to, to investigate the prevalence of anthracycline induced cardiomyopathy, one needs an accurate definition of anthracycline-induced cardiomyopathy. Commonly, the terms anthracycline cardiotoxicity and anthracycline-induced cardiomyopathy are used inter-changeably which complicates matters further. In the early studies, the focus was on clinical and/or electrocardiographic cardiac toxicity but with improvements in imaging cardiac analysis and biomarker sensitivity the focus has shifted to imaging parameters of cardiac toxicity as well as cardiac biomarkers. This is also reflected in the studies investigating the prevalence and incidence of anthracycline cardiac toxicity that follow the same pattern in how they define toxicity.

Currently, most expert consensus documents suggest a definition of cardiac toxicity or cancer-therapy related LV dysfunction (which includes anthracycline induced cardiotoxicity) occurs if the LVEF decreases by >10% from baseline and to a level below the lower limit of normal(48) or LVEF <53%(53,54), regardless of symptoms. Having said that, the same expert documents, do acknowledge the inadequacies of LVEF as a marker of cardiotoxicity and the presence of other imaging and biological markers such as myocardial strain and cardiac biomarkers which may be more useful in the identification of

early cardiotoxicity. Using this definition therefore, the prevalence of anthracycline-induced cardiotoxicity, based on the large prospective study by Cardinale et al, discussed above, is 9%(52).

A special mention with regards to children is needed as the effects of anthracyclines on a child's heart, for reasons that will be explored later, differ from adults and almost need to be treated as a different entity altogether. Indeed in a series of experiments in the 1990s, Lipshultz et al, have shown that young age is an independent risk factor for anthracycline cardiomyopathy(49,55). When assessing the late cardiac effects of anthracyclines in children treated for ALL, young age was an independent risk factor ($p=0.003$) that predicted increased afterload (end systolic stress)(49) and reduced LV mass(55) as assessed with echocardiography. In these experiments, the rates of congestive heart failure within a year of chemotherapy was 10%, of which some developed a recurrence of congestive heart failure in the next 3 to 15 years, with some needing transplantation(49,55). Rather interestingly, LV contractility is impaired in the initial years after chemotherapy, subsequently improves, and then starts to decline again after 6 years of follow up, suggesting that there is a very late onset incidence of cardiomyopathy and thus stressing the importance of long term monitoring in childhood cancer survivors(56). The incidence of clinical cardiotoxicity in children with cancer was looked at in an earlier, much larger study of 6493 children who received anthracycline chemotherapy(57). Clinical cardiotoxicity was defined as congestive heart failure not caused by other causes, a change in cardiac function on echocardiography that was significant enough to prompt discontinuation of anthracyclines and sudden death from presumed cardiac causes. In total, 106 children had cardiotoxicity (1.6%) with 58 of those developing congestive heart failure, 43 having deterioration in cardiac function and 5 having a presumed cardiac death. Most of these cardiac events (90%) occurred in the first year after chemotherapy(58).

However, even though the study analysed data over 16 years, the retrospective nature of the analysis and the fact that the follow up time is not stated, may explain the lower incidence noted. Subclinical cardiotoxicity therefore, is likely to be more common, as is the case in adults.

1.4.2 Pathophysiology of anthracycline-induced cardiomyopathy

Early post-mortem examinations of patients who have died of anthracycline-induced cardiomyopathy show a diffuse injury to the myocardium with loss of myocardial fibrils, reduction in myocardial cells, mitochondrial oedema and presence of dense inclusion bodies(44). Unlike the pathophysiology of anthracyclines against cancer cells where the exact mechanism of action is not entirely clear, in the heart, free radical generation seems to be the prevailing theory of toxicity(59). Doroshow et al has shown that doxorubicin enhances the production of superoxide, hydrogen peroxide and hydroxyl radicals in rat hearts despite the presence of superoxide dismutase and glutathione peroxidase enzymes, both of which are thought to be protective against oxygen free radicals, suggesting that injury may occur because the capacity of cardiac cells against oxygen free radicals is overcome(60). It is thought that compared to other tissues like the liver, the heart has a lower number of these protective enzymes, making it more prone to damage from free radicals(61). This injury seems to be facilitated by NADH dehydrogenase from cardiac mitochondria(62,63) as well as other enzymes which cause electron reduction of doxorubicin that lead to free radical generation(26).

Furthermore, the interaction of anthracyclines with iron and iron metabolism seem to be another important pathway by which free radicals are produced(26). Anthracyclines easily bind to iron to form complexes and, in the presence of oxygen, regularly oscillate between the Fe^{3+} and Fe^{2+} states, often producing free radicals as by-products which can be lethal

to cardiac cells(64). Specifically, Myers et al has shown that doxorubicin will form a 3:1 complex with ferric iron that will bind to human erythrocyte membranes and in the presence of glutathione it will cause destruction of erythrocyte ghost membranes through superoxide and hydrogen peroxide radical production(65). The same group has shown that the free radical scavenger tocopherol when given to mice as pre-treatment prior to Adriamycin administration, reduces Adriamycin-induced cardiomyopathy as seen on electron microscopy, probably by preventing lipid peroxidation, but without affecting its response against tumour cells(66). One of the strongest evidence in favour of the free radical and iron theory is the use of iron chelating agents such as dexrazoxane as cardioprotective agents. Both in the acute and chronic setting and in a variety of animal models, dexrazoxane has been shown to prevent anthracycline-induced cardiotoxicity(67–69). Furthermore, as I will discuss later, dexrazoxane has shown similar benefits in human trials(70,71) and currently has an indication as a cardioprotective agent against anthracycline-induced cardiomyopathy in certain scenarios.

Another theory to explain cardiotoxicity is the effect anthracyclines have on topoisomerase enzymes(72) Adult mice cardiomyocytes express topoisomerase IIβ but not topoisomerase IIα(73). Mice that are engineered to lack topoisomerase IIβ in their cardiomyocytes and then exposed to doxorubicin show protection against doxorubicin induced cardiomyopathy that seems to be driven from the effects of topoisomerase IIβ on mitochondrial function rather than cellular generation of reactive oxygen species(74).

Furthermore, early reports in animal studies show that anthracyclines may affect calcium regulation by increasing intracellular myocardial calcium concentrations which may impact on the contractile function of cardiomyocytes(75). In addition, calcium dysregulation may impact mitochondrial function and provide a further pathway by which free radicals are

generated within cardiac mitochondria, as inhibiting mitochondrial calcium uptake in cells treated with doxorubicin attenuates reactive oxygen species formation(76) and reducing mitochondrial ATP content(77). Therefore, similar to the role of calcium in post-ischaemic reperfusion injury(78), mitochondrial calcium overload may be exacerbating anthracycline injury.

As mentioned earlier, anthracyclines are also implicated in apoptosis as another mechanism involved in cardiac injury. In myocardial cell lines and isolated cardiac mitochondria, doxorubicin administration induces apoptotic pathways early on in the process, through caspase 3 activation (an apoptotic enzyme) and cytochrome c release (a pro-apoptotic protein)(79). In vivo, apoptotic cell death is seen in rats treated with doxorubicin which seems to be driven by activation of the Fas-mediated apoptotic pathway rather than p53(80).

The myofibrillar disarray seen on microscopy(44,53) may in part be explained by the direct effect of anthracyclines on gene expression. Both in vitro and in vivo, doxorubicin significantly reduces the levels of circulating mRNA of sarcomeric proteins(81) which again may be driven by caspase activation(82).

In children, treatment with anthracyclines at a young age, particularly ≤5 years old, is recognised as an independent risk factor for late onset cardiomyopathy and is indeed often part of recommendations for screening in survivors of childhood cancer(83). One initial theoretical explanation offered for this observation, is that doxorubicin may cause damage and loss to a critical number of cardiomyocytes which fall to a level below that needed to form a normal adult myocardium as the full number of adult myocytes develops by six months of age and any subsequent increase in myocardial size is due to increasing growth

of existing myocytes(49). More recently, Huang et al attempted to replicate the clinical scenario of late onset cardiomyopathy in children in a mouse model(84). Juvenile mice were exposed to doxorubicin doses that did not cause acute toxicity, and their hearts analysed after they reached adulthood. Juvenile doxorubicin exposure led to an abnormal vascular bed with less branching and reduced density, which was more prone to both physiological (in the form of exercise) and pathological (induced infarction) stress(84). Furthermore, the doxorubicin affected the number and function of progenitor cells(84), which are thought to be involved in myocardial regeneration(85). Both of these findings suggest that early doxorubicin exposure may affect the ability of the heart to respond to stress later on and therefore predispose to the development of late onset heart failure(84).

A simplified schematic of anthracycline induced cardiac injury is shown on the right-hand side of Figure 1.2 on Page 69.

1.4.3 Clinical presentation, diagnosis and treatment

1.4.3.1 Clinical Presentation

Historically, three patterns of cardiac presentation have been described with regards to anthracyclines; acute, early onset and late onset(86).

In acute cardiac injury, often occurring within the first few weeks, two patterns were observed in the early reports; non-specific ECG changes such as sinus tachycardia, ST segment/T wave changes and ventricular premature ectopics, and acute heart failure(9,44,87). The ECG changes noted however were primarily based on observations from ad hoc 12-lead ECGs. In an attempt to characterise this better, Steinberg et al performed 24 hour Holter monitoring during the first 24 hours post doxorubicin infusion. The frequency of arrhythmias during the first hour and the next 23 hours were noted and

compared to control periods (Holters performed on same patients at days remote to doxorubicin therapy either 1-5 days preceding a subsequent doxorubicin infusion and/or at least 16 days after last infusion)(88). In the first hour after treatment, the frequency of arrhythmia attributed to doxorubicin was noted in one of 33 studies (3%) (premature ventricular ectopics). In the next 24 hours, new arrhythmias were noted in 24% and again in the form of ventricular premature ectopics in all but two patients (6%) who developed non-sustained ventricular tachycardia (of at least 3 consecutive beats in duration)(88). In another report, 24-hour Holters detected arrhythmias in 65% of cases(89), though this likely reflects differences in study designs and definitions of arrhythmias. Interestingly, paroxysmal atrial fibrillation was noted in 10% of cases in this study(89). Unlike left ventricular dysfunction, arrhythmias caused by anthracyclines are thought to be dose independent(90).

The second pattern of acute cardiac injury due to anthracyclines manifests as acute heart failure and is defined as starting within one week of treatment, is rare, occurring in less than 1% of cases, and characterised by depressed myocardial contractility that is transient and will recover when the anthracycline is stopped(86).

Early, chronic progressive anthracycline cardiotoxicity defined as occurring within the first year of therapy (and after the first week from treatment), is more common and will present with the typical features of congestive cardiac failure such as breathlessness on exertion and peripheral oedema and imaging parameters consistent with dilated cardiomyopathy, though children may also present with a restrictive phenotype(86). In the early reports congestive heart failure would occur on average 80 days after the last administration of anthracycline, there was not much response to therapy(87), and in those cases were cardiac death ensued this would happen within 24-48 hours of presentation(9). We now

know that there are many patients who do not present so acutely but still have anthracycline cardiotoxicity with either mild symptoms or completely asymptomatic. Indeed, in one of the largest prospective trials of anthracycline-induced cardiotoxicity to date, 9% of 2625 patients developed cardiotoxicity (defined as a drop in LVEF of more than 10% from baseline and below 50%), most of which (80%) were in NYHA class I or II(52). In this cohort, 98% of cases occurred within the first year after chemotherapy with a median of 3.5 months.

Late onset chronic cardiotoxicity is defined as one that occurs more than 1 year after chemotherapy and has been observed to occur even up to 20 years after therapy(91). It has been more extensively studied in children(92) and echocardiographic studies suggest LV contractility to be significantly reduced more than 6 years after treatment for ALL(56), stressing the importance of long term follow up of cardiac function in cancer survivors who received anthracyclines. However, this has also been noted in adults where, breast cancer patients aged 66-70 treated with anthracycline regimes were 26% more likely to develop heart failure compared to patients receiving non-anthracycline regimes and this difference was more evident at 10 years from chemotherapy (38.4% vs 32.5%)(93).

Even though these 3 patterns of injury are helpful to clinicians in simplifying the clinical presentation, this view has been recently challenged as it is felt that it is rather a continuum phenomenon that starts with myocardial injury during or soon after chemotherapy, that leads to subclinical cardiac injury which if left untreated will then progress to overt heart failure(52).

1.4.3.2 Diagnosis

Diagnosis of anthracycline-induced cardiomyopathy often requires detailed history, examination and investigations. As stated earlier, currently accepted definitions of cancer therapeutics-related cardiac dysfunction (CTRCD) (which includes anthracycline-induced cardiomyopathy) that would prompt referral to a cardiologist or initiation of cardiac treatment, are based on changes in LVEF on imaging, principally echocardiography. These include a change in LVEF of ≥10% points from baseline and to a value below the lower limit of normal or to a value ≤50% in European guidelines(48,94) or a change in LVEF of ≥10% points from baseline or to a value ≤53% in North American guidelines(53,54), regardless of symptoms. This decrease in cardiac function should be confirmed with a repeat assessment a few weeks later.

The diagnosis can be straightforward if there is a baseline echocardiogram present for comparison, the deterioration in function happens not long after anthracycline therapy and there was no intervening clinical event that happened in the interim to explain this deterioration. However, following my experience in looking after these patients in the Cardio-Oncology service at UCLH, this is often not the case. Despite a level and grade IA recommendation in the latest European Society of Medical Oncology (ESMO) guidelines(94) to perform a baseline assessment of cardiac function, this does not always happen, largely due to the high demand for echocardiography within the clinical service. In addition, particularly in children and young adults, the deterioration in LV function can happen many years later casting doubt in some clinicians' minds of anthracyclines as the cause of cardiomyopathy.

For these reasons therefore, a detailed history and examination is needed to make the diagnosis. This should include detailed oncological and cardiovascular history and

symptoms, cumulative anthracycline doses, additional chemotherapy agents, radiotherapy treatment details, timing of treatment, assessment of risk factors for anthracycline related injury and clinical events during and after chemotherapy. In addition, basic cardiac investigations such as an ECG and echocardiogram are often performed. Despite that, it is not uncommon for patients to undergo a variety of tests to identify any other causes of deterioration of LV function. This may involve an assessment of their coronary arteries, especially in older patients, with either CT, functional testing for ischaemia or invasive coronary angiography. There is an increasing appreciation that cardiac MRI, with its ability to assess ventricular structure, size and function, assess ischaemia and tissue characterisation of the myocardium in one setting, is particularly useful in such patients to exclude other causes like myocarditis, infiltrative disorders, and infarction amongst others(95).

The reason as to why the diagnosis is sometimes difficult to make is because, at present, there is no single biomarker (either blood or imaging biomarker) that is specific for anthracycline-induced cardiomyopathy. Therefore it is often a diagnosis based on clinical judgement after other causes of cardiomyopathy have been excluded(48,96). Even with the use of late gadolinium enhancement (LGE) in cardiac MRI, a marker of myocardial scarring, LGE is only found in 6% of cases, even in the presence of reduced LVEF(97) observed in one study and 0 in another study in children, despite a significant proportion with reduced LVEF(98). There is however, emerging animal and human data that specific cardiac MRI biomarkers derived from T1 and T2 mapping may be more specific for anthracycline-induced cardiomyopathy which could be used in future, for diagnosing early cardiotoxicity(99,100). Furthermore, there is an increasing interest in microRNAs which are small molecules of non-coding RNA that are crucial in regulating mRNAs as potential biomarkers for various cardiac (and non-cardiac) conditions, including anthracycline

cardiotoxicity(101) with some evidence suggesting that some microRNAs are linked to reductions in LVEF(102).

1.4.3.3 Treatment

It was previously thought that damage to the myocardium from anthracyclines, dubbed as Type 1 chemotherapy related cardiac dysfunction(103), was permanent and irreversible(53) and may not respond to conventional heart failure therapy(104,105). However, evidence started to emerge from small studies showing significant improvement in cardiac function in severe epirubicin-induced cardiomyopathy in advanced breast cancer patients (LVEF 18-35%) to near normal function with Angiotensin Converting Enzyme Inhibitors (ACE-Is)(106,107). Addition of a beta-blocker to ACE-I improved function even further in another small study of 25 patients with doxorubicin-induced cardiomyopathy population with mean LVEF of 26%(108). Case reports for the benefit of beta-blockers on their own, particularly carvedilol, also started to emerge(109–111).

Cardinale's group addressed the effect of ACE-Is and beta-blockers in a prospective study of 201 patients with LVEF≤45% due to anthracyclines (other causes excluded with clinical history and other tests as necessary)(112). Enalapril and Carvedilol were started and up-titrated as tolerated and their effect on LVEF, the primary end point, monitored over time. The response was divided into 3 groups: Responders (LVEF increased up to at least normal limit of 50%), partial responders (LVEF increased by more than 10% but below the 50% limit) and non-responders (LVEF increased by less than 10% and did not reach the 50% limit). During follow up, 42% were found to be responders, 13% partial responders and 45% non-responders (mean LVEF before treatment vs after treatment of 41%, 28%, 38% vs 55%, 44%, 38% for each group). Importantly, full recovery was more likely to happen if treatment was started early and in fact, no patients showed full LVEF recovery if

therapy was started more than 6 months after the end of chemotherapy. As expected, responders had less cardiac events (4 vs 8 vs 26).

Thus, anthracycline cardiomyopathy can be reversed with treatment, especially if started early(112) and it is now generally accepted that treatment should not differ from heart failure of other causes(48) and follow international heart failure guidelines(113).

1.5 Screening and monitoring at risk patients for anthracycline cardiotoxicity

1.5.1. Screening prior to initiation of anthracycline chemotherapy

One of the challenges of anthracycline chemotherapy, is predicting who may be at increased risk of developing cardiac toxicity (Table 1). Risk factors can be separated into treatment-related and patient related(114).

Of the treatment-related factors, the association with cumulative dose received and cardiotoxicity is now well established as one of the most significant(115), with modern oncology regimes often limiting the cumulative maximum lifetime dose to 400-550mg/m^2 of doxorubicin(116). Therefore, anyone receiving a total cumulative lifetime doxorubicin dose ≥500mg/m^2 (epirubicin ≥720mg/m^2) is considered high risk according to the European Society of Medical Oncology (ESMO)(116). This is even lower at ≥250mg/m^2 (epirubicin ≥600mg/m^2) in the 2017 American Society of Clinical Oncology (ASCO) guidance(115).

Certain concomitant therapies can act as additive risks to anthracyclines to developing cardiomyopathy or heart failure(48,92,114). Radiotherapy, in particular high dose (>30Gy) mediastinal radiotherapy, acts as an additive risk factor for heart failure in Hodgkin's Lymphoma survivors(117) with a similar pattern seen in breast cancer patients treated with epirubicin and previous radiotherapy(118). In general, radiotherapy increases heart

disease mortality, by 4.1% per Gy in patients with breast cancer receiving modern radiotherapy treatment(119). This includes mortality from ischaemic heart disease, heart failure and valvular heart disease.

Combination therapy with trastuzumab, a monoclonal antibody against the Human Epidermal growth factor Receptor 2 (HER2), usually in the context of breast cancer requires specific mention. Trastuzumab significantly improves time to disease progression in the metastatic setting when added to standard chemotherapy, but with an increased incidence of heart failure (27% in anthracycline, cyclophosphamide and trastuzumab regime vs 8% in anthracycline and cyclophosphamide regime with NYHA III/IV of 16% and 3% respectively)(119). Similar trends were seen in operable breast cancer patients with rates of cardiac events of 4.1% in those who received trastuzumab versus 0.8% in those who didn't(120). Real world retrospective data from large patient cohorts also show hazard ratios of 7.19 for risk of heart failure when trastuzumab is combined with anthracyclines versus 1.4 for anthracyclines alone(121). Chemotherapy related dysfunction from trastuzumab is typically known as Type II dysfunction to differentiate it from that of anthracyclines which is known as Type I(103). The nomenclature, coined by Ewer and Lippman, suggests to almost distinct entities where Type I is dose related, typically thought to be permanent and irreversible with structural changes of myofibrillar disarray seen during electron microscopy. Type II however is not dose related, with no ultrastructural damage seen and thought to be reversible after discontinuation of trastuzumab(103). The enhanced toxicity seen with addition of trastuzumab to patients who already received anthracyclines is thought to be due to existing anthracycline damage that reduces the ability to compensate for the effects of trastuzumab on the heart(103).

A variety of patient related factors also increase risk. In addition to young age at treatment (as discussed above), older age (>65) is an independent risk factor with an increase in risk of congestive heart failure of 2.25 times in patients over 65 in one analysis(47) and a difference of 32% in the risk of severe LVEF decline in patients over 50 compared to younger patients in another analysis(122). Female sex is often quoted as a risk factor though this seems to be the case only in patients who have received it as children(55) but not as adults(114). Furthermore, pre-existing cardiovascular comorbidities like hypertension and diabetes will also increase the risk(48,92,123). More recently, genetic factors have been found to play a role with the presence of gene variants known to cause dilated cardiomyopathy, and in particular truncating variants of the Titin (TTN) gene, to be more prevalent in cancer patients who developed cancer therapy related cardiomyopathy (7.5% vs 1.1% in unselected cancer patients)(124). This was backed up with data from TTN truncating variants in mice showing more LV dysfunction compared to wild type mice(124) suggesting that genetic make-up may predispose some patients to develop cardiac injury more than others after exposure to anthracyclines.

In addition to clinical history and examination baseline cardiac investigations ought to be done in everyone prior to anthracycline chemotherapy both as a screening tool but also to have as a baseline comparison in the future. These include an ECG (IA recommendation, ESMO 2020 guidelines)(94), an assessment of cardiac function (IA recommendation, ESMO 2020 guidelines)(94), and increasingly baseline cardiac biomarkers (IIIA recommendation, Prospective Studies, strong evidence for efficacy with substantial clinical benefit, strongly recommended, ESMO 2020 guidelines)(48,94). Assessment of cardiac function can be with any number of imaging techniques with echocardiography being the most common (and generally most preferred) but with nuclear imaging and cardiac MRI as alternative options. More importantly, using a modality at baseline which will be available

for monitoring during and after therapy is strongly encouraged(48). Cardiac biomarkers include troponins and natriuretic peptides and even though the significance of these at baseline is less clear, it is not unreasonable to have a baseline value to compare for monitoring later on.

Table 1. Risk Factors for Anthracycline Cardiotoxicity		
	Risk	
Cumulative Dose Up to 400mg/m^2 Up to 550mg/m^2 Up to 700mg/m^2	Variable and dose dependent(47) 3-5% 7-26% 18-28%	
Concomitant therapy Radiotherapy Trastuzumab	Risk ratio 6.5 vs 4.5(117) 3 to 5 fold increased risk(119–121)	High dose radiotherapy and high dose anthracyclines vs high dose anthracyclines only
Young Age	82% of children <4 show a cardiac abnormality(49)	
Older Age	Twofold increase(47)	
Female sex	Unclear(55)	Risk seems to be relevant in children only
Comorbidities (e.g. diabetes, hypertension, renal failure, obesity)	Not specified(115,123)	
Genetic Factors	7.5% vs 1.1%(124)	Truncating variants of TTN gene

1.5.2 Screening and monitoring during and after chemotherapy

1.5.2.1 When to perform cardiac screening during chemotherapy?

Similar to baseline screening prior to chemotherapy, expert consensus documents from both oncological and cardiological societies recommend monitoring for cardiotoxicity from

anthracyclines during chemotherapy using assessment of clinical status, biomarkers and imaging assessment of cardiac function. The specifics of the monitoring however, are less clear and differ somewhat between consensus documents. The ESC's 2016 position paper on cancer treatments recommend assessment of cardiac function with imaging at the end of chemotherapy, especially in patients at increased risk or if further cardiotoxic therapy is planned. In high risk patients who have already received at least 240mg/m^2 an earlier assessment should be considered. Biomarker assessment with troponin or natriuretic peptides may be considered appreciating its role is not entirely clear(48). The American Society of Echocardiography (ASE) 2014 expert consensus document (written in conjunction with the European Association of Cardiovascular Imaging), takes a similar approach though a bit more specific. They recommend assessment of cardiac function and biomarkers at the end of chemotherapy and then at 6 months later if the total doxorubicin cumulative dose is <240mg/m^2 (or its equivalent). However, if a patient is receiving more than that as part of their regime, then assessment of function and biomarkers is recommended before each additional 50mg/m^2 dose (which is usually before each additional cycle)(53). To complicate matters further, in a subsection titled "early detection of subclinical LV dysfunction", it is suggested that patients should be monitored during chemotherapy with LV strain methods periodically and troponins each cycle(53). The American Society of Clinical Oncology (ASCO) 2017 guidelines recommend (strength of recommendation moderate) routine surveillance with cardiac imaging during chemotherapy may be offered in those who are felt to be at higher risk. For anthracyclines this is defined as anyone who is receiving a cumulative dose of ≥250mg/m^2, anyone receiving <250mg/m^2 but also receiving mediastinal radiotherapy or has multiple cardiovascular risk factors or age >60 or already has compromised cardiac function. No specific recommendations are made about biomarker surveillance as it is felt more studies are needed to clarify their role(115). The recent European Society of Medical Oncology

(ESMO) 2020 consensus recommendations, like the ASE document, offer more specific advice recommending (IA) assessment of cardiac function after a cumulative dose of 250mg/m^2 is reached, and then after each 100mg/m^2 beyond that, even if the total dose is ≤400mg/m^2. Periodic (every 3-6 weeks or before each cycle) assessment of troponin and natriuretic peptide biomarkers is also recommended, though at the lower level and grade of IIIC (defined as level of evidence III-from prospective studies, grade C - optional recommendation)(94). A summary of these recommendations is presented in Table 2.

Table 2. Recommendations for screening and cardiac surveillance during anthracycline chemotherapy

Society	Year	Recommendation[a]	Level and Grade[b]
ASE & EACVI expert consensus document(53)	2014	1. If total dose <240mg/m^2, cardiac function and biomarker assessment at end of chemotherapy and at 6 months post chemotherapy 2. If total dose ≥240mg/m^2, cardiac function and biomarker assessment before each additional 50mg/m^2 3. LV strain (GLS) periodically and troponins each cycle if wanting to detect early subclinical dysfunction	Not stated

ESC position paper on cancer treatments(48)	2016	1. Imaging assessment at the end of chemotherapy, especially in high risk patients. Sooner if a dose of 240mg/m^2 reached. 2. Biomarker measurement may be considered during chemotherapy	Not stated
ASCO clinical practice guideline(115)	2017	Cardiac imaging surveillance may be offered in high risk patients[c]	Evidence-based, evidence quality intermediate, recommendation strength moderate
ESMO consensus recommendations(94)	2020	1. Cardiac function assessment after 250mg/m^2 reached and then after each 100mg/m^2 beyond that 2. Periodic (every 3-6 weeks or before each cycle) assessment of biomarkers (troponin and natriuretic peptides)	IA[d] IIIC[d]

ASE – American Society of Echocardiography, EACVI – European Association of Cardiovascular Imaging. ESC – European Society of Cardiology. ASCO – American Society of Clinical Oncology, European Society of Medical Oncology

a – Cumulative doses are for doxorubicin, but same recommendation applies for equivalent doses if different anthracycline

b – note different grading systems for recommendations used. ASCO using the GLIDES methodology, ESMO using Infectious Diseases Society of America-United States Public Health Service Grading System

c – defined as cumulative dose of ≥250mg/m^2, or dose <250mg/m^2 but with mediastinal radiotherapy, or multiple cardiovascular risk factors or age >60 or already impaired cardiac function

d – see text for details

Even though there is some variability in the recommendations made by the different bodies, which probably reflects some of the inconsistencies in the available evidence, there seems to be an agreement that a cumulative dose of between 240-250mg/m^2 should trigger an assessment of cardiac function during chemotherapy, at least in patients who are deemed high risk. This is because of an observed increase in cardiac events with heart failure and/or reduction in LVEF during chemotherapy from 9% to 18% with an increase in cumulative dose from 250mg/m^2 to 350mg/m^2 in a retrospective analysis of around 600 patients(47). Using radionuclide assessment of LVEF in 28 patients receiving CHOP therapy for non-Hodgkin's lymphoma, LVEF decreases from 58% to 52% ($p<0.001$), 50%($p<0.001$) and 49%($p<0.001$) after cumulative doses of 200, 400 and 500mg/m^2 respectively(125). A more recent CMR study of 53 patients receiving low dose anthracyclines, showed that in 26% the LVEF deteriorates to below 50% 6 months after administration. No scans were performed during chemotherapy, but there was a decrease in LVEF noted at the one month scan(126).

1.5.2.2 When to perform cardiac screening after chemotherapy?

There seems to be an overall consensus that cardiac function should be assessed around 6 and/or 12 months post anthracycline chemotherapy. In the asymptomatic patient, with preserved cardiac function during chemotherapy, the ASE/EACVI report recommends cardiac function assessment 6 months post chemotherapy. Beyond that, it is suggested to

perform a clinical cardiovascular evaluation on a yearly basis with further imaging assessment left to the discretion of the treating physician(53). The ESC, suggests surveillance with echocardiography, particularly for survivors who received high dose anthracyclines, at 1 and 5 years post chemotherapy(48). ASCO, mirrors the ASE/EACVI, in that it recommends cardiac function assessment at 6 and 12 months post chemotherapy with no recommendations beyond that(115). ESMO in their recent statement, suggest assessment of cardiotoxicity with biomarkers and possibly imaging at 6-12 months, then again at 2 years post chemotherapy and periodically after that (IIIB)(94).

In children and young adult survivors of cancer, where a late onset cardiomyopathy that can happen many years later is increasingly being recognised, it was felt there was a need for cardiac surveillance to occur more long-term(83). However, the discordance in advice between various Children Oncology Groups was evident, to the extent that an International Late Effects of Childhood Cancer Harmonisation Group was formed to provide recommendations(127). They recommended surveillance in those who received a high dose (≥250mg/m^2) of anthracyclines, and surveillance to be reasonable in those receiving lower doses. Surveillance should start no later than 2 years after completion of therapy, repeated at 5 years and then performed every 5 years thereafter(127).

1.5.2.3 Screening modalities for anthracycline cardiotoxicity: Imaging

Historically, LVEF has been useful in detecting changes in cardiac function during and post anthracycline chemotherapy(128) and has since gained prominence in monitoring for cardiotoxicity. Nowadays this is commonly done using echocardiography as it is widely available, cheap, with no radiation involved and can also provide information beyond LVEF. It is therefore the imaging modality of choice recommended in most guidelines. Over the years, various ways have been developed to estimate LVEF using

echocardiography, all with their limitations. Two dimensional (2D) methods, are common, with the biplane method the most popular. However, in cancer patients 2D LVEF and volume calculation methods have a temporal and observer variation of about 10%(129). This is also the cut-off of a change in LVEF that would prompt discontinuation in chemotherapy that is often recommended and the reason why 2D methods have been questioned as a way to monitor these patients. Three-dimensional (3D) volumetric assessments reduce this variability to 6%(129). If endocardial definition allows, 3D is the preferred method to calculate LVEF in cancer patients on cardiotoxicity surveillance(48,53,94,115)

Nonetheless, LVEF may be a late feature of anthracycline cardiotoxicity. Ejection fraction is a composite measure of longitudinal, circumferential and radial ventricular contractility, and if one of them deteriorates the others may overcompensate to maintain the EF(130). Over the recent years, other parameters, and in particular myocardial deformation indices have become more relevant in detecting early subclinical LV dysfunction. In a systematic review, Thavendiranathan et al. have shown that changes in global longitudinal strain (GLS), precede and predict subsequent LV dysfunction, with a change of GLS from baseline between 10-15% being the most accurate(131). As such, a change in GLS of more than 15% is now recommended as suggesting a risk of cardiotoxicity(48,53,94,115)

Imaging modalities other than echocardiography can also estimate cardiac function and LVEF. Nuclear cardiac imaging (MUGA), was one of the first modalities to be used when monitoring for anthracycline cardiotoxicity(132). When compared to CMR in heart failure patients, it has less variability when calculating LVEF compared to 2D echo(133), with a high reproducibility(48). Its main limitations are exposure to radiation and limited information about other cardiac structures(48,53).

Cardiac MRI is often considered now the gold standard when assessing chamber volume and function in view of its true 3D volumetric analysis which avoids assumptions of geometrical shape that may be encountered in other modalities such as 2D echocardiography(53). In addition, due to its versatility, particularly with the use of gadolinium-contrast imaging, it can assess for a variety of different pathological entities in one setting, thus making it an attractive imaging tool in the cancer setting(95). Its use in the cancer patient therefore, is encouraged particularly if there is concern regarding suboptimal assessment with other imaging modalities or in borderline cases of LV dysfunction where cessation of chemotherapy may be considered(48,53). In a head to head echocardiography versus CMR study in cancer survivors, Armstrong et al show that 2D echocardiography overestimates the mean EF by 5% compared to CMR and that 11% of cases were misclassified as having a normal EF when in fact it was less than 50%, though when compared to 3D echocardiography, CMR findings were more similar(134).

Despite advanced imaging techniques with cardiac MRI, markers that may predict or diagnose early cardiotoxicity are still lacking, though emerging data suggest this may soon change. Late gadolinium enhancement in established anthracycline cardiomyopathy is infrequent (6%) though LV mass was inversely related to anthracycline dose and predicts future cardiac events(97). Myocardial extracellular volume (ECV), a marker of fibrosis calculated from native and post-contrast T1 mapping(135), increases after anthracycline chemotherapy compared to controls even if EF remains preserved(99,136). Furthermore, the intracellular lifetime of water (τ_{ic}), a marker of cardiomyocyte size(137), decreases post anthracyclines suggesting that atrophy may be responsible for the observed decrease in mass(136). More recently, in a CMR study in pigs, T2 relaxation times were found to be the earliest marker of myocardial damage, occurring as early as 2 weeks after the third (of 5) dose of doxorubicin(100).

Disadvantages of CMR include limited availability and expertise and long acquisition times amongst others(48,53). Acquisition times however can be reduced by revising protocols to suit specific conditions(138) or for specific CMR parameters(139).

1.5.2 Screening modalities for anthracycline cardiotoxicity: Biomarkers

Lipshultz et al, was one of the first to show that elevation of cardiac troponin T in children with ALL after receiving doxorubicin containing regimes with a suggestion of a correlation with LV wall thinning on echocardiography 9 months later(140). Since then, various investigators have been examining the role of troponin in anthracycline chemotherapy. In a set of elegant experiments Cardinale et al showed that if Troponin I is positive during (tested at various timepoints at each cycle) chemotherapy (which included a significant proportion of patients receiving anthracycline chemotherapy) it predicts subsequent deteriorations in LVEF, with the maximal troponin rise strongly correlated to the amount of LVEF decrease(141,142). Furthermore, the same group showed that if Troponin I is positive during (early) and one month (late) after chemotherapy, it can stratify patients for their risk of subsequent cardiac events, with a positive predictive value of 84%, if troponin positive (both early and late) and a negative predictive value of 99% if troponin negative (both early and late)(143). Auner et al showed that in 78 patients with haematological malignancies treated with anthracycline regimes, 15% have a positive Troponin T during their therapy, and troponin T positive patients show a greater reduction in their LVEF on subsequent echocardiograms post treatment (10% decrease vs 2%) though LVEF dropped to below 50% in only one patient (who was troponin +ve)(144). Furthermore, a positive troponin T is associated with diastolic dysfunction(145). One of the problems with the evidence about the role of troponins in monitoring and predicting of cardiotoxicity is the variability between the studies, with different assays, different time points of troponin measurement, different end points thus making it difficult to know exactly how to interpret

them(48,146). Having said that, it is generally recognised that a rise in troponin increases the risk of cardiotoxicity(48,53,94,115).

Natriuretic peptides, and in particular N-terminal-pro-brain-natriuretic-peptide (NT-pro-BNP) and brain natriuretic peptide (BNP), have also been looked at as biomarkers of early cardiotoxicity with promising results, however with similar and if not worse variability between studies to make accurate conclusions(146).

Attempts to create risk scores to calculate individual risk for cardiovascular complications of cancer therapy have been made, with the CCSS cardiovascular risk calculator derived from the Childhood Cancer Survivor Study (CCSS) data, being the most relevant for anthracyclines(147). Based on a retrospective analysis of the CCSS data, it is aimed at 5-year survivors of childhood cancer with current age up to 40, with estimates of heart failure up to age 50. They separate the risk into 3 groups i.e. low, moderate and high risk with incidence of heart failure of 0.5%, 2.4% and 11.7% respectively(148). Even though it was designed from retrospective data, it has been validated against different cohorts of childhood cancer survivors with similar validation cohort estimates(148). It requires various parameters including age at diagnosis, cumulative dose used if known and use of other chemotherapeutic agents and radiotherapy. Biomarker or imaging data are not required. No equivalent risk score exists for adults.

1.6 Cardioprotective strategies

Over the years, there has been a significant amount of research done to identify cardioprotective agents that could be given as primary prevention against anthracycline induced cardiomyopathy.

1.6.1 Angiotensin Converting Enzyme Inhibitors (ACEi)

ACEi are now the cornerstone in the treatment of heart failure with reduced ejection fraction (HFrEF)(113) and unsurprisingly, they have been investigated for their role in anthracycline cardiotoxicity.

Early animal studies with enalapril and captopril indicated that they may have a cardioprotective effect in Adriamycin induced acute cardiotoxicity by acting as antioxidants and affecting the activity of anti-oxidant enzymes(149). Indeed intracoronary administration of doxorubicin with simultaneous enalapril treatment in dogs improved survival, reduced LV end diastolic pressure and improved LV stroke work index(150). Furthermore, in addition to its antioxidant effects, enalapril seems to prevent mitochondrial dysfunction and depletion of cellular ATP levels caused by doxorubicin in rats(151). It may also play a part in the regulation of fatty acid metabolism by upregulating proliferator activated receptors (PPAR)(152). More recently, enalapril's cardioprotective action in a chronic doxorubicin cardiotoxicity rat model (but not in the acute model) has been shown to be via the PI3K/AKT/mTOR axis which is involved in cardiac atrophy, rather than due to its antioxidant properties(153). These laboratory studies, in addition to showing a potential role of enalapril as a cardioprotective agent they also provided an insight into potential mechanistic pathways of anthracycline-induced cardiomyopathy.

Whilst most studies have been undertaken using enalapril, other ACEi have also been examined. In neonatal rat cardiac myocytes treated with doxorubicin, delarapril was found to have a cardioprotective effect by improving the intracellular calcium handling that is impaired after doxorubicin administration(154). Captopril reduces Adriamycin induced cardiac injury as measured by a reduction in cardiac iso-enzymes CKMB and LDH in in-vivo rats(155). Similarly in hamsters treated with Adriamycin, lisinopril significantly improves cardiac function and survival rate compared to control animals(156). These

protective effects of lisinopril may, in part, be related to reduced ANP expression and myocellular apoptosis(157). Furthermore, with an increasing amount of patients receiving combination chemotherapies, particularly with trastuzumab in the breast cancer population, inhibition of the RAS system with perindopril (and indeed with aliskiren and valsartan) improves echocardiographic assessment of LV size and function in a chronic murine model of doxorubicin/trastuzumab cardiotoxicity(158). Furthermore, perindopril reduces oxidative stress caused by doxorubicin treatment in rats, though it did not prevent LV dilatation(159).

Human trials using enalapril as a primary cardioprotective agent have also been undertaken. Cardinale et al performed a randomized open label controlled trial of patients receiving high dose chemotherapy (58% receiving anthracycline regimes) to assess enalapril as a cardioprotective agent in 114 patients at high risk of cardiotoxicity. High risk of cardiotoxicity was identified by the presence of at least one positive Troponin I value taken soon after (immediately after and at 12, 24, 36, 72 hours after) the end of each chemotherapy cycle. Enalapril (mean dose 16mg/day) was started 1 month after the end of the last chemotherapy cycle and continued for 1 year. There was significant cardiotoxicity (defined as LVEF reduction by >10% from baseline and to a level <50%, the primary end point) in the control group compared to the enalapril group (43% versus 0%; *P*<0.001) and this was also associated with an increase in end-diastolic and end-systolic volumes(160). Similarly, in a randomised, single blinded placebo-controlled trial, enalapril was given just prior to initiation on anthracycline chemotherapy and its effect on LVEF after 6 months was compared to placebo. There was a significant reduction in LVEF in the control group at 6 months from baseline which was not seen in the enalapril group (LVEF: 46.31 ± 7.04 vs. 59.61 ± 5.70 %, respectively; p<0.001). This was associated with a significant increase in LVEDV, LVESV, LA diameter and E/e' velocity and a significant

decrease in E/A ratio, s' and e' velocity (all secondary end points) that were not seen in the enalapril group. Furthermore, troponin I and CKMB performed at 1 month after initiation of chemotherapy was higher in the control group(161). Enalapril as a cardioprotective agent has also been studied in children. In a randomised double blinded placebo-controlled trial, enalapril or placebo was given to children with haematological malignancies due to start anthracycline chemotherapy of >200mg/m^2. The primary outcome of reduction in cardiotoxicity which was defined as a reduction in LVEF >20% (an arbitrary value) was not reached. However, LVEF at 6 months was significantly less in the control group compared to enalapril group (LVEF enalapril 62.25% +/- 5.49, control 56.15% +/- 4.79, p<0.001). This was also associated with a significant increase in Troponin I and NT-pro-BNP at 6 months(162).

In contrast, Georgakopoulos et al. found that enalapril did not offer any cardioprotection in a randomised controlled study(163). However, cardiotoxicity was not clearly defined, and there was no reduction in LVEF in the control group at 12 months compared to baseline as there was in the Cardinale study (67.6% to 66.6% vs 62.8% to 48.3%)(160,163) and the Janbabai study(161). This difference may be due to the fact that biomarkers were not used to identify potential high risk patients. This was shown to be a useful way to identify at risk patients in the recent International Cardio-Oncology Society-ONE (ICOS-ONE) trial(164). In this trial, patients were randomised to a prevention group and received enalapril at the beginning of their chemotherapy and a troponin-triggered group where enalapril was started only after a troponin elevation was detected (performed before and after each chemotherapy cycle and at each study visit). The incidence of troponin elevation above normal was similar in the two groups with similar number of patients developing cardiotoxicity suggesting that a troponin triggered approach may be feasible way to identify at risk patients(164). When combined with carvedilol in the OVERCOME trial (preventiOn

of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive Chemotherapy for the treatment of Malignant hEmopathies), an open label randomised controlled trial of 90 patients, enalapril and carvedilol started prior to administration of chemotherapy (some of which included anthracyclines) prevent cardiotoxicity, as measured by deterioration in LVEF compared to the control group. There was a small but significant absolute intergroup difference of 3.1% in LVEF from baseline with echocardiography (p=0.04) and 3.4% with CMR in favour of the intervention group(165).

1.6.2 Angiotensin Receptor Blockers (ARBs)

Angiotensin Receptor Blockers (ARBs) have also been investigated as potential cardioprotective agents against anthracycline induced cardiotoxicity. Like ACE-Is, they act on the renin-angiotensin system. When doxorubicin is given to Angiotensin II type1a receptor knockout (AT1KO) mice and wild type mice treated with a AT1 antagonist, both groups of mice were protected against doxorubicin cardiotoxicity both in the acute and chronic settings(166).

Telmisartan is an AT1 receptor antagonist, which, in in-vivo models of doxorubicin and daunorubicin cardiotoxicity, is protective when measured both with biochemical and histopathological methods(167,168). Its cardioprotective effect was found to be comparable to that of captopril(169). In an Adriamycin model of cardiotoxicity it was found that the cardioprotective effect of telmisartan, and indeed losartan, appeared to be through increasing circulating plasma levels of Angiotensin 1-7 (Ang 1-7), a peptide formed from Angiotensin I and II that is thought to have cardioprotective properties(170). Furthermore, telmisartan has antioxidant properties that may also be partly responsible for reducing cardiotoxicity(171).

In humans treated with telmisartan prior to chemotherapy, telmisartan prevented a rise in inflammatory and oxidative stress markers (interleukin 6 and reactive oxygen species) that was seen in the placebo group, suggesting an anti-inflammatory/anti-oxidant mechanism of cardioprotection. Left ventricular systolic function as measured by strain rate initially reduced in both groups but subsequently significantly improved only in the telmisartan group(172). This effect on strain rate persisted after 18 months(173).

Valsartan improves the echocardiographic parameters of mice receiving doxorubicin and trastuzumab in a chronic murine model of cardiotoxicity to a similar extend to perindopril(158). Furthermore, valsartan prevents doxorubicin induced cardiotoxicity in rats as measured by in vivo haemodynamic parameters, electrocardiographic parameters and biochemical parameters. Interestingly, this protective effect was seen only when valsartan was given during or after doxorubicin but not when given before(174). Valsartan's cardioprotective effect against doxorubicin seems to be through reducing cellular apoptosis(175).

One of the earlier ARB human trials investigating anthracycline cardioprotection was done using valsartan. Patients with non-Hodgkin's Lymphoma due to start CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy were randomised to receive valsartan or nothing and the effect on acute cardiotoxicity was compared. Acute cardiotoxicity was evaluated using electrocardiographic (QTc and QTc dispersion), echocardiographic and biochemical (ANP and BNP) parameters. Valsartan significantly prevented transient changes in acute cardiotoxicity markers(176).

In a rat model of daunorubicin induced cardiomyopathy, rats treated with candesartan had reduced mortality, improved systolic and diastolic functions and reduced fibrosis and apoptosis(177). In the PRADA trial , whose primary outcome was change in LVEF from baseline as measured by cardiac MRI, there was a small but statistically significant reduction of LVEF in the placebo group (2.6, 95% CI 1.5-3.8) compared to the candesartan group (0.8, 95% CI -0.4, 1.9, p<0.026)(178). Interestingly, in a substudy analysis of the PRADA trial, candesartan had no effect on the observed increase in circulating biomarkers of cardiac injury. Metoprolol however, attenuated the increase in troponin I and troponin T as well as natriuretic peptides(179). The authors concluded that this suggests that candesartan's beneficial effect is primarily on its effect on remodelling whereas metoprolol's beneficial effect is on preventing acute cardiotoxicity. To further support that, in a further sub-analysis of the PRADA study, it was found that candesartan treatment reduced total cellular volume as measured by cardiac MRI(180). This rather surprising result may be explained by considering candesartan's actions on angiotensin II. By inhibiting angiotensin II, candesartan reduces its growth promoting effects on cardiac cells which leads to cardiac remodelling thus reducing total cellular volume rather than cardiomyocyte death and fibrosis(180).

1.6.3 Beta Blockers (BB)

Beta blockers, established as treatment for heart failure with reduced ejection fraction(113), have also been studied as primary prevention strategies particularly with carvedilol, nebivolol and to some extend metoprolol.

Carvedilol is a 3rd generation highly lipophilic, non-selective β-adrenoreceptor antagonist that also blocks α_1-adrenoreceptors, thus having vasodilating properties(181). In heart failure, it reduces mortality by 65%(182) and by 27% in cases of severe heart failure(183).

In view of its α_1-adrenoreceptor activity, it is also effective as an anti-anginal(184). In addition, carvedilol has anti-oxidant properties with an anti-oxidant activity that is ten times more than that of vitamin E(181).

Early animal studies, suggested a protective effect of carvedilol in rat hearts treated with doxorubicin when compared to placebo and atenolol(185). This protective effect seemed to be in part due to its effect on mitochondrial dysfunction(186) utilising primarily carvedilol's anti-oxidant rather than β-blockade properties(187,188). In-vivo animal studies confirmed improvement in cardiac function after treatment with carvedilol(189,190) as well as reduction in fibrosis and hypertrophy in histological specimens(191).

In parallel to the animal studies, case reports of improvement in cardiac function in anthracycline-induced cardiomyopathy following carvedilol administration started to emerge(109). Kalay et al was the first to show in a small single-blinded randomised controlled trial, that 6 months of 12.5mg of carvedilol daily preserves the LV size and function in patients treated with anthracycline chemotherapy compared to placebo(192). In a more recent study of 91 breast cancer patients, carvedilol 6.25mg twice daily, also prevented deterioration in LVEF that was seen in patients receiving placebo(193). Similar findings have been seen in children treated with Adriamycin for acute lymphoblastic leukaemia. Carvedilol, given for 5 days before every Adriamycin dose, resulted in a significant increase in cardiac function as measured by fractional shortening as well as inhibiting the release of cardiac troponin I even as early as one week after the last dose of chemotherapy(194). In patients with breast cancer receiving doxorubicin (maximum dose 536 mg/m^2), strain imaging with echo was used to assess early cardiotoxicity. At 6 months, there was a significant decrease in strain imaging parameters in the placebo group compared to the carvedilol group (12.5mg daily) suggesting that carvedilol potentially prevents early silent cardiotoxicity(195). Carvedilol seems to preserve these changes in

strain analysis as early as one week after the last chemotherapy was given(196). However, a lot of these studies were limited in their design by being single-blinded or open label or having small number of patients. The CECCY trial (Carvedilol Effect in Chemotherapy-induced CardiotoxicitY), was a double blind, randomised, placebo controlled trial of 200 HER-2 negative breast cancer patients that received doxorubicin (total dose 240mg/m^2) as part of their chemotherapy protocol(197). Carvedilol was slowly up-titrated to a maximum of 25mg twice daily if tolerated. The primary endpoint of a drop of at least 10% in LVEF at 6 months was not different between the two groups (14 patients (14.5%) in the carvedilol group and 13 patients (13.5%) in the placebo group (p = 1.0)). The LVEDd showed a non-significant trend of increase in the control group compared to the carvedilol. Troponin I however, a secondary outcome, was significantly attenuated in the carvedilol group. Significant changes in favour of carvedilol were also seen in indices of diastolic dysfunction(197). One potential explanation offered for these discrepancies in the primary outcome compared to previous studies is the lower dose of anthracycline received as well as a lower incidence of early onset cardiotoxicity in this study which might affect power calculations(197).

Nebivolol is a highly lipophilic, highly selective β-1 receptor antagonist (321-fold higher affinity for β-1 compared to β-2 receptor) that also has nitric oxide (NO) mediated vasodilatory properties(198). As such, it is often thought to be a more ‘’cardioselective’’ beta-blocker, at least in doses up to 10mg/day. In addition, it is also thought to have some antioxidant properties(199). Nebivolol has been studied in clinical trials as a treatment for hypertension(198), as well as in heart failure where it was found to reduce all-cause mortality or hospitalisation compared to placebo in elderly patients(200). In view of these properties, it has been investigated as a cardioprotective agent during anthracycline chemotherapy. In rat heart models of anthracycline-induced cardiotoxicity, nebivolol

reduced markers of cardiac toxicity such as troponin and CK(201). Similarly, nebivolol protected against cardiac muscle injury when assessed histopathologically as well as biochemically(202). In clinical trials of breast cancer, 5mg/day of nebivolol given 7 days prior to commencement of anthracycline chemotherapy and continued for 6 months, prevented LV dilatation compared to control and preserved LVEF significantly better than controls at 6 months (57.5±5.6% vs. 63.8±3.9%, p=0.01)(203). In an unblinded study, tissue Doppler imaging (TDI) and speckle tracking imaging (STI) are preserved in patients receiving nebivolol compared to the group not receiving nebivolol(204).

Reports of metoprolol being used early on in the treatment of anthracycline-induced heart failure, suggested improvement in cardiac function both in humans(111) and animals(205). This was potentially mediated by preventing calcium overload in cardiomyocytes(206). However, when give as a cardioprotective agent prior to development of heart failure, subsequent clinical studies have been neutral. In an open-label randomised control trial of 125 patients, there was a marginal but non-significant reduction in heart failure with metoprolol(207). In the Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA) study, a 2x2 factorial, randomised, placebo-controlled, double-blind clinical trial of candesartan and metoprolol in 130 breast cancer patients receiving anthracycline containing regimes, metoprolol, unlike candesartan, had no effect on LVEF change as measured by cardiac MRI(178).

1.6.4 Mineralocorticoid Receptor Antagonists (MRAs)

Mineralocorticoid Receptor Antagonists (MRAs) are second line therapy after ACE-I and Beta blockers for patients with heart failure and reduced ejection fraction, often given in patients with ongoing symptoms and persistent severe LV dysfunction (typically LVEF <35%)(113). In rats treated with doxorubicin and spironolactone, spironolactone prevented

increase in LV size and decrease in function caused by doxorubicin(208). In male mice, eplerenone prevents left ventricular dysfunction after doxorubicin treatment and mice that have had the mineralocorticoid receptor gene depleted, show similar protection suggesting a role of the mineralocorticoid receptor in cardiac protection(209). However, in a similar experiment involving female mice, eplerenone or indeed mineralocorticoid gene depletion did not show any cardioprotective effects(153). In a randomised, placebo controlled double blind study of 83 breast cancer patients, spironolactone started one week prior to anthracycline based chemotherapy was compared to placebo. The primary outcome was a change in LVEF. There was a significant reduction in LVEF from 67.7±6.3 to 53.6±6.8 (P <0.001) in the control group which was not seen in the spironolactone group (67.0±6.1 to 65.7±7.4, P = 0.094). When comparing the two groups using a general linear model, this difference in LVEF change was still significant (p<0.001). Diastolic function markers were also more significantly affected in the placebo group. Furthermore, the spironolactone group showed significantly less increases in cardiac biomarkers (CK-MB and troponin I) compared to the placebo group(210).

1.6.5 Dexrazoxane

Dexrazoxane is a water soluble form of the iron chelator ethylenediaminetetraacetic acid (EDTA), which can easily pass into cells and is broken down to an EDTA form that chelates iron by displacing it from the anthracyline(211). As the interaction of anthracyclines with iron metabolism and reactive oxygen species in cardiotoxicity became clearer, it was not long before dexrazoxane was investigated as a potential cardioprotective therapy. Indeed, in the 1970s and 1980s, Herman et al, performed a series of experiments showing that dexrazoxane (then known as ICRF-187) or similar agents can protect the heart from anthracyclines in a variety of different species(67–69,212,213). Speyer et al, was the first to show, in a single centre randomised-controlled

trial of 92 women with advanced breast cancer, that dexrazoxane significantly prevented subsequent deteriorations in LVEF (drop in LVEF 3% vs 16% for higher doses and 2% vs 7% for lower doses with and without dexrazoxane respectively), and clinical congestive cardiac failure(214) as well as allowing for higher doses of doxorubicin to be given(46). The anti-tumour effect of doxorubicin was not affected though there was possible more myelotoxicity in the dexrazoxane group(46). This benefit was further shown in a multicentre study of 162 advanced breast cancer patients with an odds ratio of developing cardiotoxicity (defined as clinical heart failure, or LVEF <45% or EF drop by >20% units) of 0.29 (95% CI 0.09-0.76, p = 0.006) in favour of dexrazoxane with similar non-cardiac toxicity, progression-free survival and overall survival(215). In children with sarcoma, smaller but still beneficial effects on cardiotoxicity were seen with dexrazoxane, although with a trend towards more haematological toxicity(216). The protective effects were further supported with larger phase III trials in breast cancer to the extent that a safety committee recommended an amendment to the protocol to give all patients dexrazoxane after doses of 300mg/m^2 due to excess cardiotoxicity in the placebo arm(70) and a subsequent analysis of the higher dose patients showing even more significant cardioprotective effects(217). Dexrazoxane also prevented cardiac injury as measured by troponin in children with ALL(218). Furthermore, patients who previously received anthracyclines and who required further anthracycline therapy, had fewer cardiac events (39% vs 13%) and heart failure (11% vs 1%) if given dexrazoxane(219). In addition, meta-analyses have shown a significant benefit for dexrazoxane in preventing heart failure with relative risk between 0.18 (95% CI 0.1-0.32 for clinical heart failure n = 1345) and 0.29 (95% CI 0.2-0.4 for clinical and subclinical heart failure, n = 643) with low heterogeneity (0-9%) in a Cochrane review(220) and 0.35 (95% CI 0.25-0.39) in another meta-analysis(221) with no effect on response rate or survival(220). However, its use in children with Hodgkin's Disease suggested an increased risk of secondary malignant neoplasms with a cumulative

incident rate of 3.4% vs 0.85% (p = 0.06), though overall, these were rare events (8 vs 2 events)(222). For that reason, the European Medicine Agency in 2011 restricted its use to only adult patients with advanced breast cancer who are to receive more than 300mg/m^2 and not to be used in children and adolescents(223). This has subsequently been revisited in 2017 and the European Medicines Agency has approved its use in children and adults who are to receive high dose anthracyclines and it is now only limited in children and adolescents who are to receive low dose (<300mg/m^2) anthracyclines(224) as recent evidence does not support previous concerns raised particularly for secondary malignancies and other toxicities(225).

1.6.6 Other Cardioprotective Strategies

1.6.6.1 Continuous versus bolus infusion

Continuous infusion over 48-96 hours is occasionally used to reduce the risk of cardiotoxicity. The rationale is that peak anthracycline concentrations are reduced thus potentially reducing toxicity. However, exposure is prolonged which may inhibit the ability of cardiomyocytes to recover thus this strategy remains controversial(227). Early reports in adults show reduced cardiotoxicity with continuous infusion and ability to increase the total chemotherapy dose used(228), though this seems to be less so the case in children(227).

1.6.6.2 Anthracycline analogues

Many anthracycline analogues have been created over the years to reduce the cardiac effects with epirubicin, idarubicin and mitoxantrone the only ones potentially showing a beneficial effect. Epirubicin dose that leads to cardiotoxicity appears to be higher(229) and is sometimes used in breast cancer regimes. Both idarubicin and mitoxantrone had promising preclinical trials of reduced cardiac side effects but the clinical studies have been less promising(227).

1.6.6.3 Liposomal anthracyclines

Liposomal anthracyclines and pegylated liposomal formulations aim to reduce cardiotoxicity by not allowing the drug to escape out of vascular beds in sensitive organs but still able to escape capillary beds with disrupted walls as seen in tumour sites. Clinical studies have been promising, particularly for liposomal doxorubicin and pegylated liposomal doxorubicin, studies show similar antitumour effects with significantly lower risk of cardiac toxicity, though long-term cardiac safety effects are not yet clear(230).

1.6.7 Remote Ischaemic Conditioning as a Cardioprotective Strategy

1.6.7.1 Ischaemia Reperfusion Injury and Ischaemic Conditioning

In an acute myocardial infarction due to coronary artery occlusion, the most effective strategy in reducing infarct size and improving outcomes, is reperfusion, but paradoxically reperfusion itself can induce injury, known as myocardial reperfusion injury(226). Described as early as 1960(227) and sometimes thought of as a double-edge sword(228) reperfusion injury is now believed to be made of four distinct types: 1. Reperfusion induced arrhythmias, 2. Myocardial stunning, 3. Microvascular obstruction (the “no-reflow” phenomenon) and 4. Lethal myocardial reperfusion injury(229). The latter refers to death of cardiomyocytes that were thought to be viable at the start of reperfusion, and it is felt to contribute to as much as 50% of the final infarct size(226). Despite improvements in reperfusion strategies with primary percutaneous coronary intervention (pPCI), dual antiplatelet therapy, drug eluting stents, heart failure therapies and others, real life registry data suggest that even though there has been improvement in survival and recurrent cardiac events there is a plateauing in recent years, with all-cause mortality at one year of 14% and heart failure of 6%(230). Therefore, strategies targeting lethal myocardial

reperfusion injury, such as ischaemic conditioning, may have an important role to further reducing infarct size and its complications.

Ischaemic conditioning was first described by Murry et al in their landmark experiment in 1986(231). In that experiment, 4 cycles of 5 minute occlusions of the circumflex artery in dogs followed by 5 minutes of reperfusion prior to a prolonged episode of 40 minute ischaemia by way of occlusion of the circumflex artery, reduced infarct size by 25%, and the term “ischaemic preconditioning” was coined. Clinically, “warm-up” angina, where patients who get exertional angina that disappears can subsequently exercise for longer with minimal symptoms, may be a manifestation of the preconditioning phenomenon(232). Indeed, patients who experience angina in the 24 hours prior to a myocardial infarction have smaller infarct sizes compared to patients with no prodromal angina again suggesting this may be a representation of preconditioning in humans(233). Several small clinical trials have investigated preconditioning in the context of cardiac surgery, and in a meta-analysis of those trials, ischaemic preconditioning reduces ventricular arrhythmias, inotropic requirements and intensive care stay(234). Applying it however to the clinical setting of an acute MI that occurs unpredictably, would be difficult(235).

Zhao et al, showed in 2003 that repetitive 30-second occlusions of the LAD in dogs, at the onset of reperfusion, in other words, post-conditioning, reduced infarct size comparable to a pre-conditioning regime (14% vs 15%) and significantly less than controls (25%, $p<0.05$)(236). Due to its clinical feasibility, it wasn’t long after a clinical trial in humans with STEMI was performed which showed that postconditioning reduces infarct size by 36% as measured by creatine kinase compared to controls(237). Despite that however, subsequent studies showed mixed results, with some showing reduction in infarct size when measured with biomarkers or imaging(235) whilst others, including a large

multicentre study of 700 patients showing neutral results(238). The reasons for the varied results are likely to be multi-factorial as described by Hausenloy et al and includes different study designs, patient selection, post-conditioning techniques and end-point definitions(235).

A few years after the Murry et al study(231), a further landmark study was published in 1993, where Przyklenk et al described remote ischaemic preconditioning(239). In a similar design to the Murry et al experiment, preconditioning was caused by 4 cycles of 5 minute occlusions of the circumflex artery in dogs followed by 5 minutes of reperfusion prior to an episode of prolonged (1 hour) ischaemia produced by occlusion of the left anterior descending artery, i.e. a different vascular bed. This remote preconditioning caused an infarct size of 6% versus 16% in the control group ($p < 0.05$)(239) suggesting that conditioning may be induced by an intervention done remotely, and as shown later the heart may be protected by an intervention performed remote to the organ(240). Khabander et al was the first to show that this remote stimulus can be applied non-invasively by a tourniquet or blood pressure cuff in both animals and humans(241) thus making remote ischaemic conditioning clinically an attractive non-invasive strategy to offer cardioprotection. Though the clinical studies of remote conditioning in the context of cardiac surgery and planned PCI showed mixed results(235), the ones performed during STEMI were more uniform. Bøtker et al was the first to show that RIC performed in the ambulance (i.e. pre-conditioning) increases myocardial salvage index as measured by myocardial perfusion scanning in a randomised controlled trial of STEMI patients(242). Furthermore, when assessed with cardiac MRI, RIC reduced infarct size, myocardial oedema, as well as high sensitivity troponin T in 84 patients with STEMI treated with pPCI(243). In addition, in a larger study of over 500 patients treated with thrombolysis RIC reduces infarct size by 32% and 19% as measured by enzymatic markers (Troponin T and

CK-MB respectively)(244). RIC performed post reperfusion (post-conditioning) also reduced enzymatic (CK-MB) infarct size in patients with anterior-STEMI(245). Whether these promising results would translate into long term benefits was unknown until 2014 when, in 333 patients with STEMI, RIC reduced a composite of major adverse cardiac and cerebrovascular events (hazard ratio 0.49, 95% Confidence Interval 0.27-0.89, p = 0.018) with a median follow-up of 3.8 years(246). This however was not shown in a subsequent study at 6 month follow-up, though this was not the primary end-point(247). In a further randomised controlled trial of 441 patients with a primary end point of cardiac mortality or hospitalisation for heart failure with a median follow up of 2.1 years, RIC improved outcomes compared to control with a hazard ratio of 0.35 (95% CI 0.15-0.78)(248).

In a landmark trial (CONDI-2/ERIC-PPCI) published during the course of my MD, 5115 patients with STEMI were randomised to receive either RIC (n = 2546) or control (n = 2569) in addition to standard care, in a multi-centre international single blinded randomised controlled study with a primary outcome defined as cardiac death or hospitalisation for heart failure at 12 months follow-up(249). At 12 months, there was no difference in the pre-specified end-point between the two groups (8.6%(control) vs 9.4%(RIC), HR 1.1, 95% CI 0.91-1.32, p = 0.32 for RIC vs control). Thus this large, appropriately powered study, concluded that the effect of remote ischaemic conditioning, despite previous promising results from smaller trials and repeatedly positive results from pre-clinical studies, does not translate into beneficial clinical outcomes at one year and therefore unlikely to be incorporated into routine clinical practice(249,250). Importantly it was shown that the mortality in the control group was extremely low (2.7%) indicating that the patients in this study were very low risk and it could be argued that such a study needs to be undertaken in high risk patients(251).

1.6.7.2 Mechanism of action of ischaemic conditioning, reperfusion injury and their relation to anthracycline cardiotoxicity

Alongside the clinical trials described above, a vast amount of data has been accrued over the years in an attempt to understand the mechanisms behind ischaemic conditioning and reperfusion injury. It is now appreciated that pre-, post- and remote conditioning share some common signalling pathways, are highly complex and therefore the exact mechanisms of action of each are yet to be fully elucidated(235).

Using preconditioning as an example as shown in Figure 1.1, the pathways can be, very simplistically, thought to occur in a linear fashion; a trigger is released during the preconditioning period which acts as a stimulus on a mediator that propagates the signal during the sustained ischaemic insult to an end effector that delivers the cardioprotective effect(252,253). Several stimuli have been described including repeated brief episodes of ischaemia and reperfusion, exercise, heat stress, hypothermia)(229) as well as triggers or autocoids that bind to receptors on the cell surface that activate appropriate signalling pathways(235). Examples of autocoids include adenosine(254), bradykinin(255), opioids(256), acetylcholine(257) and endothelin(258) amongst others, which in the presence of their respective inhibitor the conditioning effect is abolished. Once bound to their cell surface receptors, these autocoids activate a variety of signalling intracellular pathways, the mediators. These are complex cytosolic pathways, with more than one likely activated at any one time and often interacting with each other(253). Though there are others, three commonly described pathways are the Nitric-Oxide cyclic guanosine 3′,5′-monophosphate Protein Kinase G (NO-cGMP-PKG), the Reperfusion Injury Salvage Kinase (RISK) pathway and Survivor Activator Factor Enhancement (SAFE) pathways.

As described in detail by Burley et al the NO-cGMP-PKG pathway involves activation and increase in intracellular cGMP by autocoids like NO leading to activation of PKG with effect on calcium homeostasis and the mitochondrial K_{ATP} channel leading to cardioprotection(259).

The RISK pathway (first described by Yellon and colleagues) which is made up of a variety of pro-survival kinase proteins such as Akt and Erk 1/2, which when activated by autocoid and non-autocoid factors including ischaemic conditioning, have an effect on mitochondria and particularly the mitochondrial permeability transition pore (MPTP) which is inhibited as well as activating other anti-apoptotic and anti-autophagy mechanisms that lead to reduced cell death caused by ischaemic reperfusion injury(260).

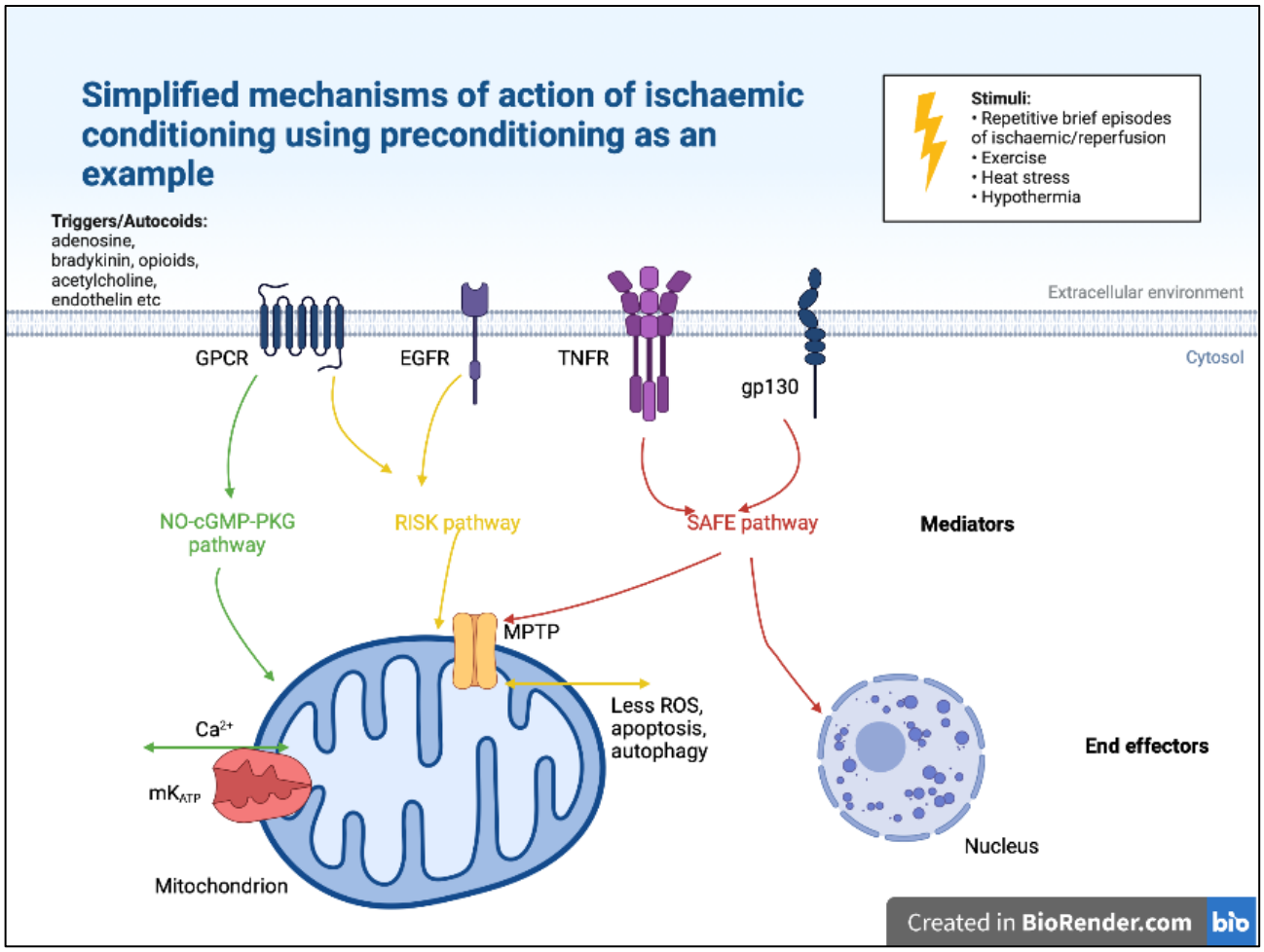
In the SAFE pathway (first described by Lecour and colleagues), which seems to be independent from the RISK pathway and activated via Tumour Necrosis Factor α and interleukin 6 (IL 6) and mediated by Janus Kinases (JAKs) and Signal Transducer and Activator of Transcription 3 (STAT 3) with effects on both the nucleus and mitochondria to protect the cell(261).

The mitochondria appear to be the most important effectors in the conditioning process. Most signalling pathways have been found to end or involve the mitochondria and in particular the MPTP which controls release of various substances in the cytosol such as cytochrome C that can lead to cell death(253).

To explain the remote part of ischaemic conditioning it is thought that a neurohumoral pathway is needed that links the remote organ and the heart(235). Evidence for a humoral component was shown when the preconditioning effect was maintained after

preconditioned blood from one animal was transfused to a preconditioning-naïve animal(262). The exact humoral molecule responsible has yet to be identified. Furthermore, interruption of the limb-to-heart neural pathway at different levels abolishes the protective effects of remote ischaemic conditioning thus suggesting the requirement of an intact neural pathway(235).

Figure 1.1. A simplified schematic illustration of conditioning using preconditioning as an example. Certain stimuli will trigger autocoids such as adenosine that act on cell surface receptors to trigger a variety of intracellular transducer pathways, the mediators. Final common pathways act on end-effectors, typically the mitochondrion, to trigger cardioprotective mechanisms through less reactive oxygen species generation, less apoptosis and less autophagy. Ca^{2+}, Calcium ion; EGFR, epidermal growth factor receptor; GPCR, G-protein coupled receptor; gp130, glycoprotein 130; mK_{ATP}, mitochondrial potassium adenosine 5 triphosphate channel; MPTP, mitochondrial permeability transition pore; NO-cGMP-PKG, Nitric-Oxide cyclic guanosine 3′,5′-monophosphate Protein Kinase G; RISK, Reperfusion Injury Salvage Kinase; ROS, reactive oxygen species; SAFE, Survivor Activator Factor Enhancement TNFR, tumour necrosis factor receptor. Adapted from Heusch 2015 and Yellon et al 2003(257,258).

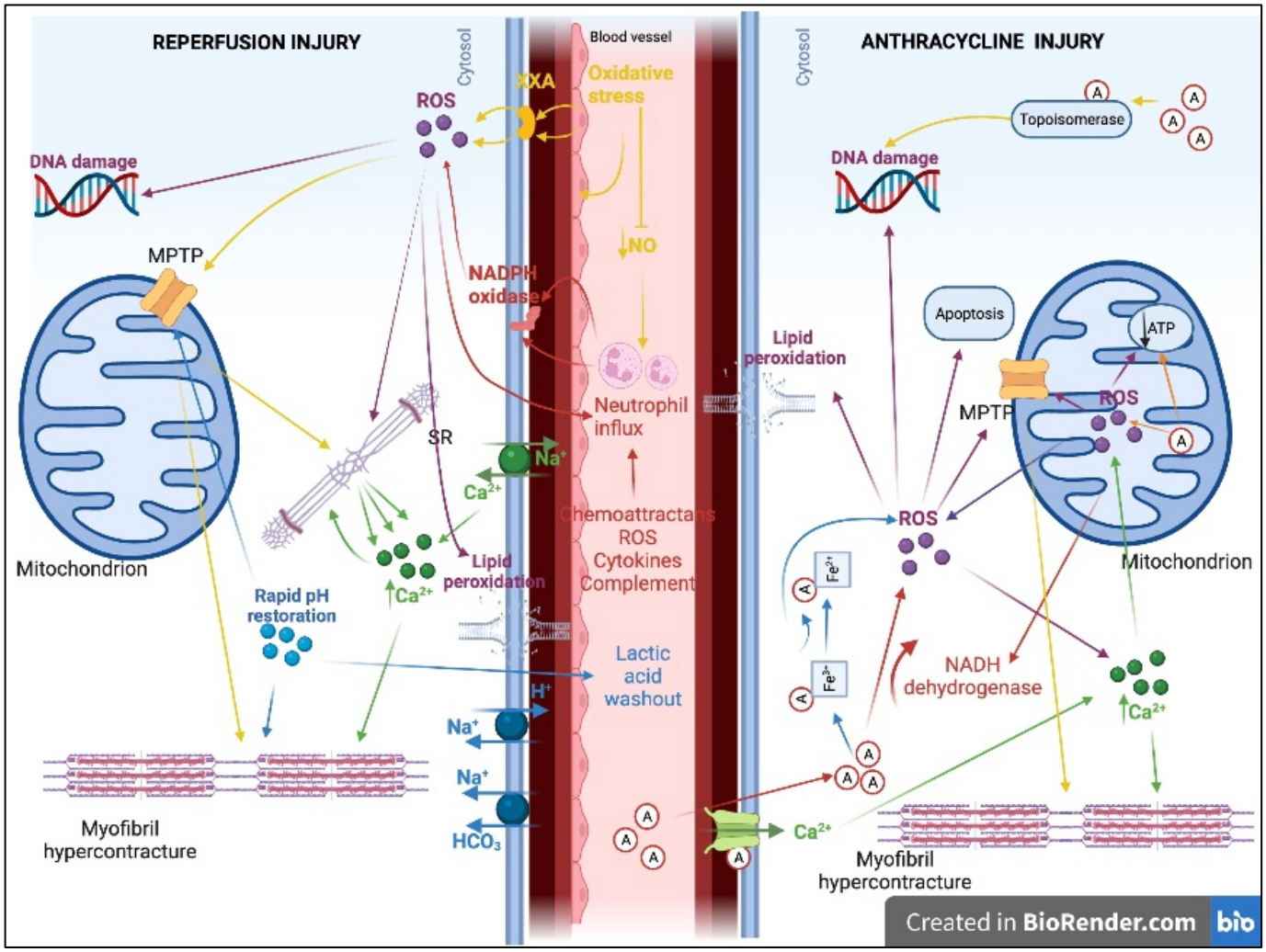


Whilst attempting to understand the pathophysiological mechanisms behind ischaemic conditioning we have gained an insight into the pathophysiology behind ischaemic reperfusion injury (IRI) (Figure 1.2). As already mentioned, IRI is made of four parts: stunning, arrhythmias, microvascular obstruction and lethal reperfusion injury, the latter being the target of conditioning. Reperfusion itself generates oxidative stress which via the generation of oxygen free radicals causes myocardial injury(226). Furthermore, reperfusion leads to increased intracellular calcium concentrations that cause hypercontracture of cells but also open the MPTP, which can be detrimental for the cell(226). A similar mechanism is thought to occur with the rapid restoration of pH that occurs during reperfusion(229). In addition, inflammatory mechanisms occurring during reperfusion lead to further reactive oxygen species generation via NADPH and xanthine oxidase enzymes(226).

The link between ischaemia/reperfusion injury and anthracycline cardiotoxicity comes from the observation that both pathologies share some common pathophysiological such as calcium dysregulation, lipid peroxidation, reactive oxygen generation and mitochondrial dysfunction(263) as depicted in Figure 1.2. In reperfusion injury, oxidative stress during reperfusion leads to reactive oxygen species (ROS) formation via xanthine oxidase (XXA) released from endothelial cells. Oxidative stress also reduces nitric oxide (NO) which together with other chemoattractants, cytokines and complement activation lead to a neutrophil influx which produce ROS via NADPH oxidase. ROS have multiple effects including opening of the MPTP, dysfunction of the sarcoplasmic reticulum (SR), disruption of the cellular membrane though lipid peroxidation and direct DNA damage. Intracellular calcium overload from the ischaemic insult is further enhanced due to dysfunction of the SR and reverse function of the Na^{+}-Ca^{2+} exchanger. Rapid pH restoration facilitated by activation of Na-H and Na-HCO3 transporters further contribute to opening of the MPTP. The end result is myofibril hypercontracture and thus cardiomyocyte damage. In

anthracycline injury, anthracyclines enter the cells directly and cause ROS generation via NADH dehydrogenase from mitochondria as well as through their interaction with iron metabolism. They also cause intracellular calcium influx. Anthracyclines interact with topoisomerase causing direct DNA damage. ROS generation leads to damage through lipid peroxidation, DNA damage, apoptosis, increase in intracellular calcium, myofibril hypercontracture and mitochondrial dysfunction.

Figure 1.2. Simplified schematic comparing reperfusion injury (left) and anthracycline injury (right), see text. A, anthracycline; ATP, adenosine 5-triphosphate; Ca^{2+}, calcium ion; Fe, iron; H^+, hydrogen ion, HCO_3, bicarbonate ion; MPTP, mitochondrial permeability transition pore; Na^+, Sodium ion; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; ROS, reactive oxygen species; SR sarcoplasmic reticulum; XXA, xanthine oxidase. Adapted from Yellon et al 2007, Hausenloy et al 2013 and Sandhu et al 2014(231,234,269).



Anthracyclines may affect some of the prosurvival pathways discussed earlier. For instance, heat shock protein 20 (Hsp 20) has been found to protect the heart during IRI through anti-apoptotic mechanisms(264) and overexpression of the same protein both in

vivo and in vitro protects against doxorubicin induced apoptosis and necrosis(265). Furthermore, ERK 1/2, which forms part of the RISK pathway, is initially upregulated potentially for protective purposes but subsequently downregulated with Adriamycin and contributes to the development of heart failure(266).

In a set of experiments Maddock's group assessed the effect of doxorubicin in an ischaemia-reperfusion model. Isolated rat hearts were subjected to 35 minutes of regional ischaemia by ligation of the left coronary artery followed by 120 minutes of reperfusion with either buffer (control) or doxorubicin. Infarct size was significantly increased by doxorubicin compared to control (64.5 ± 8% vs. 47.4 ± 2.5%)(267). When Cyclosporin A (CsA), a known inhibitor of MPTP, was added to the control perfusate as expected infarct size was reduced, and interestingly when added to the doxorubicin perfusate, infarct size was also reduced to similar levels (22% vs 27%) suggesting that doxorubicin enhances IRI and acts via the MPTP(267). Interestingly Akt and Erk 1/2 of the RISK pathway have increased phosphorylation with doxorubicin suggesting that these endogenous protective mechanisms may be recruited against doxorubicin induced cardiotoxicity(267). In a similar set up, an inhibitor of mitochondrial fission and fusion (mitochondrial division inhibitor 1 (mdivi-1)) was used both in a normoxic experiment and also an ischaemia-reperfusion experiment. In the normoxic model, mdivi 1 caused a significant reduction in infarct size seen with doxorubicin (30% vs 15%, $p<0.05$), and like CsA, mdivi 1 protected against the enhanced doxorubicin damage caused during ischaemia-reperfusion suggesting that the mitochondrion is crucial in the process of both ischaemia-reperfusion and doxorubicin induced cardiotoxicity(268).

Furthermore, the role of iron in both pathologies has been explored. Recently, a form of cell death that is different to apoptosis, necrosis and autophagy and depends upon

intracellular iron, termed ferroptosis, has been described(269). It has been implicated in various pathologies (e.g. degenerative disorders, cancer, brain haemorrhage and injury, IRI) and seems to be the result of lethal lipid peroxidation(270). In a set of very detailed experiments, Fang et al show that ferroptosis is implicated in mouse models of both doxorubicin-induced and ischaemia-reperfusion-induced cardiomyopathy that is prevented by both the iron chelator dexrazoxane as well as the ferroptosis inhibitor ferrostatin-1(271).

In fact, in isolated rat hearts, one 5-minute cycle of preconditioning with global ischaemia (by clamping the aorta) followed by 10 minutes of reperfusion prior to an epirubicin infusion was found to improve some of the physiological markers of acute epirubicin toxicity as early as 1996(272). It was not for another 20 years before any similar experiments were performed. In isolated rat ventricular cardiomyocytes, simulated ischaemic preconditioning was performed using a hypoxic buffer for 30 minutes followed by a normoxic buffer (i.e. reperfusion) for 10 minutes prior to exposure to doxorubicin(273). Simulated preconditioning reduces cell death by doxorubicin (35.4 ± 1.7% vs 14.7 ± 1.5%; $p < 0.01$), most likely does it via the PI3-kinase/Akt pathway in this model, and importantly, does not reduce doxorubicin efficacy against cancer cells when the experiment is repeated using the HeLa cervical cancer cell line(273).

In an in-vivo experiment in mice, remote ischaemic preconditioning with 3 cycles of 5-minute occlusion and 5-minute reperfusion of the femoral artery was performed one hour prior to a single 20mg/kg intraperitoneal injection of doxorubicin and compared to animals receiving a sham procedure. Survival was significantly improved with RIC vs sham after 85 days ($p = 0.007$). Even though LVEF was similar between groups, LV mass decreased in the control but not in the RIC group. Markers of fibrosis and apoptosis were also better in the RIC group(274). Furthermore, a regime of repeated RIC (rRIC) (four cycles 5-minute

ischaemia and reperfusion using a tourniquet on hindlimb of mice), starting 30 minutes prior to administration of 10mg/kg of doxorubicin and continued daily for 5 days preserved heart/body weight ratio compared to the doxorubicin only group. Troponin I collected 6 days after doxorubicin administration was significantly elevated with doxorubicin but attenuated with rRIC and LVEF as assessed by echocardiogram on day 6 showed a modest but significant difference in favour of rRIC (LVEF doxorubicin 47.5%, doxorubicin+rRIC 51.6%, $p < 0.05$)(275). Like Gertz et al(274) markers of fibrosis (myocyte cross sectional area and collagen content) and apoptosis (presence of apoptotic nuclei) were also improved(275).

Recently, Gallan-Ariola et al explored RIC as a cardioprotective strategy in a more clinically relevant experiment. Using a large animal (pig), RIC consisting of 3 cycles of 5 minute occlusion and reperfusion of the hind leg using a tourniquet prior to a series of 5 injections of 0.45mg/kg of doxorubicin given 2 weeks apart, thus mimicking to some extent clinical practice. The intra-coronary (LAD) route was chosen, which though highly aggressive and not clinically translatable, minimises systemic myelosuppression. Cardiac function was assessed at baseline and then at weeks 6, 8, 12 and 16 using cardiac MRI. LV function starts to deteriorate from the 4th doxorubicin injection onwards and by week 16, LVEF is significantly lower in the non-conditioned group compared to the RIC group (32.5% ± 8.7 vs 41.5 % ± 9.1; $p = 0.04$). T1 relaxation time, a marker of oedema and fibrosis, was significantly higher in the non-conditioned group at week 16 (ΔT1 20% vs 11%, $p = 0.04$) which correlated with histological analysis of collagen staining(276). In a second experiment looking at early cardiotoxicity, animals were sacrificed after 3 doxorubicin injections and despite normal cardiac function at MRI, there was evidence of severe mitochondrial morphological abnormalities that were attenuated with RIC(276)

hence suggestive that the protective effect of RIC occurs early on in the disease process and continues to offer protection several weeks after doxorubicin therapy is finished.

Conclusion

Based upon the potential for RIC to demonstrate protection in the pre-clinical setting, it was felt appropriate to design a clinical study to ascertain whether this phenomenon would protect patients who receive anthracyclines as part of their chemotherapy regime. As such, the ERIC ONC (Effect of Remote Ischaemic Conditioning in Oncology patients) study was designed(263), from which my MD Thesis is based on. ERIC-ONC was a randomised controlled trial comparing remote ischaemic conditioning delivered using a blood pressure cuff to the arm versus a sham-procedure in patients receiving anthracycline chemotherapy with changes in troponin as the primary outcome.

Furthermore, the best way to monitor patients for anthracycline cardiotoxicity still remains unclear, particularly with regards to cardiac biomarkers and how they relate to cardiovascular outcomes (biomarkers, imaging, clinical and electrical). A prospective analysis of that relationship was performed to identify a potential model of multimodality (biomarkers, imaging, clinical and electrical) monitoring. This also included testing a new potential biomarker (cardiac myosin binding protein C) as well as a rapid sequence cardiac MRI protocol.

My MD Thesis therefore has been set up to investigate two hypotheses:

1. Remote ischaemic conditioning prevents anthracycline cardiotoxicity as measured by changes in troponin in patients receiving anthracycline chemotherapy. The Null Hypothesis therefore being: remote ischaemic conditioning does not prevent anthracycline cardiotoxicity as measured by changes in troponin.

2. The relationship between troponin changes during anthracycline chemotherapy and cardiovascular outcomes in patients receiving anthracyclines. The Null hypothesis therefore being: there is no relationship between changes in troponin during anthracycline chemotherapy and cardiovascular outcomes in patients receiving anthracyclines

Chapter 2 – Methods

In this chapter I will describe the methodology used for my thesis. As the data for my different thesis analyses were collected from the same cohort of patients, I will describe the methodology as a whole for the following: patient population included, patient screening, enrolment and randomisation; study protocol; RIC/Sham intervention used, biomarkers used, imaging used, clinical events analysed and electrophysiological methods used. However, as my thesis has two parts, the effect of remote ischaemic conditioning and multimodality monitoring, I will describe the outcomes chosen and statistical methods used for each part separately.

2.1 Patient Population

The patients used for my thesis were patients that I recruited into the already ongoing ERIC-ONC study (Effect of Remote Ischaemic Conditioning in Oncology patients)(263). These included any patients aged 16-80, who were about to start an anthracycline containing chemotherapy regime at University College London Hospital (UCLH) and specifically at the Macmillan Cancer Centre at UCLH. The study was open to patients with any cancer who were able to tolerate a blood pressure cuff on either arm, but the cancer groups that agreed to participate in the study were the Sarcoma, Breast and Lymphoma groups. For the lymphoma patients, it was agreed that only patients on specific chemotherapy regimes (see below) were to be recruited. Patients were identified by the oncology teams and referred if they were deemed to be suitable candidates taking into account their comorbidities, frailty, mental state and urgency to start cancer treatment. I would then screen patients to ensure they met the inclusion and exclusion criteria. Patients were excluded if they had previous evidence of myocardial infarction, or other known cardiomyopathies or cardiac infiltrative disorders such as dilated cardiomyopathy, hypertrophic cardiomyopathy, Fabry's disease, cardiac amyloidosis or significant valvular

disease. A finding of left ventricular dysfunction either on history or subsequent baseline cardiac imaging would also have led to an exclusion as patients would have unlikely been able to receive anthracyclines. The presence of peripheral vascular disease, chronic kidney disease with eGFR <30ml/min/1.73m^2 or taking sulphonylurea were also exclusion criteria. Chemotherapy regimes were decided by the patient's oncology team and included the following protocols according to cancer group:

1. Sarcoma:
    a. Doxorubicin (Dox)
    b. Doxorubicin – Ifosfamide (D-Ifos)
    c. Doxorubicin – Cisplatin (D-Cis)
    d. Doxorubicin – Olaratumab (D-Ola)
    e. Methotrexate – Doxorubicin – Cisplatin (MAP)
    f. Vincristine-Ifosfamide-Doxorubicin (VI-Dox)
    g. Ifosfamide-Vincristine-Dactinomycin-Doxorubicin (IVA-Dox)
2. Lymphoma:
    a. Rituximab-Cyclophosphamide-Doxorubicin-Vincristine-Prednisolone (RCHOP)
    b. Cyclophosphamide-Doxorubicin-Vincristine-Etoposide-Prednisolone (CHOEP)
    c. Cyclophosphamide-Doxorubicin-Vincristine-Prednisolone (CHOP)
    d. Rituximab-Cyclophosphamide-Doxorubicin-Vincristine-Prednisolone-Methotrexate (RCHOP-Mtx)

e. Doxorubicin-Bleomycin-Vinblastine-Dacarbazine (ABVD)

3. Breast:

   a. Fluorouracil-Epirubicin-Cyclophosphamide-Paclitaxel-Carboplatin

   b. Fluorouracil-Epirubicin-Cyclophosphamide-Docetaxel-Trastuzumab

2.2 Patient screening, enrolment and randomisation

When a patient was identified by the oncology team and referred as a potential study candidate, I would record their details in a screening pro-forma and subsequently look through their electronic patient records to assess eligibility criteria. For patients who were excluded, the exclusion reason was documented on a screening pro-forma. If no exclusion criteria were identified, a patient information leaflet was given to the patients (either via post or in person via the oncology team) and a consultation was arranged (in person or over the phone) to discuss participation in the study and answer any questions. During that consultation, the study rationale, design, and potential outcomes were explained in detail. Any additional investigations (such as blood tests and imaging) and hospital visits (such as for follow-ups) that were not part of routine clinical care were emphasised. The voluntary nature of their participation as well as the option to withdraw at any time without affecting usual clinical care was also emphasised. The eligibility criteria were checked again in case there were mistakes in the electronic records. Opportunities to ask questions were offered and following from that consultation a ‘cool off’ period of at least 24 hours was given to them to think about it. If a patient agreed to participate, a subsequent meeting was arranged to sign the consent form which was placed in the patient’s folder and electronic patient records and a copy given to them and, if they agreed, to their GP. Baseline investigations were subsequently arranged.

Randomisation was performed in a 1:1 fashion by an un-blinded study member (in this case a research nurse), into a RIC or sham group using a randomisation software (MinimPy Version 0.3) that was downloaded onto a dedicated study laptop and with minimising factors of coronary artery disease, hypertension, and diabetes. A randomisation number was generated, and patient details were added into the study's recruitment log. A paper case report form was prepared as well as an online case report form using the REDCap™ database specific for the project to capture the data. The randomisation group was kept secret in both the paper case report forms (kept in separate folder) and the online form (not allowed access to that part) from any blinded study members, including myself.

2.3 Study Protocol

The study protocol is shown in Figure 2.1. At baseline, patients would have an echocardiogram, electrocardiogram, high sensitivity Troponin T, NT-pro-BNP and clinical blood tests as per their oncology team which usually included a full blood count, renal function and electrolytes. A cohort of patients, if they agreed, would also have a baseline rapid sequence cardiac MRI and the cardiac myosin binding protein C biomarker.

On the first day of chemotherapy, myself and an un-blinded member of the study team would meet the patient at the Macmillan Cancer Centre (or UCLH main hospital if chemotherapy was delivered as inpatient). Baseline clinical observations (heart rate, blood pressure and temperature) were recorded as well as current medications and any other significant clinical events since last seen. The intervention (RIC/Sham) was then performed by the un-blinded member of the team with 4 cycles of 5 minutes of ischaemia of the upper limb followed by 5 minutes of reperfusion lasting a total of 40 minutes prior to commencement of anthracycline chemotherapy and as close to the initiation of

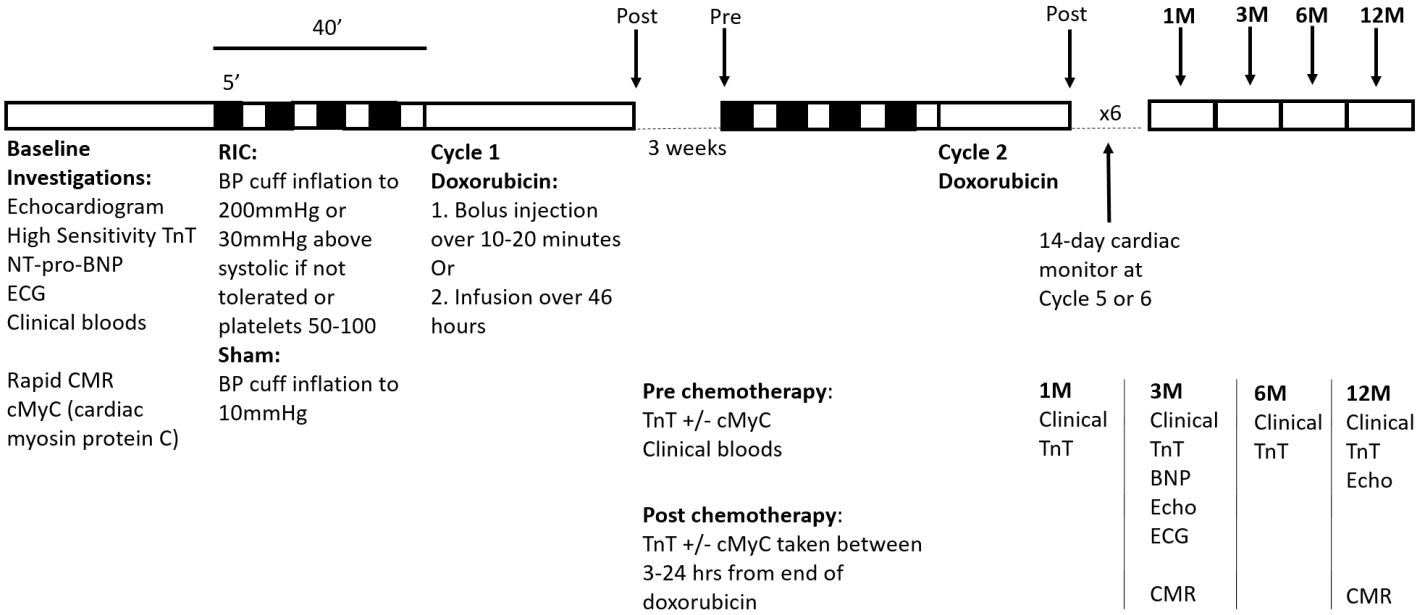
anthracycline as possible. This was usually during the same time as patients were receiving their pre-chemotherapy medications (intravenous fluids and intravenous anti-emetics). If a patient's chemotherapy regime required some chemotherapeutic agents to be given prior to anthracyclines, then the intervention was performed during the infusion of those agents but prior to anthracycline initiation, to minimise any delays (for both patient and nursing staff). After the intervention was finished, patients would have the anthracycline part of their chemotherapy either as a single bolus injection via a peripheral cannula over 10-20 minutes or as a 46-hour infusion via a peripherally inserted central venous catheter (PICC) line as per the instructions of their oncology team. Patients on a 46-hour anthracycline infusion were all sarcoma patients and as UCLH is a tertiary sarcoma centre, some patients had to travel significant distance to attend UCLH. Therefore, depending on their chemotherapy regime and local oncology care where they live, patients on an infusion would either stay at the UCLH Macmillan Centre's Ambulatory Care Unit until the infusion was finished and get disconnected at UCLH, or sent home and get disconnected at their local hospital. At the end of anthracycline chemotherapy, and ideally between 3-24 hours, a post chemotherapy blood test was performed for TnT ± cMyC. Therefore, patients who were on an infusion and were disconnected locally, did not have a post-chemotherapy sample taken.

After a break of typically 3 weeks, patients would return for the next cycle of chemotherapy. Pre-chemotherapy investigations included blood tests for TnT ± cMyC as well as routine blood tests as per usual clinical care which included full blood count, renal function, and electrolytes. Clinical observations were recorded as well as any current medications and any significant clinical adverse or other events since last seen. The protocol was then repeated as in the first cycle with post-chemotherapy blood samples taken as before. Patients would typically have a total of 6 cycles of chemotherapy. At their

penultimate or ultimate cycle, a 14-day cardiac monitor patch was attached. If a patient had less than the 6 cycles that was initially planned, then the cardiac monitor was attached on the final cycle. If there was an unexpected cessation of chemotherapy (e.g. if there was disease progression or significant side effects required chemotherapy regime to change) then the cardiac monitor was attached at that time if still within one month post chemotherapy or not attached at all.

At the end of all anthracycline chemotherapy cycles, patients would enter the follow-up stage of the study. Follow-up was performed at 4 time-points: 1, 3, 6 and 12 months post anthracycline chemotherapy. Each follow-up consisted of a clinical assessment (and recording of major clinical and adverse events) and TnT blood test (all time-points) as well as echocardiogram, NT-pro-BNP, ECG, ± CMR (3 month only) and echocardiogram ± CMR (12 month only).

Figure 2.1. Study Protocol.



2.4 Procedures

2.4.1 Intervention Protocols

Remote ischaemic conditioning was performed by applying a blood pressure cuff onto a patient's arm connected to an automated RIC machine and controlled by a specific RIC software on a study laptop that was purpose-built as previously published(277) by an un-blinded member of the study. The BP cuff was inflated to a pressure of 200mmHg (or 30mmHg above systolic if not tolerated or if platelets were between 50-100) for 5 minutes followed by 5 minutes of reperfusion for a total of 4 cycles with the whole protocol therefore lasting a total of 40 minutes as previously described(277). If platelets were <50 on the day then the RIC was omitted on that occasion. The Sham protocol was similar to the RIC protocol but the cuff was inflated to a maximum pressure of 10mmHg only(277). RIC/Sham starting times were recorded as well as doxorubicin start and end times. Any complications or malfunctions were noted.

2.4.2 Blood sampling

Blood sampling for cardiac biomarkers was performed at the pre-specified time-points with blood collected either from a peripherally inserted intravenous cannula or from a PICC line using standard aseptic non-touch techniques as per UCLH policy(278).

2.4.2.1 Cardiac Troponin T

Blood sample for high sensitivity troponin T (TnT) was collected at baseline, pre-chemotherapy at each cycle and between 3-24 hours post-chemotherapy for each cycle as well as at 1, 3, 6 and 12 month follow-up using standard SST serum bottles. Every effort was made to take the post-chemotherapy sample within the pre-specified time frame, however if patients were unwilling to wait a minimum of three hours, then the blood sampling was performed as late as possible from the end of chemotherapy. Troponin T was measured using the high sensitivity TnT assay, Elecsys, Roche with a limit of detection (LoD) of 5ng/L, a 10% precision (CV) of 14ng/L, and a total imprecision (% CV) at the 99th percentile of 8%(279).

2.4.2.2 N-terminal-pro-Brain Natriuretic Peptide

Blood samples for NT-pro-BNP were collected at baseline and 3 months post chemotherapy using standard SST serum bottles with a level of <125pg/ml in the non-acute setting and <300pg/ml in the acute setting unlikely to be related to the development of heart failure(113).

2.4.2.3 Cardiac Myosin Binding protein C (cMyC)

Blood samples for cMyC were collected at baseline, pre-chemotherapy at each cycle and between 3-24 hours post-chemotherapy for each cycle together with the TnT for the 22

patients who consented to have blood sampling for cMyC. After collection the tubes were rested for 30 minutes, centrifuged at 1000g for 10 minutes and frozen at -80^{o}C within 1 hour of collection, until assayed in a blinded fashion by a dedicated laboratory as described before(280,281). The cMyC assay (EMD Millipore on the Erenna® platform) has a LoD 0.4 ng/L and a lower limit of quantification (LoQ) (20% CV) of 1.2ng/L.

2.4.3 Imaging

2.4.3.1 Echocardiography

Echocardiography was performed at baseline, 3 months and 12 months post anthracyclines using GE machines by blinded echo-physiologists on routine clinical echocardiographic lists at UCLH using a standardised UCLH Cardio-Oncology protocol. Cardiac function and assessment of LVEF was performed using the biplane Simpson's method (of elliptical discs), by a single, experienced cardiac physiologist who was blinded to randomisation and retrospectively reviewed all echocardiograms to reduce inter-observer variability. If assessment of LVEF using the biplane method was not possible then a visual estimate of the EF was provided. The remaining echocardiographic parameters were taken from the clinical echocardiographic report.

2.4.3.2 Rapid sequence cardiac MRI (CMR)

CMR scans were performed at Chenies Mews Imaging Centre at baseline, 3 months and 1 year at a similar time (ideally on the same day if feasible) to the echocardiogram. If patients declined to have the baseline CMR they would still be eligible for the randomised controlled trial if but were not offered any subsequent follow-up CMR scans. The CMR protocol was a non-contrast study specifically to assess cardiac structure, volume and function in less than 20 minutes, using a 1.5T scanner, modified from the INCA (Peru) study protocol(139) and consisted of:

1. Localisers: Pilot 2 chamber, 5 slice short axis stack and a transverse white blood single shot fast spin echo for anatomical evaluation
2. Volume, structure and function assessment: four, two, three chamber and aortic valve segmented k-space cine acquisitions.
3. Short axis cine stack. Cine images acquired using a balanced, segmented steady – state free precession, covering the whole left ventricle. Typical image parameters were: time to echo (TE) of 1.1ms, time of repetition (TR) of 2.6ms, a flip angle of 80, Grappa factor 2, voxel size 1.8mm x 1.8mm x8mm (6mm for long axis) an x-y spatial resolution of 1 to 2mm/pixel, a slice thickness of 8-mm and 2-mm inter-slice gap.
4. Tissue mapping: One mid-segment short axis for native T1 mapping using modified look-locker inversion (MOLLI) recovery and native T2 mapping to assess for tissue characterisation
5. Real-time short-axis cine stack to assess how much extra time it adds to protocol if needed to be used in cases of poor breath-holding or poor gating (optional).

CMR analysis was done using a dedicated software (cvi42, Circle Cardiovascular Imaging, Calgary Canada). Analysis was performed by myself and checked with a supervising CMR Consultant Cardiologist. In each CMR examination, the end-diastolic phase was selected as the first phase of the acquisition. The end-systolic phase was identified by determining the phase in which the LV intra-cavity blood pool will be at its smallest by visual assessment at the midventricular level. LV endocardial and epicardial borders were manually traced in both the end-diastolic and end-systolic phases in the short-axis view.

In both end-diastole and end-systole, the most basal slice for the LV was selected when at least 50% of the LV blood pool was surrounded by myocardium. LV papillary muscles

were included as part of LV mass and volume analysis. For the myocardial maps, a single septal region of interest was manually traced. If any extra-cardiac abnormalities were seen, these were reported and checked with the patients' medical records to see if already known. If not, they were flagged up to the appropriate oncology team. Total scan time was reported with and without the optional real-time short axis stack.

2.4.4 Arrhythmia monitoring

A 12-lead ECG was performed at baseline and at 3 months post chemotherapy. Furthermore, extended cardiac monitoring was performed using an adhesive patch with a 1-lead ambulatory ECG (Zio patch, iRhythm technologies). The patch was attached onto the patients left chest wall according to the manufacturer's instructions, at the penultimate or ultimate chemotherapy cycle. The patch continuously records beat-to-beat cardiac recording for up to 14 days. Furthermore, patients can trigger a recording whenever they feel a symptom and are also asked to keep a paper log of symptoms to allow correlation with the final report. At the end of the monitoring period, the patient would return the patch to the company for analysis, which is performed using proprietary machine-learned algorithms (Zio ECG Utilization Service System (ZEUS System)), and a report generated that is reviewed by a cardiac technician(282). Patients were asked to wear the patch for 14 days or for as long as tolerated. The reports provided by the company were reviewed and interpreted by myself. The total days worn was noted, as well as the minimum, mean and maximum heart rates. Any clinically significant arrhythmias were flagged up to the oncology team and appropriate action, such as initiation of medication, taken accordingly.

2.5 Outcomes

The outcomes were separated into two main parts: 1) Outcomes for the effect of remote ischaemic conditioning, and 2) Outcomes for the multimodality monitoring of anthracycline

cardiotoxicity using TnT as marker of cardiotoxicity. Due to the very long study protocol and follow-up period, in the time allowed for my MD and prior to needing to return back to clinical training, data collection was only possible up to the 6 month follow-up time point but not the 12 month time-point. Therefore, analysis of any outcomes was performed up to the 6 month follow-up.

2.5.1 Outcomes for the Effect of remote ischaemic conditioning

2.5.1.1 Main outcome

The main outcome investigated was the change in serial high sensitivity troponin T as a marker of cardiac injury over time, during anthracycline chemotherapy and up-to 6 months follow-up post cessation of anthracycline chemotherapy. Specifically, the change in high sensitivity troponin T over time was analysed with TnT as an absolute value, change of TnT from baseline (ΔTnT) and as a binary value (Positive vs negative) and a comparison between the two groups was performed.

2.5.1.2 Secondary outcomes

2.5.1.2.1 Echocardiographic parameters

The main echocardiographic outcome assessed was the change in LVEF (ΔLVEF) from baseline to 3 months post chemotherapy. Furthermore, the change in GLS was also assessed from baseline to 3 months post chemotherapy. Specifically, the change in GLS was assessed using the ΔGLS as well as the relative percentage change in GLS from baseline to 3 months post chemotherapy. Other echocardiographic outcomes assessed included LV size parameters (LVEDd, LVEDs, IVSd, LVPWd), tissue Doppler parameters (IVS S' velocity, IVS E' velocity, Lateral S' velocity, Lateral E' velocity, E/E' ratio), Doppler parameters (E velocity, A velocity, deceleration time, E/A ratio) and RV function

parameters (TAPSE, RV S' velocity) using their change from baseline (i.e. Δ) as the outcome assessed.

2.5.1.2.2 NT-pro BNP

The biomarker NT-pro-BNP was a further secondary outcome and specifically the change in NT-pro-BNP (ΔNT-pro-BNP) from baseline to 3 months post chemotherapy.

2.5.1.2.3 Clinical outcomes

Clinical outcomes from recruitment to 6 months follow-up was a further secondary outcome. Specifically, clinical events were defined as major adverse cardiovascular and cancer events (MACCE) as follows: myocardial infarction, heart failure or asymptomatic LV dysfunction needing hospitalisation or initiation of heart failure medications, life-threatening tachyarrhythmias needing treatment or bradyarrhythmias requiring pacing, cardiac or cancer death. A composite end-point therefore was defined if patients developed myocardial infarction, or heart failure, or life-threatening tachyarrhythmias needing treatment, or bradyarrhythmias needing pacing, or cardiac or cancer death, whichever occurred first during chemotherapy or follow-up.

Furthermore, the incidence of cancer progression and serious adverse events were recorded as part of the serious adverse reporting of the study protocol. Serious adverse events were defined as any events which resulted in death, or were life-threatening, or led to hospitalisation or prolongation of hospitalisation, or led to persistent significant disability or incapacity or any other event deemed to be an important medical occurrence. Cancer progression was defined if there was disease progression for the first time since initiation of anthracycline-containing chemotherapy. If there was further disease progression

subsequently (usually in the context of second (or more) lines of chemotherapy), this was not recorded as an event.

2.5.1.2.4 Arrhythmia Incidence

The incidence of arrhythmia during chemotherapy was a further outcome. This was assessed using the cardiac monitor device attached to patients towards the end of their chemotherapy.

The arrhythmias recorded were grouped into supraventricular and ventricular. Ectopic beat (supraventricular or ventricular) frequency was separated into rare (<1% of total beats), occasional (1-5% of total beats) and frequent (>5% of total beats) and recorded for single, double or triple ectopics. Non-sustained VT was defined as more than 3 consecutive ventricular beats with a rate of more than 100bpm and duration of less than 30 seconds as per European Heart Rhythm Association(283). Non-sustained SVT was defined as more than 3 consecutive supraventricular beats with a rate of 100bpm and duration less than 30 seconds. The presence of one or more non-sustained SVT or VT was noted and the frequency of patients who had one or more episodes of SVT or VT recorded. The presence of atrial fibrillation was recorded separately, though none was seen. The presence of any higher degree (i.e. 2nd degree or more) heart block was recorded, including if transient and night-time.

2.5.2 Outcomes for monitoring of anthracycline cardiotoxicity

One of the key aspects of a cardio-oncology service is to identify patients at risk of cardiotoxicity. Assessment of risk ideally should start pre-chemotherapy and continued during and after therapy. To investigate whether there is a relationship between changes in troponin T concentrations during chemotherapy and subsequent cardiovascular

outcomes (left ventricular function, clinical events and arrhythmia incidence), data from all the patients recruited in the randomised controlled trial were analysed in a prospective observational manner. The patients were used as one cohort and any relationship between troponin and cardiovascular outcomes were analysed as one cohort but also according to their randomisation group. Furthermore, the relationship between troponin changes during chemotherapy and baseline cardiac risk factors was also investigated. The outcomes of the analysis were thus divided into two parts – identifying patients at risk prior to initiation of chemotherapy and identifying patients at risk during and after chemotherapy. Furthermore, a comparison between TnT and the cMyC biomarker was performed as well as an assessment of a rapid CMR protocol as a monitoring tool of cardiac function.

2.5.2.1 Identifying patients at risk prior to initiation of chemotherapy

International societies such as the ESC(48) and ASCO(115) recommend screening patients prior to chemotherapy and assessing baseline cardiac risk factors such as age, smoking status and presence of cardiovascular comorbidities. Identification of multiple cardiovascular risk factors leads to increased risk which, depending on the type of cancer and if other alternative therapies exist that do not affect oncological outcome, may lead to avoidance of anthracyclines(115). Retrospective studies suggest that the presence of two or more cardiovascular risk factors leads to the highest risk of subsequent development of cardiovascular disease in cancer survivor(284) but there is lack of data from prospective studies.

Cardiovascular risk scores are routinely used, particularly in primary care, to estimate the risk of developing cardiovascular disease in the future based on current risk factor profile and help guide lifestyle modification and lipid-lowering therapy. In the UK, one such score currently recommended and in use is the QRISK@3 score(285). The QRISK score, now in

its third version (QRISK@3), has been validated as a risk prediction tool to estimate the 10-year risk of cardiovascular disease, given as a single percentage risk, by incorporating a variety of different risk factors(286). Similarly, the ESC advocates use of a similar score called HeartScore, to estimate the 10-year risk of cardiovascular disease(287). The baseline risk factors that are incorporated in these scores include many (but not all) of the risk factors that are known to increase the risk of anthracycline cardiotoxicity and subsequent cardiomyopathy (namely age, smoking status, and presence of cardiovascular comorbidities such as hypertension). It has already been shown that elevations of cardiac biomarkers such as troponin early during chemotherapy and follow-up predict the development of cardiac dysfunction(141–143).

Therefore, using the patients recruited in the randomised controlled trial, I investigated whether there is any relationship between peak TnT during or after chemotherapy and baseline cardiovascular risk factors as measured by the QRISK@3 score.

The 32 patients from the randomised controlled trial were used as one cohort for this analysis. However, to ensure there are no differences between each group, the statistical analysis was repeated for each randomisation group separately.

Patients had their QRISK@3 score calculated using the online calculator https://qrisk.org. Using the pre-chemotherapy TnT values, the peak TnT was identified by looking at the TnT values at each time point from baseline up to the 6 month follow up. If more than one time-point had the same peak TnT value then the time-point that was the earliest was taken.

2.5.2.2 Identifying at risk patients during and after chemotherapy using biomarkers

2.5.2.2.1 Troponin T as a binary categorical variable

As I have described in section 2.5.2, in general the use of cardiac biomarkers, and particularly troponins, for monitoring during (and less so after) chemotherapy is encouraged, but gaps in the available evidence still exist. Specifically, unanswered questions that remain include when to perform them, which biomarkers are the most useful, how frequently to perform them, how to interpret them and what actions to be taken especially when biomarkers are elevated(48,53,94,115). In many, particularly early, publications investigating the use of troponin as a tool to detect early anthracycline cardiotoxicity and its relation to subsequent cardiac events, the analysis of troponin was performed by treating troponin as a binary categorical variable (i.e. positive vs negative)(141–145). In one of the most quoted and seminal studies on the subject with the largest number of patients to date (n = 703) by Cardinale et al(143), patients were grouped depending on whether they had a positive or negative troponin early (during chemotherapy) or late (one month after). Therefore, I performed an analysis of the troponin data by treating TnT as a binary categorical variable, and more specifically in a similar fashion to the study by Cardinale et al(143).

The 32 patients from the randomised controlled trial were used as one cohort for this analysis. However, to ensure there are no differences between each group, the analysis was repeated for each randomisation group separately. Using the pre-chemotherapy TnT values, the highest TnT was identified during chemotherapy and recorded as positive if it was ≥15ng/L (the 99th percentile upper reference limit for the presence of myocardial injury for the assay used at University College London Hospitals) or negative if <15ng/L and defined as the Early TnT (E-TnT). Similarly the highest TnT value during the follow-up period (up to the 6-month time-point) was identified and defined as the Late TnT (L-TnT).

The patients were grouped according to their E-TnT and L-TnT value and three groups were identified: E-TnT and L-TnT negative (TnT -/-), E-TnT negative and L-TnT positive (TnT -/+) and E-TnT and L-TnT positive (TnT +/+). The three TnT groups were then compared for any differences.

2.5.2.2.2 Troponin T relationship with total anthracycline dose

As described in sections 2.5, the total anthracycline dose received (in mg/m^2) is one of the strongest risk factors for the development of anthracycline cardiotoxicity and subsequent cardiomyopathy to the extent that oncological societies recommend limiting total dose to no more than 550mg/m^2 in one guideline(94) and with screening recommended when total dose reaches as low as 240mg/m^2 in some guidelines(48,53,94).

However, whether there is any relationship between total cumulative anthracycline dose received and troponin during or after chemotherapy has not been previously investigated. Therefore, I sought to investigate whether there is any relationship between peak TnT during or after chemotherapy and total anthracycline dose received.

The 32 patients from the randomised controlled trial were used as one cohort for this analysis. However, to ensure there are no differences between each group, the statistical analysis was repeated for each group separately. As before, using the pre-chemotherapy TnT values, the peak TnT was identified by looking at the TnT values at each time point from baseline up to the 6-month follow up. If more than one time-point had the same peak TnT value then the time-point that was the earliest was taken. The relationship between peak TnT and total anthracycline dose received was then assessed.

2.5.2.2.3 Cardiac Myosin Binding Protein C

Cardiac myosin binding protein C (cMyC), first described by Offer et al in 1973(288), is a 140 kilodalton protein that resides on the thick filaments of heart muscle(289). In its phosphorylated state it enhances diastolic function(290) and cardiac inotropy(291) and protects the heart during ischaemic-reperfusion injury(292). The protein is encoded by the MYPBC3 gene located on chromosome 11p11.2(293). Mutations in this gene have been associated with Hypertrophic Cardiomyopathy (HCM) and are now recognised to be one of the most common mutations accounting for about 40-50% of all HCM gene mutations(294).

The myosin binding protein family has 3 isoforms; a slow skeletal, a fast skeletal and a cardiac one which is exclusively expressed in the heart(295) and found in abundance in the heart(296). Importantly, it is increasingly recognised that cMyC is released into the circulation following a myocardial insult, typically following myocardial infarction, in both animals(297) and humans(297,298). As such, it is being explored as a potential new biomarker of myocardial injury. In its favour as a biomarker is, to some extent, its release kinetics following myocardial injury. In a porcine MI model, where a branch of the left anterior descending artery is ligated to mimic MI, cMyC is detected at 30 minutes and 3 hours post ligation, peaks at 6 hours and returns to baseline by 12 hours. TnI and TnT are also detected at 3 hours and peak at 6 hours, but only cMyC is statistically significantly elevated at 3 hours(299). In STEMI patients, cMyC and its smaller but easier to detect 40kDa fragment, peak significantly earlier compared to TnT(300). However, time of onset of injury in STEMI can be difficult to ascertain, so release kinetics of cMyC and troponin have been investigated during iatrogenic MI for HCM alcohol ablation procedures. During alcohol ablation, cMyC accumulates 6 times faster than TnT (slope 25.8 ± 1.9 vs 4.0 ± 0.4 ng/L/min)(300) as well as peaking at 4hrs compared to 6 hrs for TnT(299). Furthermore, the

clearance of cMyC is much faster than TnT with a decay half-time of 5.5+/-0.8hrs vs 22+/-5hrs, p<0.0001(300).

With the development of a high sensitivity assay(280) for cMyC, it has been possible to directly compare its diagnostic performance against high sensitivity TnI and TnT. In 1,954 patients presenting with symptoms suggestive of acute MI, the diagnostic accuracy of cMyC for acute myocardial infarction was similar to high sensitivity troponin I and T. It was however superior to high sensitivity troponin T in early presenters (<3 hours) ((AUC cMyC vs. hs-cTnT 0.915 (0.887–0.941) vs. 0.892 (0.857–0.922, p=0.022))(281). The authors attribute this to cMyC's abundancy in the myocardium, its location on the sarcomere and its loose association with myosin and actin. Equally importantly, cMyC correctly triaged more patients into rule-in or rule-out groups compared to either high sensitivity Troponin I and T thus leaving a smaller number of patients in the observation group (Net Reclassification Improvement +0.149 versus TnT, +0.235 versus TnI (*P*<0.001)(281)), thus ultimately making it potentially a better biomarker for the triage of patients in emergency departments.

In addition to being a marker of acute myocardial injury following myocardial infarction, cMyC has been investigated in aortic stenosis, where it is strongly associated with myocardial hypertrophy and fibrosis as assessed with cardiac MRI and worsening mortality(301). As well as aortic stenosis, the value of cMyC as a diagnostic and prognostic biomarker has been assessed in paediatric heart failure patients, with significantly increased levels of cMyC on admission in children with HF compared to controls(302).

To my knowledge, cMyC has not been studied in patients receiving anthracyclines. Therefore, I wanted to compare its performance against cardiac high sensitivity TnT in

patients receiving anthracycline chemotherapy. I hypothesise that, cMyC will be detected earlier compared to troponin following anthracycline chemotherapy, similar to its performance after acute coronary syndrome. Furthermore, a bigger rise compared to troponin was anticipated in view of its abundance in the myocardium. Thus, I wanted to test the hypothesis whether there is a difference between peak TnT and peak cMyC concentrations during anthracycline chemotherapy. Furthermore, to assess how cMyC compares with TnT at each cycle, I also tested whether there is a difference in the cMyC and TnT concentrations at each cycle.

Twenty-two patients from the randomised controlled trial consented to have additional blood sampling for cMyC. To assess how cMyC performs as a biomarker during chemotherapy compared to TnT, using the pre-chemotherapy samples, the peak concentration of cMyC and TnT samples were identified and the ratio of peak to baseline concentration for each biomarker was calculated and compared. Furthermore, to assess how cMyC compares to TnT at each chemotherapy cycle, the ratio of cMyC and TnT concentration to baseline was calculated for each chemotherapy cycle and the two biomarkers compared. In addition, the effect of RIC on cMyC levels was also compared, in a similar fashion to the TnT comparison described in 2.5.1.1, for absolute cMyC concentration levels as well as for the change of cMyC concentration from baseline at each cycle (ΔcMyC).

2.5.2.3 Identifying at risk patients during and after chemotherapy using imaging

2.5.2.3.1 Echocardiography

In recent guidelines an absolute drop in LVEF of 10% points from baseline and to a level below the lower limit of normal, and a relative drop in GLS of 15% from baseline are often proposed for a diagnosis of cardiotoxicity(48). I sought to assess the relationship between

cardiac biomarkers and cardiac function as assessed according to latest guidelines. The 32 patients recruited in the randomised trial were used as one cohort to assess if there is any relationship between TnT and echocardiographic parameters of LV function. However, to ensure there are no differences between each group, the statistical analysis was repeated for each group separately. The absolute change in LVEF and the percentage change in GLS was calculated for those that a baseline and 3-month post-chemotherapy LVEF and GLS was available. The peak TnT during and up to the 3-month follow-up for each individual patient was identified and recorded. The relationship between peak TnT, LVEF absolute percentage change and GLS relative percentage change was then assessed.

2.5.2.3.2 Cardiac Magnetic Resonance Imaging (CMR)

The use of CMR to assess cardiac size and function is now considered gold standard as it allows volumetric analysis of chamber size without any assumptions of geometrical shape(53). Cardiac function assessment in cancer patients is typically assessed with echocardiography and with newer techniques like 3D echocardiographic volumetric assessment, temporal and observer variability has decreased(303) with good correlation to CMR in patients with normal LV function as well as with DCM(304). However, even with 3D volumetric methods, assessment is limited if poor endocardial definition is present(305). This is particularly true for cancer patients especially if they have received surgical and/or radiotherapy treatment (e.g. mastectomy and implants for breast cancer)(306). In fact, in a retrospective analysis of CMR requests in cardio-oncology patients assessing the utility of CMR in cardio-oncology in a tertiary centre (Bart's Heart Centre) of which I was the main author and the results presented as an abstract in the Global Cardio-Oncology Summit 2019, the most common request for a CMR scan (67/199, 34%) was for assessment of LV function due to a non-diagnostic echocardiogram study(307).

However, CMR scanning can be slow, with scanning times of up-to 45 minutes for certain sequences, thus making it less suitable as a routine imaging investigation especially in cancer patients who have frequent medical appointments. Attempts to reduce scanning time have been made in order to make cardiac MRI more accessible particularly for developing countries(138,139). The TIC-TOC study investigated whether ultrafast CMR mapping without contrast could be used to assess iron overload in thalassaemia patients in Thailand(138). Scans, which included localised and pilot studies, myocardial and liver T2* and T1 mapping and 2 and 4 chamber cines, averaged 8.3 ± 2.4 minutes with analysis time of less than 1 minute. Cost was reduced 4-fold. Similarly the INCA study, assessed whether rapid contrast-enhanced cardiac MRI is feasible for wider cardiac indications in Peru, in an attempt to improve access to cardiac MRI for developing countries(139). The protocol was originally developed in the UK and included assessment of cardiac volumes and scar. One hundred patients were referred for a variety of indications, 98 of which underwent scanning. Mean scan time was 18 ± 7 minutes and findings impacted management in 56% of patients and overall rapid CMR scanning was feasible in this developing country.

Therefore, to investigate if rapid sequence CMR scanning can be used as a monitoring tool before and after chemotherapy, a pilot study was set up to see if CMR scanning with a rapid sequence protocol is feasible.

For the assessment of scan duration, 2 baseline CMR scans of patients that were later withdrawn (1 voluntary, 1 due to COVID) were included. Similarly, 4 patients who had a 12 month scan by the time of analysis were also included in the assessment of CMR duration. The whole scan cohort of 34 scans was also used to compare assessment of LVEF between CMR and echocardiography but not for any comparisons of LVEF at specific

timepoints. Comparison of LV function using LVEF between CMR and echocardiography at baseline and 3 months was performed as well as a comparison of LVEF from the whole scan cohort between the two imaging modalities.

2.5.2.4 Arrhythmia monitoring

Little is known about the presence and incidence of arrhythmias during or soon after anthracycline chemotherapy. In the early reports of anthracycline cardiotoxicity ECG abnormalities were known to occur(9,44,87) as noted on ad hoc 12-lead ECGs. Available studies specifically reporting on arrhythmogenicity during anthracycline chemotherapy are inconsistent in their methodology(88,308,309). Furthermore, the definition of an arrhythmia also varies significantly with some studies using grading systems(88,309) whereas others reporting all electrical abnormalities(308).

Therefore, in an attempt to characterise this better, the patients who were recruited in the randomised controlled trial, had a cardiac monitor attached on them for a maximum of 14 days with the start day being the start of their penultimate or ultimate chemotherapy cycle. The data from the cardiac monitoring devices were used to characterise the presence of arrhythmias during chemotherapy with anthracyclines and to analyse the hypothesis whether there is any relationship between peak TnT during chemotherapy and presence of arrhythmias. The presence or absence of any arrhythmias as previously defined was then analysed for any relationship with peak TnT during chemotherapy.

2.6 Statistical analysis

All statistical analysis and graphical representation were performed with the SPSS statistical software (IBM®, SPSS®, Statistics, Version 26). Statistical significance was considered at the 5% significance level.

2.6.1 Effect of remote ischaemic conditioning

2.6.1.1 Baseline characteristics

Summary statistics data for baseline characteristics were described as mean ± standard deviation (SD) and median ± interquartile range (IQR) for continuous variables with a parametric and non-parametric distribution respectively and as absolute numbers and percentages for categorical variables. Data are presented in boxplots, line graphs and scatter graphs as well as table format as appropriate. Between group comparison of baseline patient characteristics, cancer and chemotherapy details, intervention details, echocardiographic data, blood tests and clinical observations was done using the independent samples T test for continuous data following a normal distribution, the Mann-Whtiney test for continuous data not following a normal distribution and the chi square test for categorical data.

2.6.1.2 Comparison of pre- and post-chemotherapy Troponin T (TnT) values

Pre- and post-chemotherapy TnT are presented as median ± interquartile range (IQR) as they follow a non-parametric distribution for all patients and for each group. Statistical analysis of TnT at each cycle compared to baseline was done for all patients and for each group using the paired T test as the mean difference from baseline follows a parametric distribution. Pre- and post-chemotherapy TnT comparison for all patients and for each group was performed using random effects regression (mixed effects regression) of

repeated measures with increasing chemotherapy cycles added as a fixed effect to the model.

2.6.1.3 Effect of Remote Ischaemic Conditioning (RIC) on Troponin T levels during chemotherapy

The effect of Remote Ischaemic Conditioning (RIC) on Troponin T values during anthracycline chemotherapy was analysed by comparing the intervention and sham groups using their pre-chemotherapy absolute TnT values per cycle. TnT data are presented as median and IQR as they generally follow a non-parametric distribution. Comparison between the two groups was performed with a random effects regression (mixed effects regression) for repeated measures. A Mann-Whitney test comparing the troponins at each cycle between the two groups was also performed.

The effect of RIC on TnT during anthracycline chemotherapy was also analysed using the change of TnT from baseline (ΔTnT) and compared between the two groups. ΔTnT values are presented as mean and standard deviation as they generally follow a normal distribution. Comparison between the two groups was performed with a random effects regression (mixed effects regression) for repeated measures. An independent T test comparing the troponins at each cycle between the two groups was also performed.

The effect of RIC on Troponin T trends during anthracycline chemotherapy was further analysed by treating TnT as a binary categorical variable (i.e., positive vs negative). According to the troponin assay used by University College London Hospitals (UCLH) (high sensitivity Troponin T, Roche@) the 99th percentile upper reference limit for the presence of myocardial injury as defined in the 4th Universal Definition of Myocardial Infarction(310) is 14ng/L(311). Thus a troponin positive value was defined if ≥15ng/L. TnT

data are presented as absolute numbers and percentages. Comparison between the two groups was performed with the chi-squared test at each cycle.

The effect of RIC on absolute TnT and ΔTnT according to cumulative dose received during chemotherapy was analysed and the two groups compared using random effects regression.

2.6.1.4 Effect of Remote Ischaemic Conditioning (RIC) on Troponin T levels during chemotherapy and follow-up

The same statistical analysis for the effect of RIC on Troponin T as described in section 2.6.1.3 was then repeated but this time the follow-up period (up to the 6 month time-point) was also included.

2.6.1.5 The Effect of Remote Ischaemic Conditioning (RIC) on echocardiographic parameters after anthracycline chemotherapy

The effect of RIC on echocardiographic parameters was assessed for all patients and for each group. Data are presented as mean ± standard deviation for variables following a normal distribution, median and IQR for non-parametric continuous variables and absolute numbers and percentages for categorical variables. For all patients and for each group, comparisons of how echocardiographic parameters change before and after chemotherapy were performed using a paired T test. The two groups were compared by looking at the change from baseline (i.e. Δ) for each parameter using an independent samples T test. In addition, for GLS, the relative percentage change was calculated and compared between the two groups using an independent samples T test.

2.6.1.6 The Effect of Remote Ischaemic Conditioning (RIC) on NT-pro-BNP after anthracycline chemotherapy

The effect of RIC on NT-pro-BNP was assessed for all patients and for each group individually. Data are presented as median and IQR as they follow a non-parametric distribution. For all patients and for each group, comparisons of how NT-pro-BNP changes before and after chemotherapy was made using a Wilcoxon signed rank test. The two groups were compared by looking at the change from baseline (i.e. Δ) using a Mann-Whitney test.

2.6.1.7 The Effect of Remote Ischaemic Conditioning (RIC) on Clinical Events

The time to a MACCE event and time to cancer progression was analysed and compared for each group. Data are presented as absolute numbers and percentages and as time-to-event Kaplan-Meier plots and compared using the log-rank test and Cox regression as an estimate of the hazard ratios. Kaplan-Meier plots and Cox regression analysis was performed for the composite MACCE, as well as the individual end-points of cardiovascular events, cancer deaths and cancer progression. Clinical adverse events are presented as absolute numbers and percentages and comparison between the two groups was done using the chi-squared test.

2.6.1.8 The Effect of Remote Ischaemic Conditioning (RIC) on the incidence of arrhythmias during anthracycline chemotherapy

The incidence of pre-defined arrhythmias was recorded for all patients and for each group and data are presented as absolute numbers and percentages for categorical variables and as mean and standard deviation for continuous variables. Comparisons were made using the chi-squared test and independent samples T test.

2.6.2 Monitoring for anthracycline cardiotoxicity

2.6.2.1 Identifying patients at risk prior to initiation of chemotherapy

Peak TnT and QRISK@3 data are presented as median ± IQR for continuous data not following a normal distribution and as absolute numbers and percentages for categorical data. The relationship between peak TnT and QRISK@3 score is presented with scatter and dot diagrams for all patients. Assessment of correlation was performed using Spearman's rank correlation coefficient (ρ). The relationship between peak TnT and QRISK@3 was further analysed with linear regression. Comparison of QRISK@3 scores and peak TnT between the two randomisation groups was done using the Mann-Whitney test as both variables do not follow a normal distribution. The chi-squared test was used to compare the frequency at each time-point where TnT was peak between the two groups.

2.6.2.2 Identifying at risk patients during and after chemotherapy using biomarkers

2.6.2.2.1 Troponin T as a binary categorical variable

Troponin T data are presented as mean ± standard deviation for continuous variables and as absolute numbers and percentages for categorical variables. For each TnT group (TnT -/-, TnT -/+, TnT +/+) within group early and late TnTs were compared with the paired T test as their mean difference follows a normal distribution. Simple linear regression was used to compare for any differences in early and late TnT between the different TnT groups. This analysis was performed for all patients as one cohort and then repeated for each randomisation group.

2.6.2.2.2 Troponin T relationship with total anthracycline dose

Peak TnT and total anthracycline dose data are presented as mean ± standard deviation for continuous variables following a normal distribution and median ± IQR for continuous data not following a normal distribution. The relationship between peak TnT and total

anthracycline dose is presented with scatter and dot diagram for all patients. Assessment of correlation was performed using Pearson's correlation coefficient (r). The relationship between peak TnT and total anthracycline dose was further analysed with linear regression. Comparison of total anthracycline dose between the two groups was done using the independent samples T test. Comparison of peak TnT between the two groups using the Mann-Whitney test.

2.6.2.2.3 Cardiac Myosin Binding Protein C

TnT and cMyC data are presented as mean ± standard deviation for continuous categorical data following a normal distribution and as median ± interquartile range for continuous data not following a normal distribution. Categorical data are presented as absolute numbers and percentages.

Comparison of pre- and post-chemotherapy cMyC and TnT samples with their respective baseline samples was performed using the paired T test as the mean difference from baseline followed, in general, a normal distribution. Comparison between pre- and post-chemotherapy samples was performed with random effects regression of repeated measures.

To assess how cMyC performs as a biomarker during chemotherapy compared to TnT, using the pre-chemotherapy samples, the peak concentration cMyC and TnT values were identified and the ratio of peak to baseline concentration was calculated. The peak to baseline concentration ratio of cMyC versus TnT was compared using a Wilcoxon signed rank test for all patients and for each group. Between group comparisons for peak cMyC and TnT concentrations and peak:baseline ratios were performed with an independent samples T test. A chi square test was used to compare the time-points at which the peak

biomarker concentration was detected between the two groups. Furthermore, to assess how cMyC compares to TnT at each chemotherapy cycle, the ratio of cMyC and TnT concentrations to baseline was calculated for each chemotherapy cycle and the two biomarkers compared using a Wilcoxon signed rank test at each cycle. To assess the effect of RIC on cMyC levels, the two randomisation groups were compared using random effects regression for repeated measures. An independent samples T test at each chemotherapy cycle for absolute cMyC concentration levels as well as for the change of cMyC concentration from baseline at each cycle (ΔcMyC) was also performed.

2.6.2.3 Identifying at risk patients during and after chemotherapy using imaging

2.6.2.3.1 Echocardiography

The relationship between peak TnT, LVEF absolute percentage change and GLS relative percentage change was assessed with scatter plots and correlation analysis. Wherever a significant correlation was observed, linear regression analysis was also performed. Data are presented as mean ± standard deviation if following a normal distribution and median ± interquartile range if not. Between group comparisons was performed with an independent T test for LVEF and GLS and a Mann Whitney test for peak TnT

2.6.2.3.2 Cardiac Magnetic Resonance Imaging (CMR)

CMR and corresponding echocardiographic data are presented as mean ± standard deviation for any continuous data following a normal distribution and as median ± interquartile range for any continous data not following a normal distribution. Categorical data are presented as absolute numbers and percentages. CMR parameters at baseline and 3 months were compared with a paired T test. Comparison of LV function using LVEF between CMR and echocardiography at baseline and 3 months was done using a paired T

test and similarly comparison of LVEF from the whole scan cohort between the two imaging modalities was done again with the paired T test.

2.6.2.4 Arrhythmia monitoring

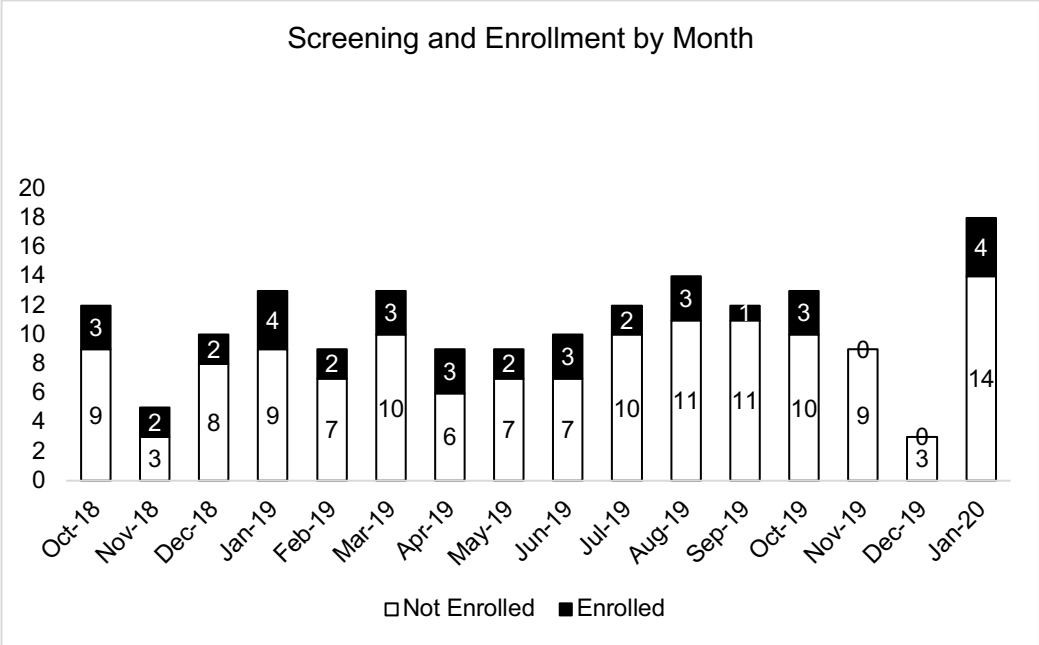
Peak TnT and arrhythmia data are presented as mean ± standard deviation for continuous data with a normal distribution, median ± interquartile range for continuous data without normal distribution and as absolute numbers and percentages for categorical data. Comparison of peak TnT levels in arrhythmia present or absent groups was done with the independent samples T test. The analysis was repeated for each individual randomisation group.

Chapter 3 Effect of Remote Ischaemic Conditioning as a Cardioprotective strategy against anthracycline cardiotoxicity

3.1 Screening and Recruitment

From October 2018 until January 2020, I screened a total of 134 patients (82 sarcoma, 48 lymphoma and 4 breast cancer). The number of patients screened and enrolled by month is shown in Figure 3.1.

Figure 3.1



The CONSORT(312–315) enrolment flow diagram is shown in Figure 3.2. Of the 134 patients screened, 97 were excluded of which 23 declined, 38 were ineligible, and 36 had other reasons for exclusion including being advised against approaching by a member of the oncology team (8) and not enough time between being referred by primary team and starting chemotherapy to perform baseline investigations (28). The latter was particularly true for lymphoma patients who would often be started with chemotherapy promptly due to clinical urgency (35% of all lymphoma referrals). Thirty seven patients were randomised to Group 1 (RIC) (n = 19) and Group 2 (Sham) (n = 18), minimising for coronary artery

disease (CAD), hypertension and diabetes. One patient from Group 1(RIC) and 2 patients from Group 2 (Sham) withdrew after randomisation but prior to starting chemotherapy. After the first cycle of chemotherapy and intervention, 2 patients from Group 1 (RIC) were withdrawn. One withdrew voluntarily for personal reasons. The second was withdrawn by the study team due to the COVID-19 pandemic as per UCL regulations during the pandemic. Non-COVID related clinical research needed to be interrupted, particularly patients at high risk of COVID in view of ongoing active chemotherapy. Both patients were excluded from the final analysis due to incomplete data. One patient from Group 2 (Sham) voluntarily withdrew at the 3 month follow-up for personal reasons but was included in the final analysis as intention-to-treat having a complete data set up to that point. Therefore, a total of 16 patients in each group were included in the final analysis.

3.2 Patient characteristics

3.2.1 Baseline characteristics

Baseline characteristics for all patients and for each group are shown in Table 3.1. Statistical analysis was done as described in section 2.6.1.1.

Mean age was 52 ± 16 with 44% females. Twenty-four (75%) patients had sarcoma, 6 (19%) lymphoma and 2 (6%) breast cancer. Thirteen (41%) patients had metastatic disease. For the majority (81%) this was a new cancer diagnosis, though for 19% this was a relapse. The most common anthracycline used was doxorubicin (94%) alone (19%) or in combination with other chemotherapy whilst the two breast cancer patients received an epirubicin-containing regime. Their Eastern Cooperative Oncology Group (ECOG) World Health Organisation (WHO) Performance status at baseline was either 0 (56%) or 1 (44%). In terms of cardiovascular comorbidities, 4 patients had hypertension (one of which also had high cholesterol) and a further 3 had high cholesterol only. Of the 32 patients, 28%

were current smokers, 22% ex-smokers and 50% non-smokers. The majority had no family history of premature ischaemic heart disease. One patient was on a beta-blocker at baseline (propranolol for migraine), 2 patients were on an ACE-I and CCB combination for hypertension, 1 patient was on an ARB and thiazide combination for hypertension and one patient was on a CCB only for hypertension. Three patients were on statins for hypercholesterolaemia (of which one patient also had treatment for hypertension). Other non-cardiac medications at baseline included protein pump inhibitors (6), prescription only pain killers (7), steroid inhalers for asthma/COPD (4) and oral steroids (1). The two groups were similar in their baseline patient characteristics.

Most patients (19) received 6 cycles of chemotherapy, 4 patients received 4 cycles, 8 received 3 cycles and 1 patient received 2 cycles. The mean cumulative anthracycline dose received was 312.4±97 mg/m^2 (median 300mg/m^2, range 150-450mg/m^2). Twenty (63%) patients received their anthracycline via a slow bolus injection through a peripheral cannula over 10-20 minutes, and 11 patients (34%) received it via a 46-hour infusion through a PICC line. One patient received two doses via an infusion and two doses via a bolus injection. Three patients (2 from Group 2 (Sham) and 1 from Group 1 (RIC)) who received mainly bolus injections and one patient (Group 2 (Sham)) who received mainly 46-hour infusion, had one dose administered via infusion or bolus injection respectively. The two groups were similar in their baseline chemotherapy characteristics.

Figure 3.2. CONSORT Diagram of screening and enrolment.

CONSORT

Enrollment

Assessed for eligibility (n=134)

Excluded (n=97)
- Not meeting inclusion criteria (n=38)
- Declined to participate (n=23)
- Other reasons (n=36)

Randomized (n=37)

Allocation

Allocated to Group 1 (RIC) (n=19)
- Received allocated intervention (n=18)
- Did not receive allocated intervention (n=1)
  - Withdrew after randomisation

Allocated to Group 2 (Sham) (n=18)
- Received allocated intervention (n=16)
- Did not receive allocated intervention (n=2)
  - Withdrew after randomisation

Follow-Up

Lost to follow-up (n=0)
Discontinued intervention (n=2)
- 1 Withdrew after cycle 1
- 1 Withdrawn by study team after cycle 1 due to COVID risk

Lost to follow-up (n=0)
Discontinued intervention (n=1)
- 1 Withdrew at 3 month follow-up

Analysis

Analysed (n=16)
- Excluded from analysis (n=2)
  - 2 patients no follow up data as withdrawn after cycle 1

Analysed (n=16)
- Excluded from analysis (n=0)

Table 3.1. Baseline Characteristics.			
	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
Patient Baseline Details			
Age (mean ± S.D.)	52 ± 16	51 ± 15	53 ± 17
Gender: **Male** **Female**	 18 (56%) 14 (44%)	 11 (69%) 5 (31%)	 7 (44%) 9 (56%)
Medical Comorbidities **Hypertension** **High cholesterol** **Other**	 4 (13%) 4 (13%) 10 (31%)	 2 (13%) 2 (13%) 4 (25%)	 2 (13%) 2 (13%) 6 (38%)
Smoking Status **Current** **Ex** **Non**	 9 (28%) 7 (22%) 16 (50%)	 6 (38%) 1 (6%) 9 (56%)	 3 (19%) 6 (38%) 7 (44%)
Family History of Ischaemic Heart Disease **Yes** **No** **Unknown**	 3 (9%) 26 (81%) 3 (9)	 2 (13%) 13 (81%) 1 (6%)	 1 (7%) 13 (81%) 2 (6%)
Baseline Medications **Beta Blockers** **ACE-Inhibitors** **ARBs** **CCB** **Thiazides** **Statins** **PPI** **Prescription pain killers** **Steroid inhalers** **Steroids** **Other**	 1 (3%) 2 (6%) 1 (3%) 3 (9%) 1 (3%) 3 (9%) 6 (19%) 7 (22%) 4 (13%) 1 (3%) 15 (47%)	 0 2 (13%) 0 2 (13%) 0 1 (6%) 3 (19%) 5 (31%) 1 (6%) 0 6 (38%)	 1 (6%) 0 1 (6%) 1 (6%) 1 (6%) 2 (13%) 3 (19%) 2 (13%) 3 (19%) 1 (6%) 9 (56%)
Cancer and chemotherapy baseline details			
Cancer Type: **Sarcoma** **Breast** **Lymphoma**	 24 (75%) 2 (6%) 6 (19%)	 13 (81%) 0 3 (19%)	 11 (69%) 2 (13%) 3 (19%)
Metastatic: **Yes** **No**	 13 (41%) 19 (59%)	 7 (44%) 9 (56%)	 6 (38%) 10 (62%)
Cancer Diagnosis Type **New** **Relapse**	 26 (81%) 6 (19%)	 12 (75%) 4 (25%)	 14 (88%) 2 (12%)
Anthracycline Type			

Doxorubicin	30 (94%)	16 (100%)	14 (88%)
Epirubicin	2 (6%)	0	2 (12%)
Chemotherapy Regime			
Dox	6 (19%)	4 (25%)	2 (13%)
D-Ifos	7 (22%)	5 (31%)	2 (13%)
FEC PC	1 (3%)	0	1 (100%)
D-Cis	5 (16%)	2 (13%)	3 (19%)
D-Ola	2 (6%)	0	2 (13%)
RCHOP	2 (6%)	1 (6%)	1 (6%)
MAP	2 (6%)	1 (6%)	1 (6%)
VI-Dox	1 (3%)	0	1 (6%)
FEC DT	1 (3%)	0	1 (6%)
CHOEP	1 (3%)	1 (6%)	0
CHOP	1 (3%)	1 (6%)	0
IVA-Dox	1 (3%)	1 (6%)	0
RCHOP-Mtx	2 (6%)	0	2 (13%)
ECOG WHO Performance Status			
0	18 (56%)	8 (50%)	10 (63%)
1	14 (44%)	8 (50%)	6 (37%)
Total Chemotherapy Cycles Received			
2	1 (3%)	1 (6%)	0
3	8 (25%)	4 (25%)	4 (25%)
4	4 (13%)	2 (13%)	2 (13%)
6	19 (59%)	9 (56%)	10 (63%)
Total Cumulative anthracycline dose received*			
Mean ± S.D (mg/m^2)	312.4 ± 99	307.9 ± 100	316.9 ± 97
Method of Administration			
Slow Bolus±	20 (63%)	8 (50%)	12 (75%)
46 Hr Infusion§	11 (34%)	7 (44%)	4 (25%)
Both	1 (3%)	1 (6%)	0
Intervention Details			
RIC/Sham full protocol received (i.e. 4 RIC/sham inflations/deflations prior to each chemotherapy cycle)			
Yes+	30 (94%)	15 (94%)	15 (94%)
No	2 (6%)	1 (6%)	1 (6%)
Total number of RIC/Sham	155	76	79

Cycles (1 cycle = 4 inflations/deflations) performed			
Time difference from RIC/Sham to Doxorubicin (Median) (minutes)	85 (n = 152)	76 (n = 74)	106 (n = 78)
*** For patients receiving epirubicin the equivalent doxorubicin dose was calculated by multiplying by 0.67 as per Zamorano et al (48).** **†Full protocol received but some deviation from protocol – see text.** **‡Three patients received one dose as an infusion – see text** **§One patient received one dose as a bolus – see text**			

Thirty patients received the full RIC/Sham protocol – i.e. 4 cycles of RIC/sham prior to each chemotherapy cycle. One patient in Group 1 (RIC) received 3 full RIC cycles but did not receive the last RIC cycle due to COVID-19 pandemic. One patient from Group 2 (Sham) received 5 full Sham cycles but did not receive the intervention during the 3rd chemotherapy cycle due to recent surgery in one arm that prohibited application of the cuff on that arm and the presence of a Peripherally Inserted Central Catheter (PICC) in the other arm. Therefore, a total of 155 successful RIC/Sham cycles were completed (1 cycle = 4 inflations/deflations of BP cuff) with 76 in Group 1 (RIC) and 79 in Group 2 (Sham). Four patients (three from Group 1 (RIC) and one from Group 2 (Sham)) had the doxorubicin infusion started prematurely prior to the intervention finishing during one of their chemotherapy cycles. Two patients (one from each group) had a transient device failure which resulted in temporary halt of the protocol in one of their chemotherapy cycles. The median time difference from intervention to starting of doxorubicin was 85 minutes and was similar in the two groups (76 minutes in Group 1 (RIC) and 106 minutes in Group 2 (Sham), p = 0.068).

3.2.2 Baseline investigations

3.2.2.1 Baseline Echocardiogram

Baseline echocardiographic data for all patients and for each group are show in Table 3.2. Normal ranges were according to British Society of Echocardiography guidelines(316,317). Statistical analysis was done as described in 2.6.1.1. Left ventricular size and function was normal at baseline with a mean LVEF of 61% and mean GLS of -19%. Right ventricular size and function was also normal as assessed visually and with a mean TAPSE of 2.2cm and RV S' velocity of 13cm/s. Mean LV Doppler and Tissue Doppler imaging (TDI) parameters at baseline were within normal range. The two groups were similar in their baseline echocardiographic parameters. There was a small difference of 2.3% in the baseline LVEF between the two Groups (Group 1 (RIC) LVEF 62%, Group 2 (Sham) LVEF 59%) which was not statistically significant (p<0.079, 95% CI -0.28-4.8.

Table 3.2. Baseline Echocardiogram

	All patients N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
LV Size Parameters			
LVEDd (cm) (Mean ± S.D)	4.5 ± 0.4	4.5 ± 0.5	4.6 ± 0.4
LVEDs (cm) (Mean ± S.D)	3.1 ± 0.3 (n = 30)	3 ± 0.3 (n = 15)	3.1 ± 0.3 (n = 15)
IVSd (cm) (Mean ± S.D)	0.94 ± 0.14	0.9 ± 0.2	0.9 ± 0.1
LVPWd (cm) (Mean ± S.D)	0.88 ± 0.16	0.9 ± 0.2	0.9 ± 0.2
LV function parameters			
LVEF (%) (Mean ± S.D)	61 ± 4	62 ± 4	59 ± 3
LVEF method Visual Biplane	10 (31%) 22 (69%)	5 (31%) 11 (69%)	5 (31%) 11(69%)
GLS (%) (Mean ± S.D)	-19 ± 2 (n = 28)	-19.2 ± 2 (n = 15)	-18.7 ± 3.1 (n = 13)
Tissue Doppler parameters			
IVS S' (cm/s) (Mean ± S.D)	9 ± 2	9 ± 2	9 ± 2
IVS E' (cm/s) (Mean ± S.D)	10 ± 4 (n = 31)	10 ± 4 (n = 15)	9 ± 3 (n = 16)

Lateral S' (cm/s) (Mean ± S.D)	11 ± 2	11 ± 3	11 ± 2
Lateral E' (cm/s) (Mean ± S.D)	12 ± 4 (n = 30)	12 ± 5 (n = 15)	12 ± 4 (n = 15)
E/E' (Mean ± S.D)	0.7 ± 0.2 (n = 30)	0.7 ± 0.2 (n = 15)	0.7 ± 0.3 (n = 15)
Doppler parameters			
E (cm/s) (Mean ± S.D)	0.70 ± 0.16 (n = 31)	0.72 ± 0.17 (n = 15)	0.67 ± 0.15 (n = 16)
A (cm/s) (Mean ± S.D)	0.66 ± 0.15 (n = 31)	0.65 ± 0.15 (n = 15)	0.68 ± 0.16 (n = 16)
Deceleration Time (ms) (Mean ± S.D)	206 ± 52 (n = 28)	198 ± 51 (n = 14)	213 ± 53 (n = 14)
E/A (Mean ± S.D)	1.1 ± 0.38 (n = 31)	1.1± 0.44 (n = 15)	1 ± 0.29 (n = 16)
RV function parameters			
RV S' (cm/s) (Mean ± S.D)	13 ± 2 (n = 27)	13 ± 2 (n = 12)	14 ± 2 (n =15)
RV TAPSE (cm) (Mean ± S.D)	2.2 ± 0.3 (n = 31)	2.3 ± 0.3 (n = 16)	2.2 ± 0.3 (n = 15)

3.2.2.2 Baseline Blood Tests

Baseline blood tests for all patients and for each group are shown in Table 3.3. Statistical analysis was done as described in 2.6.1.1. There were no significant differences between baseline haematological and biochemical markers and cardiac biomarkers between the two groups. Baseline median Troponin T (TnT) was 7ng/L (mean 8ng/L) and NT-pro-BNP was 68ng/L (mean 116ng/L). In one patient in group 1 (RIC), the baseline troponin haemolysed and therefore the post cycle 1 chemotherapy troponin was used as baseline. The value of that was <3ng/L therefore did not rise after one doxorubicin administration. In one patient again in group 1 (RIC), the baseline troponin haemolysed and there was no other sample to use for baseline for that patient.

Table 3.3. Baseline Blood Tests			
	All patients N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
Haematology			
Haemoglobin (g/L) (Mean ± S.D) (Normal range: Men: 130 – 170 Women: 115 - 155)	130 ± 18	132 ± 20	129 ± 15
Platelets ($x10^9$/L) (Mean ± S.D) (Normal range: 150 – 400)	264 ± 80	264 ± 80	264 ± 82
White Cell Count ($x10^9$/L) (Mean ± S.D) (Normal range: 3.0 - 10.0)	7.14 ± 2.14	7 ± 2.54	7.29 ± 3.38
Neutrophils ($x10^9$/L) (Mean ± S.D) (Normal range: 2.0 - 7.5)	4.54 ± 2.01	4.68 ± 1.89	4.40 ± 2.18
Biochemistry			
Sodium (mmol/L) (Mean ± S.D) (Normal range: 135 – 145)	140 ± 3	140 ± 2	140 ± 4
Potassium (mmol/L) (Mean ± S.D) (Normal range: 3.5 - 5.1)	4.5 ± 0.3 (n = 30)	4.5 ± 0.3 (n = 14)	4.5 ± 0.4 (n = 16)
Creatinine (umol/L) (Mean ± S.D) (Normal range: Men: 66 – 112 Women: 49 - 92)	76 ± 14	77 ± 12	76 ± 16
Corrected Calcium (mmol/L) (Mean ± S.D) (Normal range: 2.20 - 2.60)	2.44 ± 0.11 (n = 31)	2.43 ± 0.15 (n = 16)	2.44 ± 0.07 (n = 15)
Cardiac Biomarkers			
Troponin T (ng/L) (Median ± IQR)	7 ± 5	6 ± 5	8 ± 5

(Normal Range: 0 – 14)	(n = 31)*	(n = 15)	(n = 16)
NT-proBNP (ng/L) (Median ± IQR) Normal <400)	68 ± 63 (n = 30)	54 ± 32 (n = 14)	76 ± 123 (n = 16)
***In one patient baseline troponin haemolysed and the post chemotherapy cycle 1 troponin value was used as baseline. In another patient baseline troponin haemolysed and no post cycle 1 troponin available; see text**			

3.2.2.3 Baseline Clinical Observations

Baseline clinical observations and ECG findings performed prior to cycle 1 chemotherapy for all patients and for each group are shown in Table 3.4. Statistical analysis was done as described in 2.6.1.1. Mean blood pressure was 131/73mmHg and mean heart rate was 75 beats per minute. Most patients were in normal sinus rhythm on their baseline 12-lead ECG, whilst one patient had 1st degree heart block. Two patients did not have a baseline ECG performed. There were no significant differences between baseline clinical observations between the two groups.

Table 3.4. Baseline clinical observations

	All patients N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
Systolic Blood pressure (mmHg) (Mean ± S.D)	131 ± 16	131 ± 15	132 ± 18
Diastolic Blood Pressure (mmHg) (Mean ± S.D)	73 ± 9	72 ± 11	74 ± 7
Heart Rate (bpm) (Mean ± S.D)	75 ± 13	77 ± 13	74 ± 12
ECG Sinus Rhythm 1st Heart Block	29 1 (n = 30)	16 0 (n = 16)	13 1 (n = 14)

3.3 Effect of Remote Ischaemic Conditioning on Troponin T

3.3.1 Comparison of pre- and post-chemotherapy Troponin T (TnT) values

Pre- and post-chemotherapy TnT values were compared to assess how troponin varies before and after each cycle of chemotherapy for all patients and for each group. Troponin

T trends during each chemotherapy cycle are shown in Figure 3.3 and Table 3.5 for all patients. Statistical analysis of TnT at each cycle compared to baseline was done as described in 2.6.1.2.

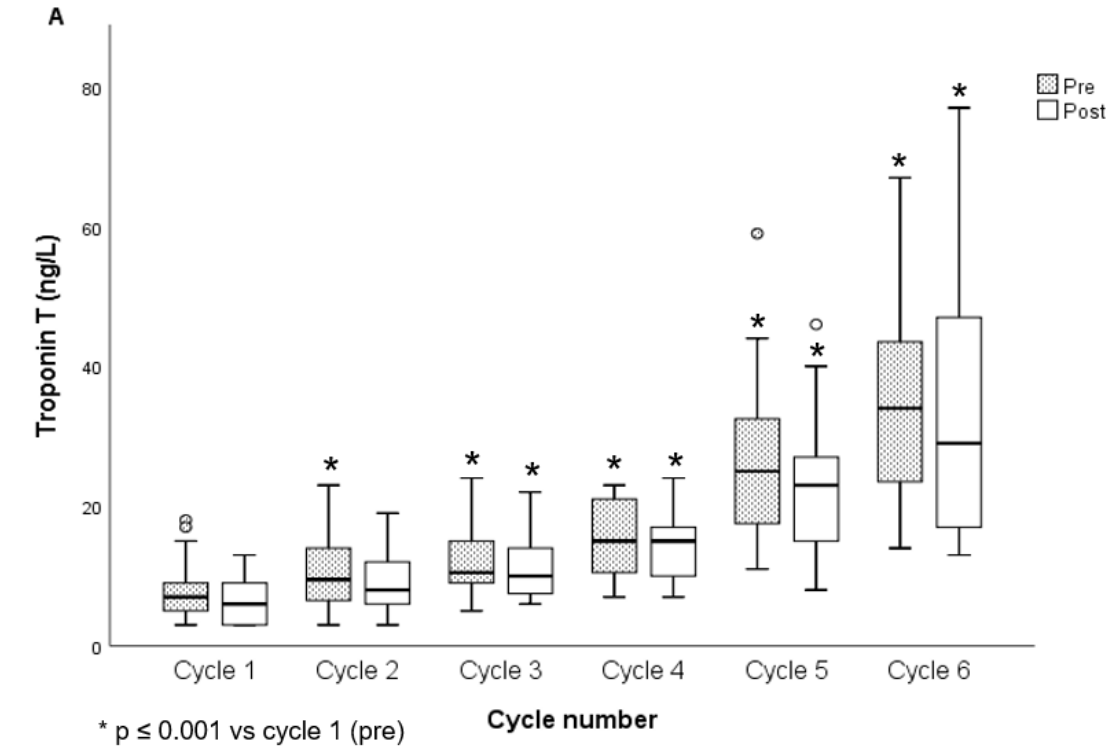
A total of 153 and 135 pre- and post-chemotherapy samples respectively were successfully analysed. In some patients, especially on a 46-hr infusion regime, disconnection of the infusion at the end of the 46 hrs would happen at their local hospital if they lived far away, thus no post-samples were taken. As patients progress through their chemotherapy, there is a general trend of an increase in their TnT (Figure 3.3). Median pre-chemotherapy TnT rises from 7ng/L at baseline to 34ng/L by cycle 6 (mean difference 27ng/L, 95% CI 20-35 ng/L, $p<0.001$ compared to baseline) and in some patients this can be as high as 67ng/L (Figure 3.3, Table 3.5). Post-chemotherapy TnT values follow a similar pattern.

Of the 140 post-chemotherapy samples collected, 3 had no chemotherapy end-time documented so the time difference from end of chemotherapy to sample collection was calculated for 137 samples (2 samples subsequently haemolysed thus not included in the overall analysis above but included in the analysis of time from chemotherapy). Post-chemotherapy troponin samples were collected at a median time of 106 minutes (98 minutes for bolus regimes, 125 minutes for infusion regimes) from the end of the doxorubicin injection/infusion. On 7 occasions where patients received a bolus injection, the end time was not recorded, thus a duration of injection of 15 minutes was assumed (the average bolus injection for injections with recorded end times was 16 minutes). Of the 140 post-chemotherapy samples collected, the majority (n = 100, 73% (n = 64 bolus regime, 36 infusion regime)) were performed less than 3 hours (180 minutes) from the end of chemotherapy. This is a deviation from the original study protocol which states

performing the post-sample between 3-24 hours post-chemotherapy and reflects patients' unwillingness to stay longer for the blood test after a long day (bolus) or often a weekend (infusion) spent at the Cancer Centre.

Random effects regression comparison between pre- and post- chemotherapy samples shows that there is no evidence of a difference between pre- and post-chemotherapy TnT ($p=0.181$). The fixed model estimates that on average pre-chemotherapy TnT is 1.9ng/L more than post-chemotherapy (95% CI -0.91-4.78). To investigate if increasing chemotherapy cycles has an effect, this was added as a fixed effect to the model and the random effects regression analysis repeated. There is still no evidence of a difference between pre- and post- chemotherapy TnT ($p=0.135$) with pre-chemotherapy TnT being on average 1.48ng/L higher than post-chemotherapy (95% CI -0.47-3.44). However, there is a significant increase of troponin with increasing chemotherapy cycles ($p < 0.001$). The mean troponin rise was as much as 27ng/L from baseline to cycle 6 further supporting the general trend of increasing Troponin T as patients progress through their chemotherapy seen in Figure 3.3.

Figure 3.3. Pre- and Post-chemotherapy Troponin T Trends for all patients. A. Boxplot of pre- (dotted) and post-(white) chemotherapy TnT per cycle. B. Line chart of median TnT per cycle for pre- (continuous) and post- (broken) samples. * p≤0.001 vs cycle 1 (pre).



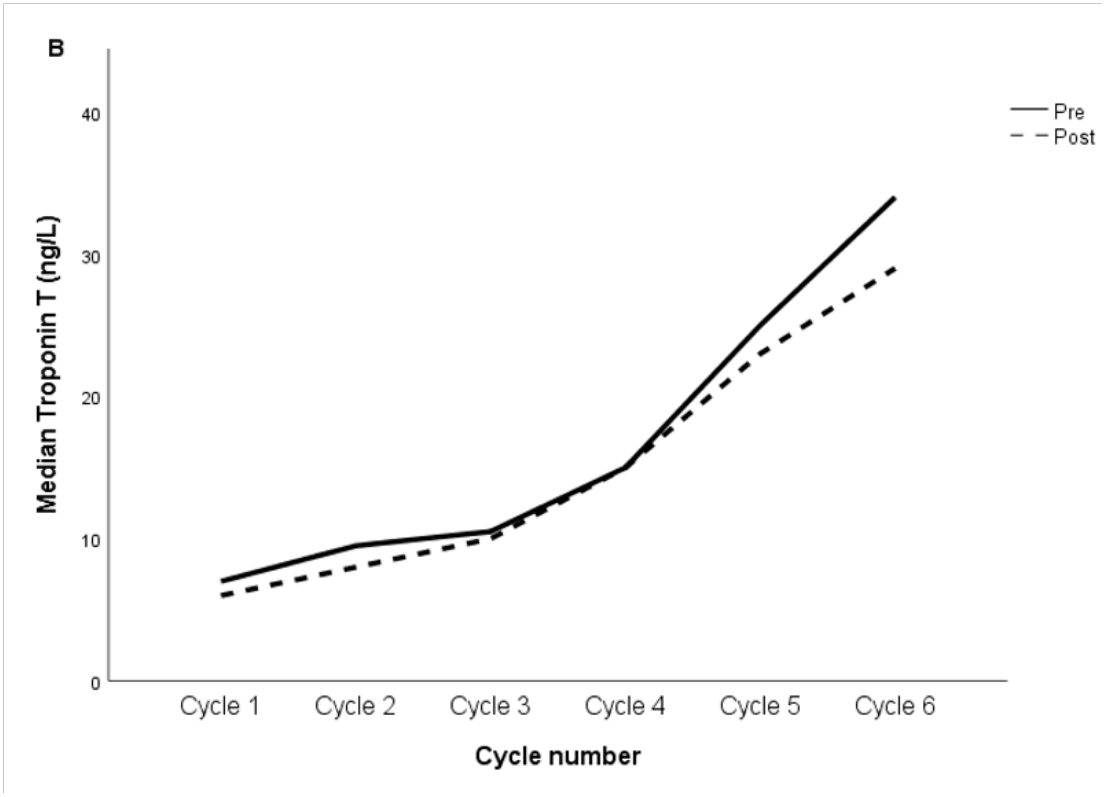


Table 3.5. Troponin T trends pre- and post-chemotherapy for all patients

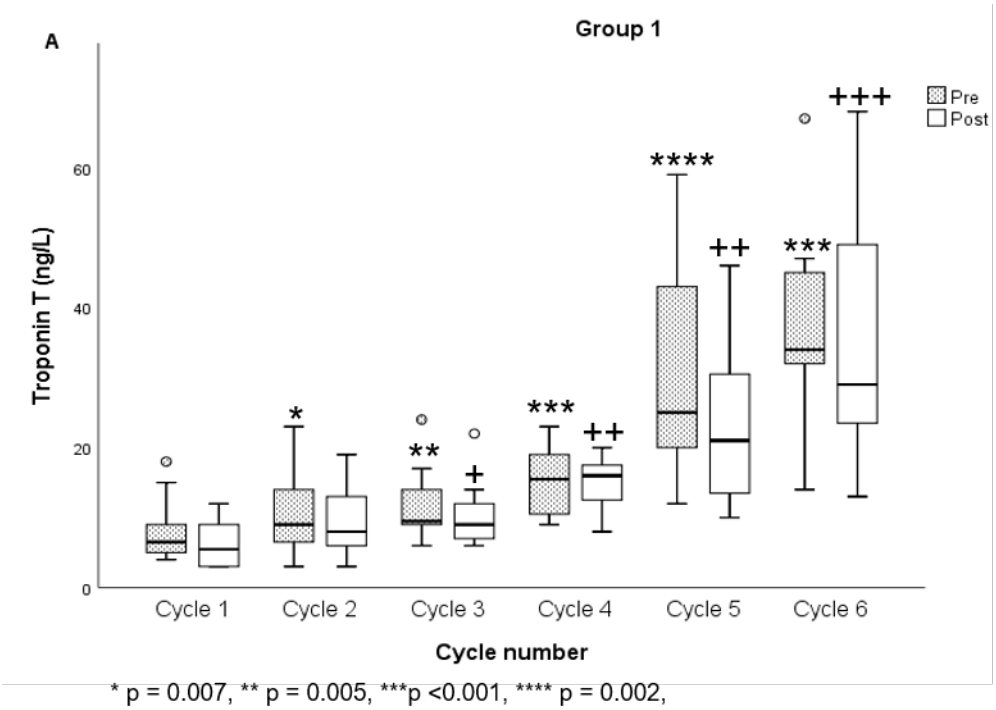
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Pre (n=30)	Post (n=29)	Pre (n=32)	Post (n=29)	Pre (n=30)	Post (n=28)	Pre (n=23)	Post (n=18)	Pre (n=19)	Post (n=15)	Pre (n=19	Post (n=16)
Median (ng/L)	7	6	10	8	11	10	15	15	25	23	34	29
IQR	4	7	8	7	7	7	11	7	18	15	26	32
Min (ng/L)	3	3	3	3	5	6	7	7	11	8	14	13
Max (ng/L)	18	13	23	19	37	22	23	24	59	46	67	77

A similar assessment was performed for each group individually as seen in Figure 3.4 (Group 1 (RIC)) and Figure 3.5 (Group 2(Sham)) and Tables 3.6 (Group 1(RIC)) and 3.7 (Group 2 (Sham)). As was seen in the analysis for all patients, there is a trend of increasing TnT concentration as patients progress with their chemotherapy. Random effects regression analysis for each group shows no difference between pre- and post-chemotherapy TnT (Group 1 (RIC) pre-chemotherapy TnT on average 2.49ng/L higher than post-, 95% CI -0.44-5.42, p=0.095, Group 2 (Sham) pre-chemotherapy TnT on average 0.64ng/L higher than post-, 95% CI -2.02-3.33, p=0.634). There is again a significant increase in troponin with increasing chemotherapy cycles ($p < 0.001$) for both groups.

Even though there is no significant difference between pre and post-chemotherapy TnT values, the post-chemotherapy TnT appear to be lower than the pre-chemotherapy value with only 21% of pre/post pairs with a post-TnT being higher than the pre-chemotherapy value. This may be a dilutional effect as many patients receive intravenous fluids depending on their chemotherapy regime. However, as the study protocol did not include a post-chemotherapy assessment of haematocrit, I am unable to assess if this is indeed a dilutional effect. Therefore, as no statistical difference was seen between pre- and post-

samples and because there may be a dilutional effect that is affecting the true post-chemotherapy TnT value and the fact that there are more missing post-chemotherapy samples compared to pre-chemotherapy, any subsequent comparisons between groups for the effect of RIC was performed using the pre-chemotherapy TnT values only (which correspond to a pre-chemotherapy TnT trough level).

Figure 3.4. Pre- and Post-chemotherapy Troponin T Trends for Group 1 (RIC). A. Boxplot of pre- (dotted) and post-(white) chemotherapy TnT per cycle. B. Line chart of median TnT per cycle for pre- (continuous) and post- (broken) samples. p values are vs cycle 1 (pre).



* p = 0.007, ** p = 0.005, ***p <0.001, **** p = 0.002,
+p = 0.04, ++ p = 0.008, +++ p = 0.004 vs cycle 1 (pre)

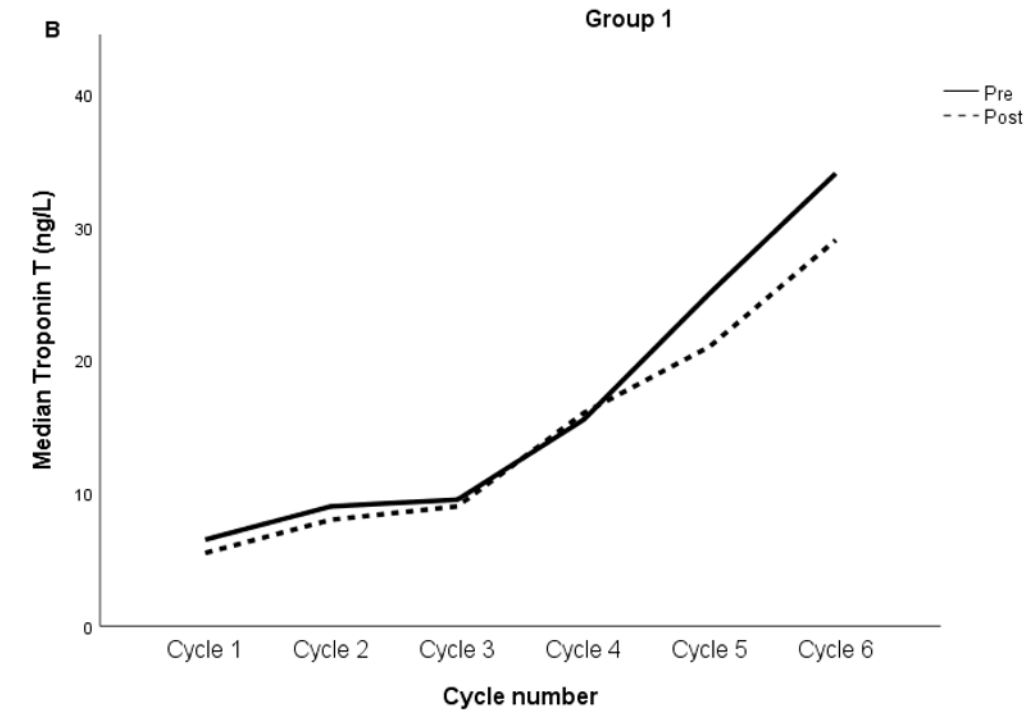
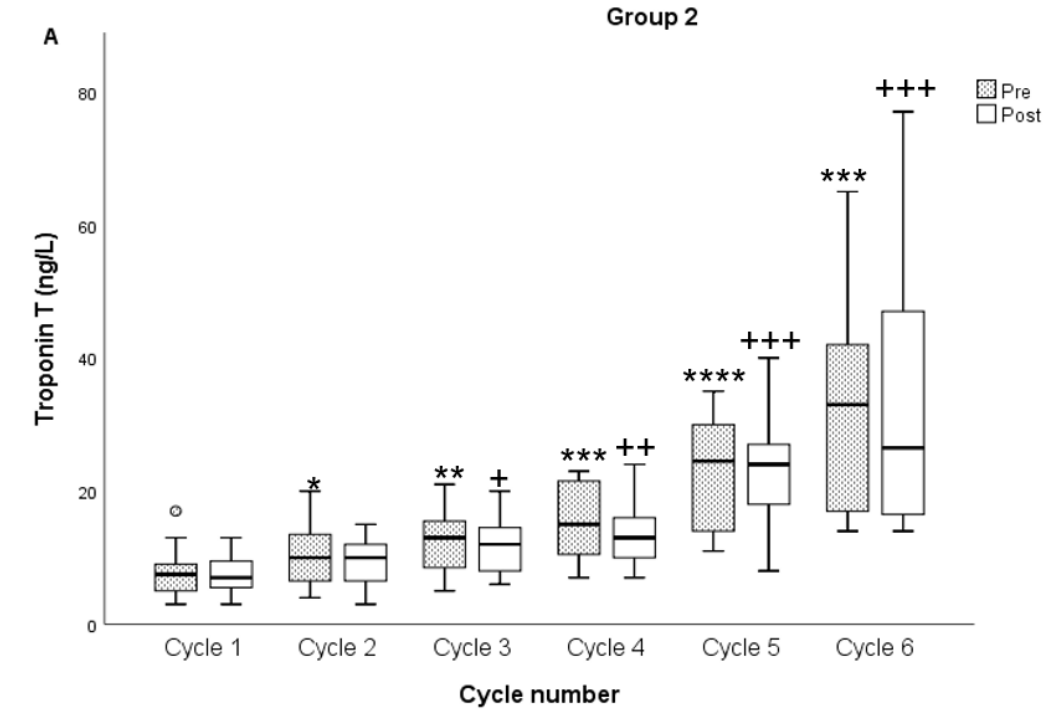


Figure 3.5. Pre- and Post-chemotherapy Troponin T Trends for Group 2 (Sham). A. Boxplot of pre- (dotted) and post-(white) chemotherapy TnT per cycle. B. Line chart of median TnT per cycle for pre- (continuous) and post- (broken) samples. p values are vs cycle 1 (pre).



* p = 0.004, ** p < 0.001, ***p = 0.002, **** p = 0.001,
+p = 0.009, ++ p = 0.01, +++ p = 0.02 vs cycle 1 (pre)

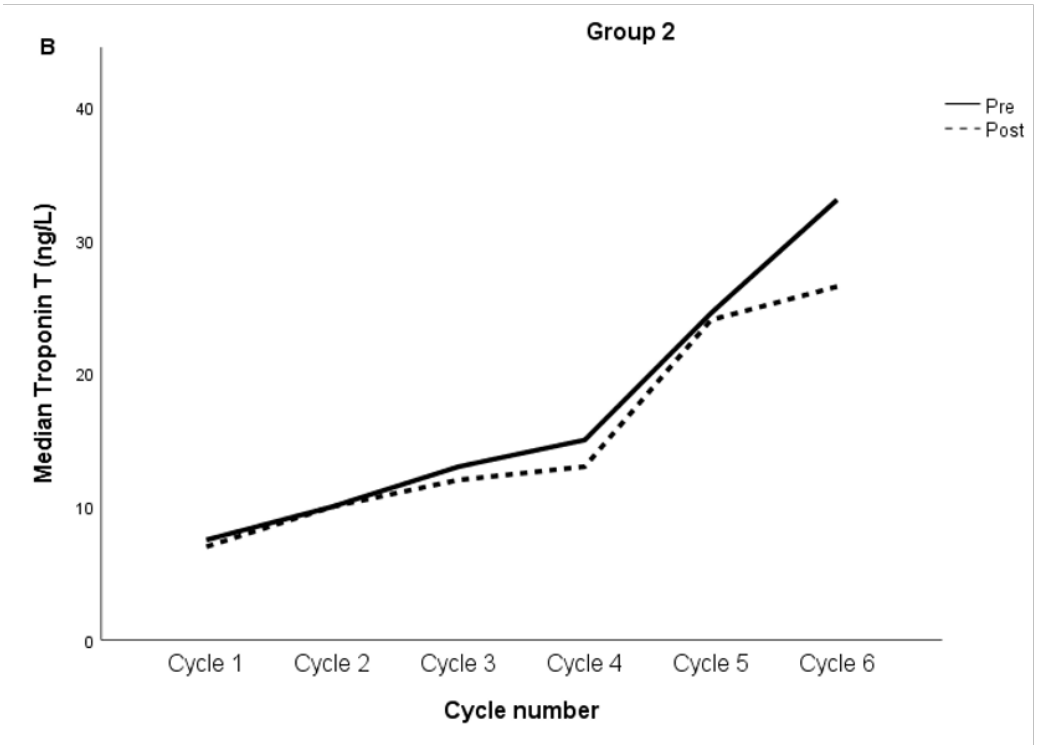


Table 3.6. Troponin T trends pre- and post-chemotherapy for Group 1 (RIC)												
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Pre (n=14)	Post (n=14)	Pre (n=16)	Post (n=14)	Pre (n=14)	Post (n=13)	Pre (n=12)	Post (n=7)	Pre (n=9)	Post (n=8)	Pre (n=9)	Post (n=8)
Median (ng/L)	7	6	9	8	10	9	16	16	25	21	34	29
IQR	5	6	8	7	6	5	9	8	25	22	15	30
Min (ng/L)	4	3	3	3	6	6	9	8	12	10	14	13
Max (ng/L)	18	12	23	19	37	22	23	20	59	46	67	68

Table 3.7. Troponin T trends pre- and post-chemotherapy for Group 2 (Sham)												
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Pre (n=16)	Post (n=15)	Pre (n=16)	Post (n=15)	Pre (n=16)	Post (n=15)	Pre (n=11)	Post (n=11)	Pre (n=10)	Post (n=7)	Pre (n=10)	Post (n=8)
Median (ng/L)	8	7	10	10	13	12	15	13	25	24	33	27
IQR	5	5	8	12	8	7	13	7	18	15	27	32
Min (ng/L)	3	3	4	3	5	6	7	7	11	8	14	14
Max (ng/L)	17	13	20	15	21	20	23	24	35	40	65	77

3.3.2. Effect of Remote Ischaemic Conditioning (RIC) on Troponin T levels during chemotherapy

3.3.2.1 Absolute Troponin T

The effect of Remote Ischaemic Conditioning (RIC) on Troponin T values during anthracycline chemotherapy was analysed by comparing the RIC and sham groups using their pre-chemotherapy TnT values per cycle (Figure 3.6, Table 3.8). Statistical analysis between the two groups was performed as described in 2.6.1.3.

Random effects regression analysis shows that there is no significant difference in the TnT values between Group 1 (RIC) and Group 2 (Sham)(Mean difference 1.5ng/L, 95% CI -2.42-5.42, p = 0.45). Adding consecutive chemotherapy cycles as a fixed effect to the model and repeating the regression analysis shows that still there is no significant

difference in the TnT values between the two groups (Mean difference 1.59ng/L, 95% CI -1.1-4.28, p = 0.245). However, the effect of increasing chemotherapy cycles appears to have a significant increasing trend ($p < 0.001$) with a mean increase in TnT of 27.4ng/L from baseline to cycle 6. A Mann-Whitney test between the two groups at each cycle supports the random effect regression analysis with no significant difference between the groups at any chemotherapy cycle (Cycle 1 p=0.834, cycle 2, p=0.925, cycle 3, p=0.518, cycle 4, p=0.757, cycle 5, p=0.413, cycle 6, p=0.462).

Figure 3.6. Troponin T Comparisons between the two groups. A. Boxplot TnT per cycle for each group (Group 1 (RIC)=dotted, Group 2 (Sham)=white). B. Line chart of median TnT per cycle for each group (Group 1 (RIC)=continuous, Group 2 (Sham)=broken).

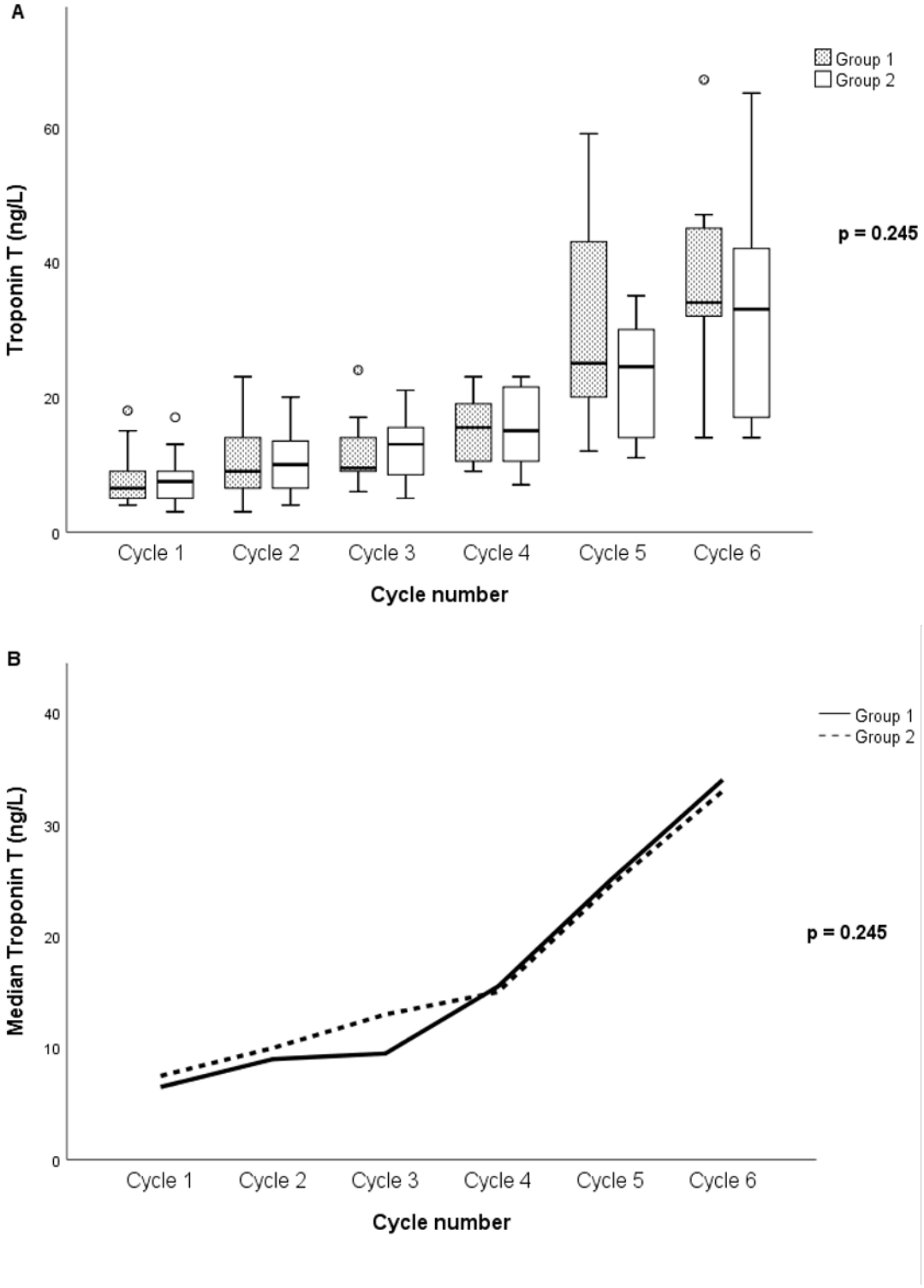


Table 3.8. Troponin T trends between Group 1 (RIC) and Group 2 (Sham) during chemotherapy

	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=16)	Group 2 (n=16)	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=12)	Group 2 (n=11)	Group 1 (n=9)	Group 2 (n=10)	Group 1 (n=9)	Group 2 (n=10)
Median (ng/L)	7	8	9	10	10	13	16	15	25	25	34	33
IQR	5	5	8	8	6	8	14	13	25	18	15	27
Min (ng/L)	4	3	3	4	6	5	9	7	12	11	14	14
Max (ng/L)	18	17	23	20	37	21	23	23	59	35	67	65

3.3.2.2 Troponin T change from baseline (ΔTnT)

The effect of RIC on TnT during anthracycline chemotherapy was also analysed using the change of TnT from baseline, ΔTnT, and compared between the two groups (Figure 3.7, Tables 3.9 and 3.10). Statistical analysis comparison between the two groups was performed as described in 2.6.1.3.

In a similar fashion to the absolute TnT values, ΔTnT values increase as patients progress with their chemotherapy (Figure 3.7A, Table 3.9) with a mean increase in TnT from baseline of 28ng/L by cycle 6 (31ng/L for Group 1 (RIC) and 25ng/L for Group 2 (Sham) (Figure 3.7B, Tables 3.9, 3.10). Random effects regression shows that there is no significant difference in the ΔTnT between the two groups with Group 1 (RIC) having on average a ΔTnT that is 2.6ng/L higher than Group 2 (Sham) (95% CI -1.9-7.1, p=0.256) when repeated measures are taken into account. Adding consecutive chemotherapy cycles as a fixed effect into the model still shows no significant difference between the groups (ΔTnT difference 2.6ng/L, 95% CI -0.62-5.63, p = 0.113), however with a significant trend with increasing chemotherapy cycles (p <0.001). An independent samples T test supports the regression analysis, with no significant difference between the mean ΔTnT at each cycle (Cycle 2: mean difference 0.3ng/L, 95% CI -2.3-2.9, p = 0.812, Cycle 3: mean difference 0.3ng/L, 95% CI -3.1-3.7, p = 0.858, Cycle 4: mean difference 1.2ng/L, 95% CI -

3.3-5.6, p = 0.586, Cycle 5: mean difference 7.5ng/L, 95% CI -4.5-19.6, p = 0.211, Cycle 6: mean difference 6.3ng/L, 95% CI -9.3-21.9, p = 0.408).

Figure 3.7. ΔTnT Comparisons between the two groups. A. Boxplot of ΔTnT per cycle for each group (Group 1 (RIC)=dotted, Group 2 (Sham)=white). B. Line chart of mean ΔTnT per cycle for each group (Group 1 (RIC)=continuous, Group 2 (Sham)=broken).

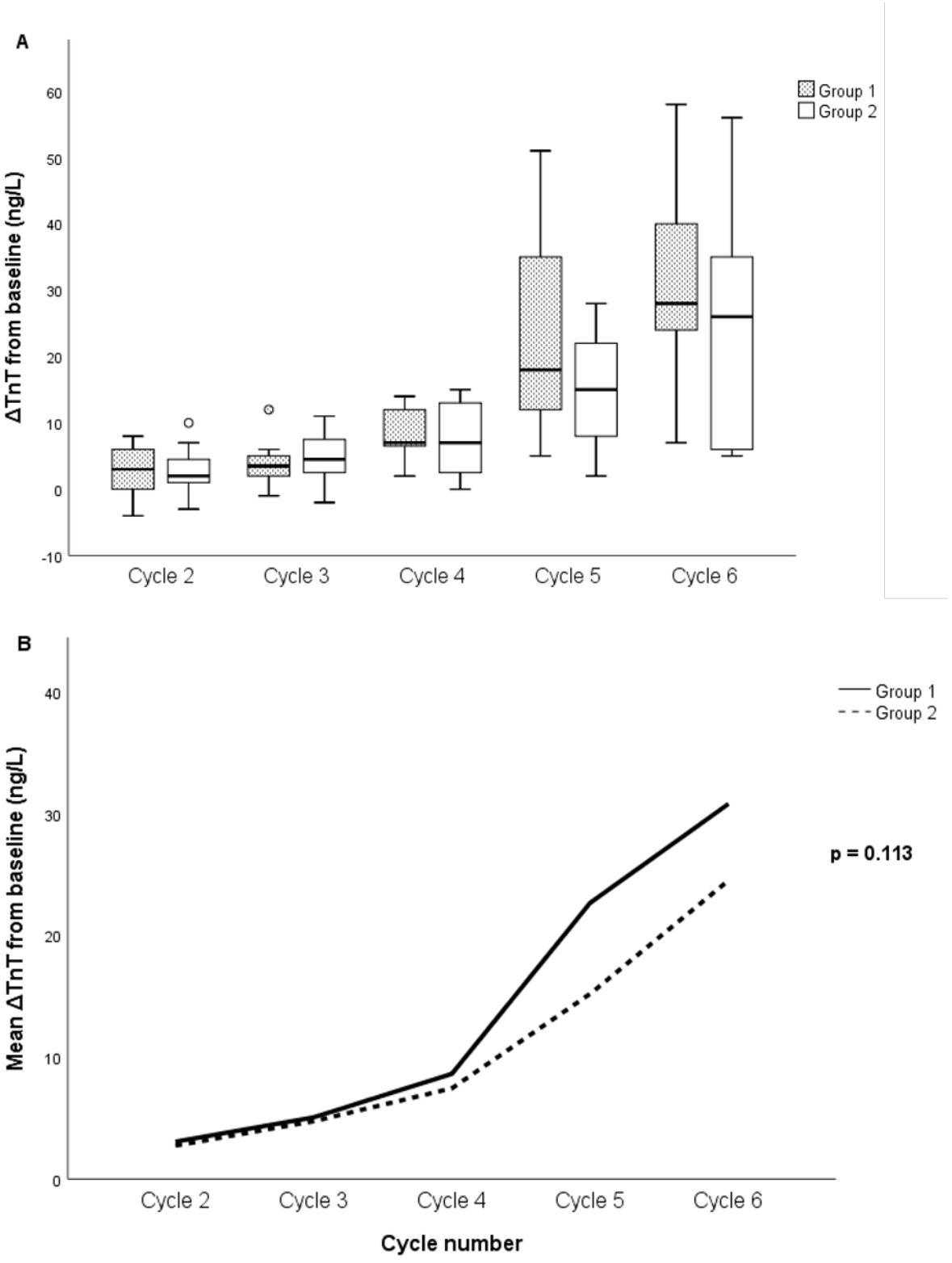


Table 3.9. ΔTnT trends for All patients during chemotherapy					
	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
	N = 30	N = 30	N = 22	N = 19	N = 19
Mean (ng/L)	3	5	8	19	28
SD	3.4	4.5	4.9	12.7	16
Min (ng/L)	-4	-2	0	2	5
Max (ng/L)	10	22	15	51	58

Table 3.10. ΔTnT trends between Group 1 (RIC) and Group 2 (sham) during chemotherapy										
	Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=11)	Group 2 (n=11)	Group 1 (n=9)	Group 2 (n=10)	Group 1 (n=9)	Group 2 (n=10)
Mean (ng/L)	3	3	5	5	9	7	23	15	31	25
SD	3.6	3.3	5.6	3.4	3.8	5.9	15.3	9.3	14.2	17.6
Min (ng/L)	-4	-3	-1	-2	2	0	5	2	7	5
Max (ng/L)	8	10	22	11	14	15	51	28	58	56

3.3.2.3 Troponin T as binary value (positive vs negative)

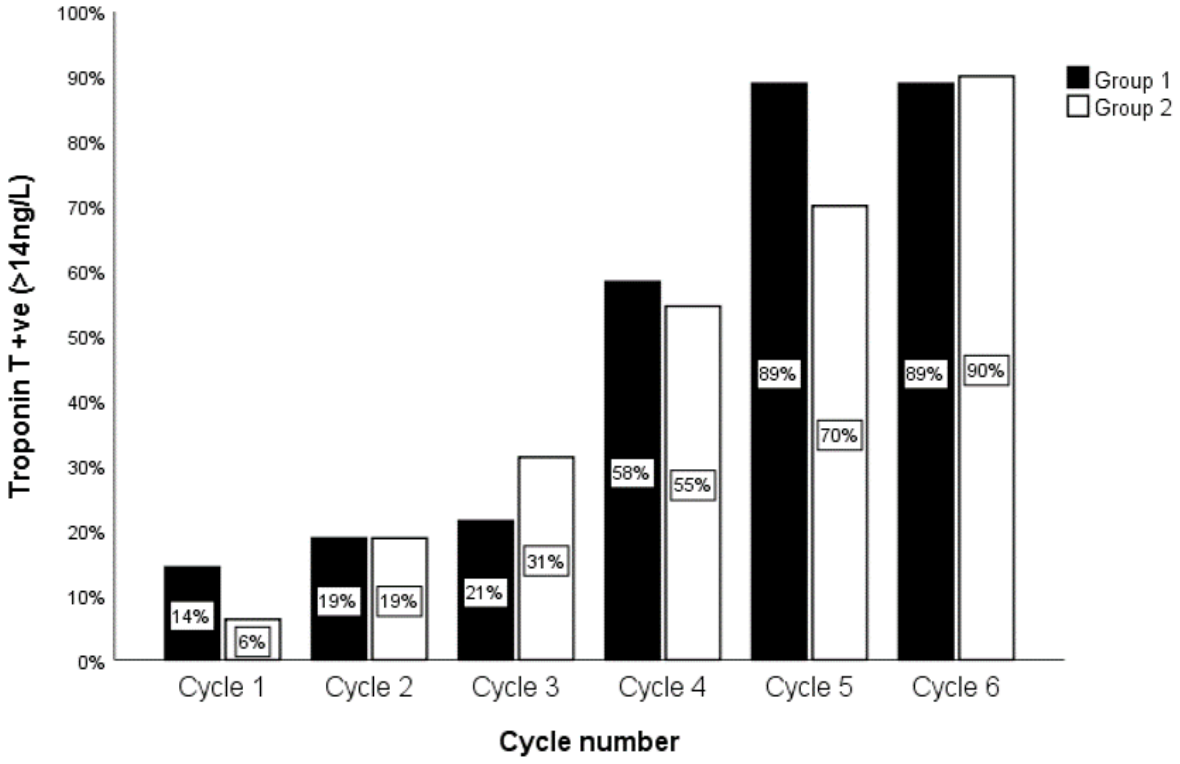
The effect of RIC on Troponin T trends during anthracycline chemotherapy was further analysed by treating TnT as a binary categorical variable (i.e. positive vs negative) (Table 3.11, 4.12, Figure 3.8) with statistical analysis of the comparison between the two groups as described in 2.6.1.3.

The number of patients with a positive TnT increases from 10% to 90% from cycle 1 to cycle 6 (Table 3.11). For Group 1 (RIC), Cycle 1 to Cycle 6 positive TnT values increase from 14% to 89% and for Group 2 (Sham), from 6 to 90% (Table 3.12, Figure 3.8). There is no significant difference in the number of positive TnT samples between the two groups at each cycle (Cycle 1, p=0.464, Cycle 2, p=1, Cycle 3, p=0.544, Cycle 4, p=0.855, Cycle 5, p=0.313, Cycle 6, p=0.937).

Table 3.11. Positive TnT trends for All patients during chemotherapy						
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
	N = 30	N = 32	N = 30	N = 23	N = 19	N = 19
TnT +ve (%)						
Yes	3 (10)	6 (19)	8 (27)	13 (57)	15 (79)	17 (90)
No	27 (90)	26 (81)	22 (73)	10 (43)	4 (21)	2 (10)

Table 3.12. Positive TnT between Group 1 (RIC) and Group 2 (sham) during chemotherapy												
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=16)	Group 2 (n=16)	Group 1 (n=14)	Group 2 (n=16)	Group 1 (n=12)	Group 2 (n=11)	Group 1 (n=9)	Group 2 (n=10)	Group 1 (n=9)	Group 2 (n=10)
TnT +ve (%)												
Yes	2 (14)	1 (6)	3 (19)	3 (19)	3 (21)	5 (31)	7 (58)	6 (55)	8 (89)	7 (70)	8 (89)	9 (90)
No	12 (86)	15 (94)	13 (81)	13 (81)	11 (79)	11 (69)	5 (42)	5 (45)	1 (11)	3 (30)	1 (11)	1 (10)

Figure 3.8. Positive TnT Comparisons between the two groups (Group 1 = RIC, Group 2 = Sham)



3.3.2 Effect of Remote Ischaemic Conditioning (RIC) on Troponin T levels during chemotherapy according to cumulative anthracycline dose received

Cumulative anthracycline dose is one of the major risk factors for anthracycline cardiotoxicity. Therefore, the effect of RIC on Troponin T according to cumulative dose received during chemotherapy was analysed and the two groups compared as described in 2.6.1.3. Figures 3.9 and 3.10 show a scatter and dot diagram of TnT and ΔTnT respectively against cumulative dose for each group.

Figure 3.9. Scatter and dot diagram with line of best fit of TnT against cumulative anthracycline dose for Group 1 (RIC) (black dots, continuous line) and Group 2 (Sham) (white dots, broken line).

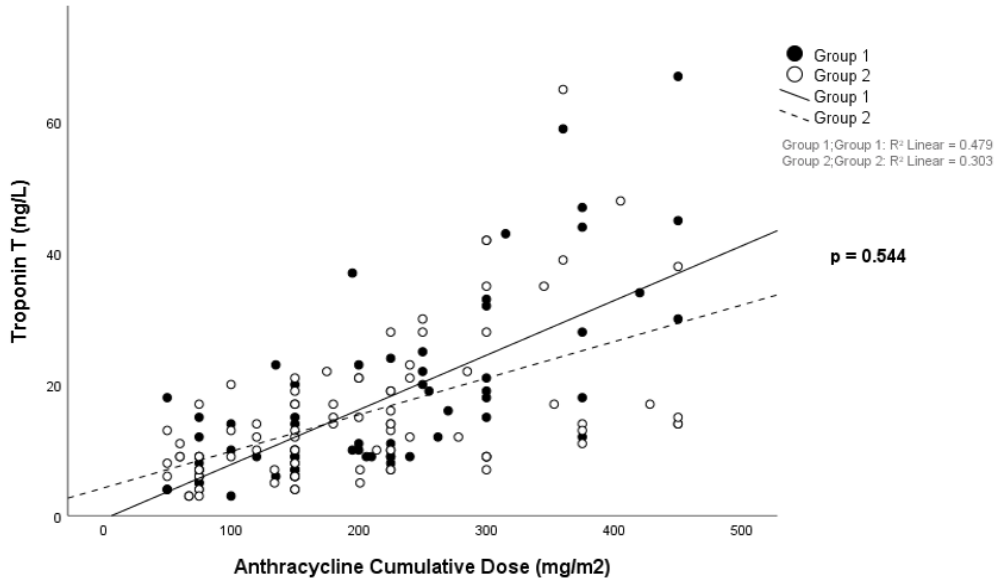
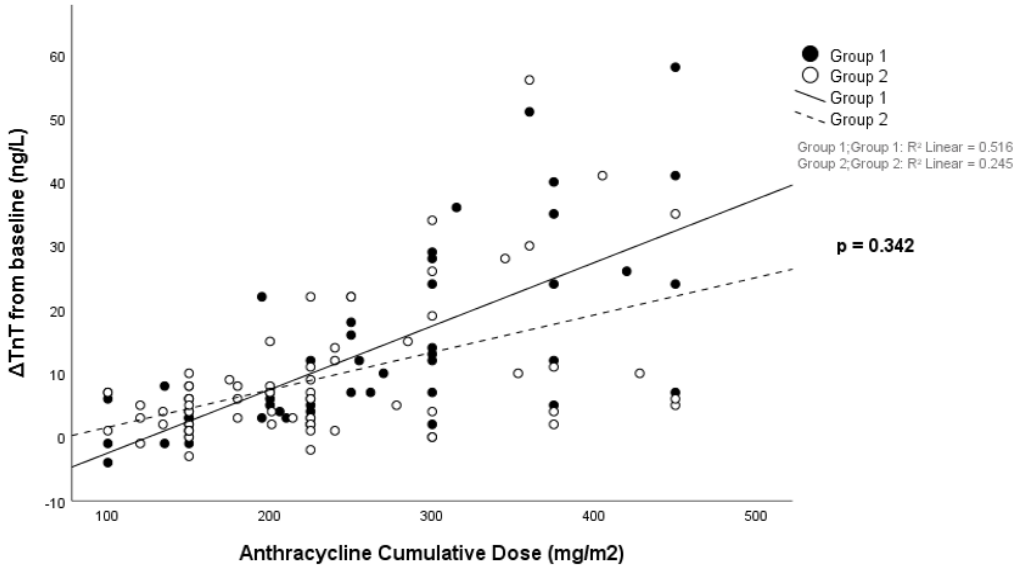


Figure 3.10. Scatter and dot diagram with line of best fit of ΔTnT against cumulative anthracycline dose for Group 1 (RIC) (black dots, continuous line) and Group 2 (Sham) (white dots, broken line).



Random effects regression analysis shows that there is no significant difference between the TnT and ΔTnT between the two groups when taking repeated measures and

cumulative dose into account. Group 1 (RIC) has on average a TnT that is 0.95ng/L (95% CI -2.1-4, p = 0.544) and a ΔTnT that is 1.73ng/L (95% CI -1.9-5, p = 0.342) higher than Group 2 (Sham).

3.3.3. Effect of Remote Ischaemic Conditioning (RIC) on Troponin T levels during chemotherapy and follow up

To assess if RIC had any effect on TnT levels taken during chemotherapy and follow up, a similar analysis was also performed but on this occasion any available TnT values that were performed during the follow up period were also incorporated.

3.3.3.1 Absolute Troponin T

Absolute TnT values during the whole study period (i.e. chemotherapy and follow-up) were compared between the RIC and sham groups (Figure 3.11, Tables 3.13 and 3.14). Comparison between the two groups was performed as described in 2.6.1.4

Table 3.13 shows the TnT trends for all patients at each follow-up time-point. Unfortunately 5 patients died during follow up (one after the 1 month follow-up, 2 after the 3 month follow-up and 2 after the 6 month follow-up). One patient withdrew after the 3 month follow up and thus no further data are available beyond that. The rest of the missing data represent patients who have not had a blood test at that particular time-point. Up to the 6 month follow- up, the majority of those missed blood tests were due to the Covid-19 pandemic (75%). Because of the prolonged follow-up protocol of the study, the missing data at the 12 month time-point are due to a combination of patients not reaching that part of follow-up (65%) and because of the Covid-19 pandemic (35%). Therefore, as n = 6 at the 12 month follow-up, the analysis of the comparison between the two groups was done up to the 6 month follow-up.

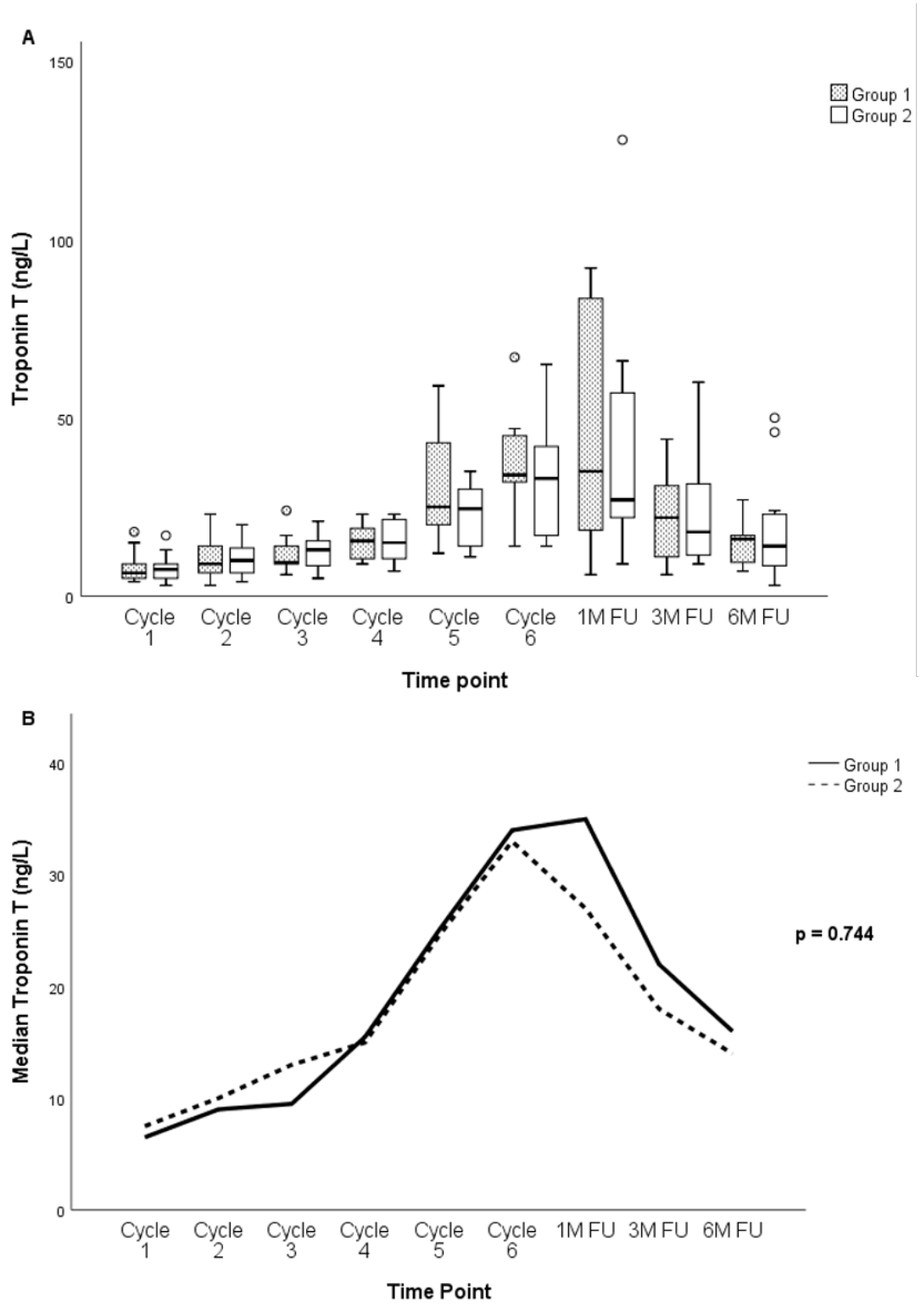
Table 3.13. Absolute TnT trends for All patients during follow-up				
	One Month	Three Months	Six months	Twelve months
	N = 29	N = 28	N = 22	N = 6
Median (ng/L)	34	20	15	13
IQR	44	22	13	19
Min (ng/L)	6	6	3	8
Max (ng/L)	128	60	50	48
Days from last cycle (median)	32	94	185	363

Table 3.14. Absolute TnT trends between Group 1 (RIC) and Group 2 (Sham) during follow-up								
	One Month		Three Months		Six Months		Twelve Months	
	Group 1 (n=16)	Group 2 (n=13)	Group 1 (n=13)	Group 2 (n=15)	Group 1 (n=11)	Group 2 (n=11)	Group 1 (n=4)	Group 2 (n=2)
Median (ng/L)	35	27	22	18	16	14	10	35
IQR	67	38	23	22	8	19	6	-
Min (ng/L)	6	9	6	9	7	3	8	21
Max (ng/L)	92	128	44	60	27	50	15	48
Days from last cycle (median)	31	33	94	93	182	187	393	331

As can be seen from Figure 3.11 and Tables 3.13 and 3.14, TnT remains elevated one month after anthracycline chemotherapy with a median of 34ng/L, after which it starts to fall. The box-whisker plots however, particularly at the one month time-point, are fairly wide for both groups suggesting there is a lot of variability between the patients' TnT values.

Random effects regression analysis shows that there is no difference in the TnT values between the two groups during chemotherapy and follow up, with Group 1 (RIC) having on average a TnT value of 1.29ng/L higher than Group 2 (Sham) (95% CI -3.45-6.05, p=0.592) when repeated measures are taken into account. Adding time (i.e. the different time-points) as a fixed effect in the model and repeating the analysis, there is still no significant difference between the two groups with Group 1 (RIC) having a TnT that is 0.62ng/L higher than Group 2 (Sham) on average (95% CI -3.13-4.37, p=0.744) but, as before, there is a significant trend of time ($p<0.001$). A Mann-Whtiney test supports the regression analysis, with no significant difference between the two groups at any of the follow-up time-points (1 month, p=1, 3 month, p=0.945, 6 month, p=0.699). A separate random effects regression analysis only for the follow-up periods also shows there is no significant difference between the two groups during follow-up (p=0.8) but with a significant trend for the effect of time ($p<0.001$), further supporting the previous analyses.

Figure 3.11. Absolute TnT values comparisons between the two groups during chemotherapy and follow-up. A. Boxplot of TnT per time-point for each group (Group 1 (RIC)=dotted, Group 2 (Sham)=white). B. Line chart of median TnT per time-point for each group (Group 1 (RIC)=continuous, Group 2 (Sham)=broken).



3.3.3.2 Troponin T change from baseline (ΔTnT)

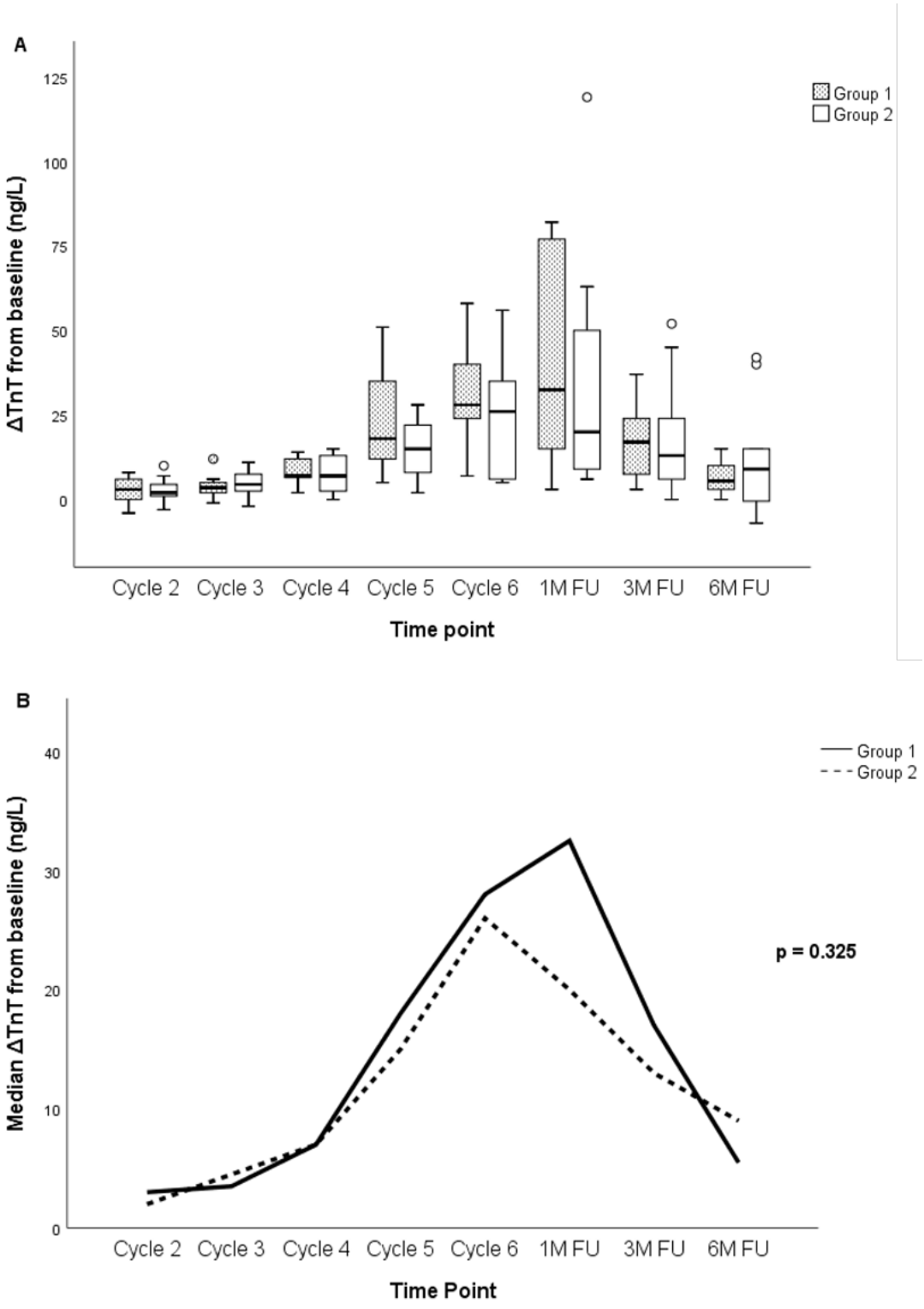
A similar analysis was performed for the TnT change from baseline (ΔTnT) during chemotherapy and follow up and a comparison made between Group 1 (RIC) and Group 2 (Sham)(Figure 3.12, Tables 3.15 and 3.16). Comparison between the two groups was performed as described in 2.6.14. As with the absolute TnT analysis, the 12M time-point was not included in the statistical analysis due to a small N number.

Similar to the absolute TnT trends, when analysing the difference from baseline (ΔTnT) as seen in Figure 3.13 and Tables 3.15 and 3.16, ΔTnT values remain elevated one month after chemotherapy with a median increase of 24ng/L from baseline (Group 1 (RIC), 33ng/L, Group 2 (Sham), 20ng/L), after which they start to decrease, though with wide boxplot in both groups suggestive of significant variability between patients. Random effects regression analysis shows that there is no difference in the ΔTnT values between the two groups during chemotherapy and follow up, with Group 1 (RIC) having on average a TnT value of 2.65ng/L higher than Group 2(Sham) (95% CI -2.69-7.99, p=0.328) when repeated measures are taken into account. Adding the different time-points as a fixed effect in the model and repeating the analysis, there is still no significant difference between the two groups with Group 1(RIC) having a TnT that is on average 2.12ng/L higher than Group 2(sham) (95% CI -2.12-6.36, p=0.325) but, as before, there is a significant trend of time (p<0.001). A Mann-Whtiney test supports the regression analysis, with no significant difference between the two groups at any of the follow-up time-points (1 month, p=0.395, 3 month, p=0.736, 6 month, p=0.860). A separate random effects regression analysis only for the follow-up periods also shows there is no significant difference between the two groups during follow-up (p=0.78) but with a significant trend for the effect of time (p<0.001), further supporting the previous analyses.

Table 3.15. ΔTnT trends for All patients during follow-up				
	One month	Three months	Six months	Twelve months
	N = 27	N = 26	N = 21	N = 6
Median (ng/L)	24	14	7	4
IQR	49	52	13	17
Min (ng/L)	3	0	-7	-3
Max (ng/L)	119	52	42	31

Table 3.16. ΔTnT trends between RIC and sham group during follow up								
	One month		Three months		Six months		Twelve months	
	Group 1 (n=14)	Group 2 (n=13)	Group 1 (n=11)	Group 2 (n=15)	Group 1 (n=10)	Group 2 (n=11)	Group 1 (n=4)	Group 2 (n=2)
Median (ng/L)	33	20	17	13	6	9	3	23
IQR	63	42	19	19	8	16	6	-
Min (ng/L)	3	6	3	0	0	-7	-3	14
Max (ng/L)	82	119	37	52	15	42	5	31

Figure 3.12. ΔTnT Comparisons between the two groups during chemotherapy and follow-up. A. Boxplot of ΔTnT per time-point for each group (Group 1(RIC)=dotted, Group 2(Sham)=white). B. Line chart of mean ΔTnT per time-point for each group (Group (RIC) 1=continuous, Group 2(Sham)=broken).



3.3.3.3 Troponin T as binary value (positive vs negative)

Treating TnT as a binary variable with a TnT≥15ng/L as positive, the analysis was repeated and a comparison was made for TnT values during chemotherapy and follow-up between the two groups. Troponin positive values for all patients is shown in Table 3.17 and for each group are shown in Table 3.18 and Figure 3.13. Comparison between the two groups was performed as described in 2.6.1.4.

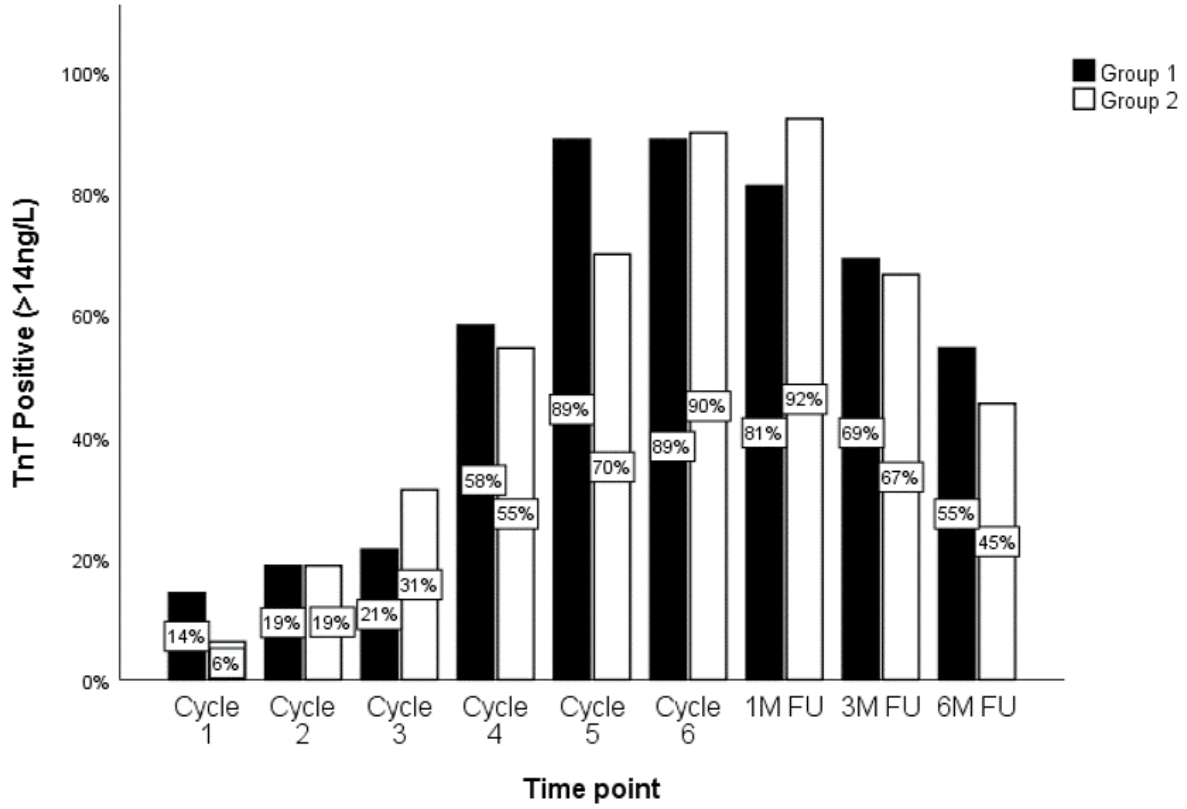
As can be seen from Figure 3.13 and Tables 3.17 and 3.18, TnT values remain positive one month after chemotherapy in up to 86% of patients (Group 1(RIC) 81%, Group 2 (Sham) 92%) after which they start to decrease, though even after 6 months from chemotherapy 50% of patients (Group 1(RIC), 55%, Group 2(Sham), 45%) still have a positive TnT. A chi-squared comparison at the follow-up time-points shows that, similar to the analysis during chemotherapy, there is no significant difference in the number of positive TnT samples between the two groups at each follow-up time-point (One month, p=0.39, Three month, p=0.885, Six month, p=0.67).

Table 3.17. Positive TnT trends for All patients during follow-up

	One month	Three months	Six months	Twelve months
	N = 29	N = 28	N = 22	N = 6
TnT +ve (%)				
Yes	25 (86)	19 (68)	11 (50)	2 (33)
No	4 (14)	9 (32)	11 (50)	4 (67)

Table 3.18. Positive TnT trends between Group 1 (RIC) and Group 2 (Sham) group during follow-up								
	One month		Three months		Six months		Twelve months	
	Group 1 (n=16)	Group 2 (n=13)	Group 1 (n=13)	Group 2 (n=15)	Group 1 (n=11)	Group 2 (n=11)	Group 1 (n=4)	Group 2 (n=2)
TnT +ve (%)								
Yes	13 (81)	12 (92)	9 (69)	10 (67)	6 (55)	5 (45)	0	2
No	3 (19)	1 (8)	4 (31)	5 (33)	5 (45)	6 (55)	4	0

Figure 3.13. Positive TnT Comparisons between the two groups during chemotherapy and follow-up (Group 1 = RIC, Group 2 = Sham)



3.3.4 Effect of Remote Ischaemic Conditioning on Troponin T during anthracycline chemotherapy Results Conclusion

In summary, due to the small N number, **the results can only be used as a hypothesis generating exercise.** Using the analysis shown above we can conclude that, anthracyclines cause an increase in TnT as patients progress with their chemotherapy that seems to persist for at least one month after the cessation of chemotherapy. RIC has no significant effect on this rise in troponin.

3.4 The Effect of Remote Ischaemic Conditioning (RIC) on echocardiographic parameters after anthracycline chemotherapy

Table 3.19 shows echocardiographic parameters for all patients and for each group before and three and twelve months after anthracycline chemotherapy. As with the TnT data, the 12 month data include only 8 patients who had an echocardiogram due either not having reached that part of follow-up yet or due to being unable to perform the scan because of the Covid-19 pandemic. Therefore, the 12 month time-point was not included in the statistical analysis. Statistical analysis and comparison between the two groups was performed as described in 2.6.15.

Table 3.19. Echocardiographic parameters before and after chemotherapy (Group 1 = RIC, Group 2 = Sham)				
	Baseline	Three months	Twelve Months	
LV Size Parameters				
LVEDd (cm) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 4.5 ± 0.4 4.5 ± 0.5 4.6 ± 0.4	 4.6 ± 0.5 (n=28) 4.5 ±0.5 (n=13) 4.6 ± 0.7 (n=15)	 4.6 ± 0.4 (n=8) 4.6 ± 0.3 (n=5) 4.7 ± 0.5 (n=3)	
LVEDs (cm) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 3.1 ± 0.3 (n=30) 3 ± 0.3 (n=15) 3.1 ± 0.3 (n=15)	 3.1 ±0.4 (n=26) 3.1 ± 0.4 (n=11) 3.1±0.4 (n=15	 3.0± 0.5 (n=8) 3.2± 0.3 n=5) 2.7 ± 0.6 (n=3)	
IVSd (cm) **All**	 0.94 ± 0.14	 0.91 ±0.22 (n=27)	 0.9 ± 0.1 (n=8)	

Group 1 **Group 2** **(Mean ± S.D)**	0.9 ± 0.2 0.9 ± 0.1	0.96 ± 0.26 (n=13) 0.87 ± 0.14 (n=14)	0.86 ± 0.1 (n=5) 0.97 ± 0.06 (n=3)	
LVPWd (cm) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 0.88 ± 0.16 0.9 ± 0.2 0.9 ± 0.2	 0.9 ±0.2 (n=27) 0.95 ±0.18 (n=13) 0.86 ± 0.22 (n=14)	 0.89 ± 0.1 (n=8) 0.84 ± 0.09 (n=5) 0.97 ± 0.06 (n=3)	
LV function parameters				
LVEF (%) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 61 ± 4 62 ± 4 59 ± 3	 60 ± 5 (n=28) 60 ± 5 (n=13) 60 ± 5 (n=15)	 58 ± 8 (n=8) 57 ± 5 (n=5) 59 ± 3 (n=3)	
LVEF method **All** **Visual** **Biplane** **Group 1** **Visual** **Biplane** **Group 2** **Visual** **Biplane**	 10 (31%) 22 (69%) 5 (31%) 11 (69%) 5 (31%) 11 (69%)	 12 (43%) 16 (57%) 4 (31%) 9 (69%) 8 (43%) 7 (57%)	 3 (38%) 3 (62%) 1 (20%) 4 (80%) 2 (67%) 1 (33%)	
GLS (%) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 -19 ± 2 (n=28) -19.2 ± 2 (n=15) -18.7 ± 3.1 (n=13)	 -18.3 ± 2.3 (n=21) -18.8 ± 1.7 (n=10) -17.9 ± 2.8 (n=11)	 -16.9 ± 2.8 (n=5) -16.7 ± 3.2 (n=4)	
Tissue Doppler parameters				
IVS S' (cm/s) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 9 ± 2 9 ± 2 9 ± 2	 8 ± 2 (n=26) 9 ± 2 (n=12) 8 ± 2 (n=14)	 9 ± 2 (n=8) 9 ± 3 (n=5) 8 ± 2 (n=3)	
IVS E' (cm/s) **All** **Group 1** **Group 2** **(Mean ± S.D)**	 10 ± 4 (n=31) 10 ± 4 (n=15) 9 ± 3 (n=16)	 8 ± 3 (n=24) 9 ± 3 (n=12) 8 ± 2 (n=12)	 8 ± 2 (n=8) 8 ± 2 (n=5) 8 ± 2 (n=3)	
Lateral S' (cm/s) **All[+]** **Group 1** **Group 2[+]** **(Mean ± S.D)**	 11 ± 2 11 ± 3 11 ± 2	 10 ± 2 (n=25) 9 ± 2 (n=12) 10 ±2 (n=13)	 10 ± 3 (n = 8) 10 ±3 (n=5) 10 ± 3 (n=3)	 p = 0.001
Lateral E' (cm/s)				

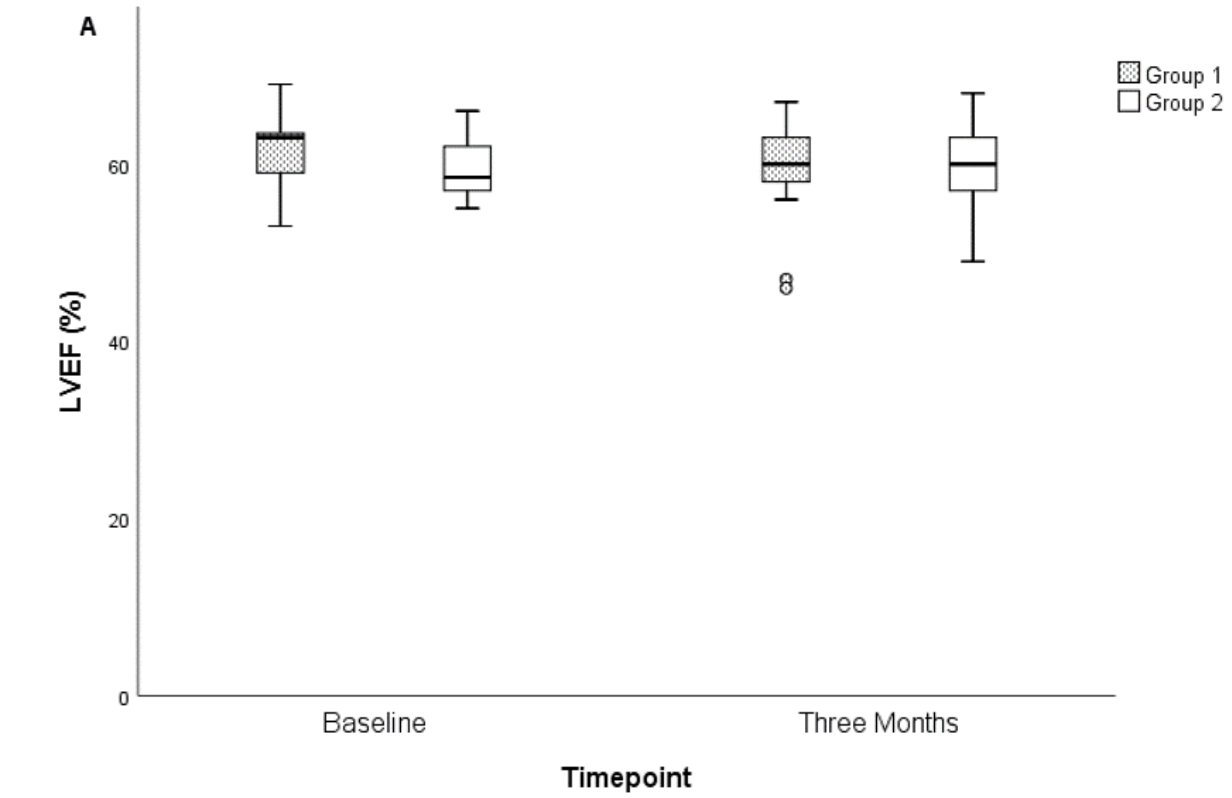
All§ **Group 1** **Group 2** **(Mean ± S.D)**	12 ± 4 (n=30) 12 ± 5 (n=15) 12 ± 4 (n=15)	10 ± 4 (n=24) 11 ± 5 (n=12) 9 ±3 (n=12)	10 ± 3 (n=8) 11 ± 4 (n=5) 9 ± 2 (n=3)	p = 0.022
E/E' **All** **Group 1** **Group 2** **(Mean ± S.D)**	0.7 ± 0.2 (n=30) 0.7 ± 0.2 (n=15) 0.7 ± 0.3 (n=15)	0.7 ± 0.2 (n=22) 0.7 ± 0.3 (n=10) 0.8 ± 0.2 (n=12)	1.5 ± 2.2 (n=8) 2 ± 2.8 (n=5) 0.76 ± 0.03 (n=3)	
Doppler parameters				
E (cm/s) **All** **Group 1±** **Group 2** **(Mean ± S.D)**	0.70 ± 0.16 (n=31) 0.72 ± 0.17 (n=15) 0.67 ± 0.15 (n=16)	0.59 ± 0.13 (n=24) 0.54 ± 0.13 (n=10) 0.62 ± 0.13 (n=14)	0.6± 1.9 (n=8) 1.7 ± 2.4 (n=5) 0.6 ± 0.04 (n=3)	P = 0.031
A (cm/s) **All** **Group 1** **Group 2** **(Mean ± S.D)**	0.66±0.15 (n=31) 0.65±0.15 (n=15) 0.68±0.16 (n=16)	0.63± 0.17 (n=24) 0.66± 0.22(n=10) 0.61±0.13 (n=14)	1.2± 1.8 (n=8) 1.7 ± 2.3 (n=5) 0.7 ± 0.06 (n=3)	
Deceleration Time (ms) **All** **Group 1** **Group 2** **(Mean ± S.D)**	206 ± 52 (n=28) 198 ± 51 (n=14) 213 ± 53 (n=14)	221 ± 81 (n=23) 213 ± 99 (n=10) 227 ± 68 (n=13)	206 ± 58 (n=8) 208 ± 58 (n=5) 203 ± 70 (n=3)	
E/A **All** **Group 1** **Group 2** **(Mean ± S.D)**	1.1 ± 0.38 (n=31) 1.2 ± 0.44 (n=15) 1 ± 0.29 (n=16)	0.99 ± 0.32 (n=24) 0.92 ± 0.39 (n=10) 1 ± 0.27 n=14)	0.94 ± 0.24 (n=8) 0.98 ± 0.29 (n=5) 0.86 ± 0.11 (n=3)	
RV function parameters				
RV S' (cm/s) **All** **Group 1** **Group 2** **(Mean ± S.D)**	13 ± 2 (n=27) 13 ± 2 (n=12) 14 ± 2 (n=15)	13 ± 2 (n=20) 13 ± 3 (n=11) 12 ± 2 (n=9)	13 ± 2 (n=5) 13 ± 3 (n=3)	
RV TAPSE (cm) **All** **Group 1** **Group 2** **(Mean ± S.D)**	2.2 ± 0.3 (n=31) 2.3 ± 0.3 (n=16) 2.2 ± 0.3 (n=15)	2.2 ± 0.4 (n=26) 2.2 ± 0.4 (n=13) 2.2 ± 0.3 (n=13)	1.9 ± 0.2 (n=7) 2 ± 0.2 (n=4) 1.8 ± 0.1 (n=3)	
Days from last				

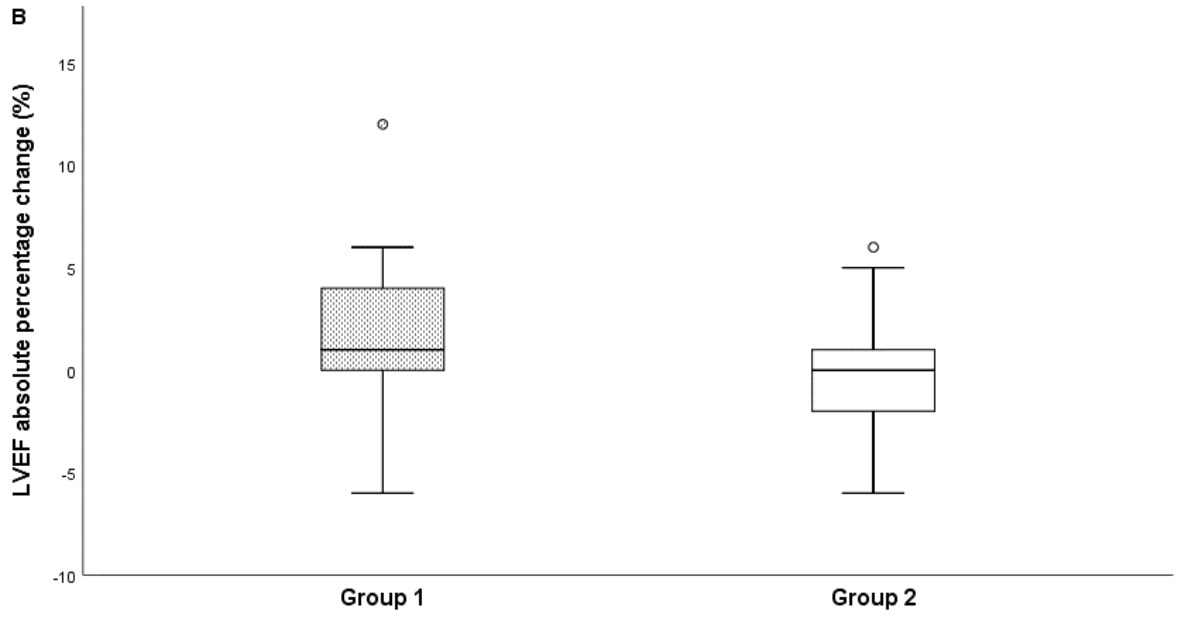
chemotherapy cycle All Group 1 Group 2 (Median ± IQR)		93 ± 33 (n=28) 105 ± 31 (n=13) 93 ± 36 (n=15)	394 ± 76 (n=8) 423 ± 88 (n=5) 365 (n=3)	
[+]All patients and Group 2 baseline vs 3 months, [§]All patients baseline vs 3 months, [±]Group 1 baseline vs 3 months, [**] LV Mass mean change from baseline Group 1 vs Group 2				

In total, 28 patients had echocardiograms at the 3 month time-point (missed scans due to: 1 death, 1 withdrawal, 2 Covid-19 pandemic) with a median time from last chemotherapy cycle of 93 ± 33 days (Group 1 (RIC), 105 ± 31, Group 2 (Sham), 93 ± 36).

Overall, there was no significant change in the mean LVEF from baseline (LVEF 61%) to 3 months post chemotherapy (LVEF 60%) with a mean decrease of 0.7% (95% CI -0.83-2.26, p=0.351). Figure 3.14 shows LVEF at baseline and three months post-chemotherapy for each group. For Group 1 (RIC), there is a small mean decrease from baseline to 3 months post chemotherapy of 1.8% (95% CI -0.956-4.496, p = 0.183) which is non-significant. For Group 2 (Sham), there is a small increase in the LVEF from baseline to 3 months post chemotherapy -0.2% (95% CI -2.1-1.7, p = 0.82) which is non-significant. When comparing the mean ΔLVEF from baseline between the two groups, there is no significant difference (mean ΔLVEF difference 1.96%, 95%CI -1.1-5.03, p=0.197) (Figure 3.14B).

Figure 3.14. A. LVEF changes from baseline to three months post chemotherapy for Group 1 (RIC) (dotted) and Group 2 (Sham) (white). B. LVEF absolute percentage change for Group 1 (RIC) (dotted) and Group 2 (Sham) (white).





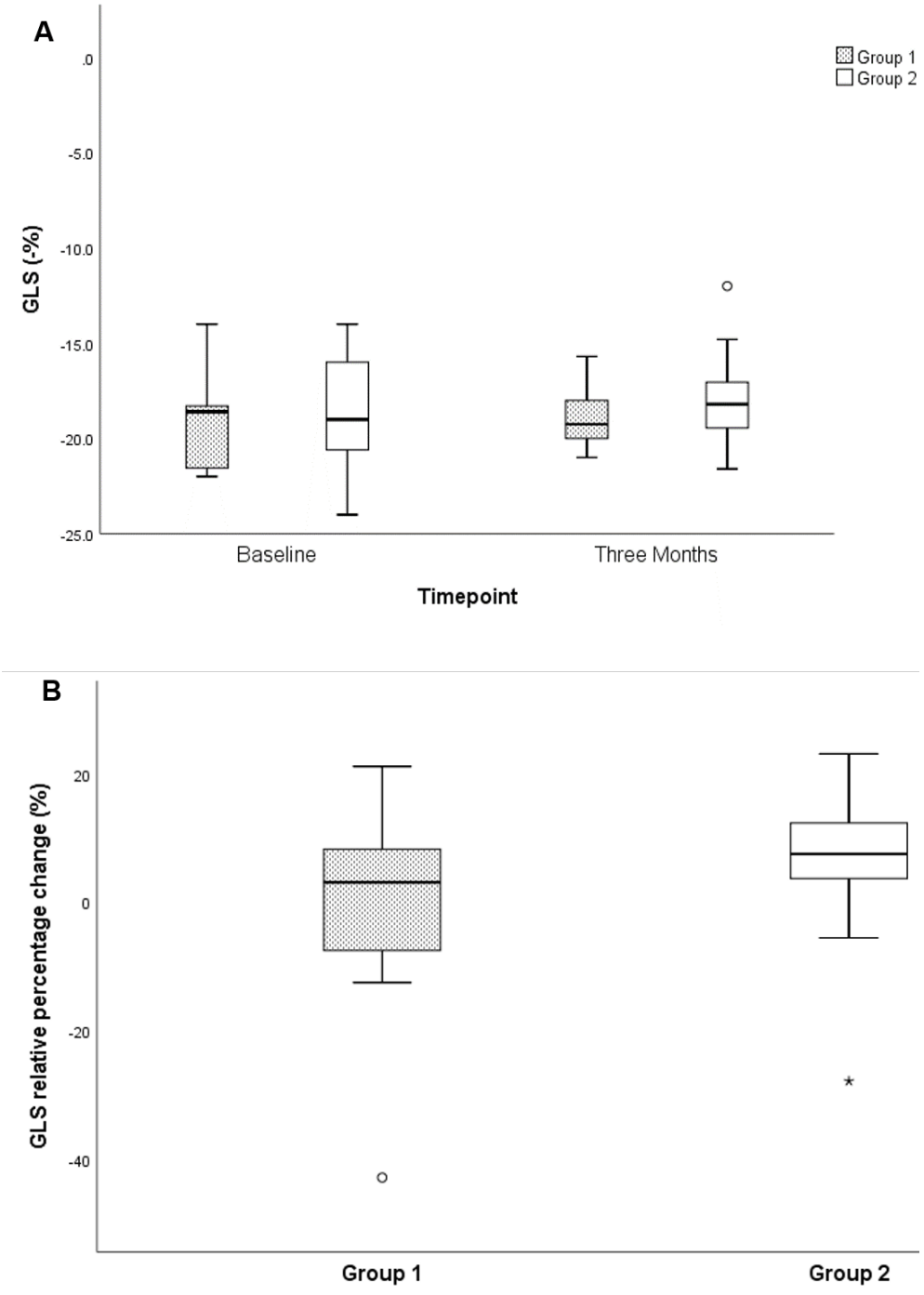
Similarly, there was no significant change in the mean GLS from baseline (GLS -19.1%) to 3 months post chemotherapy (GLS -18.5%) with a mean change (decrease) of -0.6% (95%CI -1.9-0.68, p=0.338). Figure 3.15 shows the GLS at baseline and 3 months post chemotherapy for each group. There is no significant difference for either group for the GLS at baseline and 3 months (Group 1 (RIC) GLS baseline vs 3 months -18.9% vs -18.8%, mean difference -0.1%, 95% CI -2.2-2, p=0.916; Group 2 (Sham) GLS baseline vs 3 months -19.2% vs -18.1%, mean difference -1.2%, 95% CI -3-0.67, p=0.181). When comparing the mean ΔGLS from baseline between the two groups, there is no significant difference (mean ΔGLS difference 1.1%, 95% CI -1.5-3.7, p=0.403). When considering the GLS relative percentage change from baseline, Group 1 (RIC) has a mean relative change of -1.2% and Group 2 (Sham) a mean relative change of 5.1% with a mean difference between the two groups of 6.4% (95% CI -9.6-22.4, p=0.411) that was not statistically significant (Figure 3.15).

One patient (Group 1 (RIC)) had a drop in his LVEF by 12% to 46% and a corresponding drop in his GLS of 2.9% to -15.7% (corresponding to a 16% relative change in GLS) with no heart failure symptoms but needing initiation of heart failure medication. A second patient (Group 2 (Sham)) had a drop in his LVEF by 6% to 49% and a corresponding drop in his GLS of 3.6% to -12% (corresponding to a 23% relative change in GLS) with associated heart failure symptoms and was initiated on heart failure medication by the study team. A third patient (Group 2 (Sham)) had no change in his LVEF (66% to 68%) but with an associated drop in his GLS of 3.2% to -14.8% (18% relative change in GLS) with no associated heart failure symptoms and is being monitored for any further deterioration. A fourth patient (Group 1 (RIC)) had a 21% change in his GLS (from –21.3% to -16.8%) but with an LVEF at 3 months that was unchanged at 63% (64% at baseline) who is also monitored for any further deterioration.

Of the other parameters listed in Table 3.19, only lateral S' and lateral E' velocity show a significant difference between baseline and 3 months for all patients with lateral S' velocity showing a decrease of 1.6cm/s (95% CI 0.75-2.5, p=0.001) and lateral E' velocity a decrease of 2.1cm/s (95% CI 0.34-3.9, p=0.022). A similar trend was seen for Group 2 (Sham) for the lateral S' velocity only (mean decrease 1.7cm/s, 95% CI 0.9-2.5, p=0.001) but not for Group 1 (RIC). In Group 1(RIC) there was a change in E velocity with a mean decrease of 0.1cm/s (95%CI 0.01-0.2, p=0.031) between baseline and 3 months. When comparing the changes from baseline between the two groups there are no significant differences in any of the parameters.

In summary, due to the small N number, the results can only be used as a hypothesis generating exercise. RIC does not appear to have an effect on LV function as measured by LVEF and GLS with no significant difference in the ΔLVEF and ΔGLS (absolute or relative) between the RIC and sham groups. There was also no change in EF and GLS from baseline to 3 months, however lateral S' and E' velocities were reduced compared to baseline, even though there was no difference in the change (i.e. Δ) in S' or E' velocity between the two groups.

Figure 3.16. A. GLS changes from baseline to three months post chemotherapy for Group 1 (RIC) (dotted) and Group 2 (Sham) (white). B. GLS relative percentage changes for Group 1 (RIC) (dotted) and Group 2 (Sham) (white).



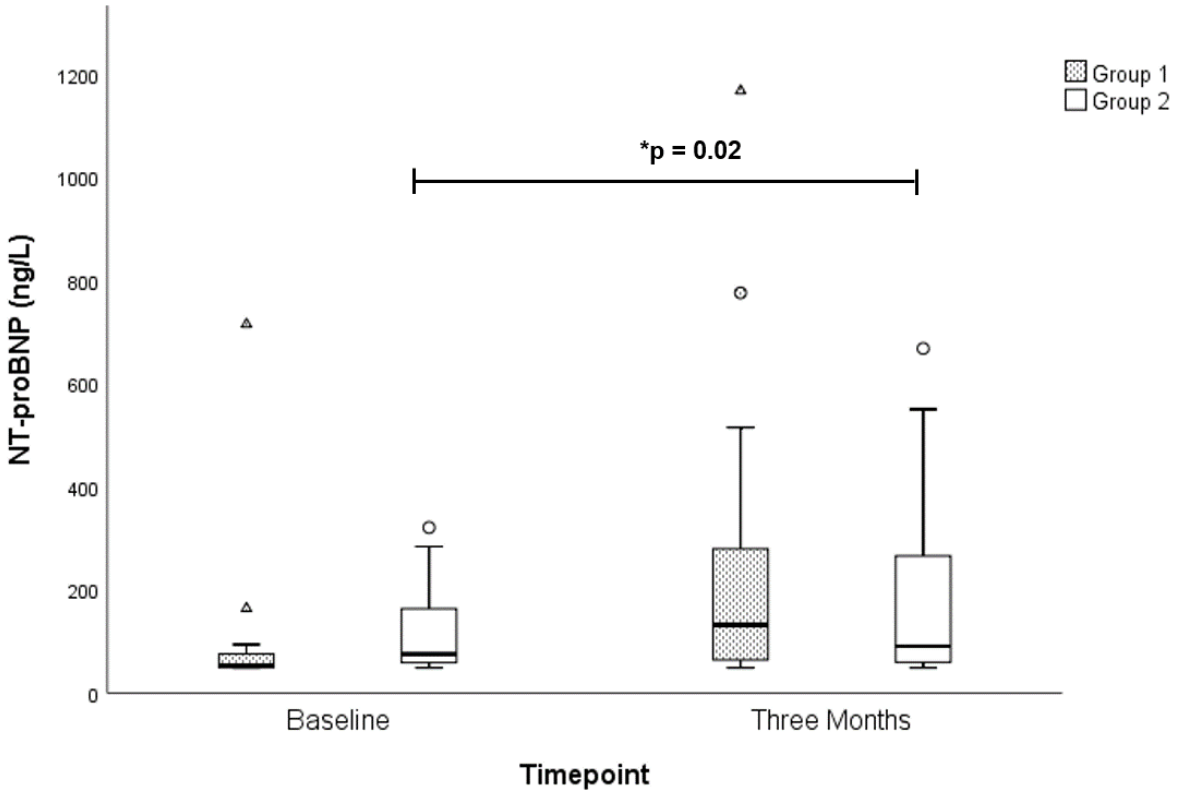
3.5 The Effect of Remote Ischaemic Conditioning (RIC) on NT-proBNP after anthracycline chemotherapy

The effect of RIC on NT-pro-BNP was assessed for all patients and for each group individually. For all patients and for each group, comparisons of how NT-proBNP changes before and after chemotherapy was made as described in 2.6.1.6. Figure 3.17 shows how NT-proBNP changes before and after chemotherapy.

There is a trend of NT-proBNP increasing from baseline to three months with a median increase from 68ng/L to 115ng/L (p=0.003) that is also significant for Group 2 (Sham) only (p=0.02) (Table 3.20, Figure 3.17). However, when comparing the mean ΔNT-proBNP from baseline between the two groups there is no significant difference (p=0.913). This suggests that there is no effect of RIC in the NT-proBNP levels after anthracycline chemotherapy, even though there is a suggestion that NT-proBNP levels increase after anthracycline chemotherapy, albeit at levels below the 400ng/L cut-off for the upper limit of normal. Due to the small N number however, the results can only be used as a hypothesis generating exercise.

Table 3.20. NT-pro-BNP at before and after chemotherapy (Group 1 = RIC, Group 2 = Sham)			
	Baseline	Three months	
NT-proBNP (ng/L) **All*** **Group 1** **Group 2±** **(Median ± IQR)** **Normal <400)**	 68 ± 63 (n=30) 54 ± 32 (n=14) 76 ± 123 (n=16)	 115 ± 274 (n=31) 132 ± 274 (n=15) 91 ± 252 (n=16)	 p=0.003 p=0.02
***p=0.003 baseline vs three months, ±p=0.02 baseline vs three months**			

Figure 3.17. Boxplot of NT-proBNP per time-point for each group (Group 1 (RIC)=dotted, Group 2 (Sham)=white). p=0.02 vs baseline



3.6 The Effect of Remote Ischaemic Conditioning (RIC) on Clinical Events

Major adverse cardiovascular and cancer events (MACCE) were defined as described in 2.5.1.2.3.

3.6.1 MACCE and cancer progression

The time to a MACCE event was analysed and compared for each group. Table 3.21 shows the MACCE events in all patients and in each group. The analysis, as before, included events up to the six-month follow-up appointment from the end of chemotherapy. Thus, any events from the start of chemotherapy (to capture events that occurred during chemotherapy) until the 6-month follow up appointment were recorded. Statistical comparison between the two groups was performed as described in 2.6.1.7 Of the 32 patients, 2 (1 from each group) had missed their 6 month follow-up appointment due to the

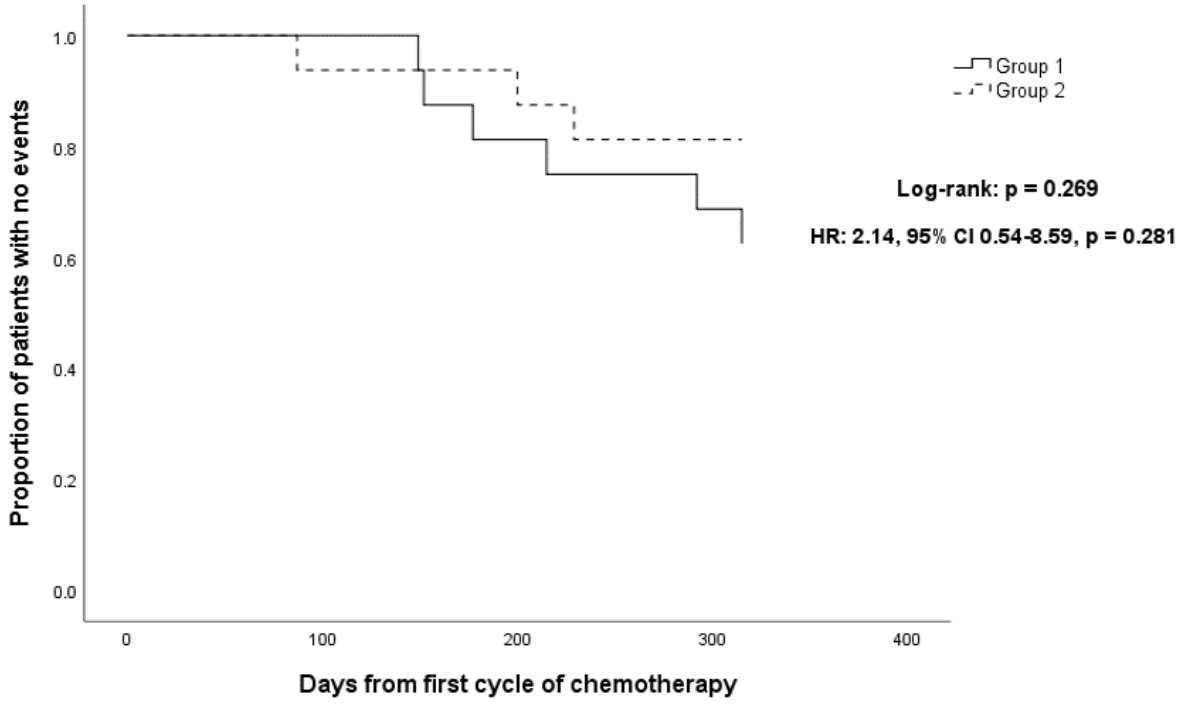
Covid-19 pandemic and 1 (Group 1 (RIC)) had not reached that time-point yet at the time of analysis. The three patients were still included in the analysis and any events were recorded up until their last follow-up. The one patient who withdrew after the 3-month follow-up was also included as intention-to-treat and any events recorded up to that appointment.

Table 3.21. Major Adverse Cardiovascular and Cancer events (Group 1 = RIC, Group 2 = Sham)

	All patients N = 32	Group 1 N = 16	Group 2 N = 16
All MACCE events (%)	9 (28)	6 (38)	3 (19)
Cardiovascular Events (%)	4 (13)	2 (13)	2 (13)
Arrhythmias (%)	2 (6)	1 (6)	1 (6)
Heart Failure (%)	2 (6)	1 (6)	1 (6)
Cancer Deaths (%)	5 (16)	4 (25)	1 (6)
Cancer Progression (%)	8 (25)	4 (25)	4 (25)

In total, 9 (28%) patients had a pre-specified MACCE event (6 (38%) in Group 1 (RIC) and 3 (19%) in Group 2 (Sham)). Of those, 4 (13%) (2 (13%) in each group) were cardiovascular events and 5(16%) (4 (25%) in Group 1(RIC) and 1 (6%) in Group 2(Sham)) were cancer deaths. Of the cardiovascular events, 2 (6%) (1 (6%) in each group) were episodes of asymptomatic non-sustained ventricular tachycardia that was picked up by the 14-day cardiac monitor at the end of chemotherapy and was significant enough to warrant initiation of treatment with beta blockers. The other 2 (6%) were a new diagnosis of heart failure or asymptomatic reduction in LV function that needed initiation of medication (1 (6%) in Group 1 (RIC) with asymptomatic deterioration in LV function (LVEF drop from 58% to 46%) and 1 (6%) in Group 2(Sham) with symptomatic heart failure and deterioration in LV function (LVEF drop from 55% to 49%)).

Figure 3.18. Kaplan-Meier plot of Major Adverse Cardiovascular and Cancer Events (MACCE) for each group (Group 1(RIC) = continuous, Group 2 (Sham) = broken).



Of the cancer deaths, 4 (3 in Group 1(RIC), 1 in Group 2(Sham)) patients had sarcoma (3 metastatic and one with local involvement but not distant metastases) at the beginning of the study. Their details are as follows: 1 patient with Stage IV lower limb rhabdomyosarcoma with lung metastases with disease progression despite first and second line chemotherapy and subsequent brain metastases; 1 patient with high grade leiomyosarcoma of the uterus with lung and peritoneal metastases treated with chemotherapy with palliative intend and subsequent disease progression despite 4th line chemotherapy; 1 patient with high grade spindle cell sarcoma of lower limb with lung metastases treated with palliative intend and subsequent disease progression despite 3rd line chemotherapy; 1 patient with poorly differentiated Stage III high grade spindle cell sarcoma of the adrenal gland with pleural and diaphragmatic involvement but no distant metastases treated with 1st line chemotherapy and pre-operative radiotherapy but with

subsequent radiotherapy induced pneumonitis and subsequent lung metastases). All 4 were felt to be expected outcomes due to their cancer diagnosis. The 5th patient had follicular T cell lymphoma with good response to initial chemotherapy who subsequently underwent autologous stem cell transplant and died due to complications post-transplant with details as follows: prolonged post-transplant admission due to severe nausea and vomiting and mucositis. Re-admitted with ongoing diarrhea and vomiting due to cytomegalovirus colitis and after 2 months in hospital deteriorated with Acute Respiratory Distress syndrome and multi-organ failure needing ITU admission and subsequent death.

As seen in Figure 3.18 there was no significant difference in the MACCE events between the two groups with a log-rank test p value of 0.269. Cox regression suggests that the risk of an event is 2.14 higher in Group 1(RIC) than Group 2(Sham) though this is not statistically significant with a 95% CI of 0.54-8.59, p=0.281. However, due to the small N number, the results can only be used as a hypothesis generating exercise.

The effect of RIC on cardiovascular events and cancer deaths separately was analysed in a similar fashion. Figures 3.19 and 3.20 show the Kaplan-Meier plots for cardiovascular events and cancer deaths respectively. There is no significant difference in cardiovascular events between the two groups with a Log-rank test p value of 0.999. Cox regression shows a hazard ratio of 1 with a 95% CI of 0.14-7.11 (p=0.999). There is no significant difference in cancer deaths between the two groups with a Log-rank test p value of 0.181. Cox regression analysis suggests that the risk of cancer death in Group 1(RIC) is 4.35 higher than Group 2 (Sham), however this was not statistically significant with a 95% CI of 0.49-38.9, p=0.189.

The effect of RIC on time to cancer progression was also analysed in a similar fashion. Time to cancer progression was defined if there was disease progression for the first time since initiation of anthracycline-containing chemotherapy. If there was further disease progression subsequently (usually in the context of second (or more) line of chemotherapy), this was not recorded as an event. There were a total of 8 cancer progressions (4 in each group) (Table 3.21). Figure 3.21 shows the Kaplan-Meier plot for time to cancer progression. There is no significant difference in time to cancer progression between the two groups with a log-rank test p value of 0.99. Cox regression shows a hazard ration of 0.99 with a 95% CI of 0.248-3.97, p=0.99.

Figure 3.19. Kaplan-Meier plot of Cardiovascular Events (MACCE) for each group (Group 1(RIC) = continuous, Group 2 (Sham) = broken).

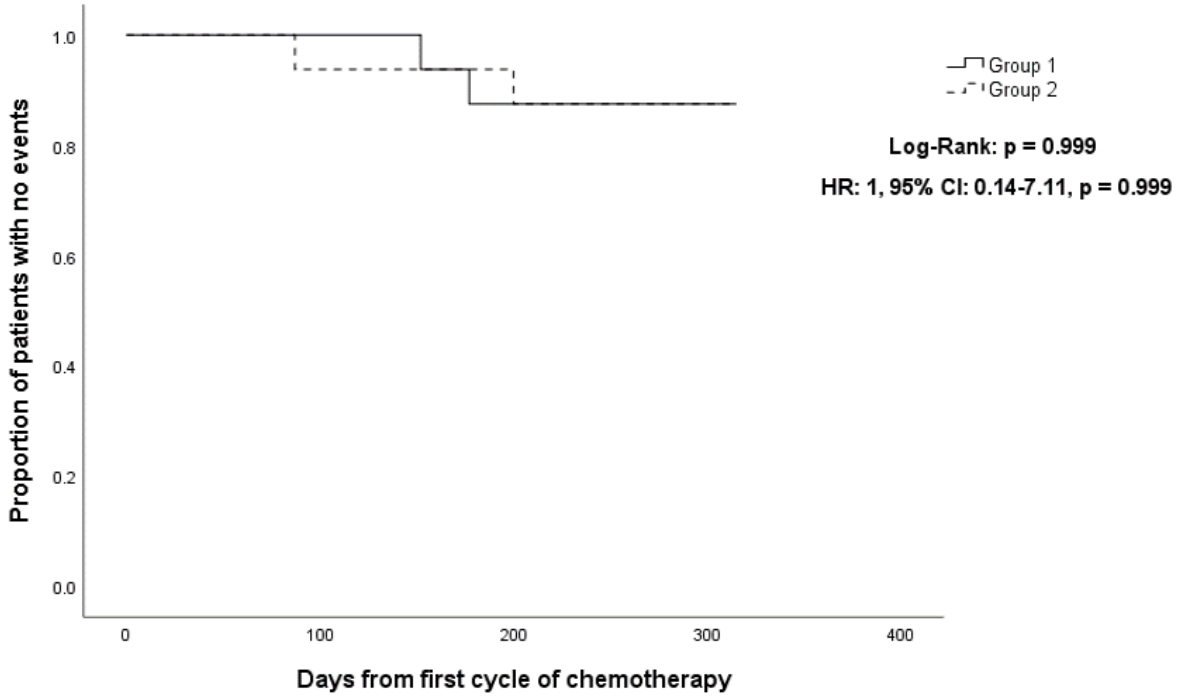


Figure 3.20. Kaplan-Meier plot of Cancer Deaths for each group (Group 1 (RIC) = continuous, Group 2(Sham) = broken).

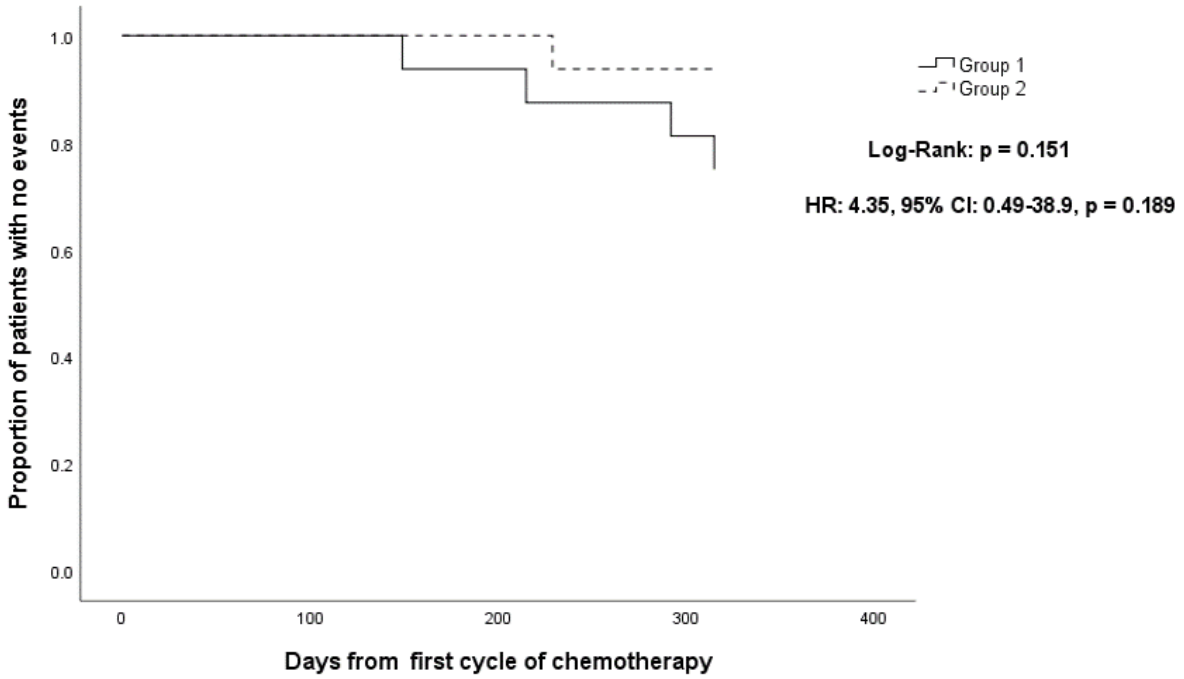
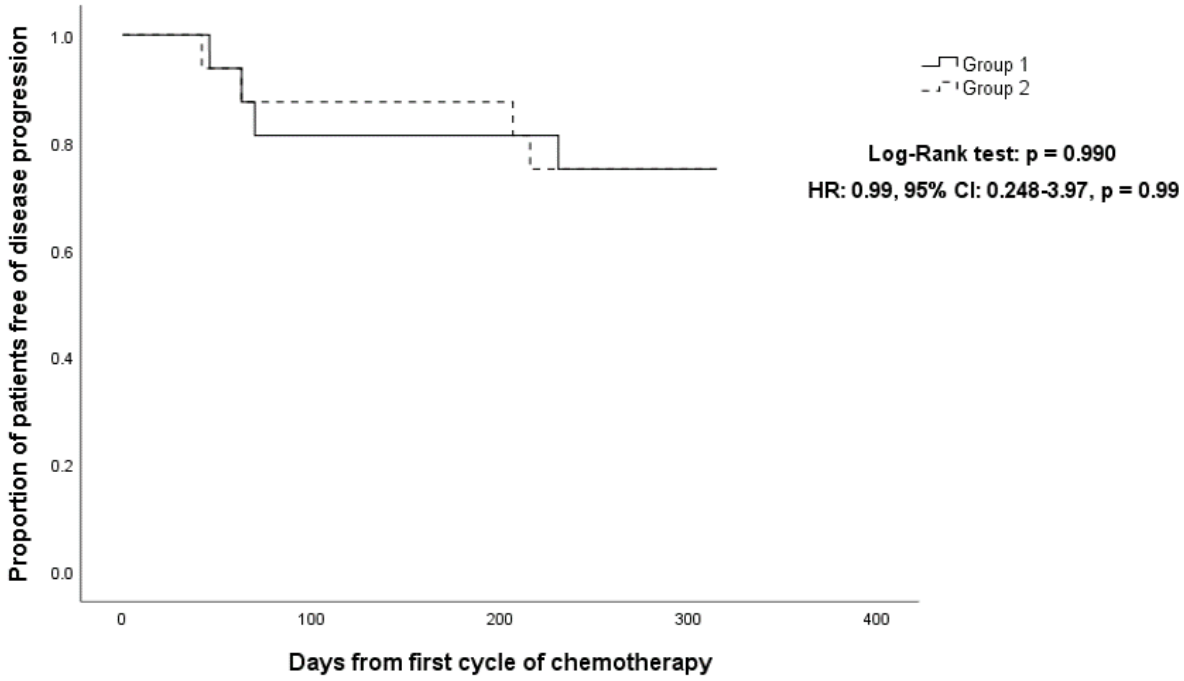


Figure 3.21. Kaplan-Meier plot of Time to Cancer progression for each group (Group 1 (RIC) = continuous, Group 2 (Sham) = broken).



3.6.2 Clinical Adverse Events

As part of the serious adverse event reporting of the study, any significant clinical events were recorded for all patients during the study. These were identified by asking patients on each visit of any serious adverse events such as important medical events or unexpected hospitalisations as well as looking at hospital records. Table 3.22 shows serious adverse event identified during the study and if each patient had any of these events. Some patients had the same adverse event more than once, so Table 3.23 shows the total number for each adverse event. Comparison between the two groups was done as described in 2.6.1.7.

In total, 27 (84%) patients had at least one serious adverse event (Group 1(RIC), 14 (88%), Group 2(Sham), 16 (81%)) with no difference between the two groups. The most common adverse event was any infection with 21 patients (66%) having an infection (Group 1 (RIC), 13 (81%), Group 2 (Sham), 8 (50%), p=0.063) with no difference between the two groups. Infections included episodes of hospitalisation due to either sepsis or fevers with or without the presence of neutropenia where no source for the fever/sepsis was identified and was in fact the commonest adverse event. A total of 17 (53%) patients were admitted at least once with sepsis or fevers with or without neutropenia and there was a trend of more admissions for Group 1 (RIC) vs Group 2 (Sham) (12 (75%) vs 5 (31%, p=0.013)(Table 3.22).

There were 52 adverse events recorded in total (Group 1 (RIC) = 30, Group 2 (Sham) = 22). Of those 52, 37 (71%) were due to any infection with no overall difference between the groups (Group 1 (RIC), 24 (80%), Group 2 (Sham), 13 (59%), p=0.1). However, when looking at sepsis or fevers with or without neutropenia, a similar trend was observed as with above, with a total of 21 (40%) events that was more common in Group 1 (RIC) vs

Group 2 (Sham) (16 (53%) vs 5 (23%), p=0.026) (Table 3.23**).** However, due to the small N number, the results can only be used as a hypothesis generating exercise.

Table 3.22. Serious Adverse Events (Group 1 = RIC, Group 2 = Sham)				
	All patients N = 32	Group 1 N = 16	Group 2 N = 16	
Any event (%)	27 (84)	14 (88)	13 (81)	
Any infection (%)	21 (66)	13 (81)	8 (50)	
Sepsis or Fever with/without neutropenia (%)*	17 (53)	12 (75)	5 (31)	p=0.013
Mucositis/periodontitis (%)	2 (6)	1 (6)	1 (6)	
Chest infection (including pneumonia) (%)	5 (16)	2 (13)	3 (19)	
Septic joint (%)	1 (3)	0	1 (6)	
Urine infection (including pyelonephritis) (%)	1 (3)	1 (6)	0	
Abscess (%)	1	1 (6)	0	
Cellulitis or wound infection (%)	4 (13)	2 (13)	2 (13)	
Infective colitis or diarrhoea (%)	1 (3)	0	1 (6)	
VTE (%)	3 (9)	2 (13)	1 (6)	
Anaemia requiring transfusion (%)	4 (13)	1 (6)	3 (19)	
Nausea, vomiting and diarrhoea needing hospitalisation (%)	1 (3)	0	1 (6)	
Other (%)	6 (19)	2 (13)	4 (25)	
*p=0.013 Sepsis or fever with/without neutropenia Group 1 vs Group 2				

Table 3.23. Total number of Serious Adverse Events (Group 1 = RIC, Group 2 = Sham)				
	All Events N = 52	Group 1 N = 30 (58)	Group 2 N = 22 (42)	
Any infection (%)	37 (71)	24 (80)	13 (59)	
Sepsis or Fever with/without neutropenia (%)*	21 (40)	16 (53)	5 (23)	p=0.026
Mucositis/periodontitis (%)	2 (4)	1 (3)	1 (5)	
Chest infection (including pneumonia) (%)	6 (12)	2 (7)	4 (18)	
Septic joint (%)	1 (2)	0	1 (5)	
Urine infection (including pyelonephritis) (%)	1 (2)	1 (3)	0	
Abscess (%)	1 (2)	1 (3)	0	
Cellulitis or wound infection (%)	4 (8)	2 (7)	2 (9)	
Infective colitis or diarrhoea (%)	1 (2)	1 (3)	0	
VTE (%)	3 (6)	2 (7)	1 (5)	
Anaemia requiring transfusion (%)	4 (8)	1 (3)	3 (14)	
Nausea, vomiting and diarrhoea needing hospitalisation (%)	1 (2)	1 (3)	0	
Other (%)	7 (14)	2 (7)	5 (23)	
*p=0.026 Sepsis or fever with/without neutropenia Group 1 vs Group 2				

3.7 The Effect of Remote Ischaemic Conditioning (RIC) on the incidence of arrhythmias during anthracycline chemotherapy

As part of the study protocol to investigate the incidence of arrhythmias in this cohort and the effect, if any, of RIC on the incidence of arrhythmias during chemotherapy, patients had a 14-day cardiac monitor patch applied on them on their penultimate or ultimate chemotherapy cycle as described in 2.4.4. Of the 32 patients, 4 (3 Group 2 (Sham), 1 Group 1(RIC)) did not have a cardiac monitor applied (2 due to Covid-19 pandemic, 1 declined, 1 had chemotherapy put on hold for several months to have surgery and subsequently decided not to have further chemotherapy, at which point was out of the time-window for the cardiac monitor to be applied). One patient had the monitor applied at

the one-month follow-up as chemotherapy ended prior to the intended 6 cycles for clinical reasons. Abnormal findings for all patients and each group are shown in Table 3.24. Comparisons were made as described in 2.6.1.8.

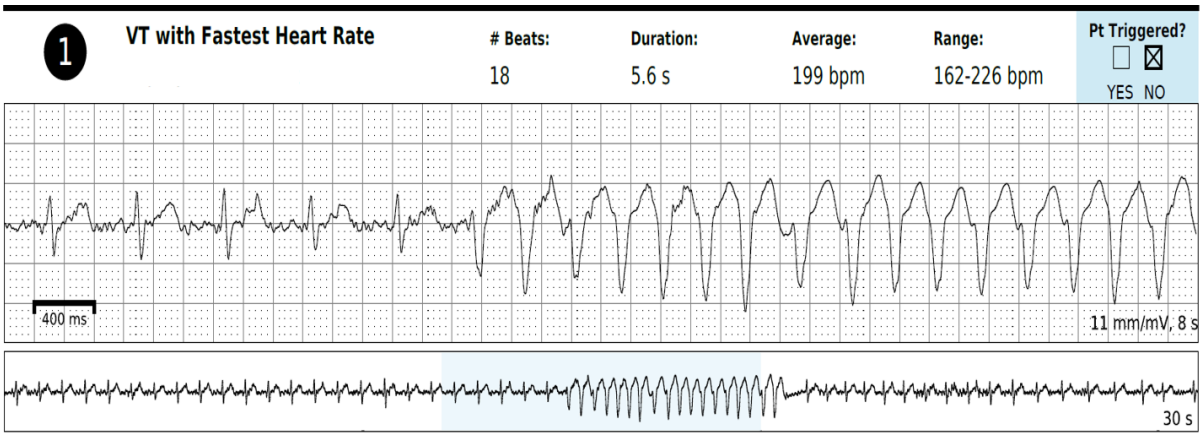
On average, the cardiac monitor was worn for 11 days. Of the 28 patients who had cardiac rhythm monitoring, 9 (32%)(Group 1 (RIC), 3 (20%), Group 2(Sham), 6 (46%) had at least one episode of non-sustained VT (defined as per the European Heart Rhythm Association (EHRA) as 3 or more consecutive ventricular beats terminating spontaneously in less than 30 seconds with a cycle length <600ms (>100bpm)(283)). In two patients (one in each group) this was deemed significant enough by the study team to require treatment with beta blockers. An example of a non-sustained VT episode that treatment was started is shown in Figure 3.22. Episodes of non-sustained supraventricular tachycardias (SVTs) were more common with 19 patients (68%) (Group 1(RIC), 11 (73%), Group 2(Sham), 8 (61%)) experiencing at least one episode. Two patients (both group 2 (Sham)) developed transient Type 2 AV block, none of which required any pacing. The burden of supraventricular and ventricular ectopics was similar in both groups as shown in Table 3.24. There were no significant differences between the two groups in any of the abnormal findings detected.

Table 3.24. Cardiac Arrhythmias during chemotherapy (Group 1 = RIC, Group 2 = Sham)

	All N = 28	Group 1 N = 15	Group 2 N = 13
Total days worn (Mean ±SD)	11 ± 4	11 ± 4	11 ± 4
Supraventricular Isolated SVEs (%)			
None	0	0	0
Rare (<1%)	26 (92)	15	11 (85)
Occasional (1-5%)	1 (4)	0	1 (8)
Frequent (>5%)	1 (4)	0	1 (8)
Couplet SVEs (%)			
None	2 (8)	2 (13)	0
Rare	26 (92)	13 (87)	13
Occasional	0	0	0

Frequent	0	0	0
Triplet SVEs (%)			
None	11 (39)	6 (40)	5 (39)
Rare	17 (61)	9 (60)	8 (62)
Occasional	0	0	0
Frequent	0	0	0
NS SVT (1 or more)(%)			
No	9 (32)	4 (27)	5 (39)
Yes	19 (68)	11 (73)	8 (61)
Ventricular Isolated VEs (%)			
None	0	0	0
Rare (<1%)	24 (86)	12 (80)	12 (92)
Occasional (1-5%)	4 (14)	3 (20)	1 (8)
Frequent (>5%)	0	0	0
Couplet VEs (%)			
None	9 (32)	6 (40)	3 (23)
Rare	19 (68)	9 (60)	10 (77)
Occasional	0	0	0
Frequent	0	0	0
Triplet VEs (%)			
None	23 (82)	14 (93)	9 (69)
Rare	5 (18)	1 (7)	4 (31)
Occasional	0	0	0
Frequent	0	0	0
NS VT (1 or more)(%)			
No	19 (68)	12 (80)	7 (54)
Yes	9 (32)	3 (20)	6 (46)
AV Block (%)			
No	26 (93)	15	11 (85)
Yes	2 (7)	0	2 (15)
2nd Type 1 (Wekenbach)	1		1
2nd Type 2	1		1
Heart Rate variability (bpm) (Mean ± SD)			
Minimum HR	51 ± 9	53 ± 7	49 ± 10
Maximum HR	158 ± 18	161 ± 13	154 ± 23
Mean HR	83 ± 10	86 ± 10	80 ± 9

Figure 3.22. Example of an episode of non-sustained VT detected by the cardiac monitor after cycle 5 (after 375mg/m^2 of doxorubicin) in a 33-year-old with pleomorphic sarcoma in the leg treated with the MAP regime and surgery.



3.8. Results Conclusion

In summary, using the analyses above we can conclude there is no significant difference between the RIC and sham groups on anthracycline cardiotoxicity as measured by TnT changes, and thus there is not enough evidence to reject the Null Hypothesis. Furthermore, RIC as applied in this protocol, does not appear to have any effect on the secondary outcomes of LV function as measured by echocardiography, MACCE and arrhythmia outcomes. In addition, RIC does not affect cancer progression and overall serious adverse events. However, numerically more patients appear to have had an infection in Group 1 (RIC) which was non-significant but there was a trend of significance when looking at admissions with sepsis or fevers with or without neutropenia in Group 1 (RIC). However, due to the small N number, the results can only be used as a hypothesis generating exercise.

Chapter 4 Multimodality monitoring of anthracycline cardiotoxicity

4.1 Identifying risk prior to initiation of chemotherapy

One of the key challenges in the field of cardio-oncology, remains identifying which patients may be at risk of developing anthracycline cardiotoxicity. Achieving this would allow identification of those patients who would benefit from closer monitoring during and after chemotherapy, intervene earlier and thus prevent the development of cardiomyopathy. Therefore, the relationship between baseline risk factors using the QRISK@3 score and peak TnT was investigated as described in 2.5.2.1 and 2.6.2.1.

Table 4.1 shows the QRISK@3 scores and peak TnT values for all patients and for each group. Median QRISK@3 score was 6.9% (Group 1 (RIC), 7.2%, Group 2 (Sham), 6.9%, p=0.749), median peak TnT was 34.5ng/L (Group 1 (RIC), 36.5ng/L, Group 2 (Sham), 31.5 ng/L, p=0.585). In the majority (63%) of patients the peak TnT was detected one month after chemotherapy, and in 20% of cases TnT peaked 3 months after chemotherapy.

Table 4.1. Baseline QRISK@3 score and peak Troponin T (TnT)

	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16	
QRISK@3 score (%) (median ± IQR) **Min** **Max**	6.9 ± 10 0.1 33.4	7.2 ± 11.3 0.2 18.8	6.9 ± 8.8 0.1 33.4	p=0.749
Peak TnT (ng/L) (median ± IQR)	34.5 ± 40	36.5 ± 65	31.5 ± 39	p=0.585
Peak TnT time-point (%) **Cycle 3** **Cycle 4** **Cycle 5** **1M FU** **3M FU** **6M FU**	 3 (9) 1 (3) 1 (3) 20 (63) 6 (19) 1 (3)	 2 (13) 0 1 (6) 10 (63) 3 (19) 0	 1 (6) 1 (6) 0 10 (63) 3 (19) 1 (6)	p=0.649

Simple linear regression was carried out to investigate the relationship between peak TnT and baseline QRISK@3 score for all 32 patients. The scatter-dot plot shown in Figure 4.2 showed only a weak correlation between peak TnT and QRISK@3 score with a Spearmann's correlation coefficient (ρ) of 0.353 with a p value of 0.047 (Pearson's correlation coefficient, r=0.155, p=0.397). Linear regression shows that there is no significant relationship between QRISK@3 score and peak TnT (p=0.397) and a slope coefficient, β, for QRISK@3 score of 0.621 (95% CI -0.857-2.1). The R^2 value was 0.024 suggesting that only 2.4% of the variability in peak TnT can be explained by baseline QRISK@3 score. The scatterplot of standardised predicted values against standardised residuals shows that the data met the assumption of homogeneity and the histogram of the residuals shows that they were generally normally distributed.

Correlation associations and simple linear regression analysis was carried out for each group individually with results that follow the same pattern as for the whole cohort and summarised in Table 4.2. Scatterplots of standardised predicted values against standardised residuals and residuals histogram for model checking were normally distributed.

Two patients developed heart failure or asymptomatic deterioration in LV dysfunction requiring medications within 6 months post chemotherapy. The first patient with symptomatic heart failure and a drop of his LVEF to 40% had a baseline QRISK@3 score of 8.6% (aged 54) and a peak TnT of 46ng/L detected 6 months post chemotherapy. The second patient who developed asymptomatic LV dysfunction with a drop in LVEF to 48% had a baseline QRISK@3 score of 11.9% (aged 58) and a peak TnT of 87 ng/L detected 1 month post chemotherapy. Total cumulative doxorubicin dose were 225 and 375mg/m^2 respectively.

Figure 4.2 Scatter-Dot plot of peak TnT(ng/L) against QRISK@3 score (%) for All patients.

ρ = Spearmann's correlation coefficient, p=0.047

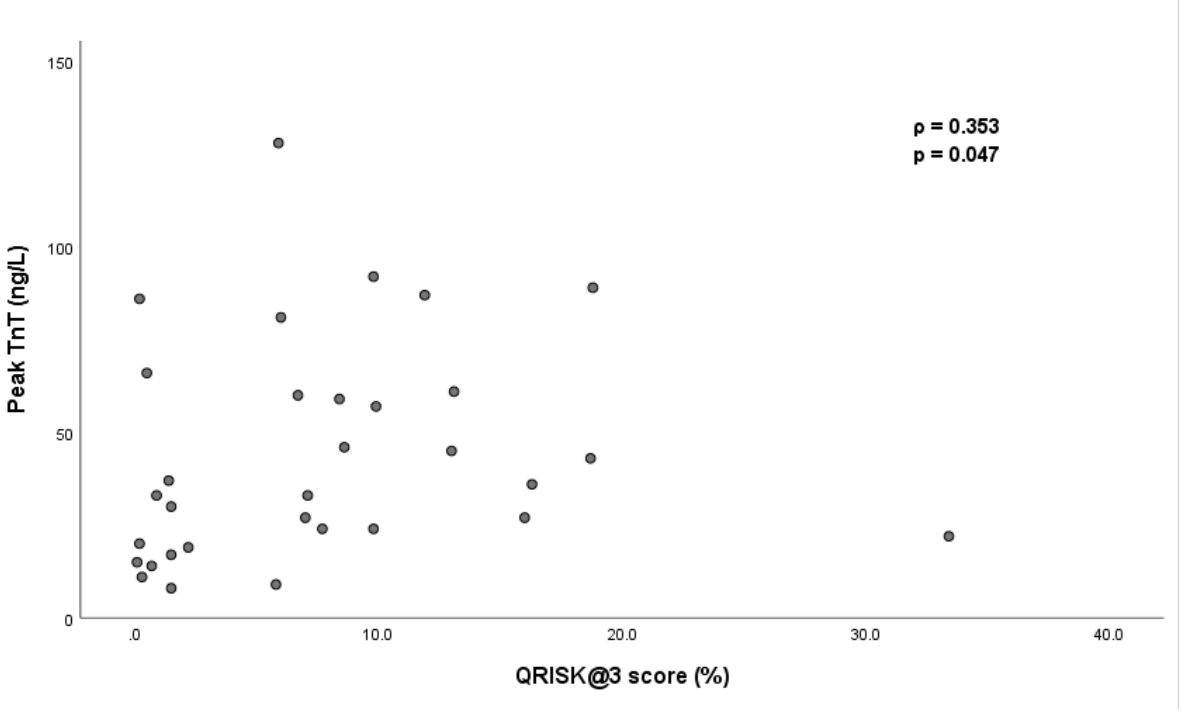


Table 4.2. Summary of simple linear regression analysis for All patients and each group			
	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
Spearmann's correlation coefficient, ρ	0.353 (p=0.047)	0.37 (p=0.159)	0.303 (p=0.254)
Slope coefficient, β **95% CI**	0.621 -0.857-2.1 (p=0.397)	1.714 -0.818-4.246 (p=0.169)	0.013 -1.989-2.015 (p=0.989)

4.1.3 Identifying risk prior to initiation of chemotherapy – Troponin T relationship with baseline cardiovascular risk scores – Results Conclusion

In summary, baseline cardiovascular risk factors as measured by the QRISK@3 score show only a weak correlation with peak TnT during and after anthracycline chemotherapy. There is no significant relationship on linear regression analysis between baseline QRISK@3 score and peak TnT values during or after chemotherapy.

4.2 Identifying at risk patients during and after chemotherapy using biomarkers

4.2.1 Troponin T as a binary categorical variable

Using cardiac biomarkers to identify patients at risk of anthracycline cardiotoxicity and subsequent cardiomyopathy during and after chemotherapy remains a hotly debated subject in the field of cardio-oncology with inconsistencies in the guidance from various international societies.

In this cohort, three different TnT patterns were identified when using TnT as a binary variable as shown in Figure 4.3. Of the 32 patients, 4 (13%) had a negative TnT throughout chemotherapy and follow-up (TnT -/-), 7 (22%) had a negative Early TnT but positive Late TnT (TnT -/+) and 21 (66%) had a positive Early and Late TnT (TnT +/+). Therefore, 87% of patients had at least one positive TnT during chemotherapy or follow-up. The mean early TnT for each group was 9±3.7ng/L (median 8.5ng/L) (TnT -/-), 10.4±3.6ng/L (median 12ng/L) (Tn-/+) and 36.4±15.4ng/L (median 37ng/L) (TnT +/+)(Figure 5.4). The mean late TnT for each group was 9.75±1.75ng/L (median 10ng/L) (TnT -/-), 24.4±10.7ng/L (median 20ng/L) (TnT -/+, $p=0.016$ vs early TnT) and 56.4±28.5ng/L (median 54ng/L) (TnT +/+, $p < 0.001$ vs early TnT). Linear regression suggested that early TnT in TnT+/+ group was 27.4ng/L and 26ng/L higher than TnT-/- and TnT -/+ respectively ($p \leq 0.001$ vs TnT -/- and TnT -/+) and late TnT in TnT+/+ group to

be 46.7ng/L and 32ng/L higher than TnT-/- and TnT-/+ respectively (p=0.001 vs TnT -/-, p=0.005 vs TnT -/+). Scatterplots of residuals against predicted values for each group suggests residuals are normally distributed around 0.

The patient who subsequently developed symptomatic heart failure with drop in LVEF to 49% belonged to group TnT -/+ and the patient who developed asymptomatic deterioration in his LV function belonged to group TnT +/+. Furthermore, the two patients who had asymptomatic non-sustained VT needing treatment both belonged to the TnT +/+ group. A correlation assessment between TnT positivity and echocardiographic and clinical cardiovascular events was not performed due to low event rate.

The above analysis was repeated for each randomisation group with results as shown in Table 4.3. There were similar trends between the RIC/sham groups and all patients in terms of absolute numbers in the different TnT groups, mean Early and Late TnT values for each TnT groups and comparisons of early and late TnT within and between TnT groups. On certain instances the statistical significance for the RIC/Sham group was different compared to the whole patient cohort analysis as highlighted in Table 4.3, though the overall trend was still similar to the whole patient cohort for those particular variables.

Figure 4.3 Early and Late TnT patterns presented in three groups: TnT -/- (Black), TnT -/+ (Dotted) and TnT+/+ (White).

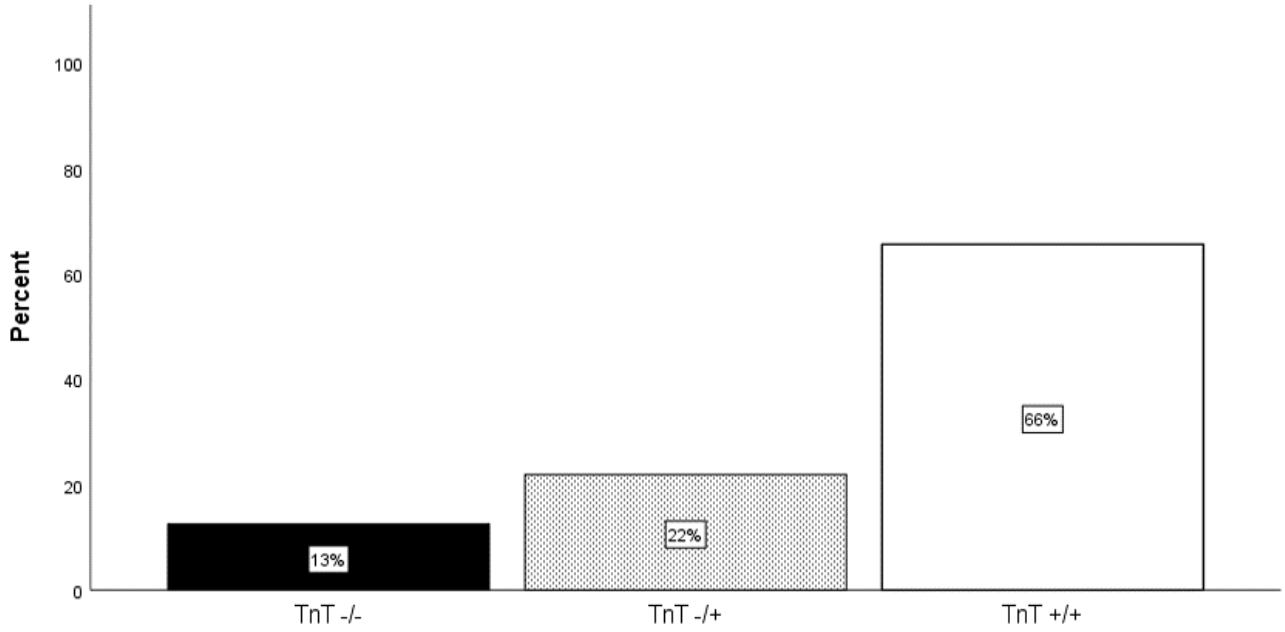


Figure 4.4. Early and Late TnT per group. *p=0.016 vs early TnT, **p<0.001 vs early TnT, §p≤0.001 vs TnT-/- and TnT -/+, ±p=0.001 vs TnT-/-, ^p=0.005 vs TnT-/+. Horizontal line represents TnT=14ng/L (upper reference limit).

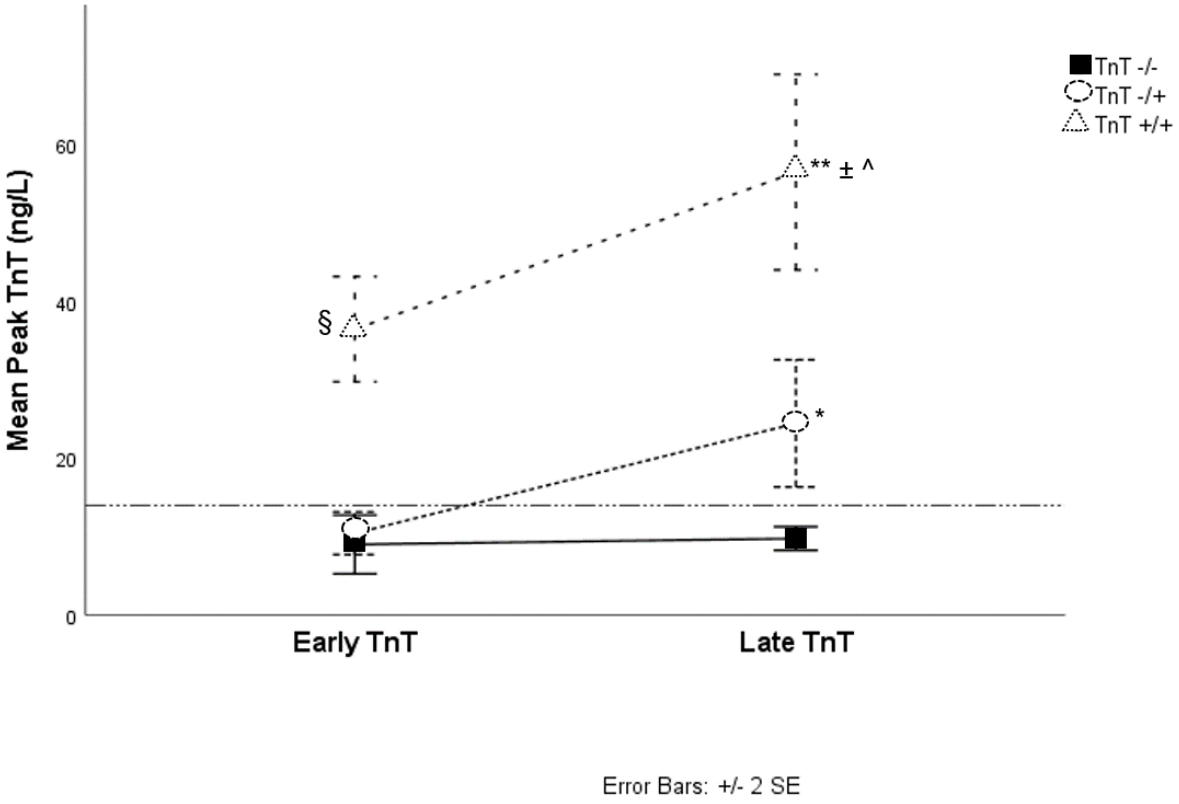


Table 4.3. Summary of Troponin T analysis for All patients and each group				
	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16	
TnT groups (%)				p=0.909
TnT -/-	4 (13)	2 (13)	2 (13)	
TnT -/+	7 (22)	3 (19)	4 (25)	
TnT +/+	21 (66)	11 (69)	10 (63)	
Mean ± S.D TnT (ng/L)				
TnT -/-				
Early	9 ± 3.7	8.5 ± 0.7	9.5 ± 6.4	p=0.846
Late	9.75 ± 1.75	9.5 ± 2.1	10 ± 1.4	p=0.808
TnT -/+				
Early	10.4 ± 3.6	10.7 ± 4.1	10.3 ± 3.8	p=0.895
Late	24.4 ± 10.7	20 ± 3.6	27.8 ± 13.7	p=0.392
TnT +/+				
Early	36.4 ±15.4	39.3 ± 14.9	33.3 ± 16	p=0.387
Late	56.4 ± 28.5	60.4 ± 26.5	52.1 ± 31.4	p=0.522

Early TnT vs Late TnT (mean difference, ng/L)				
TnT -/-	0.75 (p=0.65)	1 (p=0.5)	0.5 (p=0.91)	
TnT -/+	14 (p=0.016)	9* (p=0.099)	17.5* (p=0.084)	
TnT +/+	20 (p<0.001)	21 (p=0.006)	19 (p=0.008)	
Between TnT groups comparisons Early (B statistic, ng/L)				
TnT +/+ vs TnT -/-	27.4 (95%CI 13-41.8, p=0.001)	30.8 (95% CI 8.9-52.6, p=0.009)	23.8 (95% CI 1.1 – 46.5, p=0.041)	
TnT +/+ vs TnT -/+	26 (95% CI 14.5-37.5, p<0.001)	28.6 (95% CI 10-47.1, p=0.005)	23.1 (95% CI 5.7-40.4, p=0.013)	
Late (B statistic, ng/L)				
TnT +/+ vs TnT -/-	46.7 (95% CI 19.7-73.7, p=0.001)	50.9 (95% CI 12.1-89.6, p=0.014)	42.1* (95% CI -3-87.2, p=0.065)	
TnT +/+ vs TnT -/+	32 (95% CI 10.4-53.6, p=0.005)	40.4 (95% CI 7.5-73.1, p=0.02)	24.4* (95% CI -10-58.8, p=0.151)	
*difference compared to All patient cohort analysis in terms of statistical significance				

4.2.1.3 Troponin T as a binary categorical variable – Results Conclusion

In summary, 87% of patients have at least one positive peak TnT during or after chemotherapy. Most patients (66%) have a positive TnT during (early) and after (late) chemotherapy and 22% patients have a positive TnT only after chemotherapy. The TnT values for those patients who have an early and late positive TnT are significantly higher than patients in the other two TnT groups.

4.2.2 Troponin T relationship with total anthracycline dose

The relationship between total anthracycline dose and peak TnT was investigated as described in 2.5.2.2.2 and 2.6.2.2.2.Table 4.4 shows the total anthracycline dose and peak TnT data for all patients and for each randomisation group.

Table 4.4. Total cumulative anthracycline dose received and peak Troponin T (TnT)

	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16	
Total Cumulative anthracycline dose received* Mean ± S.D (mg/m^2)	312.4 ± 99	307.9 ± 100	316.9 ± 97	p=0.799
Peak TnT (ng/L) (median ± IQR)	34.5 ± 40	36.5 ± 65	31.5 ± 39	p=0.585

Simple linear regression was carried out to investigate the relationship between peak TnT and total anthracycline dose for all 32 patients. The scatter-dot plot shown in Figure 4.5 showed a moderate correlation between peak TnT and total anthracycline dose with a Pearson's correlation coefficient (r) of 0.422 with a p value of 0.016. Linear regression shows that there is a significant relationship between total anthracycline dose and peak TnT (p=0.016) and a slope coefficient, β, for anthracycline dose of 0.13 (95% CI 0.026-0.234) suggesting that for every mg increase in total anthracycline dose peak TnT is expected to increase by 0.13ng/L. The R^2 value was 0.178 suggesting that 17.8% of the variability in peak TnT can be explained by total anthracycline dose received. The scatterplot of standardised predicted values against standardised residuals shows that the data met the assumption of homogeneity and the histogram of the residuals shows that they were generally normally distributed.

Correlation associations and simple linear regression analysis was carried out for each group individually and summarised in Table 4.5. The analysis shows that Group 1 (RIC) in

general follows the same pattern as for the whole cohort. For Group 2 (Sham) even though the trend is similar to the whole patient cohort, correlation was weaker and regression analysis was non-significant. Scatterplots of standardised predicted values against standardised residuals and residuals histogram for model checking show they were generally normally distributed.

Figure 4.5 Scatter-Dot plot of peak TnT(ng/L) against total anthracycline dose (mg/m^2) for All patients. r=Pearson's correlation coefficient, p=0.047

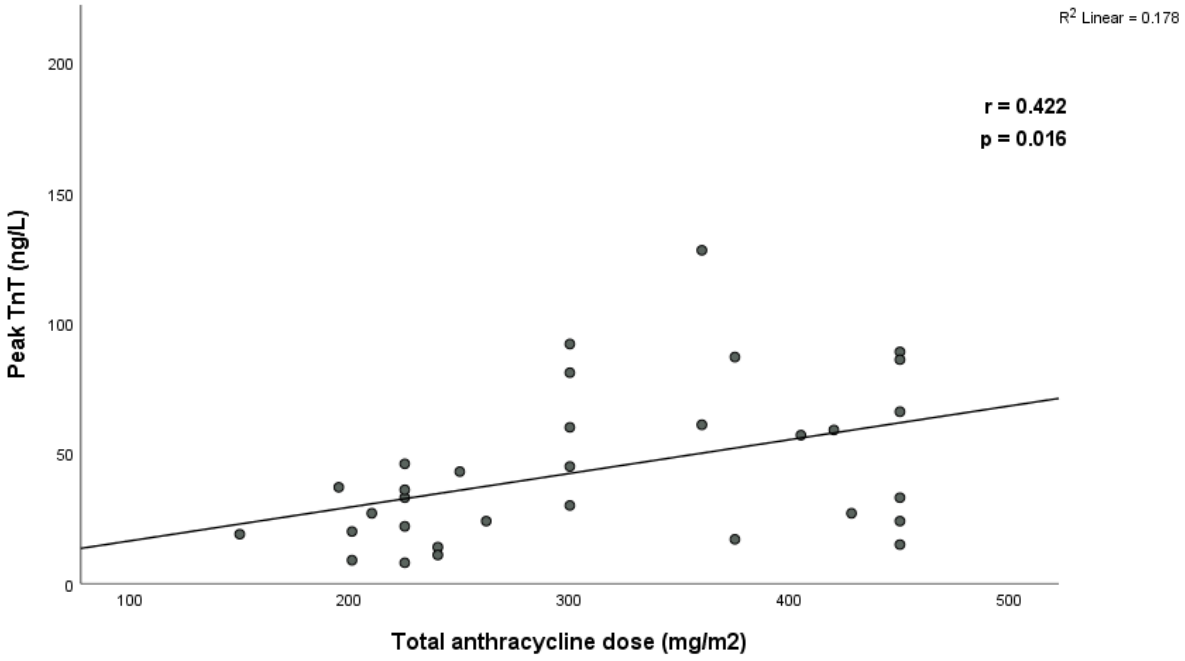


Table 4.5. Summary of simple linear regression analysis for All patients and each group			
	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16
Pearson's correlation coefficient, r	0.422 (p = 0.016)	0.568 (p = 0.022)	0.281* (p = 0.292)
Slope coefficient, β 95% CI	0.13 0.026-0.234 (p=0.016)	0.174 0.029-0.318 (p=0.022)	0.086* -0.083-0.254 (p=0.292)
*difference compared to All patient cohort analysis in terms of statistical significance			

4.2.2.3 Troponin T relationship with total anthracycline dose – Results Conclusion

In summary, when considering the results of the whole patient cohort, there is a moderate correlation between total anthracycline dose and peak TnT during or after chemotherapy and the relationship is significant on linear regression analysis, though total anthracycline dose only explains 18% of the peak TnT variability suggesting other factors are involved. However, as the results of the analysis for Group 2 (Sham), despite a similar trend, show a weaker correlation and a non-significant relationship on linear regression analysis, it is possible that the whole cohort analysis may not represent the true result and thus, we are unable to conclusively use the results of the whole cohort analysis.

4.2.3 Cardiac Myosin Binding Protein C

The role of cardiac myosin binding protein C (cMyC) in anthracycline cardiotoxicity and how it compares to TnT was assessed as described in 2.5.2.2.3 and 2.6.2.2.3.

4.2.3.1 Comparison of pre- and post-chemotherapy cMyC values

Pre- and post-chemotherapy cMyC values were compared to assess how cMyC varies before and after each cycle of chemotherapy for all patients. cMyC trends during each chemotherapy cycle are shown in Figure 4.6 and Table 4.6 for all patients. For

comparison, TnT trends for the 22 patients who had cMyC performed are shown in Figure 4.7.

A total of 101 pre- and 77 post-chemotherapy cMyC samples respectively were successfully analysed (for TnT pre: 97, post: 74). Like TnT, there is a general trend of an increase in cMyC concentration as patients progress through chemotherapy. Median pre-chemotherapy cMyC increases from 3.34ng/L at baseline to 46.93ng/L by cycle 6 (mean difference 36.8ng/L (95% CI: 18.6-55ng/L, p≤0.001) and in some patients this can be as high as 105ng/L. Post-chemotherapy cMyC values follow a similar pattern.

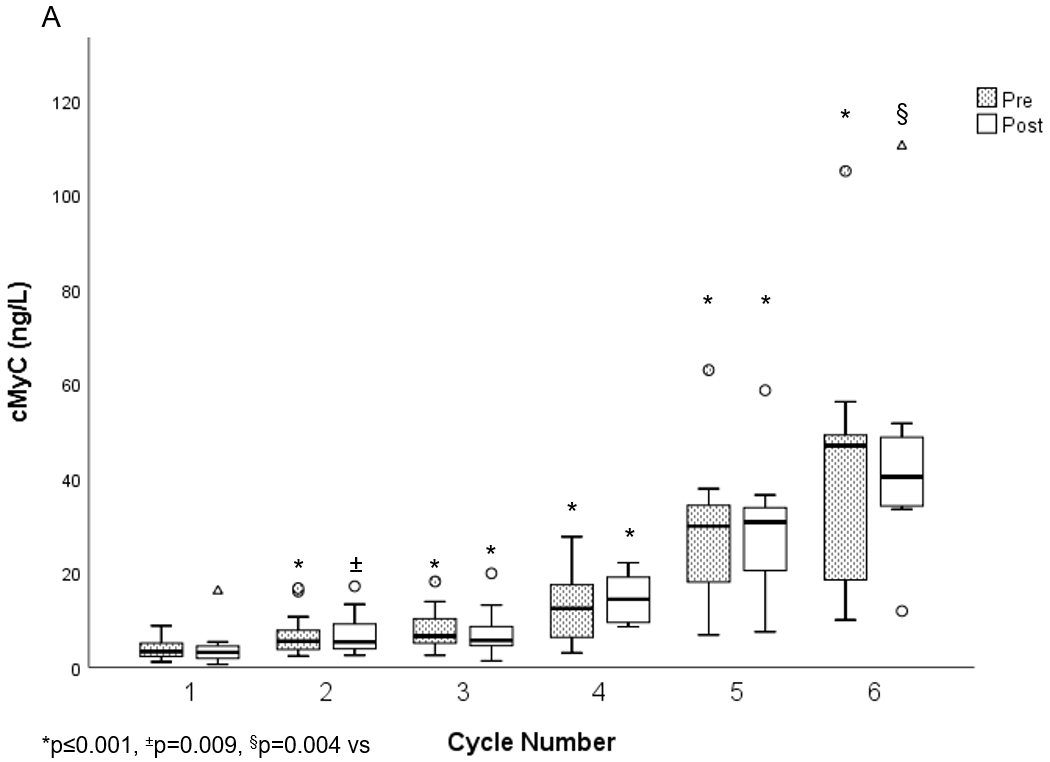
Random effects regression comparison between pre- and post- chemotherapy samples shows that there is no evidence of a difference between pre- and post-chemotherapy cMyC (p=0.768). The fixed model estimates that on average pre-chemotherapy cMyC is 0.74ng/L lower than post-chemotherapy (95% CI -5.7-4.18). To investigate if increasing chemotherapy cycles has an effect, this was added as a fixed effect to the model and the random effects regression analysis repeated. There is still no evidence of a difference between pre- and post-chemotherapy cMyC (p=0.616) with pre-chemotherapy cMyC being on average 0.82ng/L lower than post-chemotherapy (95% CI -4.04-2.44). However, there is evidence of a significant trend with increasing chemotherapy cycles (p<0.001) further supporting the general trend of increasing cMyC as patients progress through their chemotherapy as seen in Figure 4.6 and with the paired T test.

Figures 4.8 and 4.9 show boxplots of cMyC trends during chemotherapy for each randomisation group, which follow a similar pattern to the whole cohort. Random effects regression shows no difference between pre- and post-chemotherapy cMyC for either group (Group 1 (RIC) average difference 0.25ng/L (pre- higher), 95%CI -4.5-5, p=0.918,

Group 2 (Sham) average difference -1.6ng/L (pre- lower), 95% CI -4.9-1.8ng/L, p=0.354) but similar to the whole cohort, there is a significant trend of increasing chemotherapy cycles for both groups ($p<0.001$ for increasing chemotherapy cycles).

Therefore, there does not appear to be any significant difference between pre- and post-chemotherapy cMyC samples. However, as previously described in section 3.3.1, there is no post-chemotherapy haematocrit sample to assess for any dilutional effects, as patients can receive, significant amounts of intravenous fluids and this may be affecting the true cMyC level. Thus, any further analysis to compare cMyC with TnT was only performed using the pre-chemotherapy samples.

Figure 4.6. Pre- and Post-chemotherapy cMyC Trends for all patients. A. Boxplot of pre- (dotted) and post-(white) chemotherapy cMyC per cycle. B. Line chart of median cMyC per cycle for pre- (continuous) and post- (broken) samples



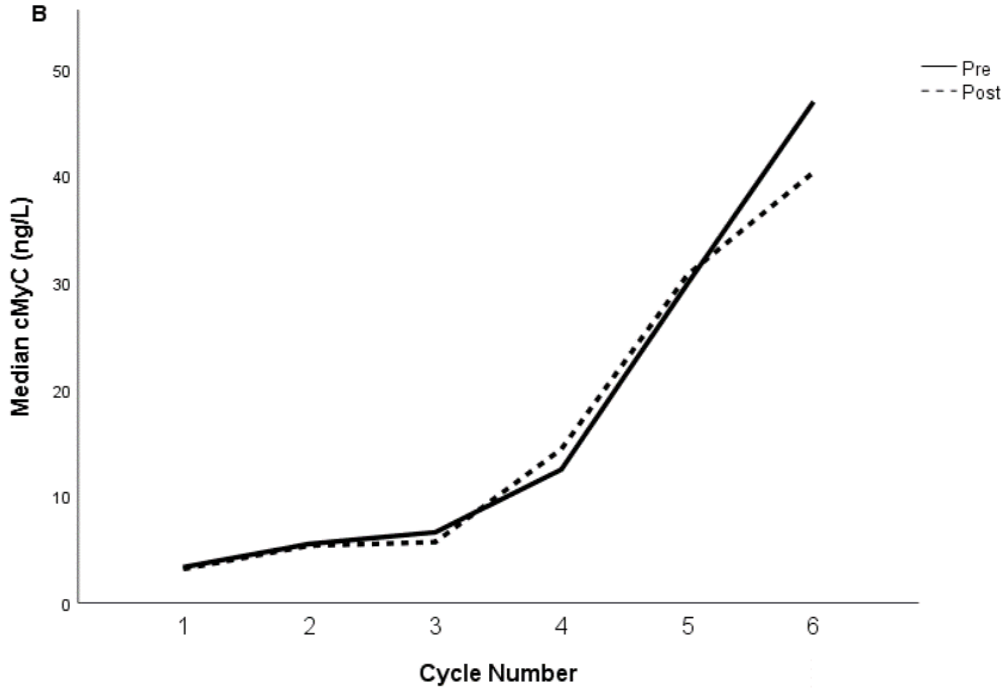
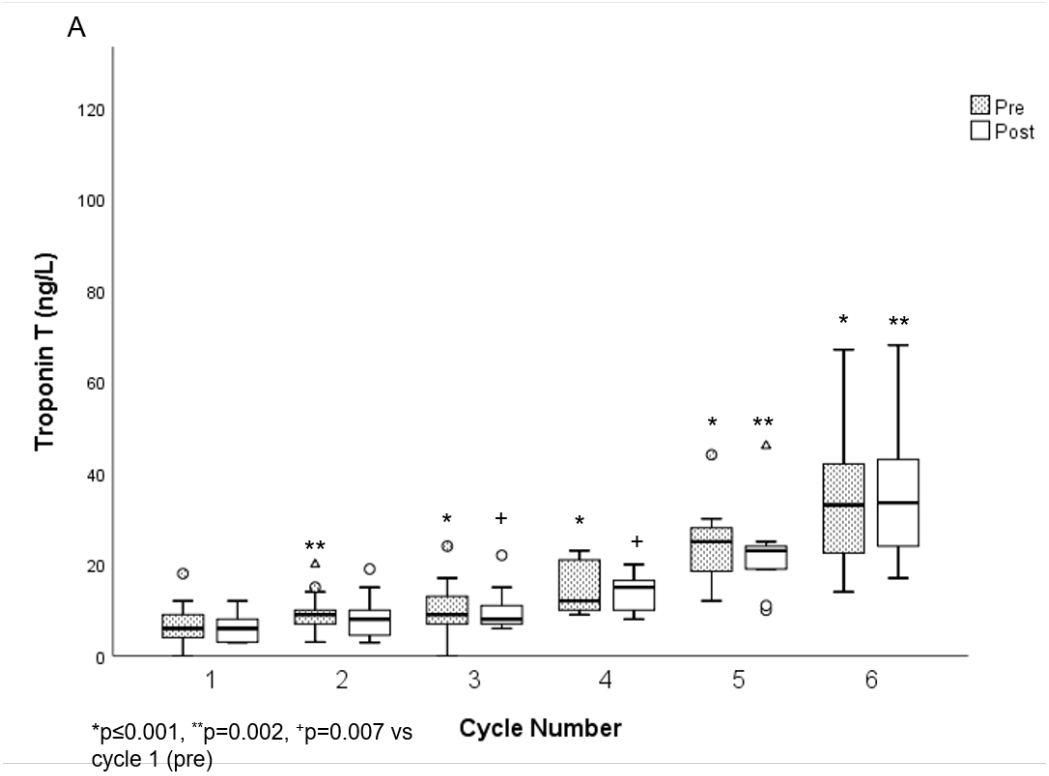


Figure 4.7. Pre- and Post-chemotherapy Troponin T Trends for all patients. A. Boxplot of pre- (dotted) and post-(white) chemotherapy TnT per cycle. B. Line chart of median TnT per cycle for pre- (continuous) and post- (broken) samples



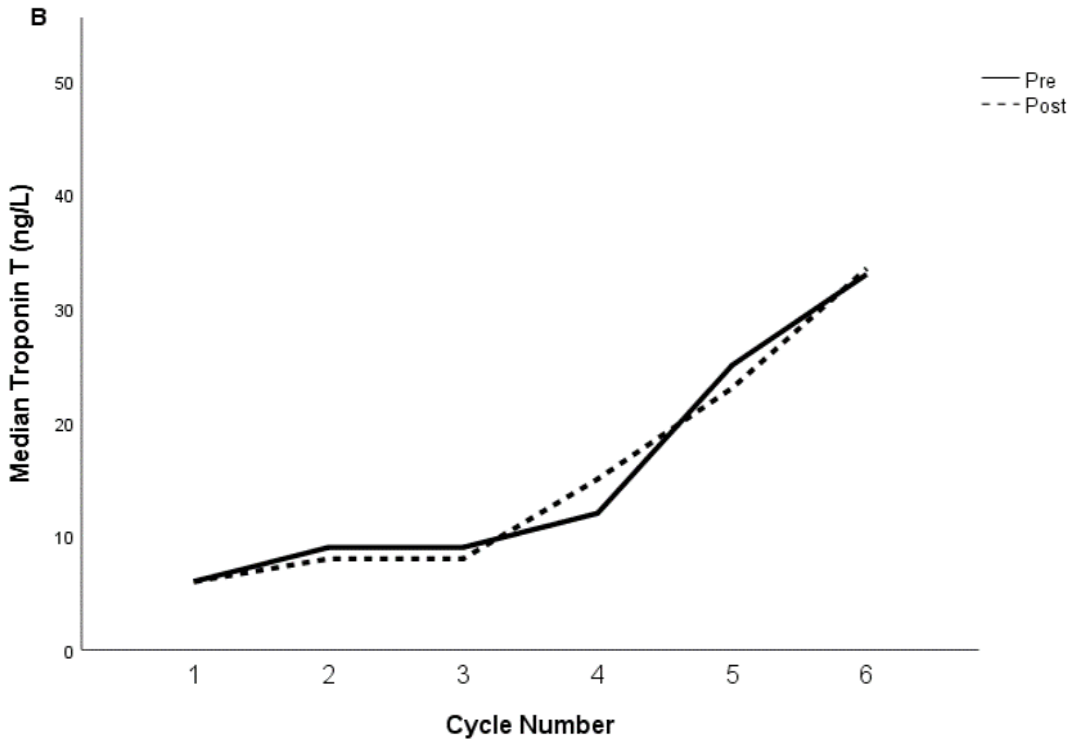


Table 4.6. cMyC trends pre- and post-chemotherapy for all patients												
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
	Pre (n=22)	Post (n=16)	Pre (n=21)	Post (n=17)	Pre (n=21)	Post (n=17)	Pre (n=15)	Post (n=10)	Pre (n=11)	Post (n=9)	Pre (n=11)	Post (n=8)
Median (ng/L)	3.34	3.17	5.52	4.6	6.61	5.9	12.49	14.4	29.88	30.7	46.93	40
IQR	3	3	14	6	5	4	14	10	24	17	32	16
Min (ng/L)	1	1	2	1	3	3	3	9	7	8	10	12
Max (ng/L)	9	16	17	17	18	20	28	22	63	59	105	110

Figure 4.8. Pre- and Post-chemotherapy cMyC Trends for Group 1 (RIC). Boxplot of pre- (dotted) and post-(white) chemotherapy cMyC per cycle for Group 1 (RIC).

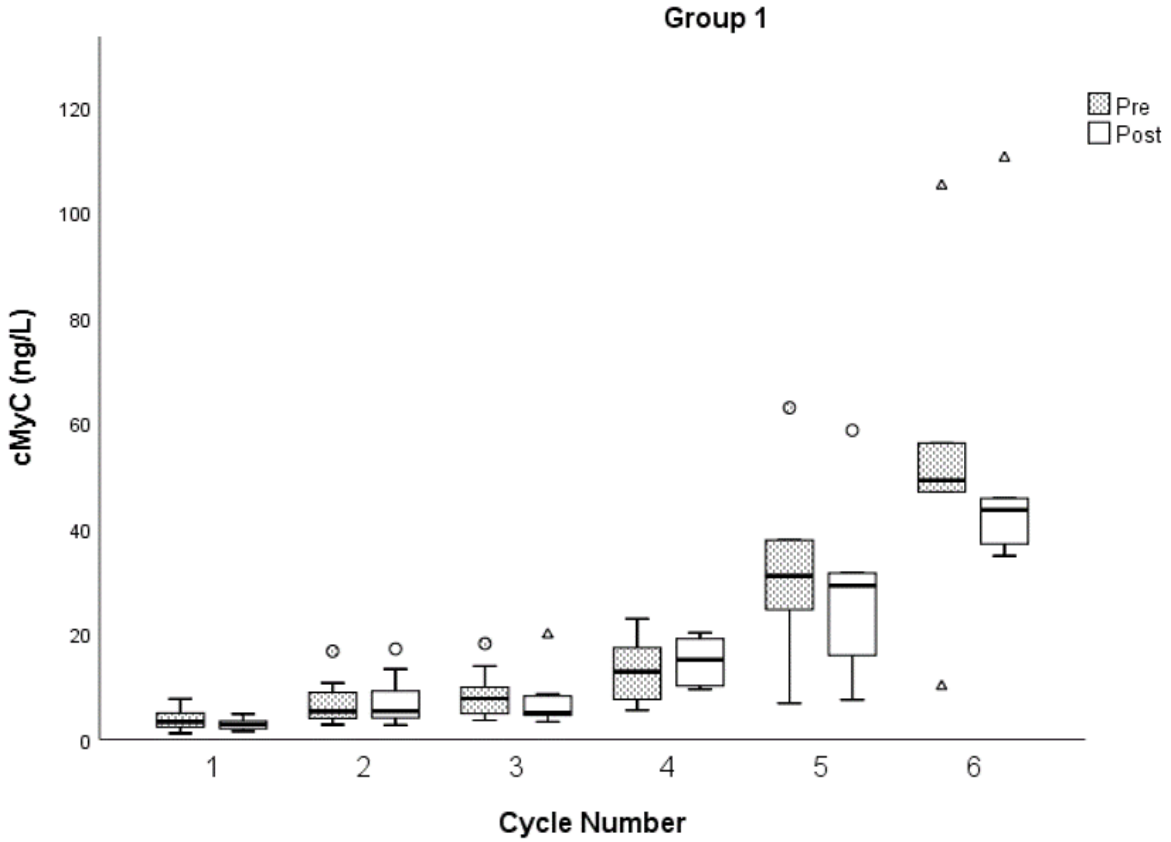
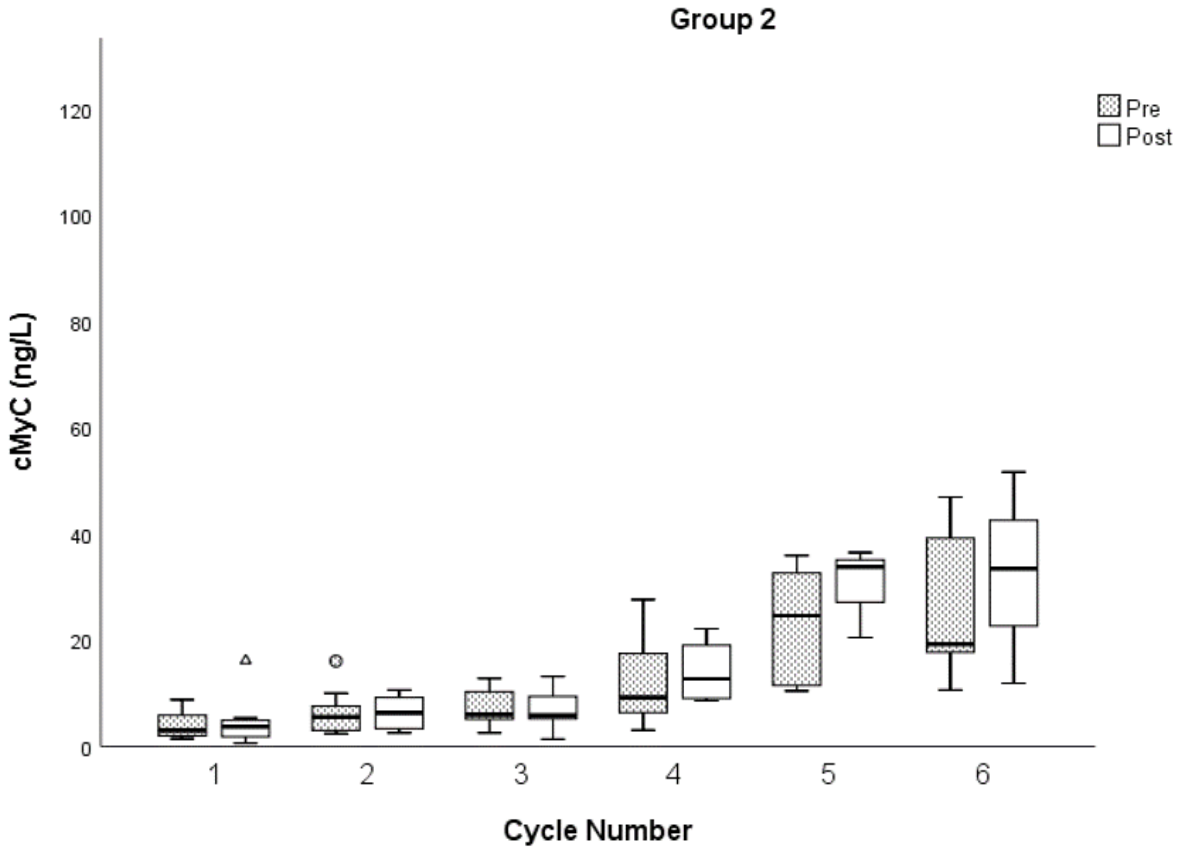


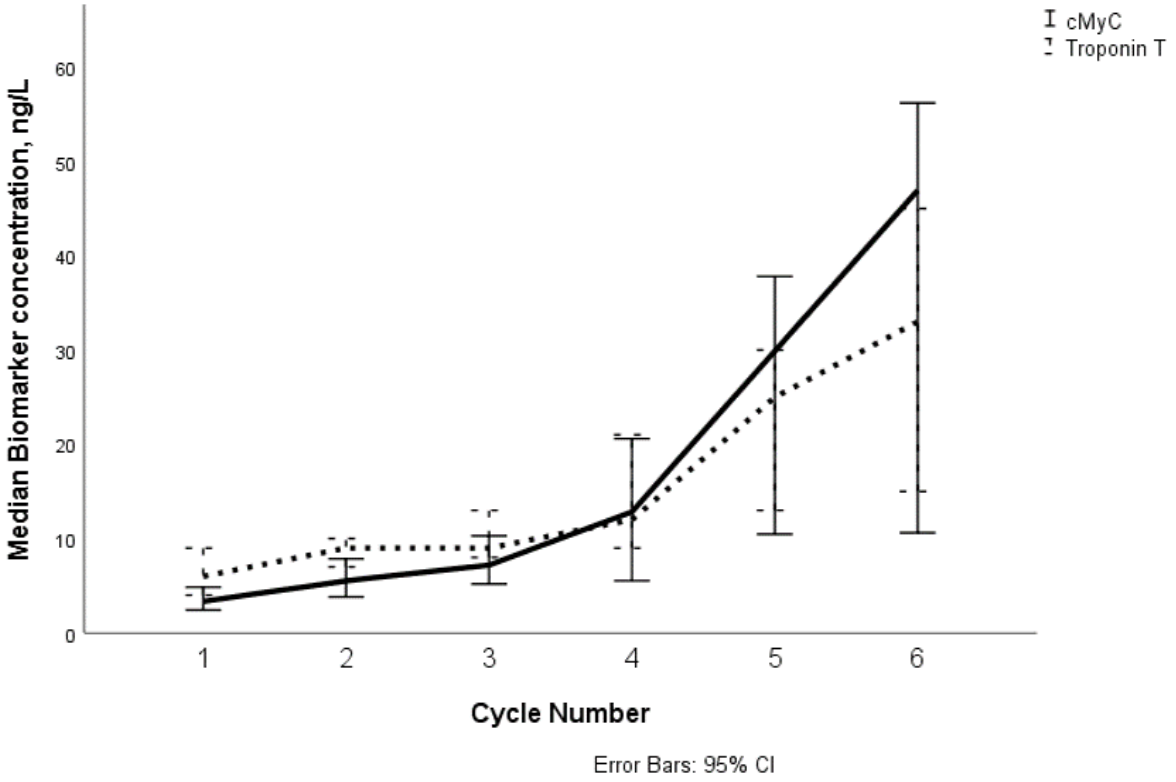
Figure 4.9. Pre- and Post-chemotherapy cMyC Trends for Group 2 (Sham). Boxplot of pre-(dotted) and post-(white) chemotherapy cMyC per cycle for Group 2 (Sham).



4.2.3.2. Comparison between cMyC and TnT

The median concentrations for both biomarkers as patients progress through their chemotherapy are shown in Figure 4.10 in ng/L (cMyC continuous line, TnT dotted line). Both cMyC and TnT follow a similar pattern of increasing in concentration with more chemotherapy cycles. Cardiac myosin binding protein C seems to have a lower baseline concentration and by cycle 3 and 4 starts to rise at a faster rate compared to TnT.

Figure 4.10. Line chart of median biomarker concentrations for cMyC and TnT in ng/L (cMyC continuous line, TnT dotted line).



Peak to baseline biomarker concentration ratio was used to compare the two biomarkers as shown in Figure 4.11 and Table 4.7. For cMyC analysis for all 22 patients was possible whereas for TnT analysis was possible for 20 patients due to two baseline TnT samples haemolysing thus precluding calculation of peak:baseline ratios. Almost half of the peak biomarker concentrations were detected at cycle 6 for both biomarkers (cMyC 46% at cycle 6, TnT 41% at cycle 6). There was a 4-fold median (mean 8-fold) increase from baseline to peak concentration for cMyC versus a 2-fold median increase for TnT (mean 4-fold) which was highly significant (Wilcoxon singed rank test Z statistic -3.8, $p<0.001$)(Figure 4.11)(Table 4.7). A similar analysis for each individual randomisation group showed similar results to the analysis of the whole cohort (Figures 4.12 and 4.13) with no differences between the two groups (Table 4.7).

Figure 4.11. Comparison between cMyC and TnT peak:baseline concentration ratios. A. Boxplot of peak to baseline ratio (cMyC dotted box, TnT white box). B. Line chart of median peak to baseline concentration ratio (cMyC continuous line, TnT broken line).

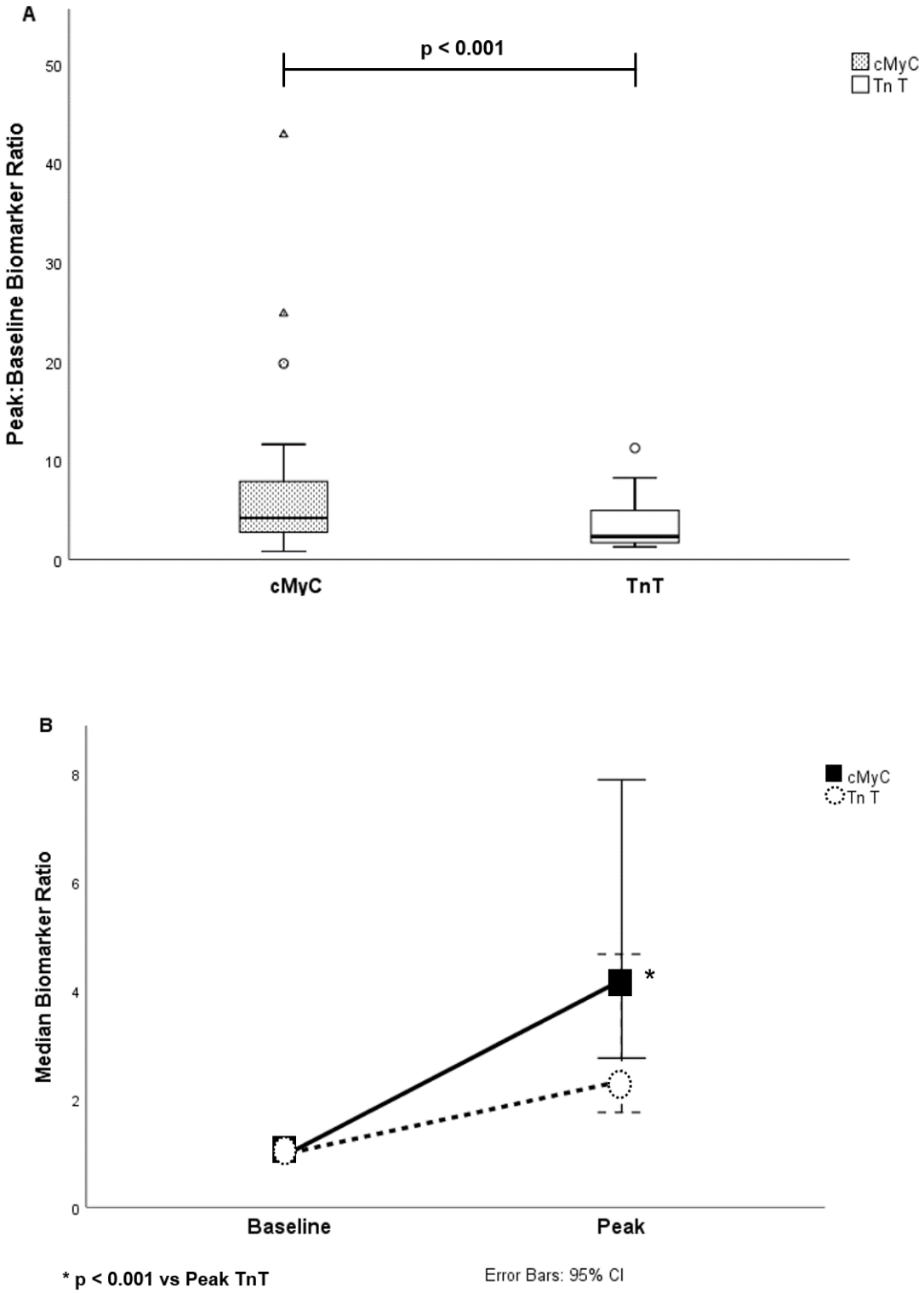


Table 4.7. cMyC and TnT comparisons for all patients and for each randomisation group				
	All N = 22	Group 1 (RIC) N = 12	Group 2 (Sham) N = 10	
Peak cMyC (ng/L) **Median ± IQR** **Mean ± SD** **Min** **Max**	 10.5 ± 39 25.45 ± 25.3 5 105	 14 ± 42 30.8 ± 30.8 5 105	 10.5 ± 29 19 ± 15.6 5 47	p=0.261
Peak TnT (ng/L) **Median ± IQR** **Mean ± SD** **Min** **Max**	 14.5 ± 26 22.18 ± 16.6 5 67	 19 ± 31 25.1 ± 19.1 6 67	 14.5 ± 24 18.7 ± 13.2 5 42	p=0.383
Peak cMyC Time (%) **Cycle 2** **Cycle 3** **Cycle 4** **Cycle 5** **Cycle 6**	 3 (14) 6 (27) 2 (9) 1 (5) 10 (46)	 2 (17) 3 (25) 1 (8) 0 6 (50)	 1 (10) 3 (30) 1 (10) 1 (10) 4 (40)	p=0.815
Peak TnT Time (%) **Cycle 2** **Cycle 3** **Cycle 4** **Cycle 5** **Cycle 6**	 4 (18) 6 (27) 1 (5) 2 (9) 9 (41)	 3 (25) 2 (17) 1 (8) 0 6 (50)	 1 (10) 4 (40) 0 2 (20) 3 (30)	p=0.237
Peak cMyC : Baseline Concentration Ratio **Median ± IQR** **Mean ± SD** **Min** **Max**	 4.2 ± 6* 8.1 ± 9.7 0.85 42.6	 4.2 ± 15.3** 10.4 ± 12.6 1.2 42.6	 4.3 ± 3.8§ 5.3 ± 2.9 0.85 11	p=0.197
Peak TnT : Baseline Concentration Ratio **Median ± IQR** **Mean ± SD** **Min** **Max**	 2.3 ± 3.4 3.7 ± 2.9 1.3 11.3	 2.4 ± 6.2 4.7 ± 3.6 1.5 11.3	 2.3 ± 2.8 2.8 ± 1.4 1.3 5.3	p=0.146
*p < 0.001 vs TnT, **p = 0.005 vs TnT, §p = 0.013 vs TnT				

Figure 4.12. Comparison between cMyC and TnT peak:baseline concentration ratios for Group 1 (RIC). Line chart of median peak to baseline concentration ratio (cMyC continuous line, TnT broken line).

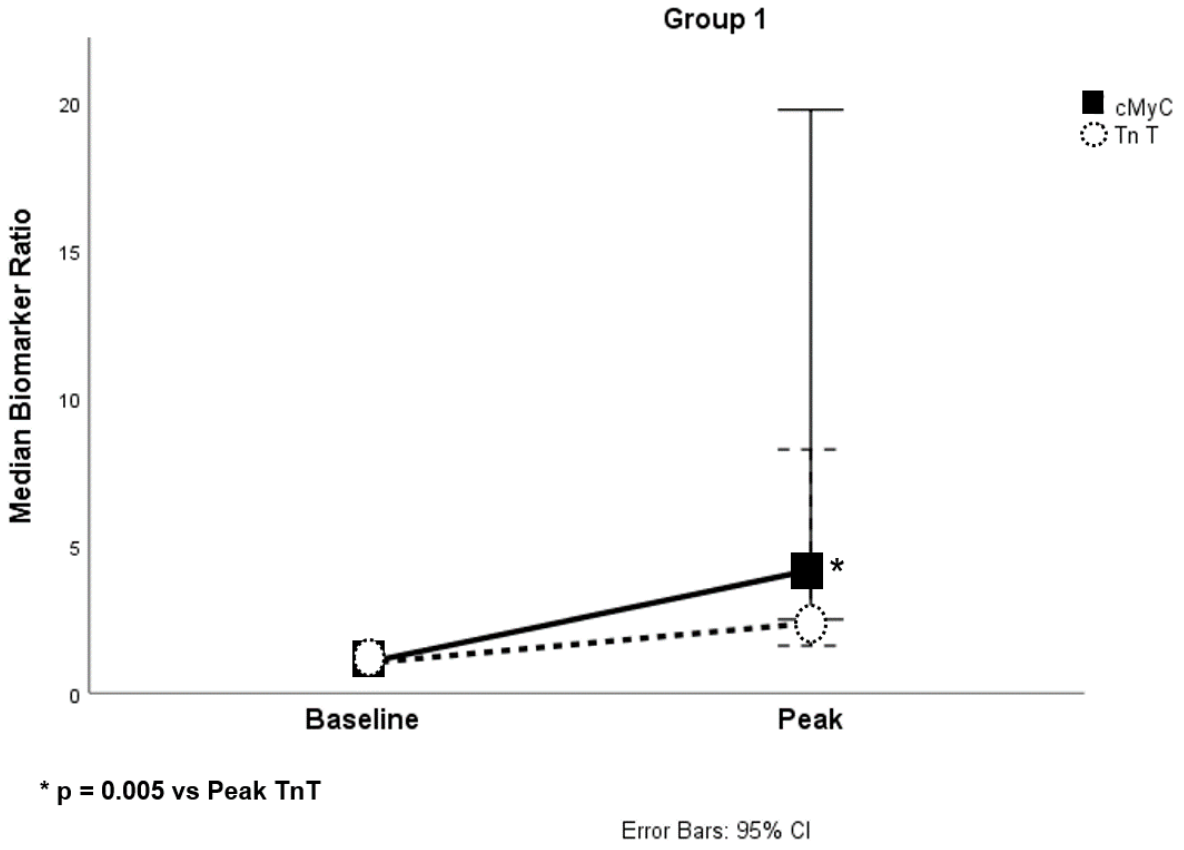
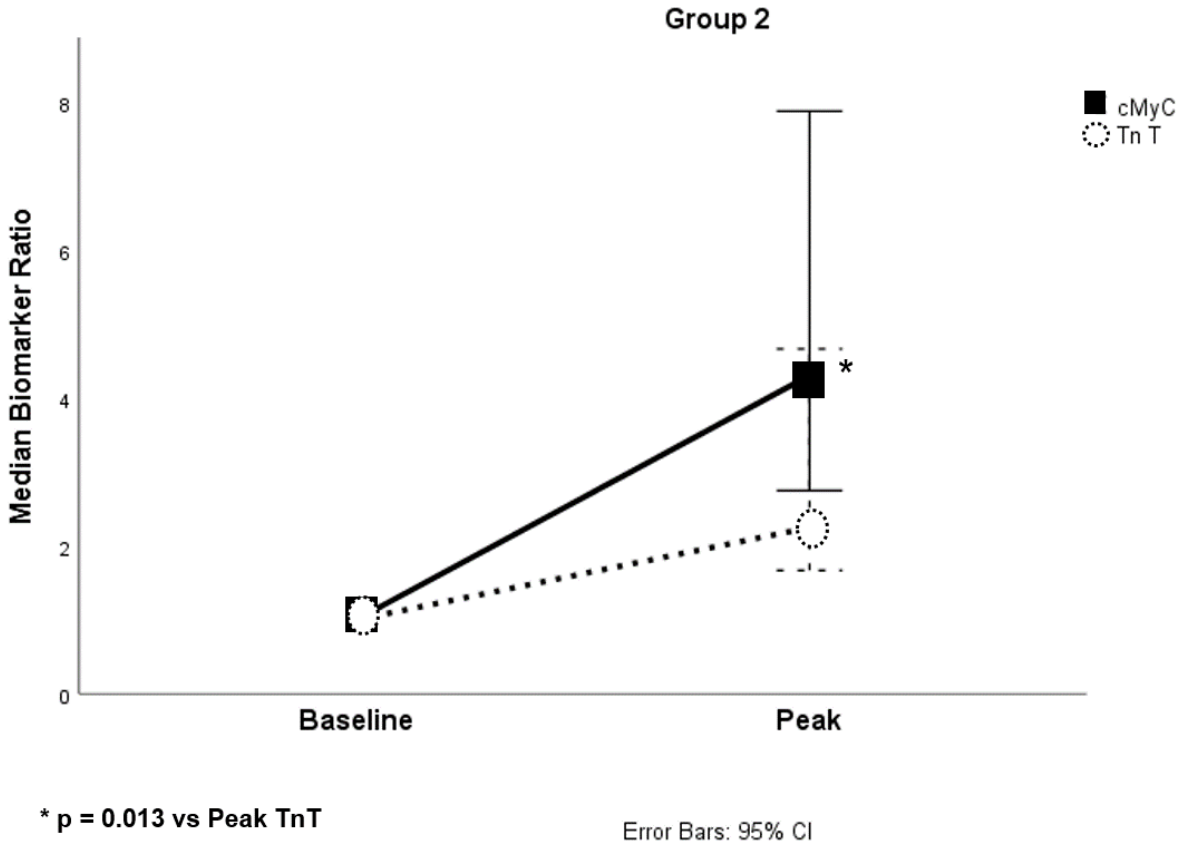


Figure 4.13. Comparison between cMyC and TnT peak:baseline concentration ratios for Group 2 (Sham). Line chart of median peak to baseline concentration ratio (cMyC continuous line, TnT broken line).



Furthermore, cMyC and TnT concentrations were also compared using their ratio to baseline at each cycle as shown in Figure 4.14 for all patients and for each group individually (Figures 4.15 and 4.16). As seen in Figure 4.14, there is a higher increase from baseline for cMyC compared to TnT at each chemotherapy cycle that is statistically significant at each cycle (Cycle 2, median cMyC concentration increases by 1.8 times vs 1.5 for TnT, p=0.007, Cycle 3, median cMyC concentration increases by 2.15 times vs 1.65 for TnT, p=0.002. Cycle 4, median cMyC concentration increases by 3.13 times vs 2.33 for TnT, p=0.005, Cycle 5, median cMyC concentration increases by 6.71 times vs 3.75 for TnT, p=0.004, Cycle 6, median cMyC concentration increases by 7.89 times vs 4.67 or TnT p=0.006).

Similarly for Group 1(RIC), there is a higher increase from baseline for cMyC compared to TnT at each cycle that is statistically significant (Cycle 2, median cMyC concentration increases by times 1.8 vs 1.58 for TnT, p=0.028 , Cycle 3, median cMyC concentration increases by 2.23 times vs 1.55 for TnT, p=0.007 , Cycle 4, median cMyC concentration increases by 3.57 times vs 2.4 for TnT, p=0.018 , Cycle 5, median cMyC concentration increases by 8.87 times vs 4.94 for TnT, p=0.028 , Cycle 6, median cMyC concentration increases by 15.7 times vs 7.72 for TnT, p=0.028).

For Group 2(Sham), even though there is a similar trend of a higher increase in cMyC from baseline compared to TnT, this is not statistically significant at each chemotherapy cycle (Cycle 2, median cMyC concentration increases by 1.83 times vs 1.33 for TnT, p=0.066 , Cycle 3, median cMyC concentration increases by 1.93 times vs 1.71 for TnT, p=0.139 , Cycle 4, median cMyC concentration increases by 3.14 times vs 1.79 for TnT, p=0.116 , Cycle 5, median cMyC concentration increases by 5.48 times vs 3.11 for TnT, p=0.080 , Cycle 6, median cMyC concentration increases by 6.81 times vs 4.33 for TnT, p=0.080).

Figure 4.14. A. Boxplot of cMyC and TnT concentration ratios from baseline per cycle for all patients. B Line chart of median cMyC and TnT concentration ratios from baseline per cycle for all patients.

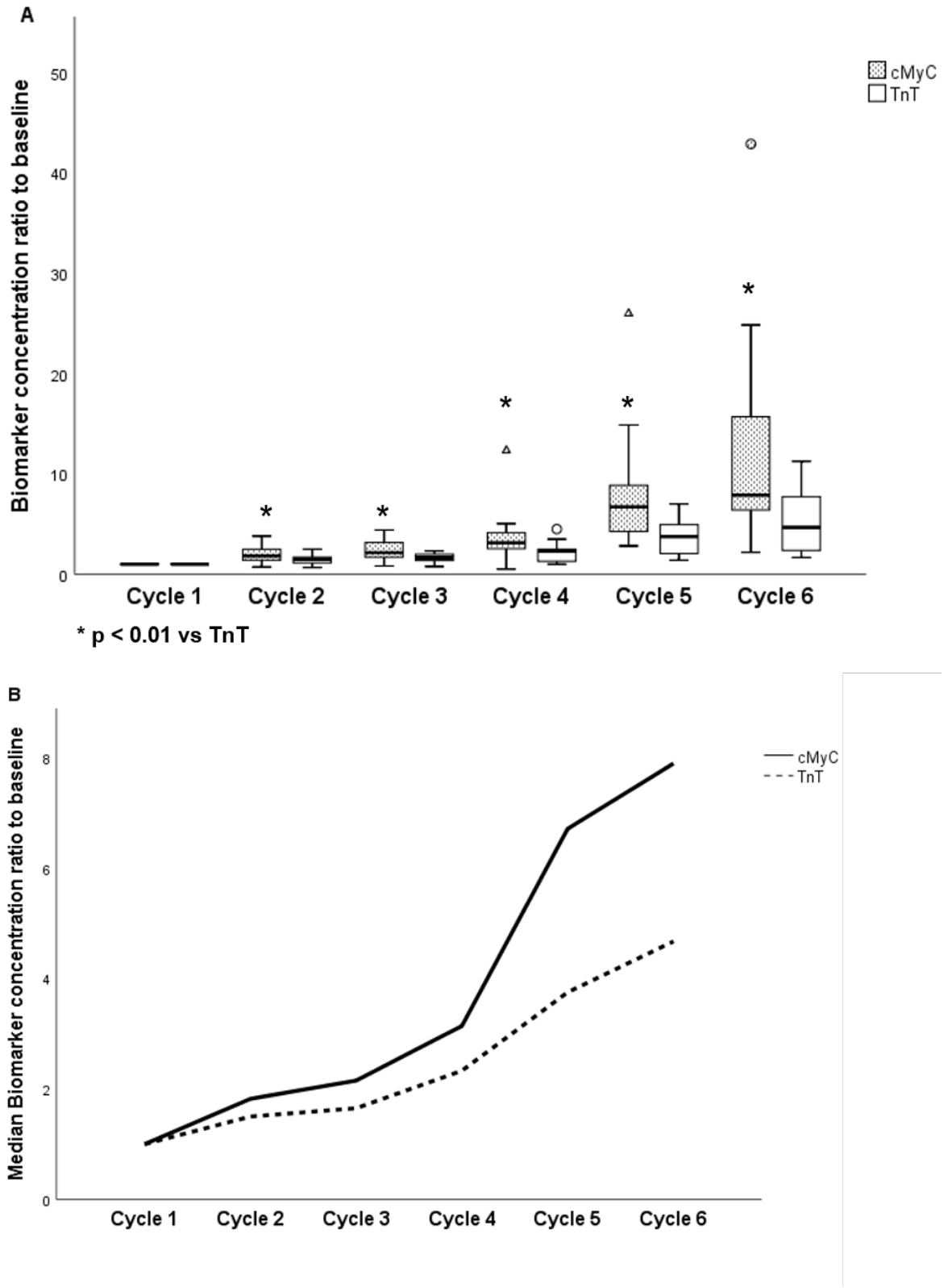
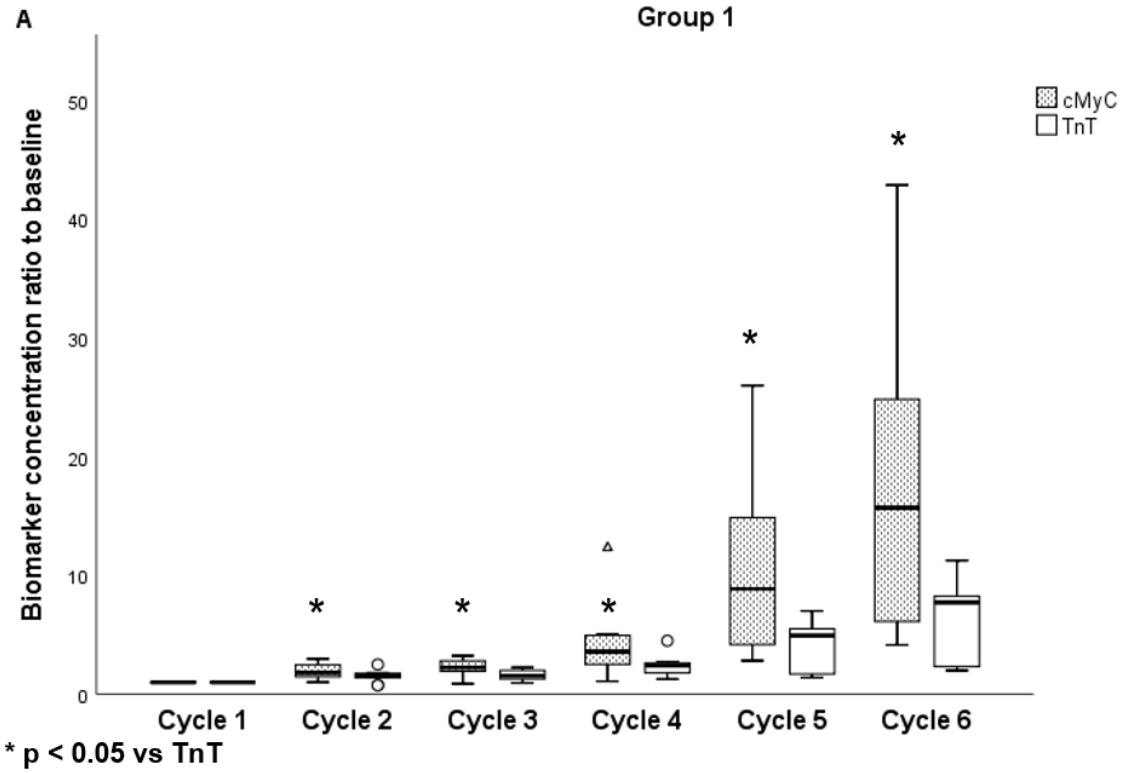


Figure 4.15. A. Boxplot of cMyC and TnT concentration ratios from baseline per cycle for Group 1 (RIC). B Line chart of median cMyC and TnT concentration ratios from baseline per cycle for Group 1 (RIC).



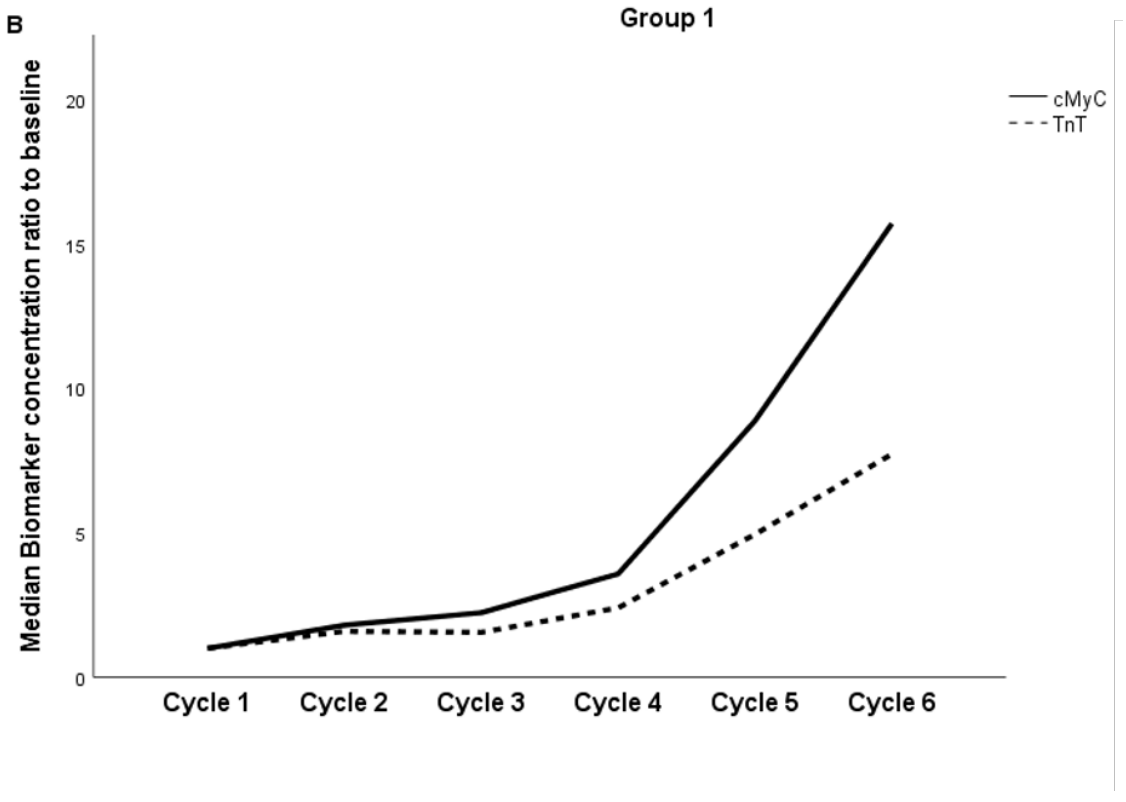
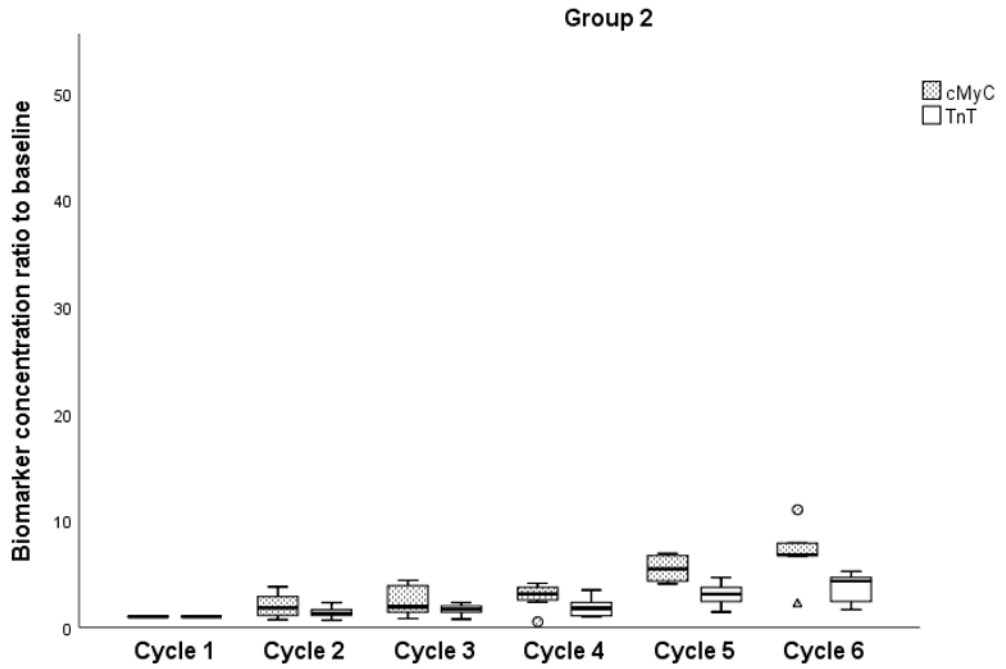
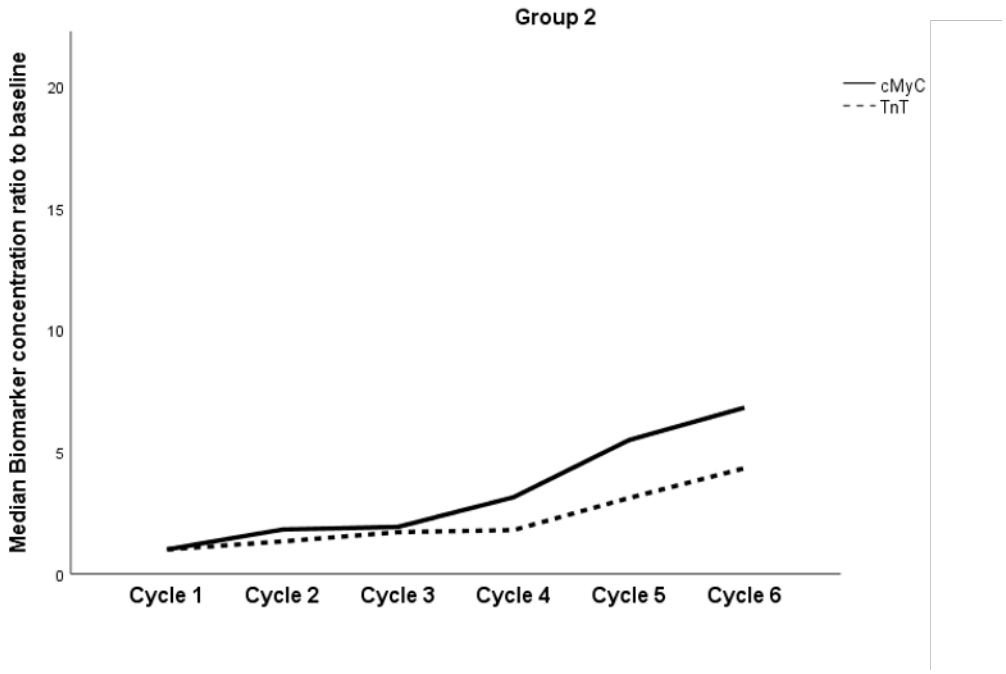


Figure 4.16. A. Boxplot of cMyC and TnT concentration ratios from baseline per cycle for Group 2 (Sham). B Line chart of median cMyC and TnT concentration ratios from baseline per cycle for Group 2 (Sham).





4.2.3.3 Effect of RIC on cMyC levels

To assess the effect of RIC on cMyC levels a similar assessment to that used in section 3.3 was performed for the 22 patients who had cMyC levels taken during chemotherapy. Looking at the absolute cMyC concentrations during chemotherapy in Figure 4.17, cMyC levels are on average 4.17ng/L higher in Group 1 (RIC) compared to Group 2 (Sham) which did not reach statistical significance using a random effects regression for repeated measures (95%CI -0.12-8.45, p=0.056) though the effect additional chemotherapy cycles was significant (p<0.001). Similarly using independent samples t-test at each chemotherapy cycle there was no difference between the two groups (Cycle 1, mean difference -0.85ng/L, 95%CI -1.97-1.8, p=0.926, Cycle 2, mean difference 0.29ng/L, 95%CI -3.58-4.16 -, p=0.88, Cycle 3, mean difference 0.96ng/L, 95%CI -2.7-4.63, p=0.59, Cycle 4, mean difference 0.49ng/L, 95%CI -8.02-8.99, p=0.904, Cycle 5, mean difference 9.35ng/L, 95%CI -12.2-30.9, p=0.352, Cycle 6, mean difference 26.02ng/L, 95%CI -8.01-60.2 , p=0.12).

However, looking at the ΔcMyC concentrations from baseline during chemotherapy in Figure 4.18, ΔcMyC concentrations are on average 5.68ng/L higher in the Group 1 (RIC) compared to the Group 2 (Sham) which just reaches statistical significance using random effects regression for repeated measures and when considering the effect of additional chemotherapy cycles (95% CI 0.41-10.86, p=0.035, p<0.001 for chemotherapy cycles). Independent samples T test at each chemotherapy cycle did not show any difference between the two groups (Cycle 2, mean difference 0.14ng/L, 95%CI -2.62-2.9 , p=0.917, Cycle 3, mean difference 0.88ng/L, 95%CI -2-3.76 , p=0.532, Cycle 4, mean difference 1.24ng/L, 95%CI -5.96-8.44 , p=0.716, Cycle 5, mean difference 10.06ng/L, 95%CI -9.85-29.96 , p=0.283, Cycle 6, mean difference 26.72ng/L, 95%CI -6.82-60.27, p=0.105). A similar analysis for TnT for the 22 patients who had cMyC analysis, did not show any

difference between the two groups for absolute TnT or ΔTnT concentrations on random effects regression for repeated measures when accounting for additional chemotherapy cycles (Absolute TnT: mean difference 1.56ng/L, 95% CI -1.31-4.43, p=0.283, ΔTnT: mean difference 2.21ng/L, 95%CI -1.27-5.69, p=0.209) suggesting that the observed difference in ΔcMyC may be due to the RIC intervention.

Figure 4.17. Effect of RIC on absolute cMyC concentrations. A. Boxplot cMyC concentrations for Group 1 (RIC, dotted) and Group 2 (Sham, white). B. Line chart of mean cMyC concentration at each cycle for Group 1 (RIC, continuous) and Group 2 (Sham, broken)

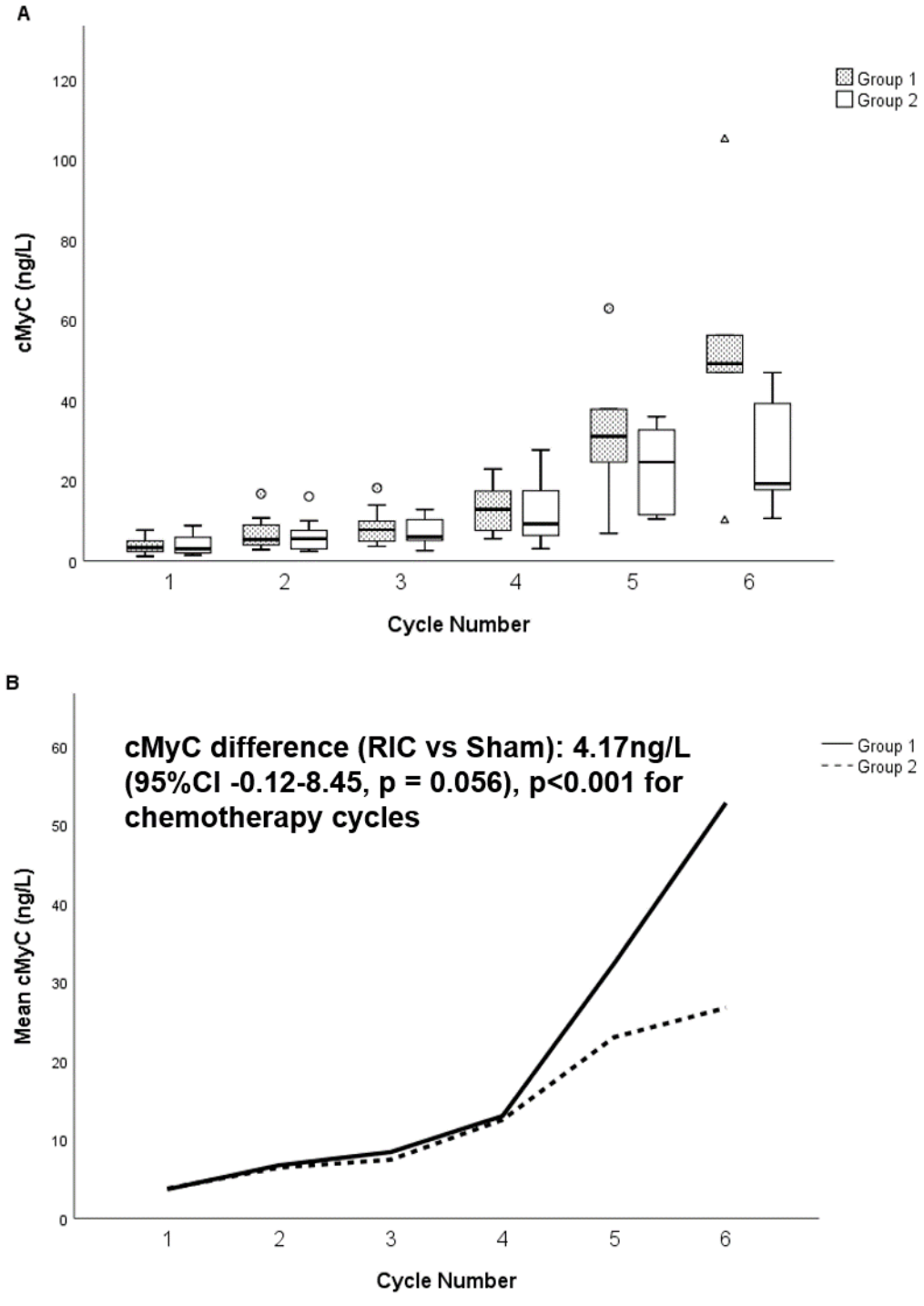
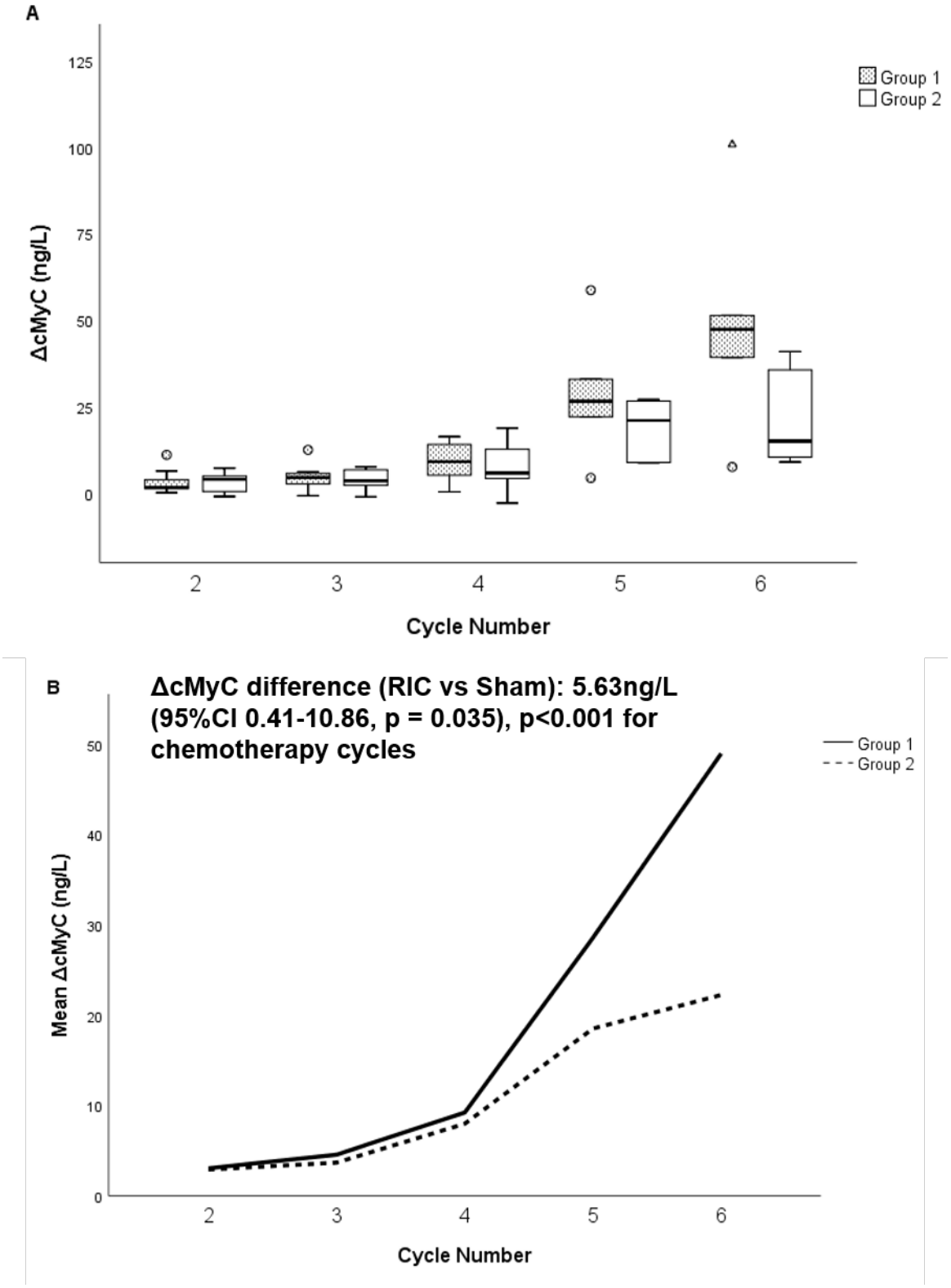


Figure 4.18. Effect of RIC on ΔcMyC concentrations. A. Boxplot ΔcMyC concentrations for Group 1 (RIC, dotted) and Group 2 (Sham, white). B. Line chart of mean ΔcMyC concentration at each cycle for Group 1 (RIC, continuous) and Group 2 (Sham, broken)



4.2.3.2.4 Cardiac Myosin Binding Protein C (cMyC) – Results Conclusion

In summary, like the analysis for TnT in Chapter 3, there does not appear to be any significant difference between pre- and post-chemotherapy cMyC samples, though the lack of a post-chemotherapy assessment of any dilutional effects makes the results more difficult to interpret.

When looking at the peak biomarker concentration, the rate of increase seems to be more with cMyC compared to TnT with a 4-fold median peak increase for cMyC compared to a 2-fold increase in median concentration for TnT that is statistically significant at the 5% level ($p<0.001$).

Cardiac myosin binding protein C seems to follow a similar trend to TnT of increasing in concentration as patients progress with their anthracycline chemotherapy cycles. When looking at the whole cohort, the rate of increase in concentration for cMyC is significantly higher compared to TnT even from Cycle 2. However, as the results of the analysis for Group 2 (Sham), despite a similar trend, show no significant difference between cMyC and TnT at each chemotherapy cycle, it is possible that the intervention may influence the results and thus, we are unable to conclusively use the results of the whole cohort analysis as they may not represent the true result.

Furthermore, when assessing the effect of RIC on cMyC concentrations, there is no difference between the two randomisation groups when looking at the absolute cMyC concentrations. However, when looking at the ΔcMyC concentration from baseline, this is higher in the RIC group suggesting that RIC increases the change in cMyC concentration from baseline.

4.3 Identifying at risk patients during and after chemotherapy using imaging

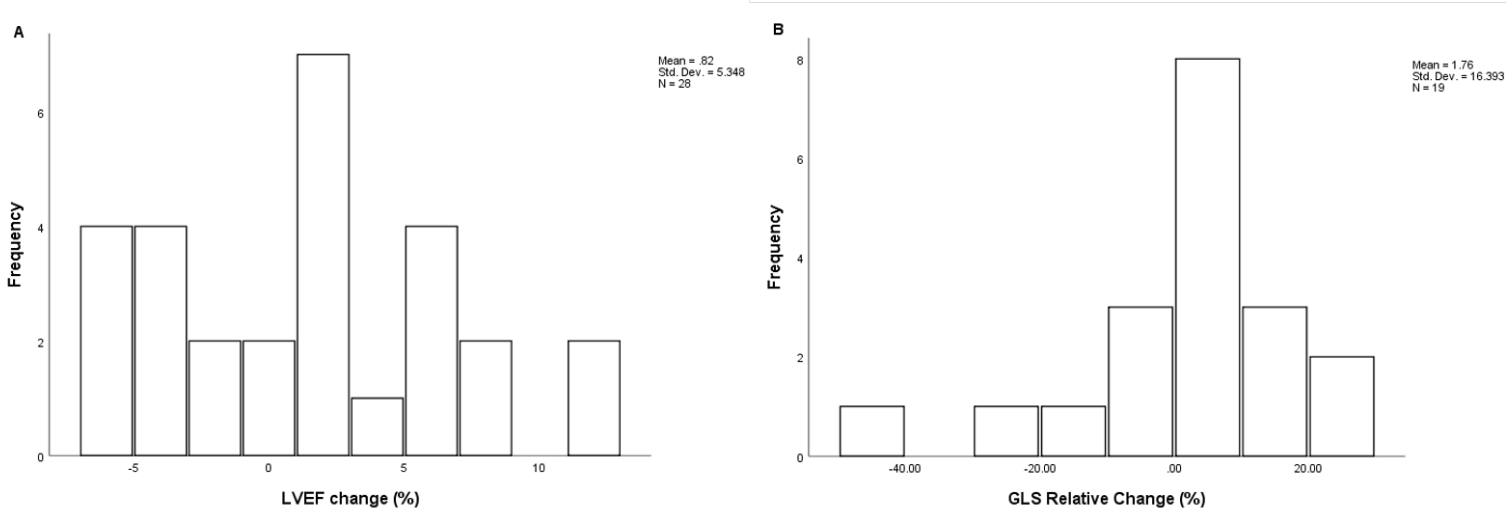
4.3.1 Echocardiography

Assessment of cardiac function using left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS) have been identified over the years as useful modalities in detecting chemotherapy induced cardiotoxicity, which includes anthracycline cardiotoxicity and measurements of LVEF and GLS are advocated in international guidelines[48,94,115,318]. The relationship between peak TnT, LVEF absolute percentage change and GLS relative percentage change was then assessed as described in 2.5.2.3.1 and 2.6.2.3.1.

Table 4.8 shows LVEF, GLS and peak TnT data for all patients and for each group. Twenty-eight patients had a 3-month follow-up LVEF (4 patients did not have a 3 month echocardiogram due to death (1), withdrawal (1), delays due to Covid (2)) whereas GLS change was assessed in 19 patients. The distribution of changes for LVEF and GLS are shown in the histograms in Figure 4.19. Most patients had minimal change in their LVEF 3 months after chemotherapy with a mean LVEF change of 0.8%. Two patients had a LVEF change of more than 10% with one developing symptomatic heart failure. The other had a 3-month LVEF of 50%, was asymptomatic and has ongoing monitoring. The mean relative GLS change was 1.8%. Four patients had a drop in relative GLS of more than 15%. Two had associated LVEF drop to less than 50% and/or heart failure needing medication. The other two are on active monitoring. Therefore, a total of 4 patients met at least one criterion for the diagnosis of anthracycline cardiotoxicity as per international guidelines. Their respective peak TnT values were 13 (subsequently rose to 46 at 6-month FU), 89, 30 and 87 ng/L with the last 3 all detected at either the one month or 3 month post chemotherapy and the first one at the last cycle of chemotherapy.

Table 4.8. Peak TnT and relationship to LVEF and GLS by echocardiography				
	All N = 32	Group 1 (RIC) N = 16	Group 2 (Sham) N = 16	
LVEF change (%)*				p=0.162
Mean ± SD	0.71 ± 3.98	2.3 ± 3.7	-0.47 ± 6.3	
Min	-6	-6	-6	
Max	12	7	12	
N	28	13	15	
GLS relative change (%)*				p=0.411
Mean ± SD	1.76 ± 16.4	-1.3 ± 17.9	5.1 ± 14.5	
Min	-42.9	-42.9	-27.9	
Max	23.8	21.3	23.1	
N	19	10	9	
Peak TnT (ng/L)				p=0.386
Median ± IQR	33 ± 42	36.5 ± 65	28.5 ± 43	
Min	8	8	9	
Max	128	92	128	
N	32	16	16	
*Negative value means improvement				

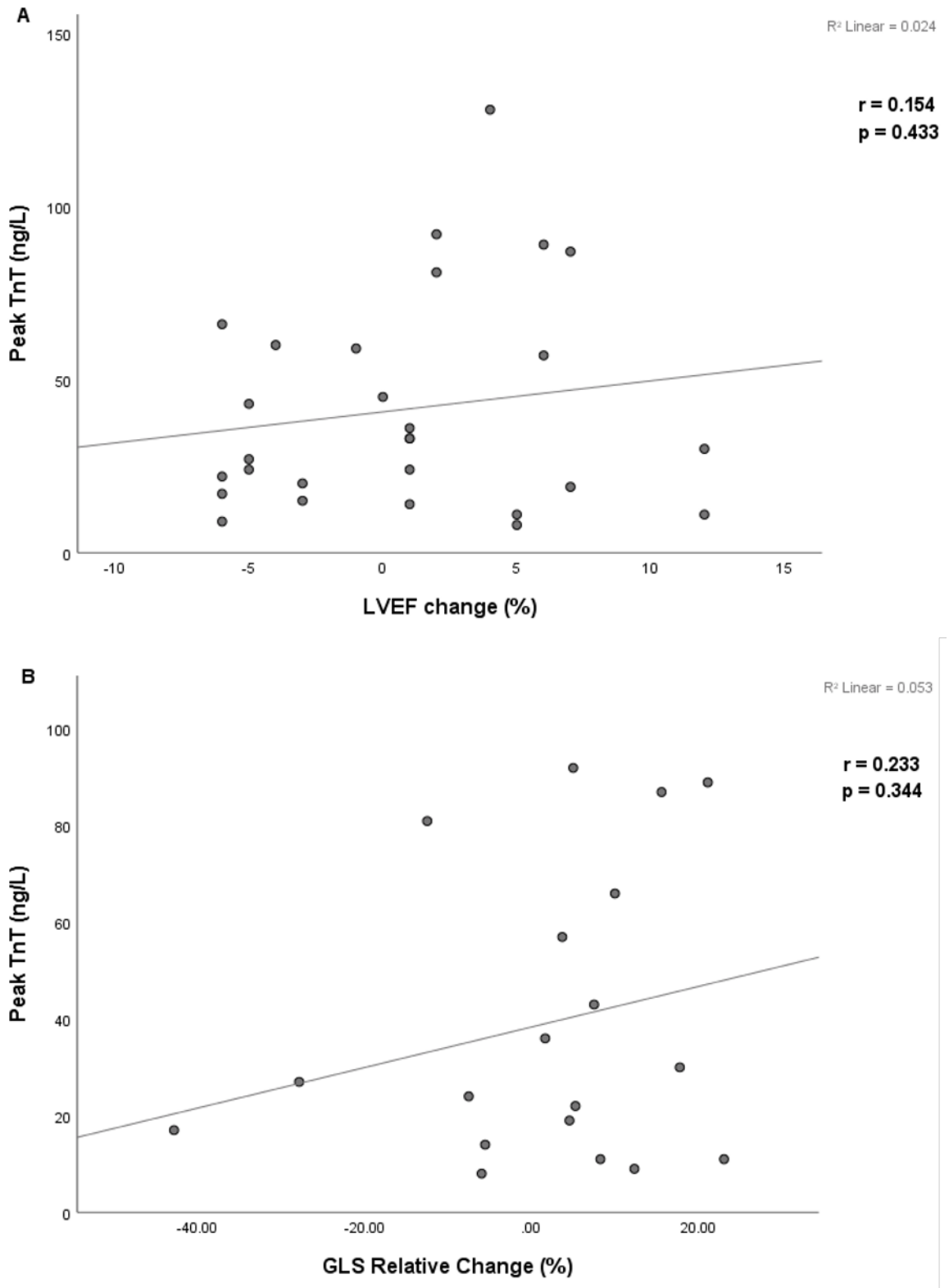
Figure 4.19. Histograms of distribution and frequencies for LVEF (A) and GLS (B) change



There is no significant correlation between peak TnT and LVEF change or GLS relative change (Figure 4.20). Pearson's correlation coefficients showed only a weak correlation for each (r=0.154 for LVEF, r=0.233 for GLS) and both were non-significant. A regression analysis was thus not performed. Similarly, analysis of the peak cMyC biomarker also did not show any significant correlation with either LVEF change or GLS relative change (r=0.01 for LVEF, r=0.264 for GLS). Three patients in the cMyC cohort had a GLS relative change of >15%.

Analysis of the two randomisation groups showed similar findings to the whole cohort analysis with no significant correlation between peak TnT and LVEF or GLS for either group (Group 1 (RIC) LVEF r=0.118, p=0.7, GLS r=0.44, p=0.203, Group 2 (Sham) LVEF r=0.111, p=0.694,GLS r=-0.023, p=0.953).

Figure 4.20. Scatterplots of peak TnT versus LVEF change (A) and GLS relative change (B)



4.3.1.3 Echocardiography – Results Conclusion

In summary, there is no relationship between peak TnT as measured during or after chemotherapy and absolute percentage change in LVEF and relative percentage GLS.

4.4. Cardiac Magnetic Resonance Imaging (CMR)

To assess if rapid sequence CMR scanning can be used as a monitoring tool before and after chemotherapy, a pilot study was set up to see if CMR scanning with a rapid sequence protocol is feasible as described in 2.5.2.3.2 and 2.6.2.3.2.

4.4.2.1 Assessment of scan duration and patient uptake.

In total, at all time-points, 34 CMR scans from 20 patients were analysed for scan duration (including 2 scans at baseline of patients subsequently withdrawn from study and 4 scans at 12M follow-up period) with a mean time of 14 ± 3 minutes with the real-time short axis stack cine included and 12 ± 2 minutes without it.

At baseline, 21 patients who participated in the randomised controlled trial and had subsequent follow-up as part of the study were offered a baseline CMR. Of those, 18 (86%) had a successful CMR scan performed whereas 3 (14%) declined. At the three-month time-point, 13 patients (62%) were offered a CMR and 8 (38%) were not offered one. Reasons for not offering included: 1. Clinically unsafe (2; one patient in decompensated heart failure and unable to lie flat, 1 patient in ITU with complications post stem cell transplant), 2. Declined baseline CMR scan (3) and 3. COVID-19 restrictions (3). Of the 13 patients who were offered a 3-month CMR scan, 2 declined the offer and one accepted the offer but no CMR slot was available. Thus, 10 (48%) CMR scans were analysed at the 3-month time-point. In the 2 patients that declined their follow-up scan, 1 patient declined all study tests and subsequently withdrew from the study at the 3-month

follow-up and the other patient did not like the experience from the baseline CMR and did not want it repeated. Taking all the CMR offers at baseline and at 3 months (34) therefore, 29 (85%) were accepted and 5 (15%) were declined.

4.4.2.2 CMR scan results before and after chemotherapy

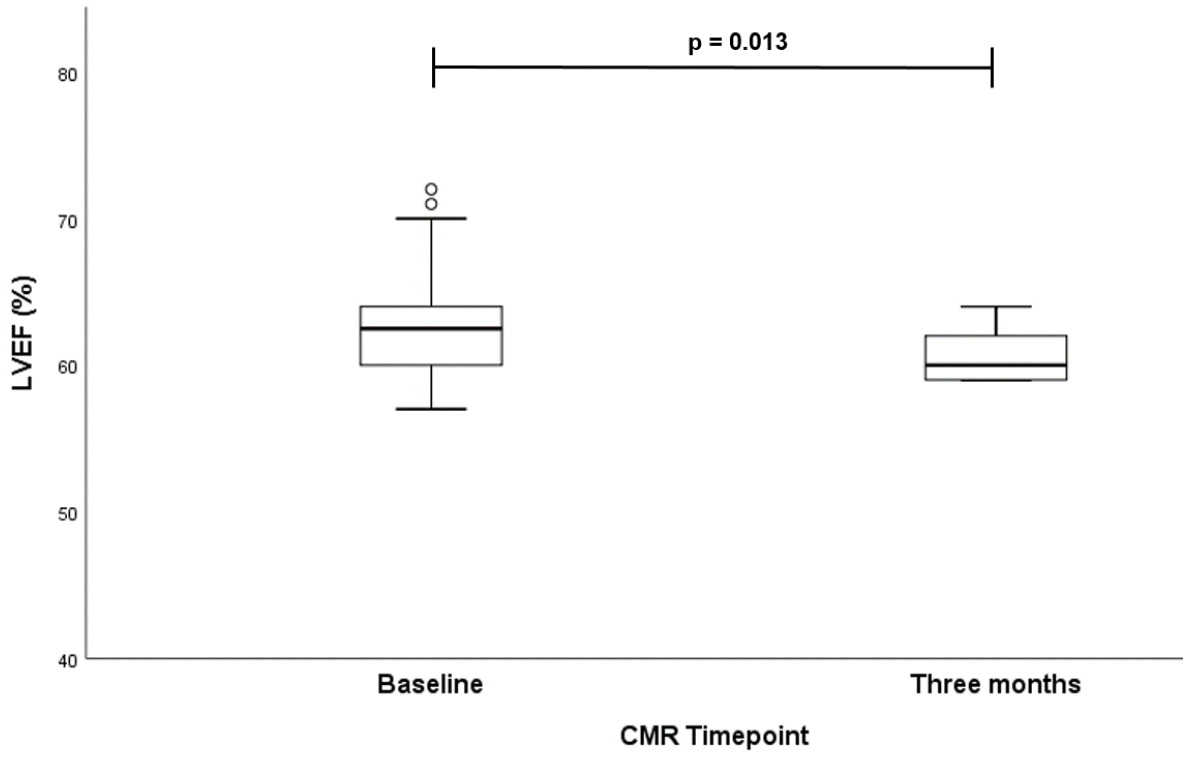
The results of the CMR analysis at baseline and 3 months, is shown in Table 5.9. There was no significant change in the LV, RV and atrial size before and 3 months after chemotherapy. Right ventricular function as measured by RVEF was unchanged. Tissue mapping remained similar before and after chemotherapy. There was a small decrease in mean LVEF of 2.9% (95% CI 0.8-5%, p = 0.013) that was statistically significant at the 5% level however still within normal range (Figure 4.21). The maximum decrease in LVEF was 7% (from 71% to 64%) and interestingly it was in a patient who had a 6% LVEF drop and a 21% relative drop in GLS by echocardiography.

Table 4.9.CMR parameters before and after chemotherapy for all patients

	Baseline (n = 18)	Three months (n = 10)
LV Parameters		
LVEDV (mls) (Mean ± S.D)	141 ± 30	143 ± 29
LVESV (mls) (Mean ± S.D)	53 ± 15	57 ± 13
LV mass (g) (Mean ± S.D)	109 ± 22	105 ± 29
LV MWT (cm) (Mean ± S.D)	8.6 ± 1.5	8.9 ± 1.5 (n=9)
LVEF (%)* **(Mean ± S.D)**	63 ± 4	61 ± 2
MAPSE (mm) (Mean ± S.D)	15 ± 3 (n=14)	15 ± 3
RV parameters		
REDV (mls) (Mean ± S.D)	138 ± 34 (n=17)	153 ± 33
RESV (mls) (Mean ± S.D)	51 ± 20 (n=17)	57 ± 18
RVEF (%) (Mean ± S.D)	64 ± 6 (n=17)	63 ± 7
TAPSE (mm)	25 ± 7 (n=16)	27 ± 4 (n=9)

(Mean ± S.D)		
Atrial Parameters		
LA area (cm^2) (Mean ± S.D)	20 ± 5	22 ± 5
RA area (cm^2) (Mean ± S.D)	19 ± 3	20 ± 5
Tissue Mapping		
Native T1 (ms) (Mean ± S.D)	1031 ± 38 (n=14)	1036 ± 38 (n=9)
Native T2 (ms) (Mean ± S.D)	45 ± 2 (n=14)	45 ±2
*p = 0.013 Three month vs baseline		

Figure 4.21. Boxplot of CMR LVEF (%) at baseline and 3 months post chemotherapy



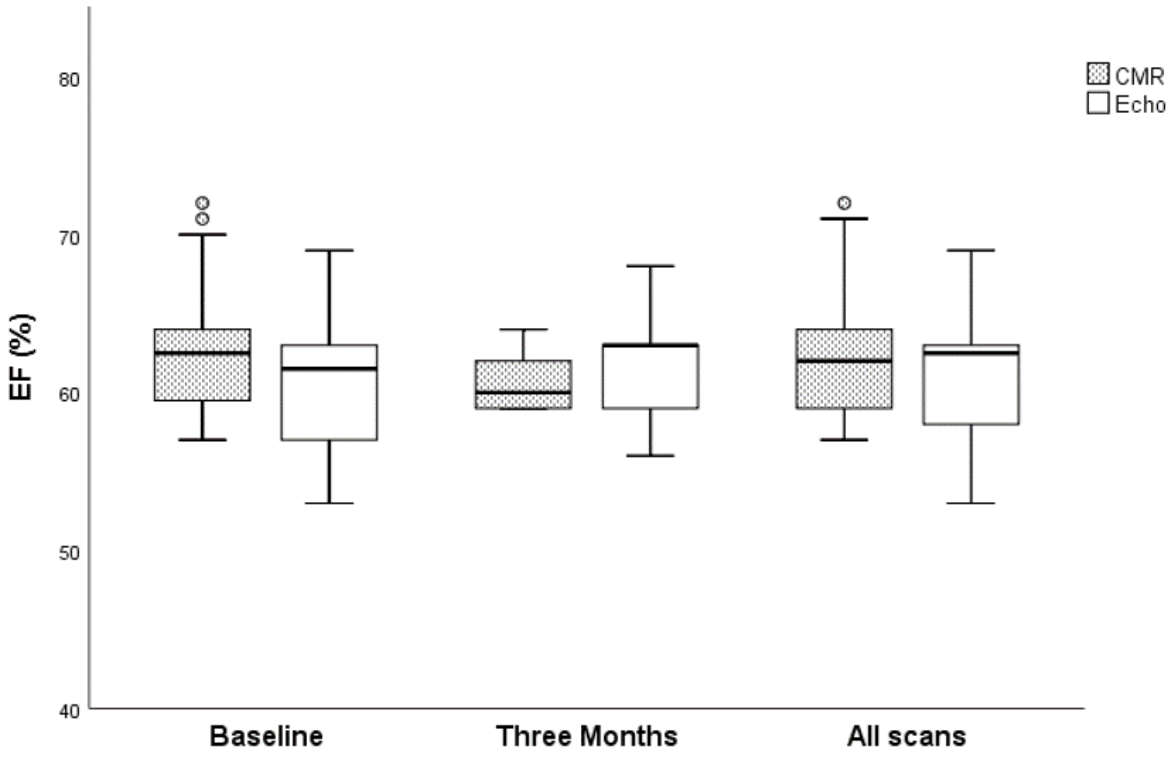
4.4.2.3 LV function by CMR versus echocardiography

A comparison of LV function analysis using LVEF by CMR and echocardiography is shown in Table 4.10 and Figure 4.22. There is no difference in LVEF at baseline or at 3 months between CMR and echocardiography as well as no difference in LVEF absolute percentage change between the two modalities. When assessing the two modalities using all CMR scans performed (including two at baseline of patients who subsequently withdrew and 4 from 12 month follow-up, total n = 34) there is no difference of between CMR and LVEF assessment.

Table 4.10. CMR versus echocardiography

	CMR	Echocardiography	
Baseline LVEF (%) (Mean ± SD) N = 18	63 ± 4	61 ± 4	p = 0.089
Three Month LVEF (%) (Mean ± SD) N = 10	61 ± 2	62 ± 3	p = 0.212
LVEF change from baseline to 3 months (%) (Mean ± SD) N = 10	3 ± 3	1 ± 3	p = 0.199
All scans LVEF (%) (Mean ± SD) N = 34	62 ± 4	61 ± 4	p = 0.2

Figure 4.22. Boxplot of CMR versus echocardiography assessment of LV function using LVEF



4.4.3 Cardiac Magnetic Resonance Imaging (CMR) – Results – Conclusion

In summary, CMR scanning with a rapid sequence protocol, as a potential tool for monitoring of anthracycline cardiotoxicity seems to be feasible, accepted by most patients and with overall similar results to echocardiography in the assessment of LV function. The mean duration of CMR using this rapid sequence protocol in cancer patients was 14 minutes. The majority (85%) of patients agreed to undertake a scan using this rapid protocol though 15% declined the offer. There is no change in the majority of CMR parameters investigated between baseline and 3 months after chemotherapy. There was a small but statistically significant drop in LVEF of 3% from baseline to 3 months, which however is unlikely to be of any clinical significance. When comparing LVEF and LVEF change as assessed with CMR and echocardiography there was no significant difference at baseline or 3 months.

4.5 Cardiac Monitoring

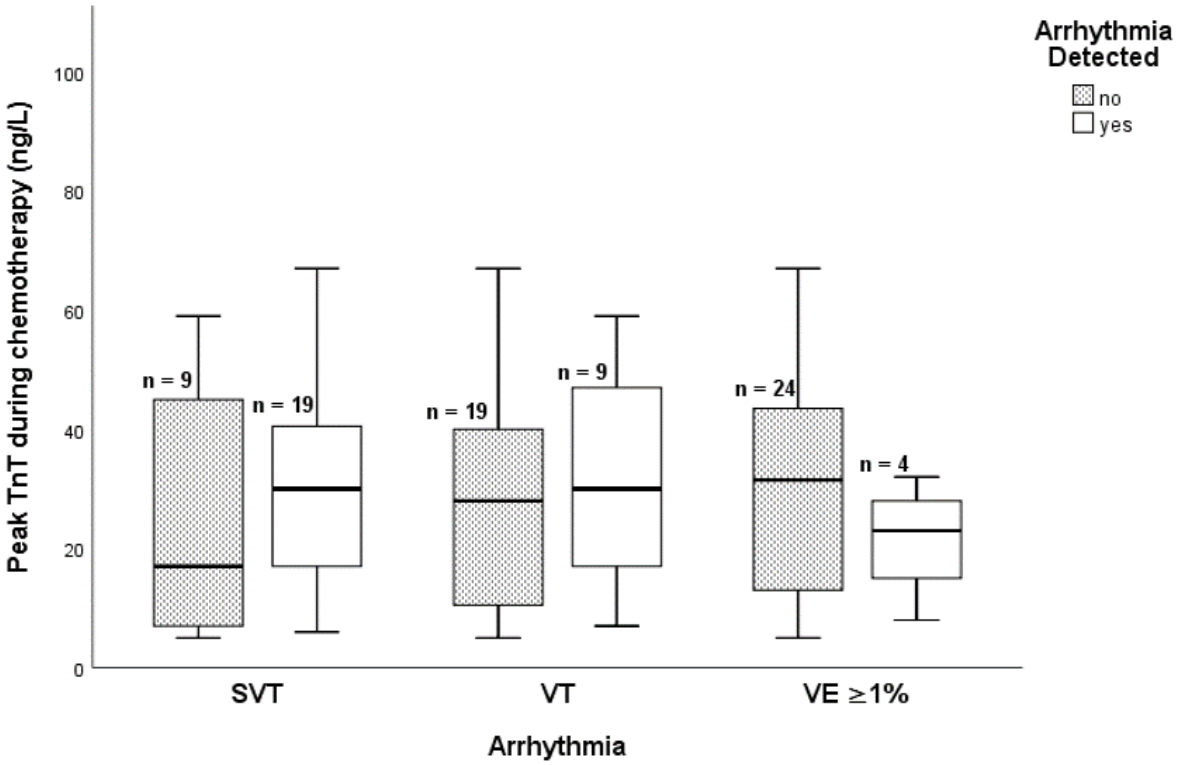
The presence or absence of cardiac arrhythmias during chemotherapy and the corresponding peak TnT during chemotherapy for all 28 patients who had a cardiac monitor attached is shown in Table 4.10. The P values represent comparison of presence versus absence of arrhythmia according to their peak troponin. There was no significant difference in peak TnT during chemotherapy between patients who had or did not have an arrhythmia in any of the arrhythmia groups examined. Figure 4.23 shows the boxplot for the 3 most frequent arrhythmias detected.

Table 4.10. Cardiac Monitoring during chemotherapy

	All patients (n = 28)	Peak TnT (ng/L) Mean ± SD	
≥1% SVEs present (%)			
Yes	2 (7)	43.5 ± 6	p = 0.261
No	26 (93)	28.1 ± 19	
≥1% VEs present (%)			
Yes	4 (14)	21.5 ± 10	p = 0.377
No	24 (86)	30.5 ± 19	
Non-sustained SVT present (%)			
Yes	19 (68)	30.7 ± 17	p = 0.539
No	9 (32)	26 ± 21	
Non-sustained VT present (%)			
Yes	9 (32)	31.6 ± 18	p = 0.647
No	19 (68)	28 ± 19	
AV Block present (%)			
Yes	2 (7)	28.5 ± 13	p = 0.958
No	26 (93)	29.2 ± 19	

Analysis of each randomisation group (RIC vs sham) showed similar results to the whole cohort. In the SVT category in Group 2 (Sham), peak TnT was higher in patients with SVT-present compared to those with SVT-absent with a mean difference of 20ng/L that just reached statistical significance at the 5% level (95% CI 0.45-39.9ng/L, p = 0.05) which was not seen in the whole cohort analysis.

Figure 4.23. Boxplot of peak TnT during chemotherapy for the three most common arrhythmias detected grouped into presence of absence of arrhythmia



4.5.3 – Cardiac Monitoring – Results Conclusion

In summary, based on the analysis of the whole cohort, there does not appear to be any relationship between peak TnT during chemotherapy and presence or absence of any arrhythmias. However, as there is a significant difference when the analysis of randomisation Group 2 (Sham) is observed with regards to the presence of SVTs that is not seen in the whole cohort analysis, this could be a source of bias and thus the results of the whole cohort cannot be taken conclusively.

Chapter 5 Discussion

In this chapter I will provide a detailed discussion of the results. The chapter will be divided into two major sections, in a similar fashion to the results section, with one focusing on the use of remote ischaemic conditioning as a cardioprotective mechanism against anthracycline cardiotoxicity and the second section focusing on monitoring during anthracycline cardiotoxicity using troponins. The discussion will focus on explaining the results and comparing them to published evidence.

5.1 The Effect of Remote Ischaemic Conditioning (RIC) as a Cardioprotective strategy against Anthracycline induced cardiac injury

There is no significant difference in TnT concentrations during and after chemotherapy between the RIC and sham groups when TnT is used a marker of anthracycline-induced cardiac injury. Random effects regression performed during chemotherapy and follow-up shows that there is no difference in TnT values between RIC and sham groups when considering TnT as an absolute number (during chemotherapy only p=0.245, during chemotherapy and follow-up p=0.744), ΔTnT from baseline (during chemotherapy only, p=0.113, during chemotherapy and follow-up p=0.325) or when considering TnT as a binary categorical variable (positive (i.e. >14ng/L) vs negative, during chemotherapy only p=0.313-0.937, during chemotherapy and follow-up p=0.39-0.887). This is despite a significant effect of additional chemotherapy cycles (i.e. time) on the regression analysis with a significant rise in troponin compared to baseline for both the RIC and sham group.

The patients were recruited into the ERIC ONC study (Effect of Remote Ischaemic Conditioning in Oncology patients undergoing anthracycline chemotherapy)(263), which is the first study to be performed in humans investigating remote ischaemic conditioning during anthracycline chemotherapy. However, the results differ from in vitro and in vivo

studies in animals that have been published before and since the ERIC-ONC study started. In one of the earliest experiments, isolated rat hearts on a modified Langerdorff system underwent a single cycle of ischaemic preconditioning consisting of 5 minutes of global ischaemia and 10 minutes of reperfusion prior to a 20 minute epirubicin infusion(272). Functional and metabolic parameters collected from the coronary effluent were then compared to a control group. Epirubicin infusion affected some functional physiological parameters such as left ventricular end diastolic pressure and LV dp/dt, which were improved with preconditioning. Interestingly, the pre-conditioning group had a higher LDH level during the epirubicin infusion that was thought to be in part due to the effect of the pre-treatment with ischaemia(272). Troponins were not measured in this experiment. I did not measure LDH but TnT, levels were higher in the RIC group, but did not reach conventional statistically significance. The troponin elevation however, is unlikely to reflect RIC induced effects as the ischaemia was done remotely and thus could not be explained by pre-treatment with ischaemia.

More recently, in vitro experiments in cardiomyocytes treated with a hypoxic buffer solution (to mimic ischaemic preconditioning) prior to exposure with doxorubicin have shown reduced cell death compared to controls(273). However, extrapolating findings from in vitro cell work to human studies can be very difficult as in vitro cell experiments are performed under very controlled conditions. In this case for instance, in addition to using a hypoxic buffer to simulate ischaemia, ventricular myocytes were derived from healthy animals, and cell death assessed histologically after using a relatively high dose of doxorubicin.

More recent and perhaps more clinically relevant in vivo animal studies may allow for better comparisons. Gertz et al performed 3 cycles of RIC with 5 minutes of ischaemia and 5 minutes reperfusion by ligating the femoral artery of mice 1 hour prior to the

administration of a single dose of intraperitoneal doxorubicin(274). Survival at 85 days was significantly improved with RIC compared to the sham group with a p value of 0.007 (hazard ratios not provided) as well as improvements in LV mass by echocardiography and markers of fibrosis and apoptosis(274). However, cardiac biomarkers such as troponins were not measured. In my analysis I found no significant difference in MACCE outcomes, which included cardiovascular events and cancer deaths whereas Gertz et al(274) reports all deaths and, although not mentioned, presumably all were cardiac deaths as the mice used did not have cancer. Analysis of survival data from my study revealed that there was no difference in the log rank and cox regression between RIC and sham groups. However, with a small N number, and with low mortality rates, it is not possible to draw definite conclusions. A larger study that is adequately powered to assess mortality rates, would allow for better comparisons. Furthermore, LV mass decreased in the mice in the sham group and was preserved in the RIC group. Similarly, I found a decrease in the LV mass in the sham group 3 months post chemotherapy whereas the mass in the RIC group if anything increased with a ΔLV mass of 22g that was statistically significant between the two groups, though the actual mass at 3 months was non-significantly different between the two groups. Having said that, it is worth pointing out that the echocardiographic calculation of LV mass was estimated from single measurements of the IVSd, LVIDd, PWd and LVIDs diameters and may not be therefore very representative of true LV mass. This could be overcome using a different imaging modality such as CMR that more accurately assesses LV mass or if using echocardiography then 3D techniques could be used(319), assuming good acoustic windows.

The route of doxorubicin administration may also be important. The intraperitoneal route, not routinely used clinically, is commonly used in animal studies as is technically simpler particularly in small animals and it does not have the same severe local side-effects due to

extravasation(320). The area under the concentration curve following intraperitoneal administration of doxorubicin however, is half compared to that of the intravenous route, likely due to first pass metabolism and partial biliary excretion(321) as well as the pharmacokinetic phenomenon of the “peritoneal-plasma barrier” (322). Thus, animal studies using the intraperitoneal route are likely to be poorly correlated to human studies using the intravenous route.

He et al performed a regime of 4 cycles of 5-minute of ischaemia and reperfusion using a tourniquet on the hindlimb of mice, 30 minutes prior to a single 10mg/kg intraperitoneal injection of doxorubicin(275). The RIC regime was repeated for 5 consecutive days and on day 6 cardiac function and troponin I were assessed. As well as better preserving LVEF, this repeated RIC regime significantly reduced levels of troponin I compared to the control group ($p < 0.05$). This was not seen in the analysis of my experiment. One possible explanation may relate to the route and dose of administration as described above. For example, an ‘average-sized’ patient with sarcoma with a body surface area of 1.9m^2 (e.g., 75kg man with height 1.75m) would typically receive 450mg/m^2 of intravenous doxorubicin over 6 cycles of chemotherapy (typically every 3 weeks) which equates to 11mg/kg total dose. Studies suggest that 50% of the intraperitoneal injection reaches the heart(321). Proportionally this still means that half of the human equivalent total dose (and full dose in the case of the Gerz et al study where 20mg/kg was used(274)) is given as a single injection to the mice. This is more likely therefore, to cause an acute myocardial injury due to acute doxorubicin toxicity that may manifest with an increase in troponin that is potentially much higher compared to the human model of smaller repetitive cumulative doses of doxorubicin. Thus, it is possible that we see a bigger effect size of an intervention (in this case RIC) because of a bigger original acute myocardial injury in the animal studies.

In an even more recent study Galan-Arriola et al designed a more clinically relevant animal model. Pigs were given 3 cycles of 5 minute ischaemia and reperfusion by occlusion of the hindlimb with a tourniquet prior to 5 intra-coronary injections of 0.45mg/kg of doxorubicin every 2 weeks(276), mimicking to some extent clinical practice. Cardiac function by CMR was significantly lower in the control group (LVEF 32.5% vs 41.5%, p=0.04) as well as CMR (T1 mapping) and histological (collagen staining) markers of oedema and fibrosis. Cardiac biomarkers such as troponin were not performed. Thus, in this more clinically relevant animal model RIC appears to be protective against anthracycline cardiotoxicity which was not observed in my study. However, important differences remain. Firstly, troponins were not assessed which was the primary outcome in my study. Secondly, the route of administration of doxorubicin may also again play a part for two reasons. The intra-coronary route for doxorubicin administration was chosen to limit the systemic myelosuppression which can be significant in pigs(323). However, in the first instance, the intra-coronary administration of anthracycline was originally developed to create severe heart failure to be used as a model for research in end-stage heart failure(324). Thus, it is possible the myocardial injury is much higher compared to the human model of intravenous administration and again it is possible we are seeing an improvement from RIC due to a bigger initial injury compared to the human model. Secondly, the intra-coronary administration of doxorubicin may be affecting myocardial contractility in a different manner compared to the systemic intravenous route. In fact, infusing doxorubicin in the LAD reduces wall contractility in the myocardium supplied by the LAD but not the remote areas and RIC improves this contractility only in the LAD region but not the remote regions (LAD RIC group contractility: 23.8%, control 6.59%, remote RIC group: 58.4%, control 45.4%)(276). This suggests that there may be a local toxic effect of doxorubicin when given via the intra-coronary route and causing a bigger injury.

Though statistically non-significant, numerically, there were more patients in the RIC group who received a doxorubicin infusion rather than a bolus (7 (44%) vs 4(25%)). Infusions over 48-96 hours are sometimes used as a cardioprotective strategy as it reduces peak concentration levels, though admittedly prolongs exposure to anthracyclines but in adults at least, there is some evidence of benefit. In a Cochrane systematic review, an infusion of 6 hours or longer reduces risk of clinical heart failure by 70% (RR 0.27, 95% CI 0.09 to 0.81, p = 0.02) in 5 randomised controlled trials of total 557 patients with no substantial heterogeneity of 2%(331). Of those 5 studies, only one however, the largest (240 patients, reached statistical significance(332). Therefore, if more patients received a less cardiotoxic regime in the intervention group, this could have made it more difficult to detect a difference from the RIC application.

Thus, it is possible the beneficial effect of RIC seen in the animal studies which is not seen in the analysis of my data is due to different methodologies and is likely that the myocardial injury as measured by troponin release in humans is nowhere near as big as in the animal models, thus making any benefit from an intervention such as RIC more difficult to detect. Repeating the study in breast cancer patients receiving anthracyclines followed by trastuzumab which is known to enhance the cardiac injury associated with anthracyclines, may be better to detect any benefit from RIC.

However, it is also possible that the RIC benefit seen in animal models of anthracycline cardiotoxicity simply does not translate into clinical studies. The recently published large, multicentre, international, randomised controlled CONDI-2/ERIC-PPCI trial investigated the effect of RIC in clinically relevant outcomes of cardiac death or hospitalisation for heart failure at 12 months in 5401 patients presenting with STEMI and treated with PPCI(249).

There was no difference between the RIC and control groups with 8.6% of events in the control group and 9.4% in the RIC group (HR 1.1, 95%CI 0.91-1.32, p=0.23)(249) despite previously promising smaller trials(250). The reasons for the failure to see any benefit were not known but potentially included inefficient conditioning protocol, RIC timing with relation to PPCI, co-medications, comorbidities and patient characteristics, infarct location and TIMI flow (though pre-specified subgroup analysis for some of these still showed no benefit with RIC)(249). Though not mentioned in the discussion section, interviews of senior authors at the ESC 2019 conference in Paris where the study was presented also mention that it is quite possible that in the modern era of STEMI care, reperfusion injury, the target of RIC, may be too small to see any meaningful translation into clinical outcomes(325). Cardiac death within 12 months was only around 3% (2.7% vs 3.1% Control vs RIC) and thus the capacity to improve on that must be very limited. One limitation identified in this study was the 1 year follow up which may be too short and a longer one may have been more useful(250). Nevertheless, the CONDI-2/ERIC-PPCI trial looked at a different cohort, with different outcomes and different pathology compared to my patient population. However, one analysis that may be relevant is the effect of RIC on troponin. In a sub-study of 2662 patients from the CONDI-2/ERIC-PPCI trial, MI size was quantified with a 48-hour area under the curve high sensitivity troponin T taken at 4 (CONDI-2) or 5 (ERIC-PPCI) time-points post PPCI. There was no difference between the two groups suggesting that there was no detectable change in acute TnT change(249). This was in contrast with some previous smaller studies where levels of troponin were reduced in patients receiving ischaemic conditioning(326–328) raising the possibility of a Type 1 Error(249), though admittedly some earlier studies also failed to show an effect of RIC on levels of troponin during myocardial infarction(242,248,329) . Thus, it is possible that troponin release as a marker of myocardial injury is not affected by RIC and thus a different marker of myocardial injury may need to be looked at.

Furthermore, the variability in patient characteristics in terms of age, different cancers, comorbidities, chemotherapy regimes and adjunctive medications to chemotherapy, may also affect the efficacy of RIC as a cardioprotective mechanism. Indeed as explained by Ferdinandy et al, the effect of other factors on the cardioprotective signalling of preconditioning was known from the 1990s and since then many factors, comorbidities and medications have been identified that potentially affect the cardioprotective effect of conditioning(330). The research understandably focused mainly on cardiovascular risk factors and medications so it is very possible that non-cardiovascular factors and non-cardiovascular medications such as chemotherapy or chemotherapy adjuncts may also have an effect on the cardioprotective signalling of ischaemic conditioning. For instance, and particularly relevant to cancer patients, there is evidence from animal studies of endogenous opioids mediating RIC induced cardioprotection as evidenced by the abolishment of the beneficial effect of RIC in the presence of the opioid antagonist naloxone(331). In humans with STEMI undergoing PPCI, addition of morphine to RIC shows similar resolution of ST elevation to RIC only, but with a significantly lower peak troponin in the morphine group(332).

As described earlier, He et al(275) and Galan-Arriola et al(276) show a preservation of LVEF as a marker of anthracycline cardiotoxicity in the animals that received RIC. In my patients there was no difference in LVEF between the RIC and sham groups from baseline to three months post chemotherapy. LVEF in RIC group dropped by 2.3%, and in the sham group it increased by 0.47% at 3 months with a mean ΔLVEF difference of 2.8% between the two groups (95% CI -1.2-6.7, p=0.162). Most of the studies in ischaemic conditioning in STEMI do not look at LVEF as an outcome but rather look at hospitalisations for heart failure which is clinically more relevant. Furthermore, patients

presenting with STEMI tend not to have a baseline echocardiogram. In one study that did look at LVEF, Munk et al assessed the impact of RIC on LV function at 30 days post STEMI treated with PPCI(333). When assessing all patients, there was no difference in 2D LVEF by echocardiography between RIC+PPCI and PPCI only groups. However, there was a difference in EF at 30 days in those with extensive area at risk (AAR) or in those presenting with anterior infarct (high AAR EF 0.51±0.07 versus 0.46±0.09; *P*=0.05, anterior MI EF 0.55±0.08 versus 0.50±0.11; *P*=0.04). Thibault et al assessed LV function recovery one year after STEMI in patients given postconditioning during PPCI and found it to be 7% better in the postconditioning group vs control (49%±13 vs 56±8, p = 0.04)(334).

The reasons why no effect of RIC on LVEF was seen in my study are unclear. One reason could be that RIC has no cardioprotective effect in anthracycline cardiotoxicity (as no effect on troponin was seen either). Another reason could be simply related to the small n number of the study as no deterioration in mean LV function was noted in the sham group either. In studies investigating cardioprotective strategies in anthracycline chemotherapy such as beta blockers or ACE inhibitors that monitor LVEF as a continuous variable, their placebo arms report mean deteriorations in LVEF varying from as little as 1% to as much as 17%(161,162,165,178,192,193,197,203,207,210,335) with only two of those studies showing no statistical significance(197,207). However, all these studies are small with the largest number of patients in the placebo arm being 96(197), assessment of LV function occurs at varying time-points post chemotherapy, and chemotherapy regimes, cancer types and doses vary. Recently, Narayan et al performed a longitudinal study in 277 breast cancer patients who received doxorubicin and/or trastuzumab and showed that LVEF will reduce by 3.6% by 1 year (6.6% if also having trastuzumab)(336). In an update study it appears that patients will have 3 different patterns of LVEF trajectory; a stable LVEF pattern (51% of patients), a pattern with a modest LVEF decline in the first 6 months

that persists throughout follow-up (40% of patients) and a pattern with a significant decline in the first year that partially recovers (9%)(337). This suggests variability between patients with regards to LVEF post chemotherapy. A further reason why no change in LVEF was noted in the sham group may be to do with whether the follow-up scan was performed too early (i.e. at 3 months). Most of the studies mentioned above that showed a deterioration in LVEF in their placebo arms, performed LV function assessment at 6 months or later, though some have shown a difference in LVEF as early as 3 weeks(210) and 3 months post chemotherapy(335). In addition, in one of the biggest prospective trials of more than 2000 patients, 98% patients who will develop a deterioration in LVEF of >10% and below 50%, will do so within the first year post chemotherapy, but the median time is 3.5 months(338).

As discussed previously, LVEF can be prone to errors with documented variabilities of up to 10%(129), thus GLS, a myocardial deformation index useful in identifying early cardiotoxicity, is subject to fewer errors with inter- and intraobserver variabilities comparable with or superior to LVEF(339) amongst both expert and trainee echocardiographers(340). Looking at GLS, there is no difference in the relative percentage change between the two groups with a mean relative percentage change difference of 6.4% (95% CI -9.6-22.4, p=0.411) at 3 months post chemotherapy. The sham group had a mean relative percentage change in GLS of 5.1% which was to some extent surprising as Thavendiranathan et al in their systematic review on the use of myocardial strain for early detection of cardiotoxicity, report bigger changes with a relative reduction in GLS of between 9-19% with 2D speckle tracking echocardiography and between 15-17% with tissue Doppler imaging(131). This was detected either during or soon after (within days to weeks) chemotherapy, but with some studies also performing echocardiograms months post chemotherapy (up to 18 months). Interestingly, Munk et al, like with LVEF, reports

improvement in GLS at 30 days post STEMI in the RIC+PPCI group in the patients with high AAR (−15.8±2.6% versus −13.5±3.2%, p=0.01 for the upper AAR quartile) but not for all patients(333). Thus, the lack of a significant drop in GLS in the sham group may also point towards study size as a potential factor affecting the results.

The effect of RIC on clinical events in patients who have received anthracycline chemotherapy was also looked at. There was no difference between the RIC and sham group in the composite end point of major adverse cardiovascular events and cancer death up to 6 months of follow-up when analysed as time to event data on Kaplan-Meier plots. Similarly, there was no difference between RIC and sham groups in major adverse cardiovascular events or cancer deaths or cancer progression when analysed independently. This was in keeping with the only study looking at the effect of RIC on cancer cells by Maulik et al(273).

When looking at serious adverse events, a similar number of patients from each group suffered at least one serious adverse event (88% vs 81%). There were more patients admitted with at least one episode of any infection in the RIC group (13 (81%) vs 8 (50%)) which was not statistically significant. Admissions due to sepsis or fevers with or without neutropenia (12 (75%) vs 5 (31%)) was statistically significant (p=0.013). A similar trend was identified when analysing the total number of serious adverse events. There was a total of 16 (53%) episodes of sepsis or fevers with or without neutropenia in the RIC group vs 5 (23%) in the sham group (p=0.026). This was an unexpected finding and with no difference in troponins between the two groups the Data Monitoring Committee advised closure of recruitment into the ERIC ONC study.

There is some data to support RIC as a protective intervention in sepsis. On a lipopolysaccharide-induced sepsis mouse model, 3 cycles of remote ischaemic conditioning using the right hind-limb prior to LPS injection improves survival at 120hrs post sepsis and reduces pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 by possibly attenuating NF-κB activation(341). In a similar experiment, RIC (6 cycles of 4 minutes ischaemia-reperfusion of femoral artery) performed either at 0, 2 or 6 hours post LPS injection in mice, improve 5-day survival (57% vs 21%, p=0.02)(342). The survival benefit seems to persist when RIC is performed repeatedly (i.e. chronic) as well as improving sepsis-induced cardiomyopathy (LVEF 32% vs 47%), reducing cardiac troponin I and protecting other systemic organs as assessed by biomarkers(343). Furthermore, benefits were seen when larger animals were also investigated(344). However, no clinical studies have yet been performed.

The reasons why an increased rate of admissions for sepsis, or fevers, with or without neutropenia, was noted in the RIC group are not clear. Although reaching formal statistical significance, both the number of events and the number of patients was small. Therefore, there remains a probability (of 5% when using 95% confidence intervals) that this observation might be attributed to chance. Furthermore, the diagnosis of “sepsis” leading to admission was a clinical one, based on history and/or clinical notes. Thus, there is the probability of inaccurate diagnosis in a clinical syndrome that was not prespecified as conforming to a precise definition.

Though statistically not significant, numerically there were more people on a doxorubicin-ifosfamide regime in the RIC group (5(31%) vs 2(13%)). Ifosfamide is an alkylating agent and when combined with doxorubicin in soft tissue sarcoma, it causes more grade 3 and 4 side effects like leukopenia (43% vs 18%), neutropenia (42% vs 37%), febrile neutropenia

(46% vs 13%) and anaemia (35% vs 5%) than doxorubicin alone(353). Differences in the chemotherapy regimes in the two groups therefore, may have contributed to the observation of more admissions with febrile neutropenia and/or sepsis.

It is however possible that RIC may enhance the systemic cytotoxic side-effect profile of chemotherapy agents, particularly with relation to myelosuppression. Haematological indices, such as neutrophil counts, were not recorded for those adverse events due to incomplete dataset as many hospital admissions were in the patients' local hospitals and not our institution.

Interestingly, when dexrazoxane was first used clinically as a cardioprotective agent, increased myelosuppression was noted when measured using white cell count and platelet levels in the dexrazoxane group but with no clinical consequences and with similar episodes of fever between the two groups(345). A similar trend in a subsequent study in children was seen, though again with no clinical consequences(216). However, a subsequent Cochrane review in 2011 showed no difference in most of those toxicities apart from grade 3 (severe) or 4 (life-threatening) abnormal white cell count at its nadir (RR 1.16, 95% CI 1.05 to 1.29, p=0.005) in favour of the control group(220) and a systematic review since then concluded that dexrazoxane may be associated with specific haematological toxicities including myelosuppression which are reversible, but the authors acknowledged that the evidence is variable and that toxicity is likely influenced by a variety of factors(225).

Despite this unexpected finding of increased admissions for infections, it might help in understanding underlying pathophysiological mechanisms and generate hypotheses. If dexrazoxane, an iron chelator, may have some effect on myelosuppression, and from this

experiment there may also be a signal for RIC to have a similar effect, then one could assume that RIC, may indeed be acting through similar pathways to dexrazoxane by affecting iron metabolism as one of the predominant mechanisms of cardioprotection. This has already been explored by Fang et al in their set of elegant experiments where dexrazoxane prevents both doxorubicin-induced and ischaemia-reperfusion induced cardiomyopathy in mice(271). Thus, rather paradoxically, RIC may still have a role to play in preventing anthracycline-induced cardiomyopathy. It is possible that, the RIC regime used was not sufficient to produce an effective cardioprotective response and a different RIC protocol may be needed, such as with more RIC cycles or with RIC performed on the day of chemotherapy and then to be continued for several days post each chemotherapy cycle. This, however, will require accurate clinical definitions to better characterise any adverse events.

5.2 Multimodality monitoring of anthracycline cardiotoxicity

The screening and monitoring of patients for anthracycline cardiotoxicity consists of three parts: 1. screening of patients prior to chemotherapy to identify those at highest risk, 2. Monitoring patients during chemotherapy to ensure patients complete their therapy without any harm and 3. Monitoring patients after chemotherapy to detect any post chemotherapy cardiotoxic effects (early and late). In fact, this is true for any cancer related therapy and is a major part of any cardio-oncology service(346). For anthracyclines in particular the protocols to undertake these three aspects of clinical monitoring remain imperfect, despite more than 50 years of research. There have been advances in understanding and identifying patients at particular risk of anthracycline cardiotoxicity(147,347). For example the CCSS score, validation cohort estimates were high ranging from 0.68 to 0.82(148) but not 100% thus all risk scores will have their failings when applied to individual patients in clinics. To that effect, I attempted to see if existing risk cardiovascular risk scores, and in

particular the QRISK@3 score for identifying the 10-year risk of developing cardiovascular disease currently in use in the UK, may be useful. The benefits of this score are that it is readily available, provides a single percentage number as risk, whilst incorporating many of the already perceived risk factors. However, there was only a weak correlation between QRISK@3 score and peak TnT during chemotherapy or follow up ($\rho = 0.35$) with no significant relationship on linear regression analysis. The most likely explanation is that it is probably too simplistic and does not include some factors that are more likely to be related to an increase in troponin, such as total anthracycline dose received. Having said that, a preliminary assessment of 52 patients from the ERIC-ONC study cohort presented as an abstract at the ESC conference 2020, showed a stronger correlation between QRISK@3 score and troponin T with a correlation coefficient of 0.618 between QRISK@3 and TnT at cycle 5(348).

The CCSS score assesses risk of cardiovascular disease due to cancer therapy (which includes anthracyclines) for survivors of childhood cancer (within 5 years of finishing therapy) and was developed using data from the large CCSS study(148). It incorporates patient's current age, age at diagnosis, use of anthracyclines and dose and use of other agents or radiotherapy to calculate a risk for the development of heart failure, ischaemic heart disease or stroke by age 50(147). However, the data used to develop it were collected retrospectively and the score cannot be used for patients who received anthracyclines as adults. Recently, in a position paper from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society, risk assessment tools for a variety of cardiotoxic agents, including anthracyclines, have been published in an attempt to assist risk stratification of patients into low, medium, high and very high risk(347). Based on available evidence and expert consensus, a risk stratification proforma is provided that

can be used to calculate risk for individual patients. For anthracyclines specifically, this incorporates the presence of elevated cardiac biomarkers at baseline, however not during or after chemotherapy(347). It will, however, require validation with prospective data (in a prospective study or real-time registry).

One important finding from my thesis is that in patients who receive anthracycline chemotherapy, TnT rises during chemotherapy and in most cases peaks about one month after chemotherapy. Treating TnT as a binary variable, shows that 87% of patients have at least one positive TnT during chemotherapy and follow-up. Furthermore, 66% of patients have a positive TnT during chemotherapy that persists up to 6 months post-chemotherapy (TnT +/+) with levels of TnT that are significantly higher compared to patients who have no positive TnT (TnT -/-) or have positive TnT during follow-up only (TnT -/+). These results follow a similar pattern to previous research. Specifically, Cardinale et al assessed 703 patients with a variety of cancers and measured their troponin I at baseline and at 5 different time-points (immediately after and 12, 24, 36 and 72 hrs after) at each cycle of chemotherapy (early) as well as at one month post chemotherapy (late)(143). The highest TnI value was recorded for each patient and three groups were identified: negative TnI (TnI-/-), early positive, late negative (TnI +/-) and positive early/late TnI (TnI+/+). This is a similar pattern to my findings except one group in my patients had a TnT that was negative early on but positive later. Cardinale et al reports 495 of 703 (70%) patients in the Tn-/- group, and only 63 of 703 (9%) in the Tn+/+ group(143) which is very different to my reported 13% and 66% respectively. A likely explanation for this difference is the troponin assays used. Newer high sensitivity troponin assays are able to measure lower values and detect smaller increases above the 99th centile upper limit of normal compared to more contemporary and point of care assays(310). Cardinale's study used the Stratus CS(143), a point of care assay(279), with a total percentage imprecision between 10-14%(349). In our

study we used a high sensitivity assay, which has a total percentage imprecision of 8% according to published data(279). In addition, Cardinale et al(143) recruited patients with different cancers with just over half of them (57%) receiving anthracyclines whereas all patients reported in this study had received anthracyclines.

Recording troponin values as positive or negative based on the 99th percentile upper reference limit of the assay being used, has the advantage of being simple to use for clinicians and easy to categorise patients into groups. However, one major limitation is the inability to quantify rises in troponin, which might provide insight to the degree of myocardial injury and therefore reflect the risk of subsequent cardiomyopathy. Furthermore, different assays will have different sensitivities in their ability to detect smaller rises in troponin above the 99th percentile as explained above. Not many studies report on what happens to troponin concentrations at each chemotherapy cycle by looking at the troponin concentration as a continuous variable as described in the current study. I found that, high sensitivity TnT gradually increases with each successive cycle from a median concentration of 7ng/L to 34ng/L ($p<0.001$) and a mean change of 28ng/L (ΔTnT) by cycle 6 that persists for one month post chemotherapy before gradually falling towards baseline. Similarly, Jones et al assessed high sensitivity TnI changes at each cycle of chemotherapy in 38 patients receiving anthracyclines and found incremental increases in TnI at each successive cycle that were significant by cycle 5 with a median increase of 30.7ng/L(350). Tzolos et al noted similar observations in 78 patients with breast cancer receiving anthracyclines, with the level of high sensitivity troponin I at each cycle being strongly correlated to the cumulative dose received(351). Furthermore, there was a 1.3fold increase in TnT by cycle 2 in 45 patients with breast cancer on an anthracycline regime with 42% of patients exhibiting an elevation in troponin at that time-point, but no other samples were performed during the rest of the anthracycline cycles(352). In 82 patients

with lymphoma on the CHOP/R-CHOP regime, high sensitivity troponin T increases significantly between cycles 2-4 and cycles 6-8 compared to baseline (12ng/L and 23ng/L vs 3ng/L)(353) and by cycle 3 in another study of 58 lymphoma patients receiving RCHOP(354), however again blood tests were not performed at every cycle. Therefore it is crucial to understand how the different biomarkers and assays behave during chemotherapy and in different clinical settings as it is likely that we will be using cardiac biomarkers during chemotherapy, particularly in patients deemed high risk, in the future(355).

Despite significant increases in TnT during chemotherapy and follow-up, I found no significant correlation between peak TnT values and change in LVEF or GLS from baseline to 3 months post chemotherapy (r = 0.1544, r = 0.233 respectively). However, the mean LVEF change and mean relative GLS change was only 1% and 2% respectively and would thus be difficult to show an association if the outcome is so small. Therefore, a larger sample size and performing LVEF beyond 3 months maybe needed to better assess for any correlations. As discussed below, overall, published evidence suggest that troponins (as a continuous variable) during chemotherapy or follow-up are associated with reductions in LVEF or development of cardiotoxicity. Cardinale et al has shown strong correlations between maximum TnI value and maximal LVEF reduction (r = 0.87 and 0.92) but only in patients who had at least one positive troponin value(141,142). In 81 breast cancer patients who received anthracyclines and subsequent taxanes and trastuzumab, high sensitivity troponin I 3 months post anthracyclines is not predictive of subsequent cardiotoxicity when used as a continuous variable but was predictive when used as a binary variable (in this case if ≥30pg/mL)(356). However, in a similar sized study with a similar cohort of breast cancer patients receiving anthracyclines followed by trastuzumab, for each increase in the standard deviation in TnI performed 3 months post chemotherapy

there was a 40% increase risk of cardiotoxicity (HR 1.38, 95% CI: 1.05 to 1.81; p = 0.020) and those patients in the highest percentile for TnI change had a 34% chance of cardiotoxicity by 15 months(357). In a small study of 40 breast cancer patients, the increment change of high sensitivity TnT may be able to predict cardiotoxicity but not the absolute peak TnT number(358). More recently in a larger study of 254 breast cancer patients treated with doxorubicin and/or trastuzumab, changes in TnT were associated with changes in LVEF with roughly a 0.6% reduction in LVEF for every doubling of the biomarker concentration as well as with subsequent development of cardiac dysfunction(359).

A recent meta-analysis assessed different biomarkers (troponins and natriuretic peptides) during chemotherapy and their ability to predict cardiotoxicity(360). In the troponin analysis, 30 studies (3049 patients) using a dichotomous (i.e., binary) analysis and 12 (811 patients) using an analysis of absolute (i.e., continuous) numbers were identified. Using the dichotomous studies, the chances of an increased troponin post treatment was much higher (OR 14.3, 95% CI 6.0–34.1) and this was highest for anthracyclines (OR 17.5, 95% CI 10.1-30.2, n=1068)(360). Similarly, in the studies using the absolute number, troponin levels were significantly higher post-chemotherapy than pre-chemotherapy (Standardised Mean Difference 1.0; 95% CI 0.6–1.3)(360). Odds ratio for troponin I and troponin T were similar. Furthermore, the likelihood of LV impairment was significantly higher if troponin was elevated, and for anthracyclines the odds ratio was 7 (95% CI 1.4-34.1, n = 326)(360). Overall, for all cancer related therapies investigated, the sensitivity and specificity of troponins to predict LV dysfunction was 69% and 87% respectively. Of note the negative predictive value was 93% thus potentially making it a useful rule out test though the positive predictive value was 52%. Importantly, heterogeneity between studies was high for a variety of factors including how LV dysfunction was defined.

Despite the majority of studies available use a binary approach when analysing troponins and their ability of to predict LV dysfunction, a recent position paper, recommends high sensitivity troponin assays to be interpreted as a continuous variable and not in a binary fashion acknowledging specifically the differences between assay characteristics as one reason for the recommendation(355). Based on the findings of the current study, I would agree with that statement as in my cohort, up to 90% of patients will have a positive troponin T at cycle 6 that persists 1-month post-chemotherapy and is still positive in 50% of patients at 6 months post chemotherapy. Thus, interpreting troponins as a continuous variable may be more useful. However, many gaps still remain in particular relating to how to interpret any changes in troponin and how much of a change, if any, is predictive of subsequent cardiac events and this is something echoed in the position paper(355). There is also no guidance whether absolute concentrations versus change in concentration from baseline (i.e., Δ) should be used hence both were analysed here.

Interestingly, in the meta-analysis above, patients who received anthracyclines, the odds ratio for troponin elevation between patients receiving <240mg/m^2 were similar to patients receiving >240mg/m^2 total dose even though there was an association between dose and LV dysfunction in studies investigating high-dose anthracyclines(360). This contrasts with the results of the current analysis where a moderate correlation between peak TnT and total dose received is suggested. This may be because the meta-analysis used troponin as a binary variable. Theoretically, you would expect a higher troponin elevation with more total dose received and thus a stronger correlation between total dose and peak troponin. I was however unable to find another study that performed a correlation analysis between dose received and peak troponin. Jones et al performed a correlation analysis between dose and troponin measurements with no correlation identified(350). However, it is not

stated whether the peak troponin was used (or indeed the total dose of anthracycline). The relationship between peak troponin achieved and total dose received requires further investigation.

Tzolos et al reports lower TnI concentrations within 24 hrs post-chemotherapy compared to pre-chemotherapy(351). This is something noted in the current analysis also and, as noted by Tzolos et al, likely to be due to dilutional effects(351). It is, however, an important finding that will likely guide future research as it suggests that the trough troponin, sampled immediately prior to chemotherapy, more accurately represents the true pre-chemotherapy troponin concentration and extent of any myocardial injury and that sampling immediately post-chemotherapy does not add to the sensitivity of detecting sub-clinical toxicity. Furthermore, we noted that the post-chemotherapy blood sample was a barrier to recruitment and thus abolishing it will likely improve patient recruitment.

The search for other biomarkers of anthracycline cardiotoxicity is an active and ongoing area of research(355). Cardiac myosin binding protein c is a novel biomarker that was chosen primarily because of its abundance in the myocardium and thus an anticipated bigger rise compared to troponin, as well as due to its earlier peak in concentration compared to troponin following myocardial injury, properties which potentially could make it a better marker to detect sub-clinical toxicity. Here I report, for the first time, the use of cMyC as a biomarker with some interesting results. Firstly, cMyC seems to follow a similar pattern to TnT in that it gradually increases in concentration as patients progress with their chemotherapy. However, cMyC appears to have a lower baseline concentration than TnT (median baseline concentration 3.34ng/L vs 6ng/L), which between cycle 3 and 4 appears to increase at a faster rate. However, for a more direct comparison between the two biomarkers, the concentration ratios to baseline for each biomarker were used. At the

highest biomarker concentration, median cMyC concentration increases 4-fold compared to 2-fold for TnT ($p < 0.001$). Furthermore, the rate of increase in cMyC concentration is higher than TnT even from cycle 2 (1.8-fold vs 1.5-fold, p=0.007), which persists and also rises faster at each cycle (cycle 6, 7.9-fold vs 4.7-fold, p=0.006). These results therefore suggest that cMyC may be a more sensitive marker than TnT to detect myocardial injury and may be able to do so at an earlier stage during chemotherapy which would make it a useful tool for monitoring patients during anthracycline chemotherapy. This is analogous to findings in patients post MI where cMyC concentrations increase to a higher degree when compared to troponin(297,298). Its usefulness as an earlier and potentially more sensitive marker of myocardial injury has been shown in patients with iatrogenic MI in the context of septal alcohol ablation for hypertrophic obstructive cardiomyopathy(299) as well as in early presenters (<3 hours) with NSTEMI(281). Whether cMyC would be useful as a predictive tool in anthracycline cardiotoxicity however needs to be correlated with cardiac function. This study found no correlation between peak cMyC concentration and LVEF or GLS change at 3 months in 22 patients. However, the results were available in a small number of patients with low clinical event rates. In 161 patients with aortic stenosis cMyC concentrations are associated with markers of hypertrophy and fibrosis on CMR imaging and in 104 patients with increased risk of mortality from aortic stenosis(301). Interestingly, cMyc concentrations were associated with indexed LV mass increase as measured by CMR (β=11.0 g/m^2 per log unit increase in cMyC after adjustment for age, sex, renal function, AV_{max}, cardiac troponin and comorbidity; 95% CI 4.7 to 17.3, P<0.001)(307). This is not surprising as cMyC is known to be abundant in the thick filaments of cardiac muscle and any changes in mass are potentially reflected in changes in cMyC concentration. Accordingly, anthracyclines are known to cause up to a 5% reduction in cardiac mass as early as 6 months post anthracycline chemotherapy as detected by CMR which were associated with worse heart failure scores without associated changes in LVEF(370).

Thus, with this amplified signal in cMyC compared to troponin seen in this study, one can speculate that changes in cMyC may potentially detect changes in LV mass thus making it a useful marker of early cardiotoxicity. A study comparing changes in cMyC concentrations in patients receiving anthracyclines versus patients receiving non-anthracycline chemotherapy whilst assessing LV mass would help in better assessing this hypothesis. Furthermore, in children admitted with heart failure, cMyC concentrations were higher overall compared to controls and were the highest in those with more severe heart failure. Mortality and readmission were predicted if cMyC concentrations remained elevated(302).

Cardiac magnetic resonance imaging (CMR) is a useful tool in cardio-oncology and is recommended in a recent position paper of the European Heart Failure Association, the European Association of Cardiovascular Imaging and the Cardio-Oncology Council of the European Society of Cardiology in assessing volumes and function particularly in patients with suboptimal echocardiographic windows(361). However, long scanning protocols, cost and lack of access make CMR less attractive to clinicians and patients alike, particularly in the cancer group where frequent scans are required for their oncological care. Based on previous work from our institution(138,362) we have devised a rapid protocol for oncology patients for the assessment of volumes, structure and function and have shown that it is both feasible and acceptable to patients. Mean scanning time was 14 minutes which can be reduced to 12 minutes without any real time short axis stack sequences and overall. In this study 34 scans were offered, and 85% were performed with no complications. Thus, similar to previous studies, quick CMR protocols specific for a particular condition or clinical scenario can be used to maximise CMR utility(138). LVEF by CMR was 63% at baseline and 61% at 3 months, which reached statistical significance ($p = 0.013$). Compared to echocardiography, there was no significant difference in the ΔLVEF from

baseline to 3 months between CMR and echocardiography (3% vs 1%). Taking all scans together, CMR LVEF was 62% vs 61% with echocardiography (p = 0.2).

The incidence of cardiac arrhythmias during anthracycline chemotherapy is not well documented. In 28 patients with a mean recording time of 11 days during the penultimate or ultimate cycle of chemotherapy, the most common arrhythmias detected were >1% burden of SVEs with a frequency of 7% and >1% burden of VEs with a frequency of 14% non-sustained SVT and VT (68% and 32% respectively) and 2 patients (7%) with transient AV block. Whether an arrhythmia was present was not related to the mean peak TnT during chemotherapy. Non-sustained SVTs were frequent but no atrial fibrillation (AF) was seen despite a previous report suggestive of paroxysmal AF in 10% of patients(89). Nine (32%) patients were found to have non-sustained VT. All were asymptomatic. However, two patients with the longest duration, 5 and 10 seconds respectively, required treatment whereas in the rest of the patients the arrhythmia lasted between 4-9 beats. One previous study reports lower VT rates of 6%(88) and none in others(308,309). It is unclear why non-sustained VT was more frequent in the current study. It is likely that the more prolonged monitoring increased the chances of recording an episode of non-sustained VT. The reasons of why VT might have occurred are also unclear. Cardiac function at that time-point was unknown as the patients would have had their next echocardiogram performed at least 3 months after the cardiac monitoring was applied according to the study protocol. Furthermore, electrolyte imbalances due to side effects of chemotherapy like diarrhoea and vomiting or inter-current illnesses such as infection that might have precipitated an arrhythmia are also unknown at that time-point. Nonetheless, an insight into patients' rhythm for a prolonged period during the last stages of anthracycline chemotherapy, which has not been investigated before, is an important analysis that will help guide clinical management and further investigations. Patients with symptoms of arrhythmia and,

particularly AF, are a common in the cancer population(363). The fact that no AF was detected in this cohort is interesting and may be related to patient population and age, cancer type and type of chemotherapy. However, equally important, the knowledge that non-sustained VT which was noted in a third of patients in this study, should prompt clinicians to be vigilant when assessing patients with symptoms of arrhythmia to detect and appropriately manage this potentially life-threatening condition.

Limitations

The main study limitation is sample size. The ERIC-ONC study, was designed as a pilot study to assess the effect of RIC on oncology patients(263). It was a hypothesis generating study exploring RIC as a cardioprotective intervention incorporating multimodality monitoring of patients receiving anthracyclines.

The sample size calculation for ERIC-ONC was 128 to detect a theoretical treatment effect of 35% with 80% power at the 5% significance level(263). The treatment effect of 35% was a hypothesised predicted effect of RIC on troponin based on studies in the context of cardiac surgery and elective PCI, with quoted event rate reductions of 26%(364), 43%(365), 18%(366) and 17%(367). Since then, research in STEMI studies has shown a beneficial effect of RIC on troponin between 16-35%(326–328), though some studies have been neutral(248,329,368) including a troponin sub-study of the large CONDI-2/ERIC-PPCI study(249).

Furthermore, the effect of doxorubicin on high sensitivity TnT concentrations in ERIC-ONC was estimated, due to lack of other studies using TnT at the time, based on a study by Katsurada et al in patients with breast cancer treated with anthracyclines and

trastuzumab(369) where a peak high sensitivity TnT of 11±7.8ng/L at 6 months was noted. However, this was after patients had received anthracyclines and trastuzumab and also that peak value corresponded to those patients who developed cardiotoxicity with a reduction in LVEF of >5% (the group without cardiotoxicity had a troponin of 4ng/L at the same time-point)(369). In addition, there is an increasing awareness of the variability in troponin changes during anthracycline therapy between patients(355). Thus, the above points demonstrate the complexities involved in performing sample size calculations in this scenario where no previous studies of RIC during anthracycline chemotherapy in humans exist. The effects of a small sample size would affect a variety of the outcomes, whether they are continuous, such as troponin and change in LVEF or categorical, such as the frequency of any clinical events as well as interpretation of p values and confidence intervals, particularly if they are borderline.

In addition, the points above also demonstrate a second limitation of the study, which is whether troponin is indeed the best main outcome to assess the effect of RIC, particularly as we are still unclear of how best to use biomarkers to predict future cardiotoxicity. In most studies investigating cardioprotective strategies for the prevention of anthracycline induced cardiomyopathy the main outcome is assessment of LV function, principally with LVEF on echocardiography, CMR or nuclear imaging(161,162,165,178,192,197,203,207,210,214,215,220,335). However, LVEF reduction can be a late feature of cardiac injury and may present late at which point myocardial damage may be irreversible and thus may not be the most suitable parameter to use(346). Cardiac biomarkers such as troponin, on the other hand, are quick, and easy to measure and very sensitive to cardiac injury. Cardiac biomarkers could be ideal as predictors of myocardial damage if a correlation between them and cardiotoxicity is proven.

Having patients with three different types of cancer, with different chemotherapy regimes will introduce variability to the results and thus make findings more difficult to interpret. However, this was a 'real world' study in patients receiving anthracyclines which despite the variability in their diagnosis would be a good cohort to test cardioprotective strategy. Furthermore, this was also a pragmatic approach to improve recruitment. Looking at published literature, both approaches are represented. The RIC and sham groups appeared well balanced with regards to cancer type and chemotherapy regimes. One way to minimise this could be to perform the analysis of the two groups after stratifying for different chemotherapy regimes, infusion vs bolus administrations, presence or absence of previous or subsequent radiotherapy and presence of cardioprotective medications at baseline. This was not performed due to the small n size but is something that will need to be considered in future larger studies.

The findings suggest that the post-chemotherapy biomarker does not add to the sensitivity of detecting a sub-clinical toxicity and that the pre-chemotherapy ("trough") sample may be more representative of underlying cardiotoxicity. It is likely the post-chemotherapy sample is further affected by dilutional problems from the administration of intravenous fluids during chemotherapy but the lack of a post-chemotherapy haematocrit to assess for any dilutional effects does not allow confirmation of that hypothesis. In a recent study by Tzolos et al in breast cancer patients, high sensitivity TnT 24hrs post chemotherapy is 33% lower than pre-chemotherapy, which was unexpected and thought to be due to intravenous fluid administration and/or steroid administration during chemotherapy and that the pre-chemotherapy sample may be more reliable in assessing myocardial injury(351). With regards to the troponin samples during the follow-up period, there were unfortunately some missing data despite best efforts to minimise this. This was due to patient deaths and withdrawal but also because of the Covid-19 pandemic that meant

patients were unable to attend for blood tests. This was a particular problem for the 6-month follow-up time point with the n number reducing to 22.

An unexpected finding was the higher number of admissions due to sepsis or fevers with or without neutropenia in the RIC group. This could be explained by the small study size. However, it could also be due to the way these episodes were recorded, which was based on the discharge diagnosis but with no details with regards to specifics about definitions of sepsis, temperature recordings and other markers of infection and shock. Similarly, the absence of any other data such as electrolyte abnormalities during any arrhythmic events detected on the 14-day cardiac monitor, make it difficult to infer whether they were due to chemotherapy or other reasons.

The main limitation of my second part that investigates monitoring for cardiotoxicity particularly using biomarkers, is the fact that the analysis was performed using the whole cohort as one, and even though there was no difference demonstrated between the two groups, there is always a possibility that the intervention is influencing the outcome investigated. To minimise that, analyses were performed for the cohort, as well as for each group individually and any conclusions were only made if both the group results were similar the whole cohort results. Furthermore, the post chemotherapy echocardiograms used to assess any relationship between peak troponin and change in LVEF were performed at the 3-month time point which may be too soon post chemotherapy to see any effect of anthracyclines on cardiac function and this is another limitation. Most patients will develop cardiac dysfunction in the first year post anthracycline chemotherapy(338) and therefore a one year follow-up echocardiogram is more suitable. The same also applies for the patients who had a CMR performed who would ideally also need a CMR performed at the 1year time-point. In addition, the 3-month CMR included only 10 patients and is likely

to be too small for direct comparisons between CMR and echocardiography. Having said that, the main aim of the CMR cohort was to assess feasibility of using CMR as a monitoring tool rather than a direct comparison with echocardiography. Similarly, only 22 patients had the cMyC biomarker performed which despite some interesting results will need to be verified in a bigger cohort and be correlated with cardiac function and clinical outcomes.

Strengths

Despite the limitations outlined above, my MD thesis has some important strengths. In the first instance, this was the first experiment to assess the effect of RIC during anthracycline chemotherapy in humans. Secondly, a variety of outcomes were explored including cardiac biomarkers during chemotherapy, but also cardiac function and clinical outcomes. Thirdly, reporting of serious adverse effects and cancer outcomes was done in detail and this ensured detecting any adverse trends that may impact on clinical care. This is important as, other than the studies on dexrazoxane, several of the studies investigating cardioprotective strategies do not always report on non-cardiac or cancer/chemotherapy specific adverse effects in detail(161,162,192,203,210,335).

Furthermore, I report on the dynamic trends of troponin T concentrations at each cycle of chemotherapy (as well as follow up) using troponin as a continuous variable, something that I have found to be reported only twice in the literature so far and on both occasions using troponin I(350,351). It is important to understand how biomarker concentrations fluctuate during chemotherapy for particular biomarkers and assays if biomarkers are to be used to try and predict risk of cardiotoxicity. In addition, troponin changes were assessed in different ways, with absolute and delta concentrations, as there is no agreed consensus what correlates best with cardiac injury(350) as well as a binary variable to compare with previous research.

Conclusions and Future Directions

In conclusion, the analysis of this thesis shows that remote ischaemic conditioning does not act as a cardioprotective mechanism against anthracycline cardiotoxicity as measured by changes in troponin during and after chemotherapy. Furthermore, RIC does not seem to affect any of the secondary outcomes of cardiac function as measured by imaging, clinical and arrhythmic outcomes. The results are affected by the fact that the study has a low number of patients and thus should be seen as a hypothesis generating exercise without being able to make firm conclusions. However, the study provides valuable information for future research as it presents in a very detailed, prospective, and observational manner, changes in a variety of different cardiac parameters during the course of a patient's journey through chemotherapy and follow-up. In particularly the study demonstrates, how specific cardiac biomarkers (troponin T and the novel cMyC biomarker) rise at each cycle during chemotherapy, peak up to one-month post-chemotherapy and can remain elevated up to 6 months post-chemotherapy. There was no correlation with any secondary outcomes, however, knowledge and understanding of how biomarkers behave during chemotherapy would allow to incorporate these into prediction tools to assist clinicians in predicting anthracycline cardiotoxicity.

Future research should focus on two areas: firstly, on observational, prospective (and "real world registries") research of monitoring for anthracycline cardiotoxicity and focusing in particular on the detection of early subclinical cardiotoxicity using biomarkers and imaging. Secondly, research should focus on cardioprotection studies to identify agents that may prevent cardiotoxicity. With regards to RIC, different regimes can be investigated, such as a more prolonged RIC model where patients receive RIC at home in between chemotherapy cycles. It is possible that the RIC stimulus was insufficient to provide any

significant cardioprotection as the injury from anthracyclines is a more prolonged, chronic injury unlike the acute injury in myocardial ischaemia. This, however, will need to be designed in a manner to monitor for any potential adverse outcomes, in particular infections as observed in the current study. Furthermore, different cardioprotective drugs can be tried with other iron chelators being the most obvious. Desferrioxamine for instance may be a suitable candidate as it is less likely to cause neutropenia compared to deferiprone and has been investigated before with some promising results, albeit much less than dextrazoxane and only in animal studies(64).

For any future study, the findings of this thesis will help in the design particularly with the timings of investigations. Based on this study I would suggest, biomarkers such as troponins to be performed at baseline, and then as a trough level at the mid-point and end of chemotherapy as well as at one, three- and 6-months post chemotherapy. With regards to assessment of cardiac function by imaging, I would suggest a baseline assessment and an assessment within one month from end of treatment which would coincide with the highest rise in troponin, as well as at 6- and 12-months post chemotherapy. Furthermore, an additional assessment of cardiac function using new imaging modalities such as myocardial deformation imaging or MRI parameters should be performed at the mid-point of chemotherapy to investigate their suitability as early detectors of toxicity.

I hope, the analysis and results of this thesis should help pave the way for more clinical studies in cardio-oncology at UCL and UCLH.

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