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Abstract

Cardiovascular morbidity and mortality following anthracycline therapy poses a significant clinical challenge. Cardiovascular magnetic resonance (CMR) is a non-invasive imaging modality that offers unique insight into both the pathophysiology and evaluation of anthracycline cardiotoxicity. This thesis comprises a series of investigations in patients treated with anthracycline chemotherapy using CMR.

In Chapter 3, the phenotype of late stage anthracycline cardiomyopathy is described. Surrogate CMR markers of both focal (late gadolinium enhancement; LGE) and diffuse (elevated native myocardial T1 mapping measurements) fibrosis are associated with adverse left ventricular remodelling and impaired left ventricular ejection fraction (LVEF). In addition a higher rate of hospitalisation for heart failure was identified in patients with elevated native myocardial T1 mapping measurements. In Chapter 4, anthracycline treated patients with normal range LVEF are shown to have significant perturbations of CMR derived 2D and 3D global longitudinal strain (GLS), native myocardial T1, ECV, indexed left ventricular mass and indexed myocardial cell volume, compared with a control population of similar age, sex and LVEF. It is also demonstrated that these parameters have good to excellent levels of inter- and intra-observer reproducibility. In Chapter 5, cardiotoxicity is prospectively characterised using multiparametric CMR, echocardiography and blood biomarkers, including microRNA (miRNA). It is reported that baseline CMR derived mitral annular plane systolic excursion (MAPSE) and baseline circulating miRNA-181-5p and miRNA-221-3p are associated with poor recovery of LVEF 6 months after completion of anthracycline chemotherapy.

In conclusion, multiparametric CMR provides unique, valuable insights into the pathophysiology and cardiovascular effects of anthracycline chemotherapy, which could potentially inform risk stratification, monitoring and treatment strategies in patients receiving cardiotoxic cancer therapy.

Dedication

I dedicate this thesis to my family. To my wife Jenny, my parents David and Shirley and my children Matilda and Annabelle, without whose enduring support, I would not have completed this work.

You are the reason I am where I am today.

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Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED:  DATE: 15th November 2020

Publications

The following publications arose from this research:

1. **Harries I**, Biglino G, Baritussio A, De Garate E, Dastidar A, Plana JC, Bucciarelli-Ducci C. Long term cardiovascular magnetic resonance phenotyping of anthracycline cardiomyopathy. Int J Cardiol. 2019 Oct 1;292:248-252. doi: 10.1016/j.ijcard.2019.04.026. Epub 2019 Apr 10.

Dr Iwan Harries conceived the study, collected and analysed the data, wrote and edited successive versions of the manuscript and figures, and reviewed the manuscript critically for important intellectual content.

2. **Harries I**, Liang K, Williams M, Berlot B, Biglino G, Lancellotti P, Plana JC, Bucciarelli-Ducci C. Magnetic Resonance Imaging to Detect Cardiovascular Effects of Cancer Therapy: a Practical Guide for Cardio-Oncologists. JACC Cardio-Oncology. 2020 Jun. doi.org/10.1016/j.jaccao.2020.04.011

Dr Iwan Harries helped to conceive, designed and edited successive versions of the article and figures, and reviewed the manuscript critically for important intellectual content.

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Dr Iwan Harries helped conceive and design the research, obtained ethical approval and funding, analysed and interpreted data, and drafted and revised the manuscript critically for important intellectual content

2. Harries I, Berlot, ffrench-Constant N, Williams M, Liang K, De Garate E, Baritussio A, Biglino G, Plana JC, Bucciarelli-Ducci C. Cardiovascular Magnetic Resonance Characterization of Anthracycline Cardiotoxicity in Adults with Normal Left Ventricular Ejection Fraction

Dr Iwan Harries conceived and designed the research, collected analysed and interpreted data, and drafted and revised the manuscript critically for important intellectual content

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Other Research Output

The following studies were published during the study period:

1. Berlot B, **Harries I**, Bucciarelli-Ducci C. Connection between the heart and the gut. Heart. 2019 Apr 8. pii: heartjnl-2019-314832.
2. Milano EG, **Harries IB**, Bucciarelli-Ducci C. Young adult with Friedreich ataxia. Heart. 2019 May;105(10):797-806.
3. Dastidar AG, **Harries I**, Pontecorboli G, Bruno VD, De Garate E, Moret C, Baritussio A, Johnson TW, McAlindon E, Bucciarelli-Ducci C. Native T1 mapping to detect extent of acute and chronic myocardial infarction: comparison with late gadolinium enhancement technique. Int J Cardiovasc Imaging. 2019 Mar;35(3):517-527.
4. Rodrigues JCL, Amadu AM, Ghosh Dastidar A, **Harries I**, Burchell AE, Ratcliffe LEK, Hart EC, Hamilton MCK, Paton JFR, Nightingale AK, Manghat NE. Noctural dipping status and left ventricular hypertrophy: A cardiac magnetic resonance imaging study. J Clin Hypertens (Greenwich). 2018 Apr;20(4):784-793
5. Baritussio A, Ghosh Dastidar A, Frontera A, Ahmed N, De Garate E, **Harries I**, Diab I, Duncan E, Thomas G, Nisbet A, Bucciarelli-Ducci C. Diagnostic yield of cardiovascular magnetic resonance in young-middle aged patients with high-grade atrio-ventricular block. Int J Cardiol. 2017;244:335-339.
6. Rodrigues JC, Rohan S, Ghosh Dastidar A, **Harries I**, Lawton CB, Ratcliffe LE, Burchell AE, Hart EC, Hamilton MC, Paton JF, Nightingale AK, Manghat NE Hypertensive heart disease versus hypertrophic cardiomyopathy: multi-parametric cardiovascular magnetic resonance discriminators when end-diastolic wall thickness ≥ 15 mm. Eur Radiol. 2017 Mar;27(3):1125-1135.

The following conference abstracts were presented during the study period:

1. **I Harries**, G Biglino, B Berlot, M. Williams, V De Francesco, K Mitrousi, C Lawton, JC Plana, C Bucciarelli-Ducci. Myocardial fibrosis is associated with adverse left ventricular remodelling and heart failure hospitalisations in anthracycline-treated cancer survivors at 5 years follow-up. EuroCMR 2019.
2. **I Harries**, M Curran, K Mitrousi, M Williams, B Berlot, V De Francesco, C Lawton, C Bucciarelli-Ducci. Anthracycline treated cancer survivors with normal LVEF have significant perturbations of longitudinal strain and myocardial tissue composition. EuroCMR 2019.
3. **I Harries**, M Williams, B Berlot, K Mitrousi, V De Francesco, U Benedetto, S Lyen, C Bucciarelli-Ducci. Shot through the heart. EuroCMR 2019. *Awarded runner up prize for best case presentation.*
4. B Berlot, **I Harries**, G Biglino, C Bucciarelli-Ducci. Left ventricular volumes: the importance of contouring the basal slice. EuroCMR 2019.
5. **I Harries**, J Tan, G Biglino, AG Dastidar, A Baritussio, E De Garate, C Bucciarelli-Ducci. Native myocardial T1 correlates with left ventricular volumes and function in patients treated with anthracycline. CMR 2018.
6. **I Harries**, N ffrench-Constant, G Biglino, AG Dastidar, A Baritussio, E De Garate, C Bucciarelli-Ducci. CMR-derived strain in adult cancer survivors with a normal left ventricular ejection fraction: an age and sex matched case-control study. CMR 2018.
7. **I Harries,** AG Dastidar, A Baritussio, E De Garate, S Kestenbaum, M Williams, C Seyani, P Brady, J Chai, G Richards, J Bracken, V North, C Bucciarelli-Ducci. Gender differences in patients with late chemotherapy induced cardiomyopathy by CMR. EuroCMR 2017.
8. **I Harries,** AG Dastidar, A Baritussio, E De Garate, T Erdei, G Pontecorboli, C Bucciarelli-Ducci. Native myocardial T1 and T2 mapping in healthy volunteers: the effect of wall thickness, gender and myocardial region at 1.5T. EuroCMR 2017.

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List of abbreviations

2D	Two dimensional
3D	Three dimensional
ACEi	Angiotensin converting enzyme inhibitor
AE	Adverse event
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ARB	Angiotensin II receptor blocker
BB	Beta blocker
BNP	Brain natriuretic peptide
BMI	Body mass index
bSSFP	Balanced Steady State Free Precession
CMR	Cardiovascular magnetic resonance
CT	Computerised tomography
DNA	Deoxyribonucleic acid
ECV	Extracellular volume
EGE	Early gadolinium enhancement
ESC	European society of cardiology
FT	Feature tracking

GCS	Global circumferential strain
GLS	Global longitudinal strain
GRS	Global radial strain
HL	Hodgkin lymphoma
ICC	Intraclass correlation coefficient
ICI	Immune checkpoint inhibitors
IQR	Interquartile range
LA	Left atrium
LAD	Left anterior descending artery
LAVi	Left atrial volume indexed
LCx	Left circumflex artery
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEDV	Left ventricular end diastolic volume
LVEDVi	Left ventricular end diastolic volume indexed
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
LVESVi	Left ventricular end systolic volume indexed
LVSD	Left ventricular systolic dysfunction

MACE	Major adverse cardiovascular event
MAPSE	Mitral annular plane systolic excursion
MI	Myocardial infarction
MiRNA	Micro ribonucleic acid
MOLLI	Modified look locker inversion recovery
MRI	Magnetic resonance imaging
MUGA	Multigated acquisition
NHL	Non-Hodgkin lymphoma
NT-pro BNP	N-terminal pro brain natriuretic peptide
NYHA	New York Heart Association
PI	Principal Investigator
PICC	Peripherally inserted central cannula
PIS	Patient information sheet
RA	Right atrium
RCT	Randomised controlled trial
RCA	Right coronary artery
REC	Research Ethics Committee
RNA	Ribonucleic acid
RV	Right ventricle

SAE	Serious adverse event
SD	Standard deviation
STIR	Short-tau inversion recovery
TCM	Takotsubo cardiomyopathy
T1	T1 (longitudinal) relaxation time
T2	T2 (transverse) relaxation time
TAPSE	Tricuspid annular plane systolic excursion
Troponin I	TnI
Troponin T	TnT
VO_2 max	Maximum rate of oxygen consumption

Chapter 1 Introduction

Cancer is a leading cause of death affecting at least one in three people in the United Kingdom during their lifetime[1]. Advances in treatment and supportive care have led to improved survival for cancer patients. However, cancer therapy confers excess risk of both immediate and long term cardiovascular disease, and patients with established cardiovascular disease are more susceptible to side effects from cancer therapy. Consequently, collaboration between oncologists and cardiologists in the emerging field of cardio-oncology is required[2]. The principal aims of a cardio-oncologist are to eliminate cardiovascular disease as a barrier to cancer treatment, and to monitor and mitigate the cardiovascular side effects of cancer treatment.

Anthracyclines are an effective treatment for many forms of cancer. However, their use is limited by dose dependent cardiotoxicity, which manifests clinically as left ventricular dysfunction and heart failure[3]. The mechanism of anthracycline cardiotoxicity is incompletely understood, though the early stages are accompanied by histological changes, which seem to predate declines in myocardial function[4]. The early histological changes are reversible if anthracycline treatment is discontinued[5]. The later stages of anthracycline cardiomyopathy are characterised by myocardial fibrosis[6], impaired myocardial function, and are associated with a poor prognosis[7], all of which highlights the importance of early detection of anthracycline cardiotoxicity.

Cardiovascular magnetic resonance (CMR) is a multiparametric imaging modality that can assess cardiac anatomy, function, myocardial tissue properties (e.g. fibrosis), myocardial perfusion and blood flow in a single radiation free examination with high levels of accuracy and reproducibility, thus making it an important tool in the assessment of cardiovascular disease.

This thesis describes the role of CMR in the evaluation of anthracycline cardiotoxicity.

Chapter 2 Literature Review

2.1 Chemotherapy

The annual incidence of cancer in the United Kingdom is approximately 363,484; or one new diagnosis every two minutes[1]. However, cancer outcomes continue to improve with 50% of patients surviving cancer for 10 years or more[1]. Indeed survival trends have doubled in adults and tripled in children in comparison to the 1960's[1], leading to an increasing number of cancer survivors. Paradoxically, this has led to an increasing emphasis not only on cancer survival, but also on the long term effects of cancer treatment on survivors. Early detection, surgery, advances in supportive care and chemotherapy have all contributed to these improvements.

2.1.1 History of anthracycline chemotherapy

Anthracyclines are among the most efficacious cancer therapies ever developed and were first isolated from Streptomyces peucetius over 50 years ago[8]. Indeed, anthracyclines are listed by the World Health Organisation among the model list of essential medicines, and despite rapid progress in cancer treatment and the development of novel targeted therapies, anthracyclines continue to play a prominent role in the treatment of several forms of cancer today. Doxorubicin (Figure 2-1) and daunorubicin were the first anthracyclines to be used in clinical practice[8] and together with epirubicin and idarubicin, comprise the most common forms of anthracycline used in modern chemotherapeutic regimens.

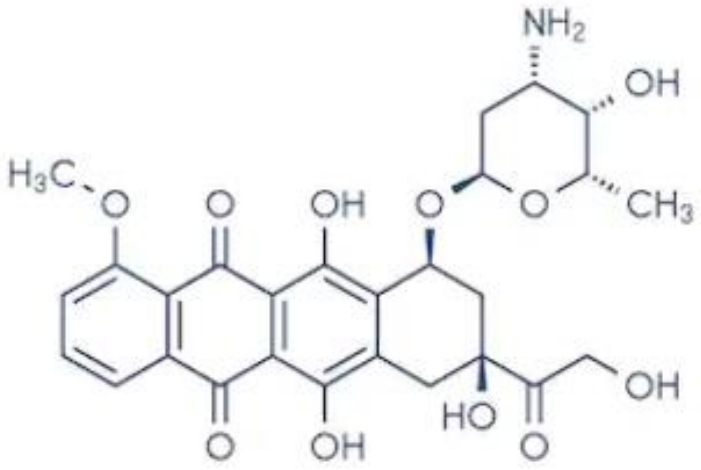

Figure 2-1 Molecular structure of doxorubicin

2.1.2 Role of anthracycline in modern chemotherapeutic regimens

Anthracyclines are a key component of chemotherapeutic regimens and continue to play an important role in the treatment of a range of malignancies, including lymphoma, leukaemia, multiple myeloma, and solid tumours of the breast, lung, gastrointestinal tract, genitourinary system and sarcoma. For example, the majority of elderly lymphoma patients are treated with anthracyclines[9], as are at least 50% of childhood cancer patients[10].

2.1.3 Mechanisms of action and cardiotoxicity

Anthracyclines exert a wide variety of effects depending on the dose, method of administration, and target cell or cellular mechanism under investigation, though their precise mechanism of action, and particularly the pathophysiology of cardiotoxicity, remains unclear[8]. The following are some of the most widely accepted mechanisms thought to underpin cardiotoxicity.

2.1.3.1 DNA intercalation

Mammalian cells readily take up anthracyclines, which become localised to the nucleus and insert between adjacent DNA base pairs by virtue of the intercalating function of the chromophore moiety[11]. Consequently, DNA and RNA synthesis in highly replicating cells is inhibited, leading to therapeutic effect in malignancy.

2.1.3.2 Reactive oxygen species/free radical formation

Free radical formation is generally accepted to be one of the main mechanisms of anthracycline induced cardiotoxicity[12]. Excessive reactive oxygen species are generated when the quinone moiety of anthracyclines undergo redox reactions in the presence of oxyreductive enzymes such as NADH dehydrogenase[8]. The conversion of quinone to semiquinone also yields free radicals that react with oxygen, leading to the accumulation of superoxide and peroxide radicals[13]. Free cellular iron may also increase levels of reactive oxygen species with doxorubicin-iron complexes resulting in increased cellular stress and mitochondrial dysfunction[13]. Cardiomyocytes appear particularly susceptible to doxorubicin induced oxidant stress due to the increased reliance of these mitochondria dense cells on oxidative substrate metabolism[14]. Animal models have confirmed the importance of modulating reactive oxygen species. Overexpression of manganese dependent superoxide dismutase decreased apoptosis and improved left ventricular function, whereas deletion of manganese dependent

superoxide dismutase increased apoptosis and reduced ventricular function in mice treated with doxorubicin[15].

2.1.3.3 Topoisomerase II β

DNA toposiomerases are enzymes that help to regulate DNA replication, recombination and transcription by introducing temporary single or double stranded DNA breaks[16]. Two isoenzymes of topoisomerase II are expressed in humans: topoisomerase II α and topoisomerase IIβ. Topoisomerase II α is highly expressed in proliferating cells, whereas topoisomerase IIβ is highly expressed in quiescent cells, such as cardiomyocytes. Doxorubicin binds to topoisomerases. When bound to topoisomerase IIα, the cell cycle is arrested and apoptosis is induced[17]. Binding of doxorubicin to topoisomerase IIβ in cardiomyocytes also leads to mitochondrial dysfunction and apoptosis[8]. Indeed, a topoisomerase IIβ knockout murine model reported an absence of doxorubicin induced cardiotoxicity[18].

2.1.3.4 Other mechanisms

Other proposed mechanisms of anthracycline induced cardiotoxicity include disruption of neuregulin-ErbB receptor signalling[8], transcriptional changes in intracellular adenosine triphosphate production, downregulation of messenger RNA expression for sarcoplasmic reticulum, depression of glutathione peroxidase activity, and respiratory defects associated with mitochondrial DNA damage[12].

2.2 Definition of cardiotoxicity

No universally accepted definition of 'cardiotoxicity' exists, partly because it can express an extremely broad variety of manifestations, depending on the agent, population, timing and investigative methodology employed. However, recent societal consensus statements have attempted to standardise the definition of cardiotoxicity relating to depressed cardiac function as an LVEF drop of >10 percentage points to a value below the lower limit of normal, typically 53% measured by echocardiography [2, 19].

2.2.1 Incidence and timing of anthracycline cardiotoxicity

Cardiotoxicity encompasses a broad clinical spectrum that ranges from asymptomatic but detectable structural or functional change or elevation of cardiac biomarkers, through to symptomatic heart failure. Consequently, the incidence varies according to the definition

employed, as well as the agent and population being studied. Using the most widely used definition of anthracycline cardiotoxicity (a drop in LVEF of >10 absolute percentage points to a value below the limit of normal), the incidence of cardiotoxicity ranges from 1% for epirubicin to 48% for high dose (>700mg/m^2) doxorubicin[2]. Median time from administration to diagnosis is 3.5 months with 98% of cases reported to be detectible within the first year[20]. Table 2-1 summarises prospective adult human studies of anthracycline cardiotoxicity in chronological order, including the definition and incidence of cardiotoxicity and main study findings.

Table 2-1 Prospective adult human CMR studies of cardiotoxicity in chronological order

Author and Year	*n*	Mean Age/Sex	Treatment	Follow up Imaging	Definition and incidence of cardiotoxicity	Findings
Wassmuth et al. 2001 [93]	22	43; 77% women	First course: Doxorubicin 67 mg/m^{2}or Epirubicin 76 mg/m^2	3 and 28 days after treatment initiation	LVEF < 55% occurred in 27% (*n* = 6)	Significant decrease in LVEF 67.8 ± 1.4% to 58.9 ± 1.9%. LVEF decreased significantly at 28 days in patients with an increase in relative enhancement of > 5 on day three
Chaosuwannakit 2010 [110]	40	52; 70% women	Doxorubicin 215 mg/m^2 or Daunorubicin 265 mg/m^2 or Trastuzumab 920 mg	4 months after treatment initiation	Not reported	Significant decrease in LVEF 58.6 ± 6.3% to 53.9 ± 6.4%. Significant increase in aortic stiffness (p < .0001)
Fallah-Rad 2011 [117]	42	47; 100% women	Epirubicin or adriamycin and Trastuzumab	12 months after treatment initiation	LVEF decline of 10% to < 55% occurred in 24% (*n* = 10); determined by echo.	Significant decrease in LVEF 66 ± 5% to 47 ± 4% in 10 with cardiotoxicity. All of whom exhibited subepicardial LGE.
Drafts et al. 2013 [82]	53	50; 58% women	50 – 375 mg/m^2 Doxorubicin equivalent	1, 3 and 6 months after treatment initiation	Of those with LVEF > 50% at baseline (*n* = 47), 26% (*n* = 12) had LVEF <50% after six months	Significant decrease in LVEF 58 ± 1% to 53 ± 1%. Significant decrease in mean mid wall circumferential strain (-17.7 ± 0.4% to -15.1 ± 0.4%; p = .0003) No new LGE.
Jordan et al. 2014	65	51; 86% women	55% (*n* = 36) received median 240 mg/m2 doxorubicin equivalent, 38% (*n* = 25) received a monoclonal antibody	3 months after treatment initiation	Not reported	Significant decrease in LVEF 57 ± 6% to 54 ± 7%. Significant increase in T1 weighted signal intensity 14.1 ± 5.1a.u. to 15.9 ± 6.8a.u.. No LGE.
Lunning et al. 2015 [102]	10	59; 40% women	300 mg/m^2 Doxorubicin	3 months after treatment completion	LVEF decrease by > 10% occurred in 50%	Significant decrease in FT-GCS (p = .018). Trend to FT-GLS decrease (p = .073) 30% exhibited one new or progressive segment of LGE

Nakano et al 2016 [118]	9	62.3; 100% women	67% had epirubicin. 100% had Trastuzumab	3, 6 and 12 months after treatment initiation	No cardiotoxicity	Significant decrease in LVEF (68.4 ± 6.6% to 61.7 ± 8.7) at 6 and 12 months (62.9 ± 7.8%) but not at 3 months (65.4 ± 8.7%). Significant decrease in SENC-GLS and SEND GCS at 6 months but not at 3 or 12 months.
Barthur et al 2017 [119]	41	52; 100% women	56% received anthracycline. 100% received 18 cycles of Trastuzumab	6, 12 and 18 months	No cardiotoxicity	Significant decrease in LVEF at 6 (60.4 ± 4.2% to 58.3 ± 5.1%) and 12 (57.9 ± 4.8%) months but not at 18 months. Significant decrease in RVEF at 6 (58.3% to 53.9%) and 12 (55%), which had recovered at 18 months (56.6%)
Muehlberg et al 2018 [120]	23	59; 52% women	360 – 40 mg/m2 Doxorubicin equivalent	48 after treatment initiation and on treatment completion	LVEF decrease by > 10% occurred in 39% (*n* = 9)	Significant decrease in subgroup with cardiotoxicity (63.5 ± 5.8% to 49.9 ± 5.0%) but not in those without (59.2 ± 10.3% to 58.3 ± 7.8%). At 48hours, subgroup who developed cardiotoxicity had significantly lower native T1 times than those who did not develop cardiotoxicity.
Ong et al 2018 [84]	41	52, 100% women	56% (*n* = 23) received anthracycline. 100% received Trastuzumab	6, 12 and 18 months after treatment initiation	2.4% (*n* = 1) experienced cardiotoxicity	Significant decrease in LVEF at 6 months (60.4% to 58.4%) and 12 months (57.9%) but not at 18 months (60.2%). Significant decrease in FT-GLS and FT-GCS at 6 and 12 months but not at 18 months.

CMR= cardiovascular magnetic resonance, FT = feature tracking, GCS = global circumferential strain, GLS = global longitudinal strain, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, SENC = strain encoded imaging, RVEF = right ventricular ejection fraction

2.3 Cardioprotective strategies

Cardiomyocyte survival depends on the balance between activity in cytotoxic and cytoprotective pathways and an increased understanding of these pathways may inform novel treatments to reduce anthracycline induced cardiotoxicity[14]. A variety of potential cardioprotective strategies have been explored. These range from modified anthracycline preparation and administration, antioxidants and free radical scavengers, to established treatments of left ventricular dysfunction in other settings, such as angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB) and beta blockers.

2.3.1 Doxorubicin analogues and administration methods

Doxorubicin analogues, such as epirubicin and liposomal doxorubicin, are recognised to be relatively less cardiotoxic than doxorubicin[10]. A meta-analysis of 55 randomised controlled trials (RCTs) confirmed that the risk of clinical cardiotoxicity was significantly lower with epirubicin in comparison to doxorubicin (OR 0.39 95% confidence interval: 0.20-0.78), and liposomal in comparison to non-liposomal doxorubicin (OR 0.18 95% confidence interval: 0.08 – 0.38), without compromising efficacy. Similar results were reported for subclinical cardiotoxicity. Furthermore, bolus infusion was associated with a significantly higher risk of clinical and subclinical cardiotoxicity in comparison to continuous infusion (OR 4.13 95% confidence interval 1.75-9.72) in adults[10]. However, this effect does not appear to extend to paediatric populations. A study of 102 children randomised to bolus versus continuous infusion of doxorubicin for acute lymphoblastic leukaemia (ALL) showed no difference in ventricular dimensions, ventricular function, wall thickness, ventricular mass or event free survival rates at a median of 8 years follow up[21]. Despite these findings, Smith et al. concluded that the evidence was not sufficiently robust to support routine clinical use of cardioprotective agents or liposomal doxorubicin, calling instead for an improvement in cardiac monitoring in oncology trials[10].

2.3.2 Dexrazoxane

The cardioprotective effect of dexrazoxane was originally thought to be mediated by iron chelation but the absence of similar effects for other iron chelators, such as deferasirox [22] argues against this hypothesis. Current thinking is that dexrazoxane confers cardioprotection by binding to topoisomerase II and influencing topoisomerase IIβ. Indeed this property of

dexrazoxane also led to concerns over a non-statistically significant increase in secondary malignant neoplasms reported by a meta-analysis of five paediatric RCTs (absolute incidence 2.7% *vs.* 1.1%, p = .06)[23].

Meta-analysis of RCT's indicates that administration of dexrazoxane in combination with doxorubicin or epirubicin to adult patients significantly reduces rates of clinical cardiotoxicity (OR 0.21 95% confidence interval 0.13-033) and subclinical cardiotoxicity (OR 0.33 95% confidence interval 0.20 – 0.55), in comparison to each agent alone[10]. This effect was replicated in a paediatric meta-analysis of five RCTs and 12 non-randomised studies that reported a statistically significant reduction in both clinical cardiotoxicity (RR 0.29) and subclinical cardiotoxicity (RR 0.43) associated with dexrazoxane use[23].

2.3.3 Antioxidants

Due to the proposed involvement of free radical and reactive oxygen species in the pathophysiology of anthracycline induced cardiotoxicity, there has been considerable interest in the potential role of antioxidants as cardioprotective agents. Indeed, animal studies have reported encouraging results. For example, antioxidant treatment can cause Akt activation and it has been shown that intracoronary Akt1 gene delivery protected left ventricular function and left ventricular growth in rats treated with doxorubicin[24]. However, these findings have not been replicated in human studies. The largest study to date randomised 54 patients receiving doxorubicin to either N-acetylcysteine or placebo and found no difference in the incidence of clinical heart failure (12.5% *vs.* 10%, p = .77)[25].

A study of 20 children with ALL or NHL treated with anthracycline reported a significant difference in the percentage left ventricular fractional shortening occurring in 10 patients treated with coenzyme Q10, compared with those not treated with Coenzyme Q10, as well as a decrease in left ventricular wall thickening in the latter group, suggesting a cardioprotective effect for conenzyme Q10[26], though these findings are yet to be replicated in larger studies.

2.3.4 Angiotensin converting enzyme inhibitors (ACEi), Angiotensin receptor blockers (ARB) and Beta blockers (BB)

Carvedilol is a non-selective βantagonist that reduces the risk of death and hospitalisation for cardiovascular causes in patients with left ventricular systolic dysfunction[27]. Consequently, its role in the prevention and treatment of anthracycline cardiomyopathy has been the subject

of several studies. In one of the first studies, 70 adult female patients were randomised to 6.25mg carvedilol monotherapy or placebo prior to commencing doxorubicin treatment. In contrast to those in the placebo group, the carvedilol group showed no significant reduction in echo derived strain parameters one week after initiation of chemotherapy[28]. However, a recent meta-analysis of nine RCTs comprising 717 patients concluded that preventive use of carvedilol may be associated with a reduced incidence of LVSD, better diastolic function, and lower troponin I levels, though validation by larger studies over longer periods was recommended[29]. Two studies have reported similar findings for carvedilol in paediatric populations[30, 31] and one study of adult females suggested a class effect by reporting that nebivolol conferred cardioprotection by maintaining LVEF in comparison to placebo at six months[32].

Metoprolol and candesartan combination therapy was recently evaluated in 120 patients receiving adjuvant anthracycline containing regimens with or without trastuzumab for breast cancer by the 2x2 factorial, randomised, double blind, placebo controlled PRADA (PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy) trial[33], which was one of the first RCTs to employ CMR to determine the primary endpoint of change in LVEF from baseline to end of chemotherapy. Interestingly, candesartan provided cardioprotection by maintaining LVEF (percentage point drop in candesartan group 0.8 *vs.* 2.6% in placebo group, p = 0.026) but metoprolol did not[33].

The OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtheapy for the treatment of Malignant hEmopathies) evaluated prophylactic combination enalapril and carvedilol to control in 90 adult patients with normal LVEF[34], and used a combination of echocardiography and CMR to determine the absolute change in LVEF from baseline. LVEF decreased significantly in the control group but did not change in those receiving carvedilol and enalapril, which resulted in a -3.1% difference by echocardiography (p = .035) and -3.4% difference (p = .09) in the 59 patients who underwent CMR. This also translated to a significant reduction in the composite end point of death or heart failure (6.7% *vs.* 22%, p = .036)[34]. Furthermore, patients with acute leukaemia had a greater degree of LVEF decline than patients undergoing autologous stem cell transplant (mean -6.4% *vs.* -1.0%)[34].

The double blind, placebo controlled MANTICORE trial (Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research) evaluated the role of 2 - 8mg perindopril (*n* = 33), 2.5-10mg bisoprolol (*n* = 31) or placebo (*n* = 30) in 94 adult female patients receiving trastuzumab with or without doxorubicin or epirubicin for breast cancer, using CMR derived LVEDVi and LVEF as the primary outcome measures[35]. Trastuzumab mediated increases in LVEDVi were similar in the bisoprolol (+8ml ± 9ml/m^2), perindopril (+7 ± 14ml/m^2), and placebo (+4ml ± 11ml/m^2) groups (p = .36)[35]. Trastuzumab mediated decline in LVEF was attenuated in the bisoprolol group (-1 ± 5%), relative to the perindopril (-3 ± 4%) and placebo (-5 ± 5%) groups (p = .001) and perindopril and bisoprolol were independent predictors of maintained LVEF on multivariable analysis[35]. Furthermore, these treatments were well tolerated with no serious adverse events during the study. However, the authors concluded that trastuzumab mediated left ventricular remodelling (the primary outcome measure) was not prevented.

Enalapril monotherapy initiated one month after the completion of chemotherapy and continued for one year was evaluated in an RCT of 114 patients (56 enalapril, 58 control) with raised troponin I after high dose anthracycline chemotherapy[36]. 43% of control subjects and 0% enalapril subjects (p < .001) experienced a > 10% decline in LVEF to < 50%, measured by echocardiography. A large subsequent longitudinal study (median follow up 5.2 years, IQR 2.6 - 8.0 years) of 2625 patients by the same group reported that 98% of cardiotoxicity cases were detectable in the first year (median time to detection 3.5 months after chemotherapy completion), and that early detection and prompt initiation of heart failure therapy were important determinants of recovery of cardiac function[20].

2.3.5 Statins

Statins are known to decrease atherosclerosis related morbidity and mortality, and are reported to have potent anti-inflammatory and antioxidant effects[37]. In a small study of patients receiving anthracycline, 20 patients were randomised to 40mg atorvastatin for six months and 20 to control. Echocardiography derived LVEF decreased significantly six months after chemotherapy in the control group (63 ± 7% to 55 ± 10%, p < .0001) but did not change in the atorvastatin group (61 ± 8 *vs.* 63 ± 9, p = .144). However, the number of patients experiencing an LVEF < 50% (five patients in the control group *vs.* one in the atorvastatin group) did not meet statistical significance (p = .18)

2.3.6 Aldosterone antagonists

One randomised study of 25mg per day of spironolactone (*n* = 43) compared with placebo (*n* = 40) reported non-significant declines in LVEF in the placebo group (67.7 ± 6.3 to 53.6 ± 6.8) compared with the spironolactone group (67.0 ± 6.1 to 65.7 ± 7.4; *p* = .094), in tandem with preservation of diastolic function in the intervention group[38] .

2.3.7 Other strategies

In general terms, physical activity is reported to have small to moderate beneficial effects on health related quality of life and cardiorespiratory fitness in women receiving treatment for breast cancer[39]. One small non-randomised study examining the effects of exercise training in breast cancer patients receiving anthracycline reported that decreases in VO_2 max were attenuated by exercise training (15% *vs.* 4%, *p* = .01) and fewer patients in the exercise training group met functional disability criteria (7 *vs.* 50%, *p* = .01), though no between group differences in resting LVEF were observed[40]. Similar significant attenuation of NT-proBNP release and improvement of resting LVEF was reported following a single bout of vigorous intensity exercise 24 hours prior to doxorubicin in the intervention group (*n* = 11) in comparison to controls (*n* = 13)[41]. Indeed, an RCT is underway to investigate the impact of exercise training on cardiotoxicity and cardiac health outcomes[42].

2.4 Detection of anthracycline cardiotoxicity

2.4.1 Biopsy

Endomyocardial biopsy is the only method to provide direct histopathological evaluation of the effects of anthracycline treatment on the myocardium and was originally described as early as 1978[4, 43]. However, the inherent risks of cardiac biopsy mean that it is not feasible for the routine evaluation of patients receiving potentially cardiotoxic treatment. Consequently, the non-invasive imaging evaluation of the effects of cardiotoxic therapies have become established in clinical practice and will be discussed in turn.

2.4.2 Imaging

Cardiovascular imaging has emerged as the preferred method to evaluate and monitor cardiac function during anthracycline chemotherapy. A variety of imaging techniques exist; each have relative advantages and disadvantages. It is important to note that the data provided by each

method, for example LVEF, are not interchangeable[44] and it is therefore recommended that the same modality be used for serial evaluation wherever possible[2].

2.4.2.1 Echocardiography

Echocardiography is the cornerstone of cardiac function imaging evaluation during potentially cardiotoxic chemotherapy. It is widely available, relatively cheap and provides detailed information on chamber volumes, systolic function, haemodynamic parameters, intracardiac pressure and flow. The most widely used parameter for assessing left ventricular systolic function in cardiovascular medicine is LVEF and this has been used as a marker for cardiotoxicity for over 30 years[45]. The most widely accepted definitions of cardiotoxicity is an asymptomatic drop of > 10% to a value < 53%[19]. However, measuring LVEF with echocardiography has important limitations. Obtaining sufficient image quality can be difficult, particularly in patients who have had left sided breast surgery, irradiation or breast reconstruction/prostheses. Furthermore, these methods are angle dependent, and rely on geometric assumptions and operator expertise[46]. The coefficient of variation of two dimensional LVEF is reported to be approximately 12%[47, 48], which hampers the ability of this method to detect the often subtle changes that accompany cardiotoxic therapy. It is important to note that the threshold to detect asymptomatic cardiotoxicity is 10%[2]. Using 3D echocardiographic methods, the reproducibility and accuracy of LVEF increases[48] but the determination of LVEF by all methods is influenced by prevailing haemodynamic conditions and geometric assumptions and perhaps most importantly, a reduction in LVEF may only occur when tissue level damage is extensive[49]. Consequently, increasing interest has emerged in the role of non-invasive markers of early myocardial damage, such as deformation or 'strain' techniques. The role of myocardial deformation imaging is becoming established in a variety of clinical scenarios with a growing body of evidence supporting its use in the setting of cardiotoxicity from cancer therapy[49]. Indeed, LVEF based and myocardial deformation based techniques are being evaluated by an ongoing randomised controlled trial[46]. Myocardial deformation techniques also have limitations, which include interobserver, intra-observer and inter-vendor variability, which lead to recommendations for serial evaluation to be performed using the same equipment and by the same operator, where feasible[2].

2.4.2.2 Multigated Acquisition (MUGA)

Multigated acquisition is a non-invasive imaging technique that uses radioactive tracers to evaluate the cardiac blood pool using a gamma camera, which provides accurate and reproducible data on LVEF[50]. Advantages include broad availability in certain healthcare systems, such as the United States of America, and reproducibility[2]. However, limitations include the use of ionizing radiation, absence of haemodynamic information or data on cardiac anatomy, and susceptibility to a range of artefacts.

2.4.2.3 CMR

CMR provides accurate, reproducible data on cardiac volumes, systolic function, flow and tissue characteristics (oedema, scar, fibrosis, vascularity, adiposity) in a single radiation free examination with versatile applications in cardio-oncology, which will be discussed in detail in section 2.5.

2.4.3 Biomarkers

A range of serological cardiotoxicity biomarkers have been investigated in the context of cardiotoxicity. However the utility of serological biomarkers is hampered by the difficulty in establishing a normal reference range, different assays, determining when during treatment is the optimal time to sample and also what action should be taken if an abnormal result is detected[2].

2.4.3.1 Troponin

Cardiac troponin is widely used in clinical practice and its release has been associated with an adverse prognosis in a range of cardiovascular diseases[51]. In general terms, a troponin rise is observed in approximately one third of patients receiving anthracycline treatment[52, 53]. New elevation of troponin I has been associated with a greater incidence of cardiac events following chemotherapy, particularly if persistently elevated at three months[53], and in single centre studies, is reported to identify trastuzumab treated breast cancer patients who are at risk of cardiotoxicity and unlikely to recover baseline cardiac function despite supportive medical therapy[54]. Furthermore, the magnitude of troponin rise has been reported to correlate with the degree of cardiomyocyte loss in a prospective study of breast cancer patients[55]. The utility of cardiac troponin in the context of cardiotoxic chemotherapy is further complicated by the observation that baseline elevation of troponin can occur due to

malignancy itself. For example, Cardinale et al. reported that 19% of Troponin I elevations occurred prior to anthracycline treatment[54], Auner et al. reported baseline elevation of troponin T in 3.8% of 78 patients with haematological malignancy and Lipshulz et al. reported that 10% of 119 paediatric patients with haematological malignancy had baseline elevation of troponin T [56]. More recently, high sensitivity troponin assays have become widely available with several studies reporting detectable temporal changes in the context of anthracycline treatment. A study of 43 breast cancer patients receiving anthracycline and trastuzumab reported that elevation of high sensitivity cardiac troponin I at three months was an independent predictor of cardiotoxicity at six months[57]. A larger study of 81 female patients treated with anthracyclines, taxanes and trastuzumab by the same group described that elevation of high sensitivity troponin I on completion of anthracycline was predictive of subsequent cardiotoxicity[58]. In one of the largest reports on the role of biomarkers in predicting cardiotoxicity, Zardavas et al. reported that baseline elevation of troponin I or T was associated with an increased risk of trastuzumab related cardiac dysfunction in 452 patients with breast cancer[59]. Furthermore, Ky et al. reported that interval changes in high sensitivity Troponin I were associated with a greater risk of cardiotoxicity in a multicentre study of 78 breast cancer patients that evaluated eight different biomarkers[60]. Finally, Lipshulz et al reported that high sensitivity troponin T levels were associated with abnormally reduced left ventricular mass and end diastolic wall thickness four years later[61] in a paediatric study of 119 patients treated with anthracycline.

2.4.3.2 Natriuretic peptides

Evaluation of brain natriuretic peptide (BNP) and its inactive N-terminal amino acid fragment (NT-pro-BNP) has become well established in the evaluation and monitoring of heart failure because they are indicative of ventricular volume overload or wall stress, and provide important diagnostic, therapeutic and prognostic information[62]. Data on the utility of natriuretic peptides in the context of anthracycline chemotherapy are conflicting. For example, a prospective study of 100 breast cancer patients receiving anthracyclines, taxanes and trastuzumab reported a significant correlation between NT-proBNP elevation and mortality at one year[63]. Another study of 52 patients with a variety of cancer diagnoses and chemotherapeutic treatment regimens reported that a persistently elevated NT-proBNP early after administration of chemotherapy was strongly associated with the subsequent

development of diastolic cardiac dysfunction[64]. In one of the largest studies to date, conducted in a paediatric population of 205 patients with haematological malignancy, Liphultz et al. reported that elevation of NT-proBNP was associated with adverse left ventricular remodelling four years later[61]. By contrast, several studies have reported the absence of an association between natriuretic peptides and cardiotoxicity [57, 58 60] and therefore, their role in the context of cardiotoxic chemotherapy remains to be defined.

2.4.3.3 MicroRNA

MicroRNAs (miRNAs) are a novel class of short non-coding RNAs that regulate elemental cellular processes and gene expression, and play a critical role in cardiovascular biology[65]. They are implicated in the pathogenesis of several cardiovascular diseases and, owing to their relative stability in plasma and ease of quantification, have evolved to become an appealing diagnostic and therapeutic biomarker[66]. Leading on from animal data[67], several reports of dysregulation of miRNA in the setting of cardiotoxicity in human studies have emerged. In a pilot study of 33 children, Leger et al. reported temporal dysregulation of miRNA 29-b and miRNA-499 isolated from plasma following anthracycline treatment. Furthermore, the degree of upregulation correlated both with anthracycline dose, and the extent of cardiac injury evaluated by high sensitivity troponin T[68]. Oatmen and colleagues conducted a study of 12 paediatric patients treated with anthracycline chemotherapy using CMR and an unbiased, custom microarray of 84 miRNAs extracted from serum, compared with age matched reference normal patients[69]. They reported that the functional domain of miRNAs associated with myocardial differentiation and development fell over three fold on completion of anthracycline. Furthermore, eight miRNAs were significantly downregulated after completion of anthracycline in patients with the greatest declines in LVEF (> 10%, $p < .05$). In a study of 55 breast cancer patients treated with anthracycline, miRNA-1 was associated with changes in LVEF and showed a greater area under the curve (AUC) than high sensitivity troponin I to discriminate between those who did and did not develop cardiotoxicity[70]. By contrast, a separate study of 58 breast cancer patients reported that despite promising preliminary animal data, miRNA-208a failed as a biomarker of doxorubicin induced cardiotoxicity because it was not detectable in any patient at any timepoint. Overall, MiRNA represent an appealing biomarker for cardiotoxicity, given their stability, ease of quantification, presence in multiple

bodily fluids and critical role in cardiovascular biology. However their precise role merits further evaluation[71].

2.5 CMR

2.5.1 CMR imaging sequences

CMR uses a variety of imaging sequences to provide comprehensive information about the cardiovascular system that is tailored to the clinical question posed. The CMR protocol is typically chosen based on the information provided by the referring physician and it is therefore important that all relevant background information is included, and the clinical question clearly formulated. Each sequence provides specific anatomical, tissue characteristics, functional, flow or perfusion data. Typically, a combination of sequences is selected to create the CMR protocol (Figure 2-2).

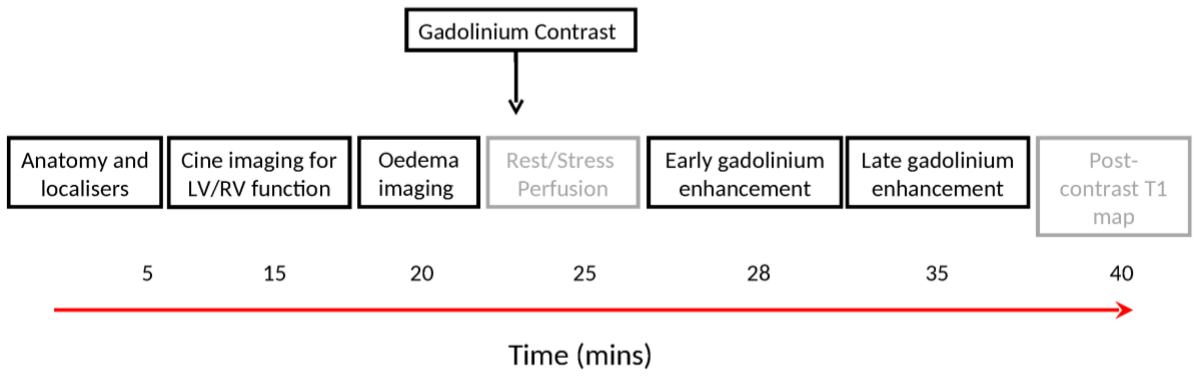

Figure 2-2. Typical composition of a CMR imaging protocol

CMR protocols are composed of different imaging sequences and tailored to the clinical question posed. Example additional imaging sequences are represented in grey. Native (non-contrast) and contrast enhanced sequences occur prior to and following gadolinium administration, respectively. CMR = cardiovascular magnetic resonance. LV = left ventricle, RV = right ventricle

Guideline directed indications for CMR are discussed later in this chapter and recently published appropriate use criteria[72] provide a useful reference to those seeking to request

CMR. Each CMR imaging sequence, along with its application in the setting of anthracycline cardiotoxicity, will be described in turn. The Society for Cardiovascular Magnetic Resonance (SCMR) have published comprehensive guidelines for describing CMR techniques and principles[73], principles of CMR reporting[74], CMR image interpretation and post-processing[75].

2.5.1.1 Cardiac Anatomy - dark blood (T1 and T2 weighted) imaging, white blood and fat suppression sequences

CMR protocols typically begin with cross sectional axial and/or coronal images of the thorax and upper abdomen that provide anatomical information about not only the cardiovascular system but also the remainder of the thoracic and upper abdominal anatomy (Figure 2-3). Major incidental extracardiac findings, which are of particular relevance in cardio-oncology populations, were reported to occur in 12% of CMR studies in a recent meta-analysis[76]. These findings may require additional testing and can contribute to contextualising the underlying cardiovascular diagnosis.

In addition to extra-cardiac findings (Figure 2-4), anatomical sequences can provide both direct anatomical information e.g. pericardial thickening following radiation (Figure 2-4) and indirect information relating to the haemodynamic consequences of underlying disease e.g. pleural effusions (Figure 2-4). Incidental findings, such as primary or secondary malignancy may also be encountered (Figure 2-4).

Anatomical sequences help to describe tissue properties using the signal intensity in each sequence, for example, fluid appears bright on T2 weighted images, which can be of particular use in the assessment of inflammatory pericardial or pleural fluid collections.

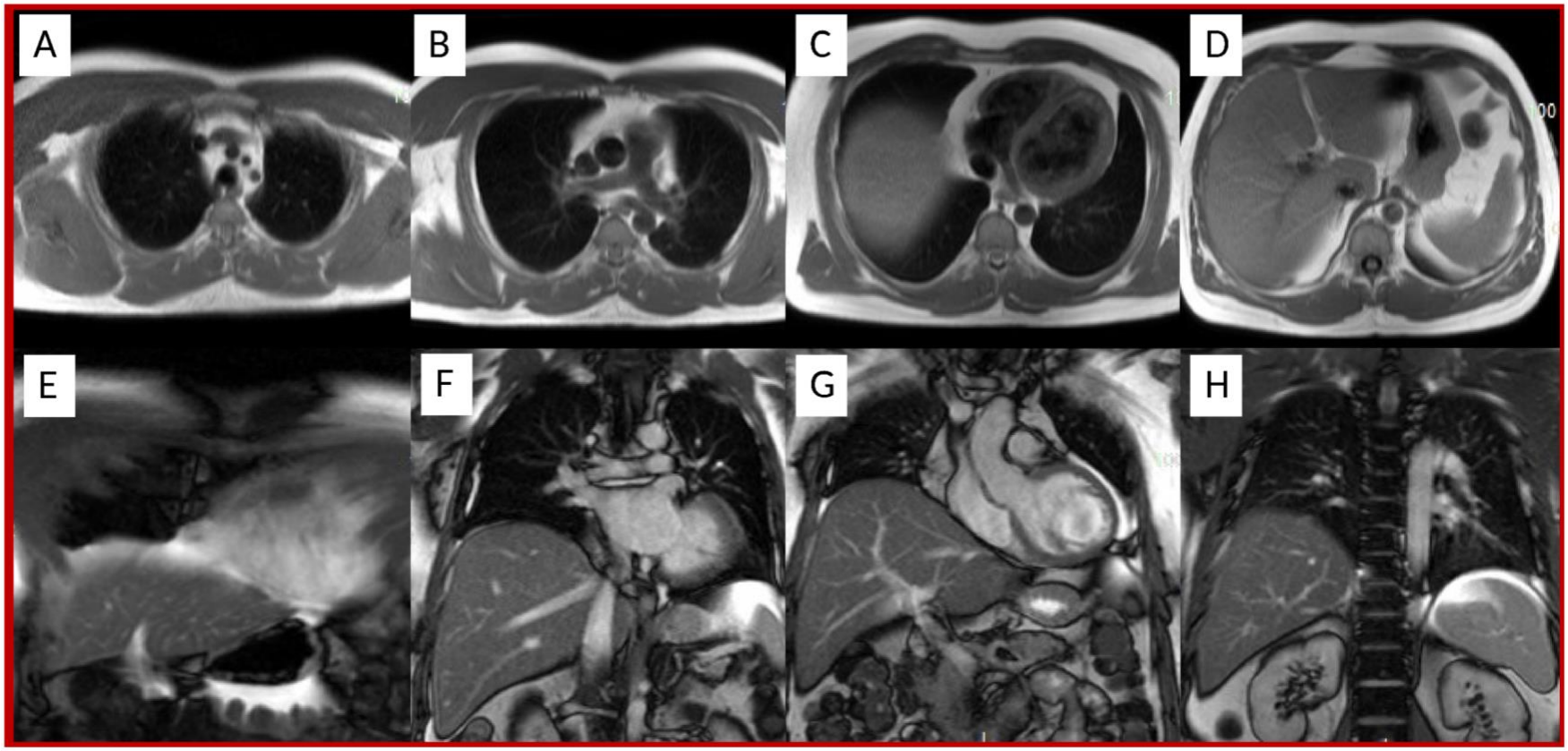

Figure 2-3 Example anatomical CMR sequences

Sequential axial black blood (A-D) and coronal white blood (E-H) CMR images through the thorax and upper abdomen.

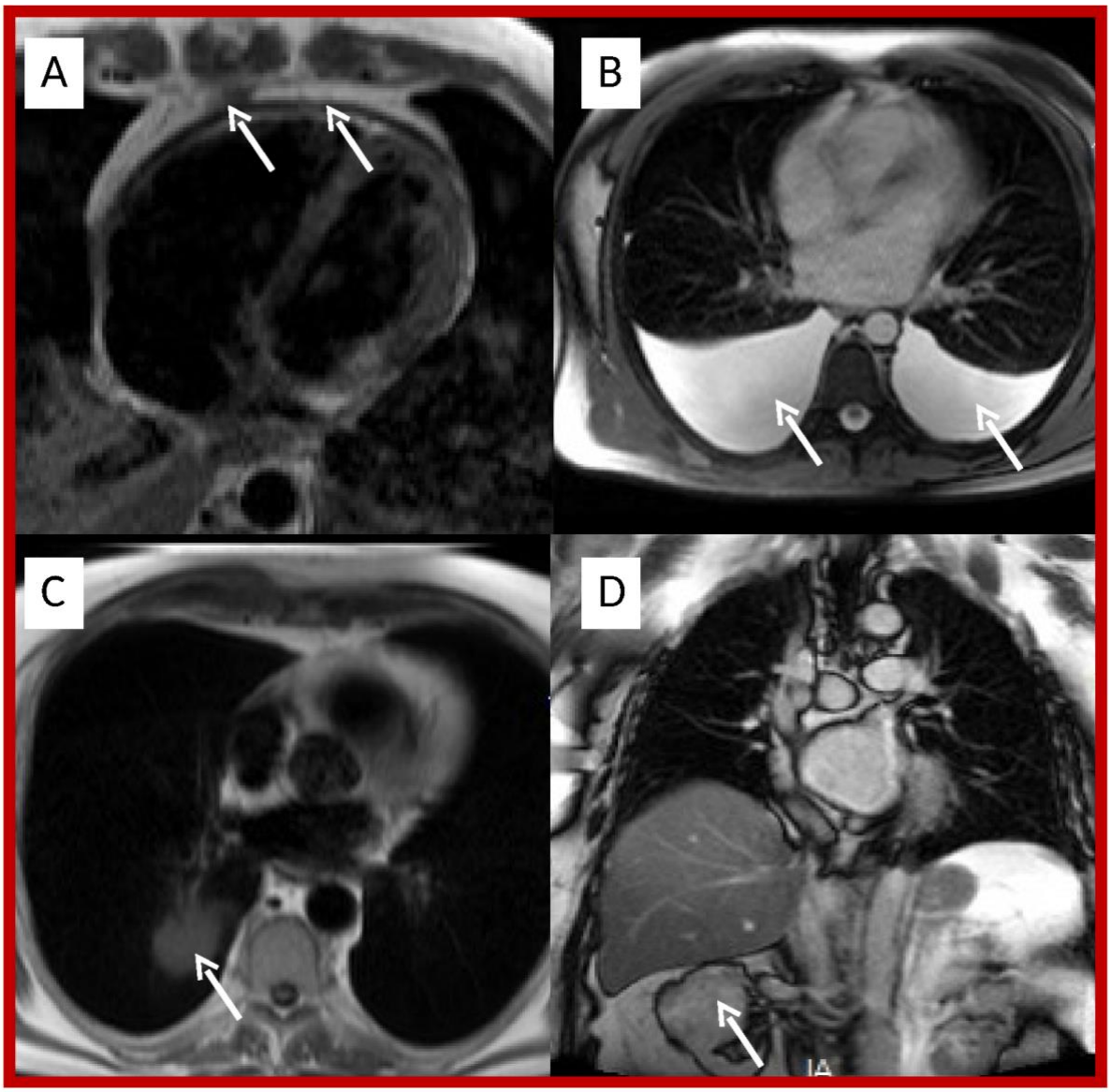

Figure 2-4 Abnormalities detected on anatomical sequences

Panel A: T1 weighted black blood turbo spin echo demonstrating diffuse pericardial thickening (arrowed), panel B: white blood T2 weighted axial sequence demonstrating bilateral pleural effusions (arrowed) due to constrictive cardiomyopathy (same patient as panel A). Panel C: incidental right lung tumour (arrowed). Panel D: incidental right renal tumour (arrowed).

2.5.1.2 Cardiac function, ventricular volumes and mass using bSSFP cine sequences

CMR is the gold standard for the assessment of ventricular volumes and ejection fraction (EF) with high levels of accuracy and reproducibility, both in health and disease in the left[77,44] and right ventricle[78]. Balanced steady state free precession (bSSFP) cine sequences provide high resolution, time resolved images that allow excellent discrimination of the endocardial and epicardial borders, which are subsequently contoured to provide three dimensional (3D) estimations of ventricular volumes, function and mass. The left ventricle is conventionally assessed in three long axis planes and a stack of contiguous short axis 'slices' from the left ventricular base in the atrioventricular plane, to the apex (Figure 2-5). The long axis planes are similar to those obtained by echocardiography; four chamber (4c), three chamber (3c) and two chamber (2c) (Figure 2-5). The right ventricle can also be evaluated in the same short axis stack (Figure 4) or alternatively with an axial stack with similarly high levels of interobserver and intra-observer reliability reported for each method[79].

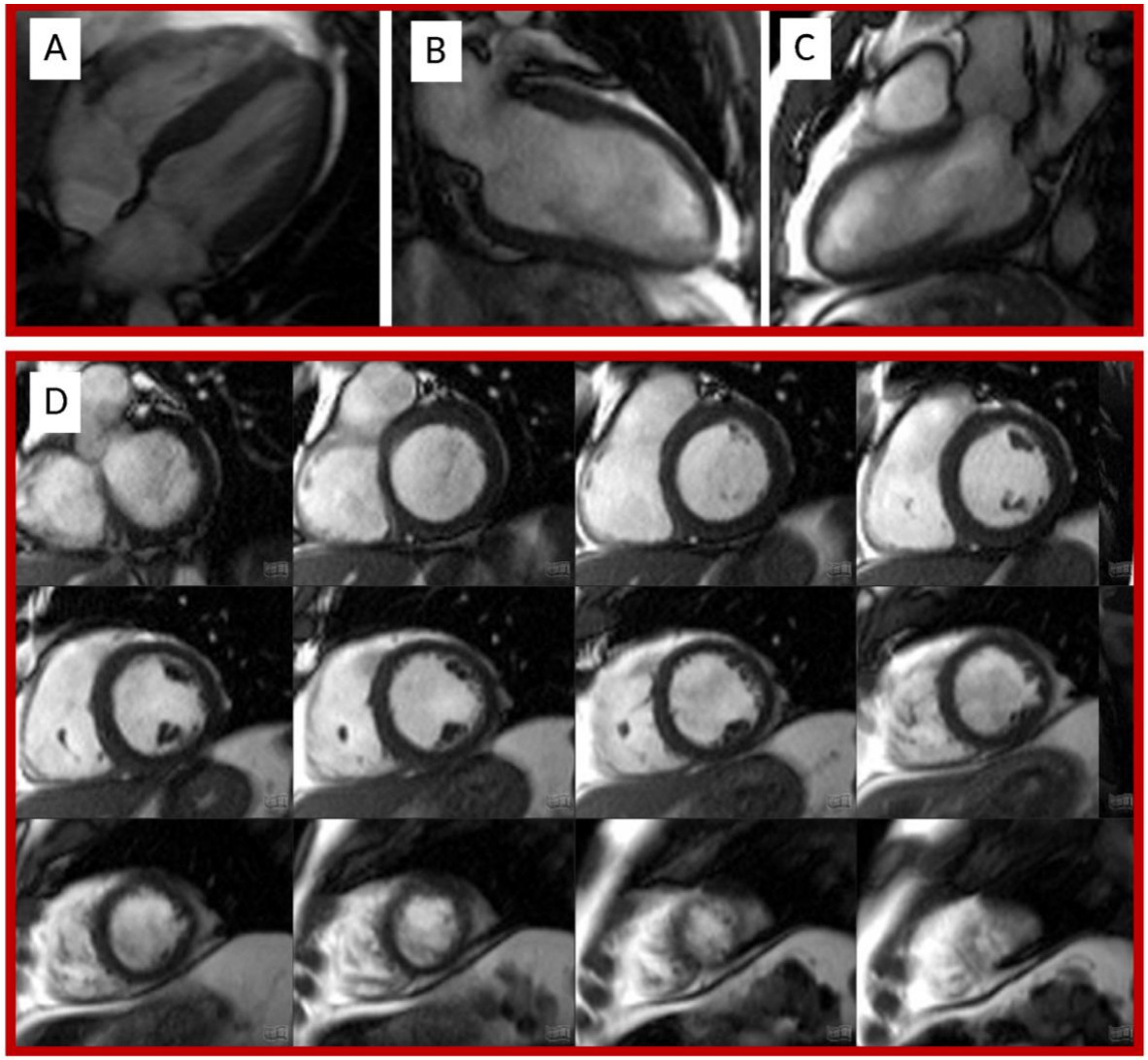

Figure 2-5 Still ‘cine’ images of the left ventricle

Example long axis views (A: four chamber, B: two chamber C: three chamber) and a stack of short axis ‘slices’ from base to apex (D).

Endocardial borders are contoured at end diastole and end systole in each of the short axis (or axial) slices to provide three dimensional volumes that do not rely on the geometric assumptions of two dimensional (2D) methods. However, it should be noted that volume estimation by CMR relies on geometric assumptions and whilst the papillary muscles are part of the myocardium, in clinical practice they are often included as part of the blood pool and thus contribute to ventricular volume[75]. Regional wall motion can be assessed either subjectively or objectively (using wall thickening or myocardial strain) and is conventionally reported by dividing the left ventricle into 17 equally weighted segments, as recommended by the American Heart Association (AHA) writing group on myocardial segmentation and registration for cardiac imaging[80].

Measurement of left ventricular volumes, ejection fraction and mass by CMR are highly accurate and have been demonstrated to be more reproducible (coefficient of variability for LVEF in normal subjects = 2.4%) than left ventricular volumes and mass by echocardiography (coefficient of variability for LVEF in normal subjects 8.6%) or radionuclide ventriculography[77,44]. Consequently, CMR is being employed increasingly in cardio-oncology research trials for precise serial evaluation of cardiac structure and function[33,35].

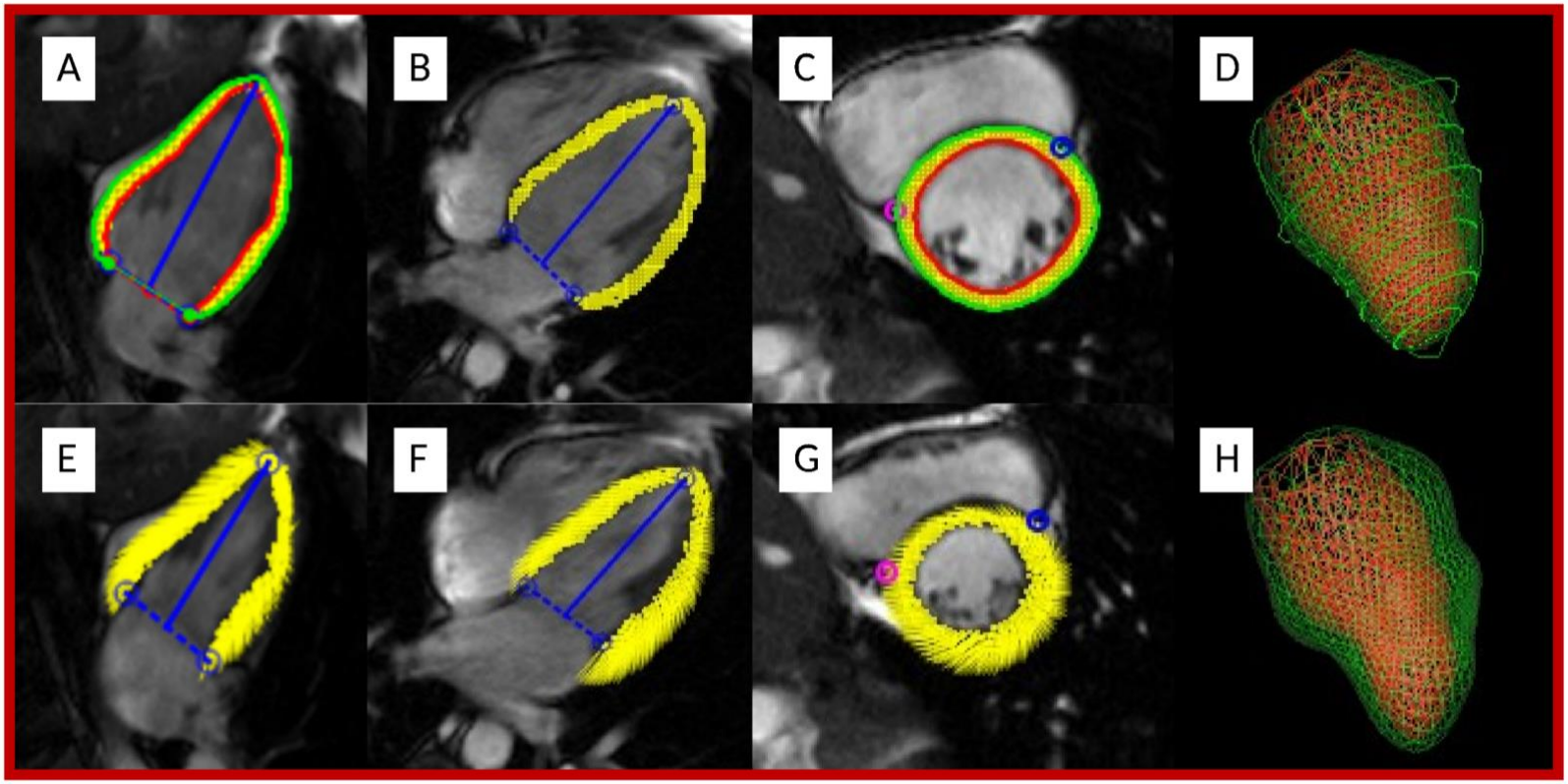

Figure 2-6 Strain overlaid bSSFP images of the left ventricle

Diastole (Panel A-D) and systole (E-H). A and E = two chamber view, B and F = four chamber view, C and G = mid-ventricular short axis view D and H = composite 3D reconstruction. Red line = endocardial border, green = epicardial border and yellow = tracked myocardial features. bSSFP = balanced steady state free precession

The left ventricular myocardium is an architecturally complex structure that deforms in longitudinal (along the long axis; apex to base), circumferential (along the circular axis) and radial (towards the centre of the ventricle) planes. Methods to quantify myocardial deformation, or strain, rely on the principle of tracking distinctive features over successive images and several CMR sequences have been developed for this purpose. These include tagging, displacement encoding with simulated echoes (DENSE), phase velocity mapping (PVM), strain encoded imaging (SENC) and latterly, feature tracking (FT). An advantage of FT strain is that it can be calculated from standard cine sequences (Figure 2-6), whereas the other methods require dedicated sequences to be acquired. There is emerging evidence of a significant incremental prognostic role for CMR strain when added to LVEF and LGE techniques in both ischaemic and dilated cardiomyopathies[81]. A systematic review of echocardiography derived myocardial deformation confirmed the value of these parameters for the early detection of myocardial changes and prediction of anthracycline cardiotoxicity[49], indeed the utility of strain guided echocardiography monitoring of patients receiving cardiotoxic chemotherapy in comparison to standard care is currently being evaluated by the SUCCOUR randomised controlled trial[46]. Whilst the CMR derived strain literature is in its infancy, initial reports confirm detectable temporal changes in circumferential[82,83] and longitudinal[84] strain in patients receiving cardiotoxic chemotherapy, including anthracycline. Indeed, the sensitivity of strain in comparison to LVEF based techniques mean that it has the potential to play an important role in the early detection of cardiotoxicity, though large scale confirmatory evidence is currently lacking. In comparison to speckle tracking echocardiography, CMR based feature tracking shows good agreement and appears to have comparable levels of reproducibility[85]. However, it must be noted that strain has limitations. The range of vendors (hardware and software), acquisition techniques and post-processing algorithms mean that normal reference ranges are difficult to establish. Furthermore, inter- and intra-observer variability, as well as interstudy reproducibility continue to present barriers to widespread clinical use.

2.5.1.3 Myocardial and pericardial oedema/inflammation using T2 weighted imaging and native T2 mapping:

CMR is the reference standard imaging technique to detect myocardial hyperaemia and oedema using both T2 and T1 weighted imaging sequences[86,87]. Regions of oedema and

inflammation exhibit higher signal intensity or enhancement on these sequences. The T2 weighted short tau inversion recovery (T2 STIR) sequence is commonly used, though T2 mapping and early gadolinium enhancement (T1 weighted) are also employed. Some sequences are assessed qualitatively e.g. T2 STIR by adjudicating signal intensity in different myocardial segments, whereas others are assessed quantitatively e.g. by drawing 'regions of interest' that provide a T2 mapping value in milliseconds (e.g. T2 mapping). Native myocardial T2 mapping is reported to be the most reproducible method in the setting of oedema associated with myocardial infarction[88] and the utility of parametric mapping methods was recognised by the updated Lake Louise criteria for the evaluation of non-ischaemic myocardial inflammation[89]. A small human study showed evidence of myocardial oedema in two of seven patients immediately after receipt of anthracycline using CMR[90] and recent animal[5] and human[91] studies reported the potential utility of native myocardial T2 mapping as a biosignature of early anthracycline associated cardiotoxicity.

2.5.1.4 Detection of myocardial hyperaemia/oedema - T1 weighted early gadolinium enhancement (EGE) imaging:

Following the administration of a gadolinium based contrast agent, which shortens the T1 and T2 relaxation time of tissue where it accumulates, T1 weighted EGE sequences have historically been used to evaluate myocardial oedema by exploiting the enhanced vasodilatation and vascular permeability of acutely inflamed tissue. EGE sequences have been used as a marker of myocardial injury in patients with myocarditis[87] and formed part of the original Lake Louise diagnostic criteria. In a study of 22 patients using EGE sequences, an increase in myocardial signal intensity three days after the administration of anthracycline predicted subsequent decline in LVEF at 28 days[93].

2.5.1.5 Myocardial scarring/fibrosis - Late gadolinium enhancement (LGE) sequences

LGE is the cornerstone of CMR tissue characterisation and its presence predicts adverse outcomes in a variety of cardiomyopathies, including coronary artery disease[95], non-ischaemic cardiomyopathy[96], hypertrophic cardiomyopathy[97], cardiac sarcoidosis[98], myocarditis[99] and cardiac amyloidosis[100]. Gadolinium preferentially accumulates in areas of interstitial expansion (typically scar and fibrosis) giving these areas a hyperintense (bright) appearance in comparison to surrounding healthy myocardium, which appears of lower signal intensity

(darker), because of the comparative absence of contrast. The accumulation pattern of contrast in the myocardium mirrors the pathophysiology of the underlying disease, defining ischaemic patterns (subendocardial to transmural, following the ischaemic necrotic wave front), and non-ischaemic patterns (mid-wall, epicardial) Figure 2-7. However, anthracycline related cardiac dysfunction may exhibit diffuse, rather than focal fibrosis. For example, a study of 10 explanted hearts with advanced anthracycline cardiomyopathy (1 death and 9 following cardiac transplant) demonstrated interstitial fibrosis in all patients; six with multifocal fibrosis, three with diffuse fibrosis and one with focal fibrosis[6]. Consequently, diffusely fibrosed myocardium has uniform signal intensity and there may be an absence of normal reference myocardium. Thus, the most common finding in the setting of cancer therapeutics related cardiac dysfunction is the absence of LGE[101] and less commonly mid-myocardial[102, 103], epicardial and right ventricular insertion point[104] LGE. Importantly, and in contrast to other populations, the presence of LGE has not been associated with adverse clinical outcomes in cardio-oncology populations. Furthermore, its relationship with adverse left ventricular remodelling and clinical outcomes has not been described.

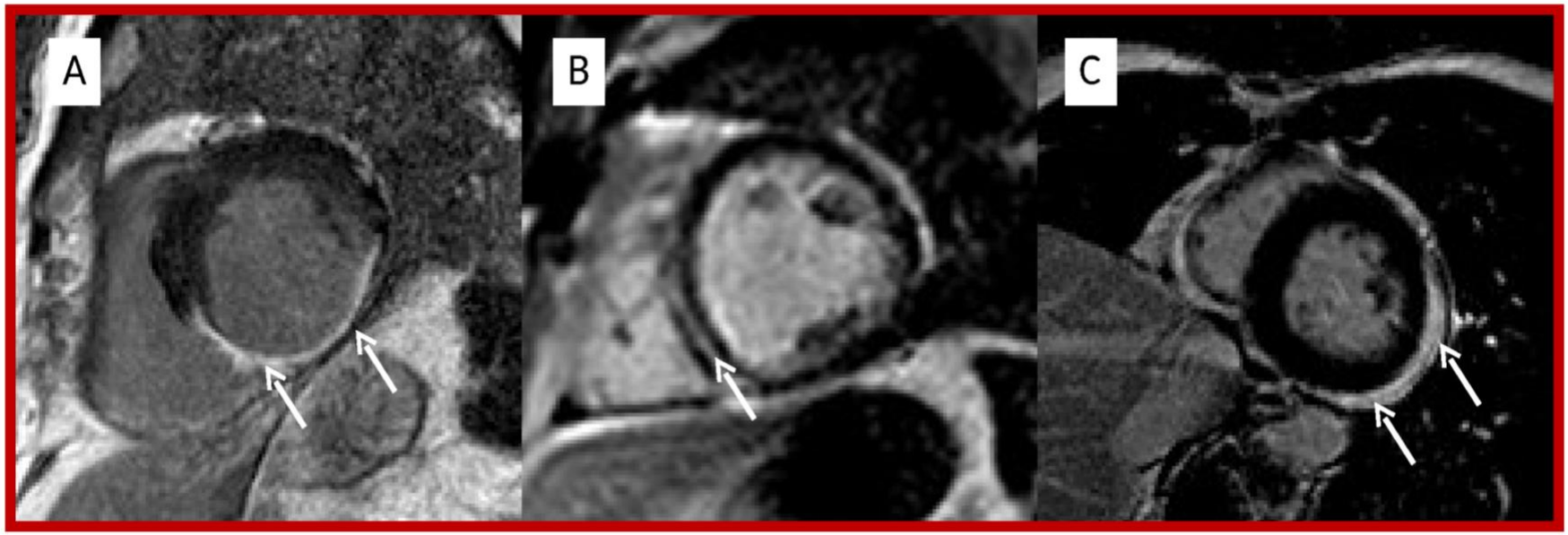

Figure 2-7 Patterns of myocardial scarring in post-contrast images using LGE

T1 weighted post contrast short axis mid ventricular images: A: ischaemic LGE (arrowed) due to transmural infarction in the right coronary artery territory; B: mid-myocardial LGE in dilated cardiomyopathy (arrowed) and C: subepicardial LGE (arrowed). LGE = late gadolinium enhancement.

2.5.1.6 Intracellular and interstitial myocardial fibrosis and oedema/inflammation - Native and post-contrast myocardial T1 mapping sequences.

Novel, advanced tissue characterisation sequences such as native T1 mapping and extracellular volume (ECV) fraction estimation may be able to address the perceived shortcomings of LGE by quantitatively assessing the extracellular space (Figure 2-8). Indeed, these techniques have shown excellent reproducibility and robust validation against biopsy proven collagen volume fraction and extracellular space reported in explanted hearts[105] and biopsied patients with dilated cardiomyopathy[106]. Using this technique, an increased ECV, used as a surrogate marker of myocardial fibrosis, was reported in a cohort of patients receiving anthracyclines, as compared with age and sex matched controls[107]. It has also been shown to occur independently of underlying cancer or cardiovascular comorbidities three years after anthracycline treatment[108]. Whilst these techniques hold promise to detect the presence of subtle, diffuse fibrosis their precise role in the identification, monitoring and prognosis of anthracycline cardiotoxicity is yet to be precisely defined.

In addition to detecting late cancer therapy related fibrosis, native myocardial T1 mapping and ECV can be elevated due to inflammatory myocardial processes, including early after cancer treatment[91]. Therefore, native T1 mapping and ECV should not be considered only to represent diffuse fibrosis, but rather to represent an estimation of the extracellular volume.

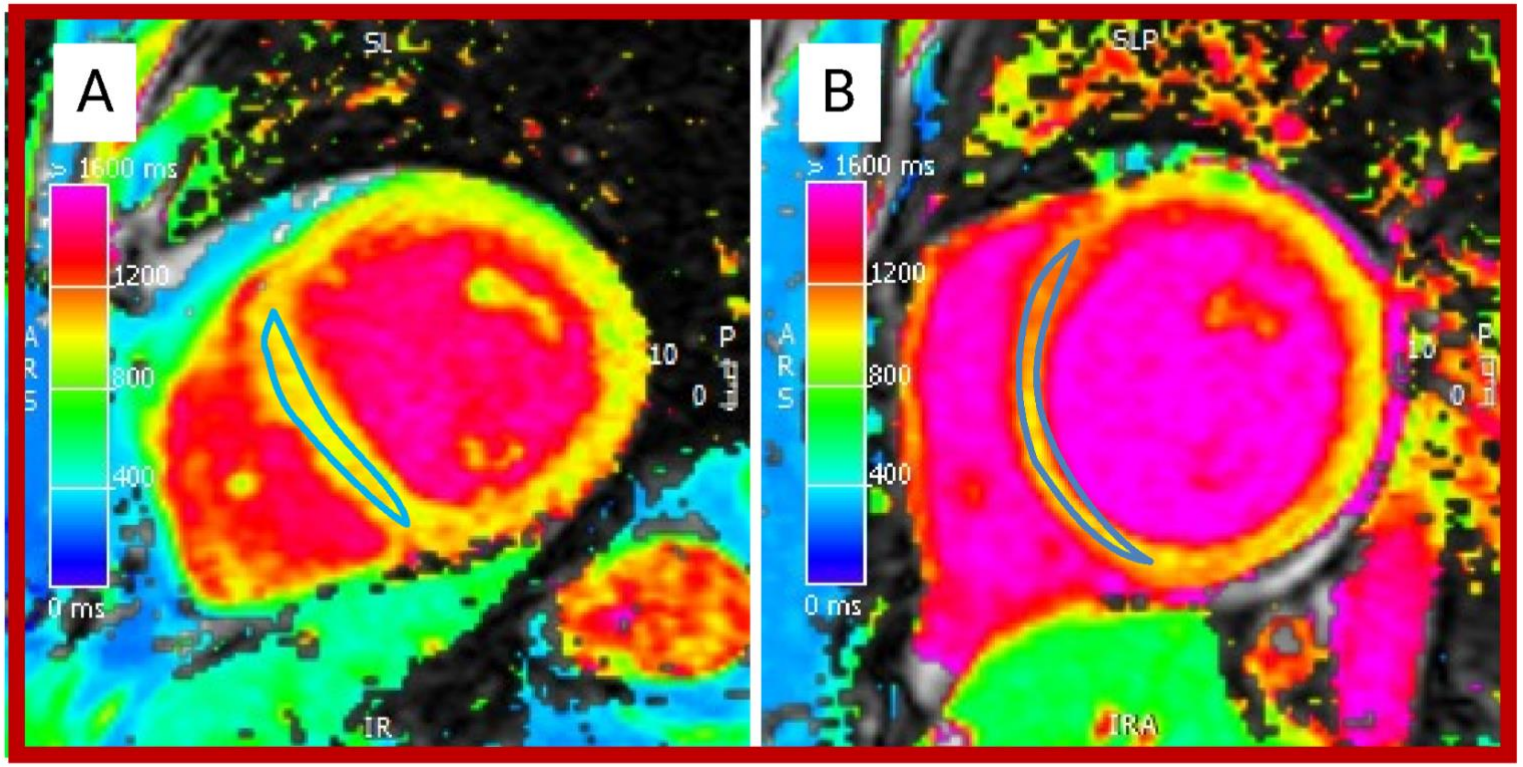

Figure 2-8 Short axis mid ventricular native myocardial T1 maps

A: normal native myocardial measurements (1010ms) and B: abnormal measurements (1136ms) in the mid-ventricular septum. Colour scale is evident on the left hand side.

2.6 CMR to detect the cardiovascular effects of anthracycline

2.6.1 Myocardial dysfunction and heart failure

Left ventricular dysfunction that manifests clinically as heart failure is the most concerning complication of anthracycline chemotherapy, principally because it carries a poor prognosis, particularly when it is detected late[7]. The mechanisms underpinning cardiotoxicity were discussed in section 2.1.3. From a clinical perspective, two types of cardiotoxicity were originally described[19]. Type I toxicity is attributable to anthracycline e.g. Doxorubicin, is dose dependent, may be irreversible and is associated with typical histological changes, whereas type II toxicity is typically attributed to agents such as monoclonal antibodies e.g. Trastuzumab, occurs independent of dose, is largely reversible following interruption, and is not associated with histological changes. Latterly, the division of cardiotoxicity into two distinct types appears overly simplistic with a significant degree of overlap recognised e.g. reversibility of anthracycline cardiotoxicity if identified early[20].

Whilst the non-invasive evaluation of LVEF has several limitations, it is the most widely accepted strategy for monitoring cardiac function in oncology populations. With this in mind, it is important to understand that different mechanisms can drive changes in LVEF, which is a product of end diastolic and end systolic left ventricular volumes. One study of 120 adults reported that among patients with declines in LVEF meeting criteria for cardiotoxicity, 19% were due to an isolated fall in end diastolic volume, thought principally to represent volume depletion[111]. Furthermore, declines in LVEF driven by an increase in end systolic volume can be caused both by extrinsic factors such as increased afterload, as well as intrinsic myocardial dysfunction[112]. These factors ought to be borne in mind when interpreting the results of CMR in patients receiving cancer therapy.

In a porcine model of anthracycline toxicity, Galan-Arriola[5] demonstrated that T2 relaxation time prolongation was the earliest CMR parameter to change. This occurred at week six (two weeks after the third dose of intracoronary Doxorubicin), corresponded with intracardiomyocyte oedema at tissue level and importantly, identified cardiotoxicity at a reversible stage. Other parameters such as T1 mapping, ECV and left ventricular wall motion only occurred at a later stage, when histological changes became irreversible. These data are

yet to be replicated in a human population but highlight the potential utility of T2 mapping to inform treatment and monitoring strategies.

The precision of CMR in measuring dynamic changes in ventricular volumes and ventricular mass make it an important tool to monitor and identify early subtle cardiac changes associated with cancer therapy employed by a number of prospective studies (Table 2-2) [113].

CMR is effective in the evaluation of cardiomyopathy late after anthracycline chemotherapy, which is particularly relevant when managing the increasing population of paediatric and adult cancer survivors. Indeed, CMR identified a higher prevalence (14%) of cardiomyopathy (left ventricular ejection fraction < 50%) in 114 asymptomatic childhood cancer survivors assessed 27.8 years after anthracycline chemotherapy compared with 2D or 3D echocardiography[113]. In fact, 11% of the study population were misclassified as having a normal LVEF by 2D echocardiography in comparison to CMR, with the higher cut off of an LVEF of 60% improving the detection of cardiomyopathy. In another study of 62 asymptomatic childhood cancer survivors assessed a median of 7.8 years after anthracycline treatment, the prevalence of cardiomyopathy (ejection fraction < 55%) in the right and left ventricles was 81% and 79%, respectively[115]. A subsequent study by the same group using 2D echocardiography, 3D echocardiography and CMR reported the prevalence of cardiomyopathy (LVEF <55%) as 78% in 71 survivors of childhood cancer[116].

Left ventricular mass is another CMR derived marker of interest for detecting cardiotoxicity. An inverse association between anthracycline dose and left ventricular mass was reported in a study of 91 patients with cardiomyopathy a median of 88 months after chemotherapy[104], which is in keeping with the report of Armstrong et al. who found that 48% of patients had left ventricular mass two standard deviations below normal. Furthermore, in the former study, left ventricular mass demonstrated a strong inverse association with major adverse cardiovascular events and a recent report[55] provided additional pathophysiological insight by suggesting that this decrease may be due to cardiomyocyte apoptosis.

Table 2-2 Prospective adult human CMR studies of cardiotoxicity in chronological order

Author and Year	*n*	Mean Age/Sex	Treatment	Follow up Imaging	Definition and incidence of cardiotoxicity	Findings
Wassmuth et al. 2001 [93]	22	43; 77% women	First course: Doxorubicin 67 mg/m^{2}or Epirubicin 76 mg/m^2	3 and 28 days after treatment initiation	LVEF < 55% occurred in 27% (*n* = 6)	Significant decrease in LVEF 67.8 ± 1.4% to 58.9 ± 1.9%. LVEF decreased significantly at 28 days in patients with an increase in relative enhancement of > 5 on day three
Chaosuwannakit 2010 [110]	40	52; 70% women	Doxorubicin 215 mg/m^2 or Daunorubicin 265 mg/m^2 or Trastuzumab 920 mg	4 months after treatment initiation	Not reported	Significant decrease in LVEF 58.6 ± 6.3% to 53.9 ± 6.4%. Significant increase in aortic stiffness (p < .0001)
Fallah-Rad 2011 [117]	42	47; 100% women	Epirubicin or adriamycin and Trastuzumab	12 months after treatment initiation	LVEF decline of 10% to < 55% occurred in 24% (*n* = 10); determined by echo.	Significant decrease in LVEF 66 ± 5% to 47 ± 4% in 10 with cardiotoxicity. All of whom exhibited subepicardial LGE.
Drafts et al. 2013 [82]	53	50; 58% women	50 – 375 mg/m^2 Doxorubicin equivalent	1, 3 and 6 months after treatment initiation	Of those with LVEF > 50% at baseline (*n* = 47), 26% (*n* = 12) had LVEF <50% after six months	Significant decrease in LVEF 58 ± 1% to 53 ± 1%. Significant decrease in mean mid wall circumferential strain (-17.7 ± 0.4% to -15.1 ± 0.4%; p = .0003) No new LGE.
Jordan et al. 2014	65	51; 86% women	55% (*n* = 36) received median 240 mg/m2 doxorubicin equivalent, 38% (*n* = 25) received a monoclonal antibody	3 months after treatment initiation	Not reported	Significant decrease in LVEF 57 ± 6% to 54 ± 7%. Significant increase in T1 weighted signal intensity 14.1 ± 5.1a.u. to 15.9 ± 6.8a.u.. No LGE.

Lunning et al. 2015 [102]	10	59; 40% women	300 mg/m^2 Doxorubicin	3 months after treatment completion	LVEF decrease by > 10% occurred in 50%	Significant decrease in FT-GCS (p = .018). Trend to FT-GLS decrease (p = .073) 30% exhibited one new or progressive segment of LGE
Nakano et al 2016 [118]	9	62.3; 100% women	67% had epirubicin. 100% had Trastuzumab	3, 6 and 12 months after treatment initiation	No cardiotoxicity	Significant decrease in LVEF (68.4 ± 6.6% to 61.7 ± 8.7) at 6 and 12 months (62.9 ± 7.8%) but not at 3 months (65.4 ± 8.7%). Significant decrease in SENC-GLS and SEND GCS at 6 months but not at 3 or 12 months.
Barthur et al 2017 [119]	41	52; 100% women	56% received anthracycline. 100% received 18 cycles of Trastuzumab	6, 12 and 18 months	No cardiotoxicity	Significant decrease in LVEF at 6 (60.4 ± 4.2% to 58.3 ± 5.1%) and 12 (57.9 ± 4.8%) months but not at 18 months. Significant decrease in RVEF at 6 (58.3% to 53.9%) and 12 (55%), which had recovered at 18 months (56.6%)
Muehlberg et al 2018 [120]	23	59; 52% women	360 – 40 mg/m2 Doxorubicin equivalent	48 after treatment initiation and on treatment completion	LVEF decrease by > 10% occurred in 39% (n = 9)	Significant decrease in subgroup with cardiotoxicity (63.5 ± 5.8% to 49.9 ± 5.0%) but not in those without (59.2 ± 10.3% to 58.3 ± 7.8%). At 48hours, subgroup who developed cardiotoxicity had significantly lower native T1 times than those who did not develop cardiotoxicity.
Ong et al 2018 [84]	41	52, 100% women	56% (n = 23) received anthracycline. 100% received Trastuzumab	6, 12 and 18 months after treatment initiation	2.4% (n = 1) experienced cardiotoxicity	Significant decrease in LVEF at 6 months (60.4% to 58.4%) and 12 months (57.9%) but not at 18 months (60.2%). Significant decrease in FT-GLS and FT-GCS at 6 and 12 months but not at 18 months.

CMR= cardiovascular magnetic resonance, FT = feature tracking, GCS = global circumferential strain, GLS = global longitudinal strain, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, SENC = strain encoded imaging, RVEF = right ventricular ejection fraction.

2.6.2 Current international guideline recommendations on the role of CMR in cardio-oncology.

CMR is becoming increasingly incorporated into societal cardio-oncology guidelines. The role identified for CMR in the most recent publications is described in detail below. In brief, CMR is particularly useful when LVEF is difficult to obtain by other means[158] (e.g. poor echocardiographic window due to left sided breast surgery, lung disease or obesity), when results obtained by other means are suboptimal, borderline or conflicting[19] and where discontinuation of chemotherapy is being contemplated[19]. Furthermore, CMR is particularly useful in the assessment of pericardial disease[2, 19], infiltrative diseases and cardiac masses[2, 19], as well as the aetiology of left ventricular dysfunction and presence of scarring or fibrosis[2].

Table 2-3 International guideline recommendations on the use of CMR in oncology populations

European Society of Cardiology (ESC) Position Paper on cancer treatments and cardiovascular toxicity[2]
• 'CMR is useful to determine the cause of left ventricular dysfunction and to clarify left and right ventricular function in challenging cases (i.e. borderline or contradictory results from other imaging modalities)'. • 'CMR also serves to evaluate the pericardium, especially in patients with chest irradiation'. • 'LGE may be useful to detect scarring or fibrosis, which may have prognostic implications in the context of impaired left ventricular function. • CMR is an excellent test for the comprehensive evaluation of cardiac masses and infiltrative conditions.
Expert Consensus for Multimodality Imaging Evaluation of Adult Patients during and after Cancer Therapy: American Society of Echocardiography and the European Association of Cardiovascular Imaging[19]
• CMR may be particularly useful in situations in which discontinuation of chemotherapy is being entertained and/or when there is concern regarding echocardiographic or equilibrium radionuclide angiographic calculation of LVEF. • If the quality of the echocardiogram is suboptimal, CMR is recommended. • CMR should be considered in the evaluation of primary tumours of the heart with or without compromise of the pericardium or when the diagnosis of constrictive pericarditis remains uncertain after a careful echocardiographic evaluation. • Standard precautions for CMR safety need to be followed, including consideration of electromagnetic interference. This may be particularly relevant in patients with breast cancer, in whom tissue expanders placed for breast reconstruction may represent a hazard.
Prevention and Monitoring of Cardiac Dysfunction in Survivors of Adult Cancers: American Society of Clinical Oncology Clinical Practice Guideline 2017[158]
• Imaging modalities such as CMR or multigated acquisition (MUGA) may be considered if an echocardiogram is not available or technically feasible (e.g., poor image quality as a result of body habitus, chronic lung conditions, or history of mediastinal surgery), with preference given to CMR.

2.6.3 Problems and Safety in CMR

CMR emerged as an important tool to evaluate the cardiovascular system nearly two decades ago but significant barriers to its widespread use in routine clinical practice persist. CMR remains a comparatively time consuming and complex investigation that is typically performed in academic centres. It requires a cooperative patient, ideally in sinus rhythm who can hold their breath to provide high quality images. However, the advent of novel technologies such as compressed sensing real time cine CMR, self gating and artificial intelligence have the potential to simplify and reduce image acquisition time up to twentyfold, without compromising image quality[159]. The cost and general availability of MRI scanners is an additional problem that may partially be addressed by collaborative use of resources and establishment of referral pathways between smaller and larger institutions. Furthermore, most MRI scanners installed in the last 10 years have the capacity to undertake cardiac imaging and data indicate that use of CMR increased in the United States during this period[160], with over 600 centres submitting claims for CMR services, according to 2018 billing data furnished by the Centers for Medicare and Medicaid Services.

There are an increasing number of physicians (cardiologists and radiologists) training and certifying worldwide and CMR expertise at both clinician and technologist level is increasing. To increase availability of technology and expertise, the development of technologies that allow remote supervision[161] of CMR scanners may also have an important role to play in training staff in geographically remote locations. Shortened, simplified protocols coupled with increasing automation of acquisition, processing and analysis, as well as the potential for remote collaboration, image analysis and interpretation will also increase the accessibility of this important tool to clinicians working in a variety of healthcare settings.

Technical challenges in CMR can often be overcome. For example, CMR may be facilitated in claustrophobic patients with a prone position, use of an eye mask, pre-procedural sedative or general anaesthetic (generally paediatric populations), the accompaniment of a friend or relative or by using a large bore scanner. Difficulty breath holding can be overcome by reducing the number of slices or phases and thus the time required for acquisition, by using a respiratory navigator or by acquiring images during free breathing or inspiration, rather than held expiration.

Image quality can be improved in patients with arrhythmia by employing arrhythmia rejection and/or correction protocols, or by using prospective ECG gating or real time acquisition. Table 2-4 describes safety concerns and proposed solutions to challenges encountered in clinical CMR.

Table 2-4 Safety concerns in CMR and possible solutions

Safety Concern	Possible solution
MRI	
Metallic foreign bodies (non-clinical)	Removal; Note intra-orbital metallic foreign bodies are an absolute contraindication to MRI scanning.
Metallic foreign bodies (clinical)	Patients with MRI conditional devices that pose no hazard in specific conditions can safely undergo MRI scanning. Consult MRI safety of specific product or device (e.g. mrisafety.com). There is increasing evidence that the risks attached to previously MRI unsafe clinical devices (e.g. legacy pacemakers, defibrillators, tissue expanders) can be mitigated and that patients can safely undergo MRI scanning [162, 163]. Consult MRI safety of specific product or device (e.g. mrisafety.com). and consult individuals/centres with expertise.
Pregnancy	Often avoided during first trimester, though risk is not known No known risk during second or third trimester
Gadolinium contrast	
Allergy	Gadolinium panel allergy testing
Anaphylaxis	Non-contrast study
Pregnancy	Contrast not generally administered in the 2nd or 3rd trimester unless clinical condition makes use absolutely necessary
Breast feeding	Restriction may not be necessary; pre-CMR breast milk collection and precautionary post-CMR breast milk suspension for 24h often recommended.
Renal impairment	Weigh benefit and risk, minimise dose of a low risk agent, avoid repeat dose for seven days
Haemodialysis	Non-contrast study; dialyse within 24h of administration if benefit felt to outweigh risk

CMR = cardiovascular magnetic resonance, MRI=magnetic resonance imaging

2.7 Study Rationale

Data on the CMR features of anthracycline cardiomyopathy are drawn from a limited number of small studies. Long term data on the presence and extent of LGE (a surrogate marker of focal fibrosis) and native T1 mapping values (a surrogate marker of diffuse fibrosis) are limited and conflicting. Furthermore, the relationship between these CMR parameters and cardiac structure and function has not been described in detail. A better understanding of these features may shed light on the pathophysiology and natural history of anthracycline cardiomyopathy and contribute to our understanding of the condition.

Excepting a small number of recognised clinical risk factors, predicting anthracycline cardiotoxicity continues to pose a significant clinical challenge. Therefore, the identification of non-invasive predictors of anthracycline cardiotoxicity, either at baseline or early during treatment would be of particular clinical value and may ultimately facilitate targeted treatment and monitoring strategies.

2.8 Study Objective

The overarching purpose of this study is to characterise the role of CMR in the diagnosis and prediction of anthracycline cardiotoxicity.

2.9 Study Aims

1. To characterise the long term CMR features of anthracycline cardiotoxicty in patients with impaired LVEF.
2. To characterise the long term CMR features of anthracycline cardiotoxicity in patients with normal LVEF.
3. To prospectively characterise the immediate and short term CMR and biomarker features of anthracycline cardiotoxicity.
4. To prospectively explore the ability of CMR and biomarkers to predict subsequent anthracycline cardiotoxicity.

Chapter 3 CMR Phenotyping of Long Term Anthracycline Cardiomyopathy

3.1 Introduction

Anthracycline cardiomyopathy is increasingly recognised as an important contributor to the morbidity and mortality of cancer survivors [164]. Consequently, international societies have published recommendations for the non-invasive monitoring of patients receiving potentially cardiotoxic agents[2, 19, 158]. CMR is the gold standard method for measuring ventricular volumes and function and has the unique capability to characterise myocardial tissue. CMR studies have focused on the early stages of anthracycline cardiomyopathy [82, 93, 102, 110]. There are limited longitudinal studies examining cardiovascular structure and function over an extended period of time [104, 165].

The early stages of anthracycline cardiomyopathy are characterised histologically by myocardial oedema, inflammation and vacuolisation[101], whereas in the later stages, diffuse myocardial fibrosis predominates [6]. Discrete foci of myocardial fibrosis can be detected by LGE sequences with broad diagnostic and prognostic applications in cardiovascular medicine[166]. There are limited, somewhat conflicting data on the presence, extent and location of focal myocardial fibrosis detected by LGE in adult patients with anthracycline cardiomyopathy, [82, 102, 117, 165] though it is generally reported to be an infrequent finding[101]. Furthermore, the qualitative nature of the analysis, which relies on the presence of normal myocardium for reference, means that the diffuse fibrosis typically associated with anthracycline cardiomyopathy may not be detectable. Novel quantitative techniques such as native myocardial T1 mapping and myocardial ECV estimation using gadolinium based contrast agents may be better able to estimate diffuse fibrosis, with accumulating evidence of abnormal values following anthracycline treatment[107, 108, 167], and correlation with exercise capacity[167] and cardiac function[107] reported in paediatric and adult populations, respectively.

3.1.1 Study Objectives

Therefore, the objectives of this study were to describe the long term phenotype of anthracycline cardiomyopathy, determine the prevalence of focal (LGE) and diffuse (elevated

native T1 measurements) myocardial fibrosis and their association with cardiac remodelling, function and clinical outcomes.

3.2 Methods

3.2.1 Study Population

Consecutive patients referred for clinically-indicated CMR at a tertiary referral centre (Bristol Heart Institute, Bristol, United Kingdom) between January 2010 and January 2017 were retrospectively screened for eligibility. Indications included investigation of dyspnoea, chest pain and to evaluate the aetiology of left ventricular dysfunction. Inclusion criteria were: age > 18 years and evidence of cardiotoxicity after anthracycline chemotherapy, which was defined either as an LVEF of < 57% or symptoms and/or signs of congestive heart failure. Exclusion criteria were: abnormal LV ejection fraction prior to therapy, concomitant cardiac disease that could cause heart failure including ischaemic heart disease (infarct pattern on LGE or inducible myocardial ischaemia during pharmacological stress), hypertrophic cardiomyopathy, moderate-severe valvular heart disease or valve replacement, history of excessive alcohol consumption (> 28 standard units per week), active thyroid disease or family history of cardiomyopathy in a first degree relative. Formal Research Ethics Committee (REC) approval was not obtained. The local institutional research and innovation department (Bristol Royal Infirmary) approved the study as a service evaluation exercise. All patients provided written consent for use of anonymised data for research purposes at the time of CMR.

3.2.2 Clinical Characteristics

The following data were collected: demographics, body surface area, body mass index, comorbidities, medications, cancer diagnosis, cancer therapy (including date, dose and type of therapy), New York Heart Association (NYHA) functional class at the time of CMR, hospitalisations for heart failure and all cause mortality.

3.2.3 CMR - volumes and function

All patients underwent CMR at 1.5 Tesla (Avanto, Siemens, Erlangen, Germany). Short axis steady state free precession (SSFP) whole left ventricular (LV) cines (typical scan parameters: 8 mm slice thickness, no slice gap, temporal resolution 38.1 ms, echo time 1.07 ms, in plane

pixel size 1.5 x 0.8 mm) were used to determine biventricular volumes, left atrial volumes, LVEF and left ventricular mass, which were indexed to body surface area, according to previously described methods [168]. All measurements were performed by experienced CMR readers (IH/CBD) blinded to clinical details using previously validated[169] threshold detection software (CMR42, Circle Cardiovascular Imaging, Calgary, Canada) by manually drawing the epicardial and endocardial borders at end systole and end diastole, taking particular care to track the mitral valve plane through the cardiac cycle.

3.2.4 CMR – LGE

LGE imaging was used to detect focal myocardial fibrosis according to previously described methods[168] after the administration of 0.1 mmol/kg Gadovist (Bayer, Reading, United Kingdom) with an inversion time progressively set to null normal myocardium. The presence, location and extent of LGE in each of the 16 American Heart Association (AHA) segments was adjudicated qualitatively by two experienced, independent CMR readers (IH/CBD) with consensus required in cases of discrepancy. Readers were not blinded to anthracycline administration history and no control patients were assessed. Patients were divided into groups according to the presence of focal myocardial fibrosis.

3.2.5 CMR – Native myocardial T1 mapping

Native myocardial T1 maps were obtained in short axis using the modified look-locker inversion recovery sequence (MOLLI) i.e. 35° flip angle, 100ms minimum TI, 80ms TI increment, 150ms time delay with 5-(3)-3 heartbeat acquisition scheme[170]. Post-contrast T1 maps were acquired using the MOLLI variant 4(1)3(1)2. Regions of interest were drawn on motion corrected T1 maps in the mid cavity interventricular septum to determine native myocardial T1 measurements using Argus software (Siemens, Germany) by an experienced CMR reader (IH), avoiding areas exhibiting LGE. Patients were divided into those with normal native myocardial T1 measurements (< 1065 ms) and those with increased native myocardial T1 measurements (> 1065 ms), according to the locally-established normal reference range of 1024 ± 41 ms for a control population of 29 subjects[171]. Secondary analysis, treating native myocardial T1 as a continuous variable was undertaken to explore correlations with cardiac morphology and function. ECV was calculated using the established formula [153]:

$$ECV = \frac{\Delta R1_{myocardium}}{\Delta R1_{blood}} \times (1 - Haematocrit)$$

where

$$\Delta R1 = \left(\frac{1}{T1_{postcontrast}} - \frac{1}{T1_{native}}\right).$$

3.2.6 Statistical Analysis

Descriptive statistics were reported for all study data, including means and standard deviations for continuous variables, and counts and percentages for categorical variables. Differences between groups were assessed with student t test and Mann-Whitney U test for normally and non-normally distributed variables, respectively. Univariable and multivariable linear regression models were built to assess the association between outcomes of interest and one or more predictors, including controlling for age and gender in some of the associations. A *p* value < .05 was taken to indicate statistical significance. The analysis was carried out in Stata (Stata v. 13, StatCorp LLC, Texas United States).

3.3 Results

3.3.1 Patient Characteristics

81 patients were eligible for inclusion (62 with LVEF < 57% and 19 with symptoms and/or signs of heart failure) Figure 3-1. Their baseline characteristics are summarised in Table 3-1. Mean age was 55 ± 14 years; 68% were women. The majority of patients were treated for breast cancer (58%) or haematological malignancy (38%). 56 of the patients (69%) had a baseline LVEF > 55% on echocardiography. Baseline data was not available for 23 patients (28%), commonly because their treatment was administered in excess of 10 years prior to CMR. Baseline echo was not undertaken in two patients (2%) due to perceived low clinical risk of cardiomyopathy. As a component of a variety of chemotherapeutic regimens, the mean cumulative equivalent doxorubicin dose was 279 ± 89 mg/m^2 (range 50 – 450 mg/m^2). The anthracycline dose did not correlate significantly with ventricular volumes or systolic function; neither did the administration of radiotherapy (*p* > .05 in all cases). Median time from therapy to CMR was 60 months (interquartile range 17–152 months)**.** Follow up commenced at the

time of referral for CMR and medical records were reviewed a median of 21 months (interquartile range 12–33 months) after CMR. During this period, 28% of the study population were hospitalised with a diagnosis of heart failure recorded in their medical records and 6% died. Overall LVEF was impaired (49 ± 12%), driven by a pathologically increased iLVESV (47 ± 23 ml/m^2) when compared with established normal reference ranges[172]. iLVEDV (87 ± 25 ml/m^2) remained within established normal reference ranges, albeit toward the upper limit[172], as did indexed left ventricular mass (iLVM) (58 ± 15 g/m^2). The predominant left ventricular phenotypes were (Figure 3-2): normal iLVM and normal iLVEDV (33%; normal phenotype), normal iLVM and high iLVEDV (22%; eccentric dilatation) and small iLVM and normal iLVEDV (21%; myocardial atrophy).

Table 3-1 Clinical characteristics according to presence of LGE and native myocardial T1 measurements

	Cohort (*n* = 81)	**LGE** (*n* = 15)	**No LGE** (*n* = 66)	***p***	**Normal T1** (*n* = 23)	**Elevated T1** (*n* = 13)	***p***
Age, years	55 ± 14	57 ± 12	54 ± 15	0.55	57 ± 9	49 ± 18	0.25
Female	55 (68)	6 (33)	49 (64)	**0.01**	18 (78)	9 (69)	0.55
Body mass index, kg/m^2	27 ± 6	28 ± 5	28 ± 6	0.82	28 ± 6	28 ± 5	0.13
Body surface area, m^2	1.9 ± 0.2	1.97 ± 0.17	1.89 ± 0.23	0.12	1.95 ± 0.23	1.84 ± 0.21	0.12
Comorbidities							
Systemic hypertension	20 (25)	3 (20)	17 (26)	0.64	3 (13)	5 (38)	0.08
Diabetes	3 (4)	2 (13)	1 (2)	**0.03**	0 (0)	1 (8)	0.18
Atrial fibrillation	2 (2)	1 (1)	1 (0)	0.74	0 (0)	0 (0)	-
Medications							
ACE inhibitor	48 (63)	11 (73)	36 (55)	0.36	12 (52)	7 (54)	0.83
ARB	5 (7)	1 (6)	4 (6)	0.98	0 (0)	1 ()	0.17
Beta blocker	48 (63)	12 (80)	36 (55)	0.13	10 (43)	9 (69)	0.10
Diuretic	35 (46)	10 (67)	25 (38)	0.07	5 (22)	7 (54)	**0.04**
Aldosterone blocker	15 (20)	4 (27)	11 (17)	0.45	1 (4)	1 (8)	0.65
Ivabradine	3 (4)	1 (7)	2 (3)	0.54	0 (0)	0(0)	-
Cancer type							
Breast	47 (58)	10 (67)	37 (56)		8 (35)	9 (69)	
Haematological	31 (38)	4 (27)	27 (41)	0.52	14 (61)	4 (31)	0.13
Other	3 (4)	1 (7)	2 (3)		1(4)	0 (0)	
Chemotherapy							
DOX, mg/ m^2	279 ± 89	310 ± 101	272 ± 85	0.23	253.6 ± 89.2	281.8 ± 61.7	0.34
Radiotherapy	43 (54)	7 (47)	36 (55)	0.50	15 (65)	7 (54)	0.66
Therapy to CMR, months	60 (17-152)	153 ± 110	96 ± 116	**0.02**	94 ± 126	93 ± 85	0.53

Values are mean ± SD , *n* (%), or median (25th to 75th percentile). ACE = angiotensin converting enzyme; ARB = angiotensin II receptor blocker; CMR = Cardiovascular magnetic resonance; DOX = Equivalent doxorubicin dose; LGE = late gadolinium enhancement.

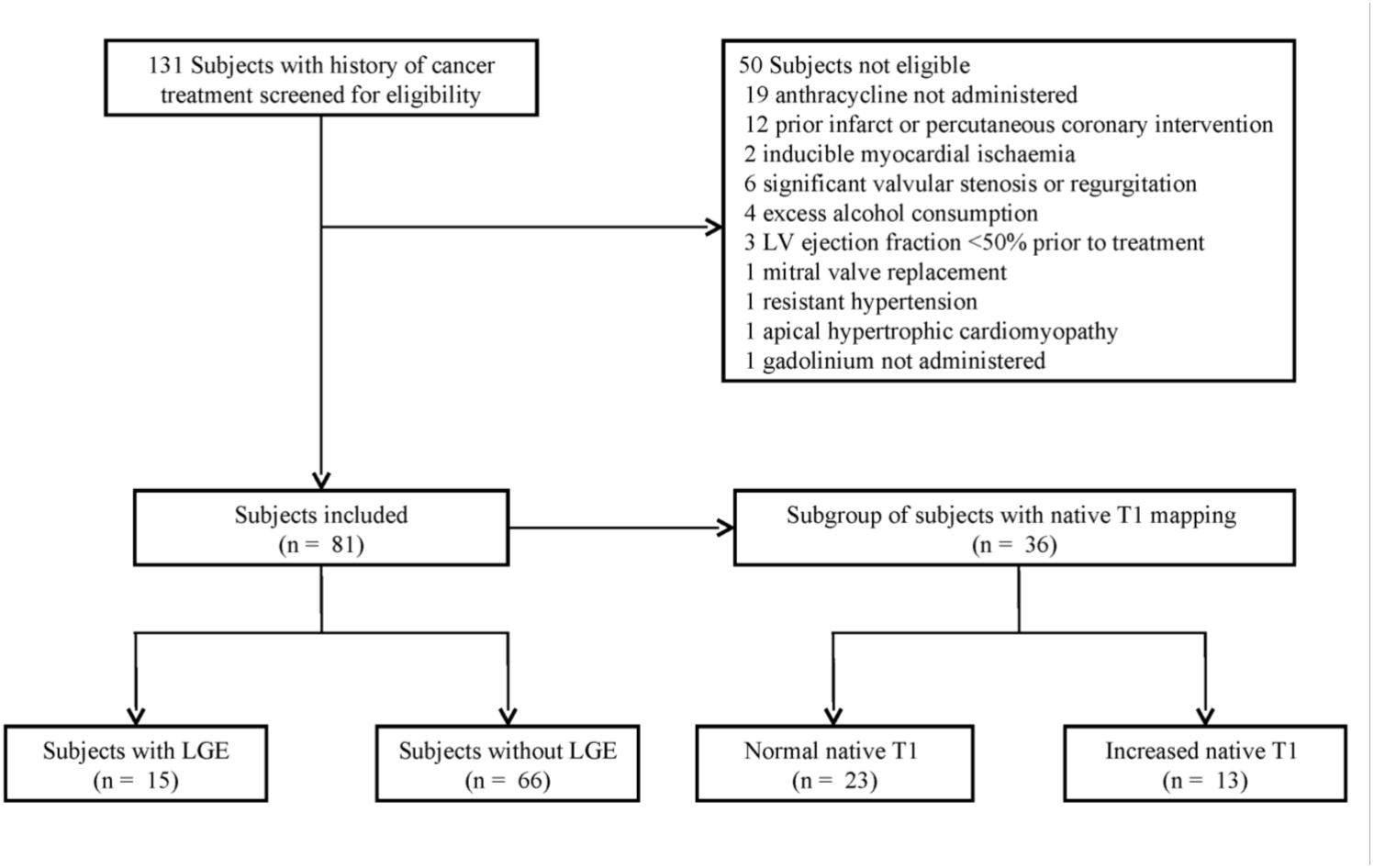

Figure 3-1 Study population, exclusion criteria and description of subgroups. n = number of patients

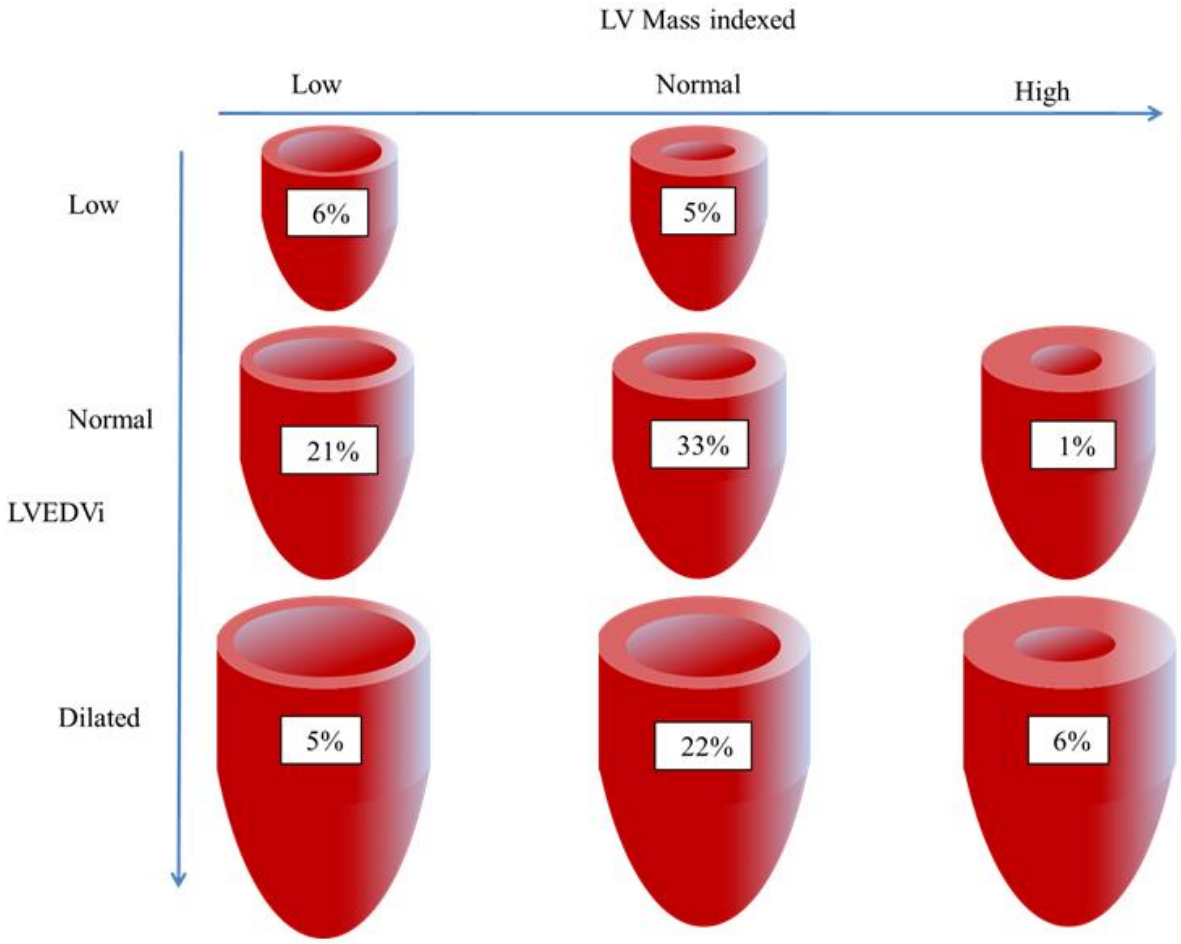

Figure 3-2 Left ventricular phenotypes in anthracycline cardiomyopathy

Percentage distribution of left ventricular phenotype according to age and sex adjusted LVEDVi (y axis), and LVMi (x axis).

3.3.2 LGE imaging for focal myocardial fibrosis

LGE was present in 19% of patients and was located exclusively in the mid-myocardium (non-ischaemic pattern) affecting a mean of 2.6 ± 1.4 segments in each patient. The commonest affected myocardial segment was the basal inferoseptal segment; 53% of patients with LGE exhibited it here (Figure 3-3).

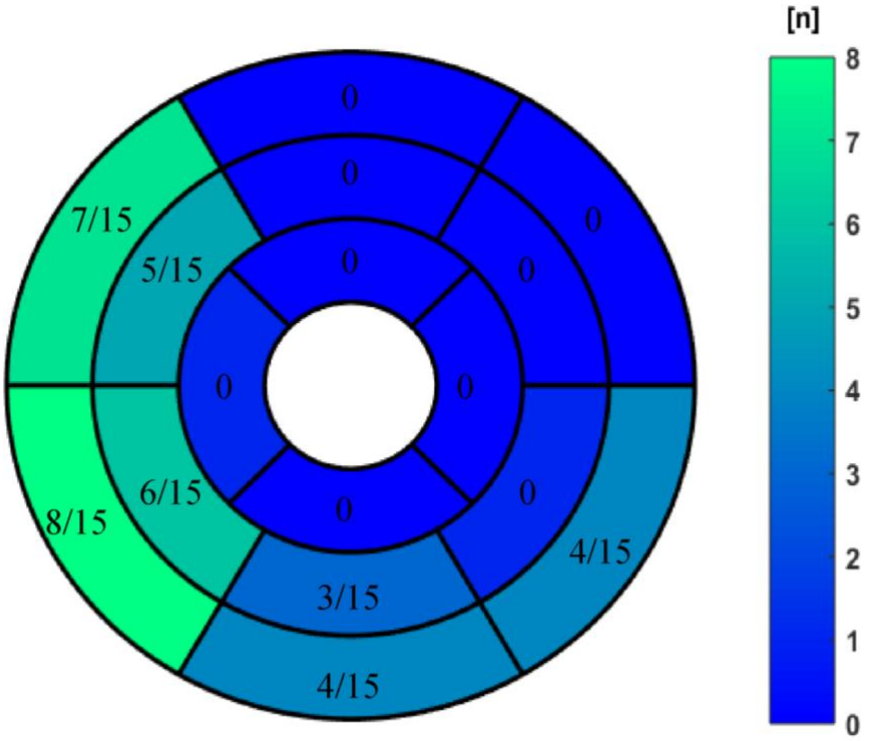

Figure 3-3 Segmental distribution of mid-myocardial LGE among patients with LGE

Segmental distribution of LGE among subjects with mid-myocardial LGE using the American Heart Association (AHA) 16 segment plot. E.g. 53% of patients with LGE had basal inferoseptal LGE. LGE = late gadolinium enhancement

Patients with LGE were more likely to be male (67 *vs.* 36%, p = .01), have diabetes (13% *vs.* 2%, p = .03) and were scanned at a greater time interval after cancer therapy (153 ± 110 *vs.* 96 ± 116 months, p = .02). No other differences in baseline

characteristics of patients with and without LGE were identified, including cumulative anthracycline dose (310 ± 101 *vs.* 272 ± 85 mg/m2, *p* = .23; Table 3-1)

Patients with LGE had significantly reduced LVEF (43 ± 11 *vs.* 50 ± 12%, *p* = .03) and significantly increased iLVEDV (102 ± 34 *vs.* 83 ± 21 ml/m^2, *p* = .03), iLVESV (61 ± 32 *vs.* 43 ± 20 ml/m^2, *p* = .03) and iLVM (65 ± 14 *vs.* 56 ± 14 g/m2, *p* = .04), compared with those without (Table 3-2). No significant differences in right ventricular volumes or systolic function, MAPSE or LAVi were observed. Hospitalisations with heart failure were similar between groups. Two patients (13%) with LGE died during follow up in comparison to three (5%) patients without (Table 3-2).

Multivariable regression analysis adjusting for age, sex, and diabetic status showed that the presence of LGE had a significant positive association with iLVEDV (*p* = .03, CI 2.15-31.0), and iLVESV (*p* = .03, CI 1.29-28.2) but not with LVEF (*p* = .17, CI 11.7-2.08) or other parameters.

3.3.3 Native myocardial T1 mapping techniques

Native myocardial T1 mapping sequences were available for analysis in a subgroup of 36 patients. 23 patients (64%) had normal native myocardial T1 measurements (1023 ± 28 ms), and 13 (36%) had increased native myocardial T1 measurements (1092 ± 20 ms) using the mean native myocardial T1 + 1 SD (1024 + 41 ms = 1065 ms) of a locally derived healthy control population as a cut point [171]. Baseline characteristics were matched (Table 3-1).

Table 3-2 CMR, clinical and outcome characteristics according to presence of LGE and native myocardial T1 measurements

	Cohort (*n* = 81)	Patients with LGE (*n* = 15)	Patients without LGE (*n* = 66)	*p*	Patients with normal T_1 (*n* = 23)	Patients with elevated T_1 (*n* = 13)	*p*
LV end diastolic volume index, ml/ m^2	87 ± 25	102 ± 34	83 ± 21	**.03**	74 ± 19	97 ± 22	**.002**
LV end systolic volume index, ml/m^2	47 ± 23	61 ± 32	43 ± 20	**.03**	35 ± 15	56 ± 22	**.005**
LV stroke volume index, ml/m^2	40 ± 9	41 ± 8	40 ± 9	.90	39 ± 8	41 ± 9	.50
LV ejection fraction, %	49 ± 12.0	43 ± 11	50 ± 12	**.03**	55 ± 9	44 ± 13	**.01**
LV mass index, g/m^2	58 ± 15	65 ± 14	56 ± 14	**.04**	50 ± 12	63 ± 17	.10
MAPSE, mm	12 ± 3	11 ± 2	12 ± 3	.41	13 ± 3	12 ± 3	.10
Left atrial volume index, ml/m^2	51 ± 18	59 ± 24	49 ± 16	.20	46 ± 14	56 ± 20	.16
Native myocardial T1 (ms)	-				1023 ± 20	1092 ± 20	**< .001**
Pre-contrast blood T1 (ms)	-				1646 ±76	1698 ± 87	0.06
Post-contrast myocardial T1 (ms)	-				505 ± 33	494 ± 50	0.46
Post-contrast blood T1 (ms)					354 ± 45	345 ± 54	0.57
Haematocrit (%)					37.5 ± 7.1	34.7 ± 7.9	.56
ECV (%)					28.5 ± 4.1	30.9 ± 2.4	**.03**
RV end diastolic volume index, ml/ m^2	68 ± 16	69 ± 17	68 ± 16	0.82	66 ± 12	73 ± 15	.26
RV end systolic volume index ml/m^2	31 ± 11	33 ± 14	31 ± 10	0.47	29 ± 8	35 ± 13	.36
RV ejection fraction, %	55 ± 9	54 ± 9	55 ± 9	0.61	57 ± 7	51 ± 11	.10
TAPSE, mm	20 ± 5	21 ± 4	20 ± 6	0.69	22 ± 5	20 ± 7	.19
NYHA Class							
I	21 (26)	4 (27)	17 (26)		9 (39)	3 (20)	
II	38 (47)	6 (40)	32 (48)		12 (53)	5 (33)	
III	19 (23)	4 (27)	15 (23)		2 (9)	3 (20)	
IV	3 (4)	1 (7)	2 (3)		0 (0)	2 (15)	
Hospitalisation for heart failure	23 (28)	5 (27)	18 (28)	0.64	2 (9)	5 (38)	**0.03**
Deaths	5 (6)	2 (13)	3 (5)	0.20	1 (8)	0 (0)	0.45

Values are mean and standard deviation (±), *n* (%), or median (25^{th} to 75^{th} percentile)
ECV = extracellular volume fraction, CMR = Cardiovascular magnetic resonance; LGE = late gadolinium enhancement; LV = left ventricle; MAPSE = mitral annular plane systolic excursion; RV = right ventricle; TAPSE = tricuspid annular plane systolic excursion.

Patients with increased native myocardial T1 measurements had significantly reduced LVEF (44 ± 13 *vs.* 55 ± 9%, *p* = .01) and significantly increased iLVEDV (97 ± 22 *vs.* 74 ± 19 ml/m^2, *p* = .002) and iLVESV (56 ± 22 *vs.* 35 ± 15ml/m^2, *p* = .005), compared with those with normal measurements (Table 3-2), differences that persisted in regression analysis controlling for age and sex (LVEF [*p* = .003, CI -20.4–-4.7], iLVEDV [*p* = .004, CI 8.8–40.9], iLVESV [*p* = .001, CI 9.9–37.6]. Regression analysis controlling for age and sex also revealed that LAVi was significantly increased in patients with increased native myocardial T1 measurements (*p* = .02, CI 2.3–24.3). Furthermore, hospitalisations for heart failure were higher (38% *vs.* 9%, *p* = .03) in patients with increased native myocardial T1 measurements. The only death occurred in the group with normal native myocardial T1 measurements. Among patients with increased native myocardial T1 measurements, 1 (8%) also had evidence of focal fibrosis by LGE.

Secondary analysis treating native myocardial T1 as a continuous variable demonstrated significant positive correlations with iLVEDV (*p* < .001, CI 0.15-0.48, R^2 0.33), iLVESV (*p* < .001, CI 0.15-0.44, R^2 0.36), LAVi (*p* = .04, CI 0.01 - 0.28, $R^2$0.12) and LV mass indexed (*p* = .01, CI 0.04-0.27, 0.18); and significant negative correlations with LVEF (*p* < .001, CI -0.24- -0.08, R^2 0.35) and MAPSE (*p* = .02, CI -0.05 - -0.004, R^2 0.14) (Figure-3-4 Panel A-F). In 31 patients with post-contrast mapping sequences available for analysis, ECV correlated significantly with iLVEDV (*p* = .003, CI 111-476, R^2 0.27) and iLVESV (*p* = .02, CI 42-378, R^2 0.18) but not with LVEF (*p* = .1, CI -187-18, R^2 0.09) or other parameters.

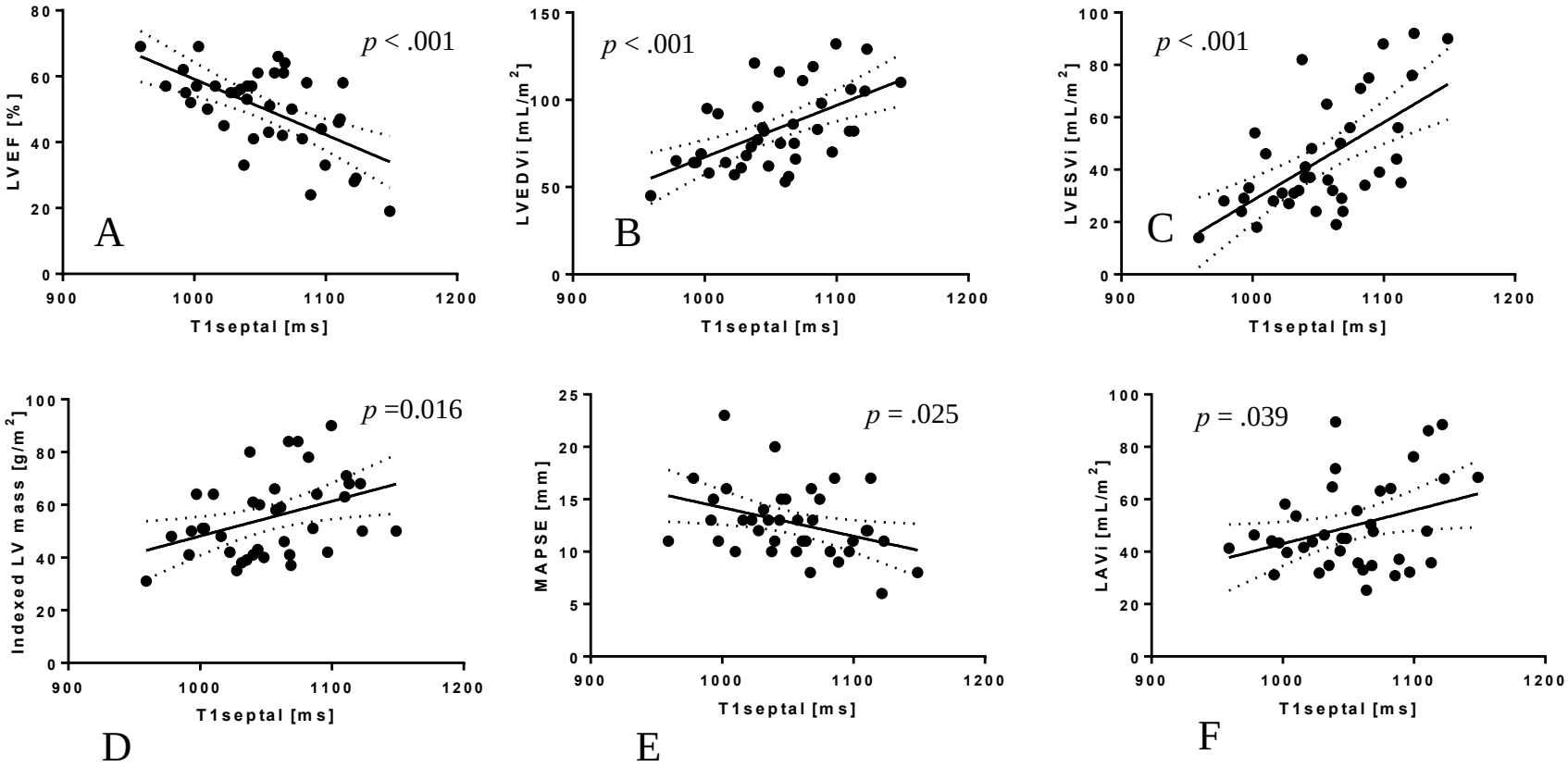

Figure-3-4 Regression analyses of absolute native myocardial T1 measurements

Regression analyses demonstrates significant negative correlations with systolic function (panel A LVEF, panel E MAPSE), and significant positive correlations with volumes and mass (panel B LVEDVi, panel C LVESVi, panel D LVMi, and panel LAVi).

3.4 Discussion

This study provides several insights into the characteristics of late anthracycline cardiomyopathy. First, the primary driver of reduced LVEF in long term anthracycline cardiomyopathy was a pathological increase in iLVESV and the predominant left ventricular phenotypes were: normal mass and normal volume, eccentric dilatation or myocardial atrophy with normal volume. Second, the prevalence of focal myocardial fibrosis at a median five years post-therapy was relatively low (19%) and distributed exclusively in the mid-myocardium with a predilection for the basal and mid-interventricular septum. Third, patients with LGE had significantly reduced LVEF compared with those without. Fourth, in subgroup analysis, the prevalence of elevated native myocardial T1 measurements was relatively high (36%) and these patients had significantly reduced systolic function (LVEF), adverse left ventricular remodelling and a higher rate of hospitalisation for heart failure compared with those with normal native myocardial T1 measurements. Fifth, native myocardial T1 measurements and ECV correlated with left ventricular volumes, and native T1 also correlated with LVEF but ECV did not.

3.4.1 Cardiac Morphology and Function

In keeping with previous reports, the predominant mechanism of cardiotoxicity in this cohort was a pathological increase in iLVESV[82, 110]. Both iLVM and iLVEDV, as well as all measures of RV volume and systolic function remained within established reference ranges[172]. Interestingly, iLVEDV was towards the upper limits of normal in the cohort as a whole but pathologically elevated in the subgroup of patients with focal fibrosis by LGE, suggesting that patients who exhibit focal fibrosis have a more severe or advanced cardiomyopathy than those who do not, though this was not associated with adverse clinical outcomes. In addition, a higher iLVM was observed in patients with focal fibrosis compared with those without. This elevation in mass is probably due to eccentric dilatation, rather than true hypertrophy, given that only 1% of the population had true LV

hypertrophy (defined by a normal iLVEDV and high indexed left ventricular mass). Whilst the comparison is not direct, this finding is at odds with a previous report correlating lower iLVM with adverse clinical outcomes [104], and may relate to the differing timing, methodology and populations of the studies. A recently published longitudinal echocardiography study of breast cancer patients described a relative increase in indexed left ventricular mass, which was sustained at three years follow up[173]. In this study, cumulative anthracycline dose was not significantly associated with ventricular volumes or systolic function, which may relate to the relatively small sample size and use of anthracycline doses in the modern clinical therapeutic range.

It is interesting to note that there was considerable heterogeneity in the left ventricular phenotypes, though the majority of the study population displayed either a normal left ventricular phenotype (normal iLVEDV and normal LVMi), eccentric dilatation (normal LVMi and elevated iLVEDV), or a phenotype of myocardial atrophy (low LVMi and normal iLVEDV), with only a minority (11%) exhibiting the so called Grinch syndrome phenotype (low iLVEDV with or without a normal LVMi) previously described by a paediatric study using echocardiography[174]. Aside from methodological differences, this may also point to differing remodelling between children and adults.

While native T1 correlated with left ventricular volumes, it did not correlate significantly with LVEF, which is counterintuitive. One potential explanation could be the influence of LV wall thickness on LVEF, which was not measured in this study but could be the topic of future investigations.

3.4.2 LGE Imaging - focal fibrosis

Commensurate with previous reports of both early[82, 165], and late[104] anthracycline cardiomyopathy, the prevalence of LGE at a median interval of five years post-therapy was low. The minority of patients with LGE exhibited significantly higher indexed LV volumes and significantly lower LVEF than those without, though no association with adverse clinical outcomes was demonstrated. This is in keeping

with the published anthracycline cardiomyopathy literature to date but inconsistent with meta-analysis of non-ischaemic cardiomyopathies as a whole, where the presence of LGE predicts adverse cardiovascular outcomes[175]. The absence of this association could in part be due to the low prevalence of LGE and small sample size in studies of anthracycline cardiomyopathy to date, but may also represent the limitation of this technique to detect the diffuse myocardial fibrosis typically associated with anthracycline cardiomyopathy[6]. It is noteworthy that patients with LGE were assessed after a significantly greater period than those without, which raises the question of whether fibrosis develops over time and may explain why early studies of anthracycline cardiomyopathy have reported such a low prevalence of LGE. However, in univariable regression modelling of LGE, time interval between chemotherapy and CMR was not significantly associated with left ventricular volumes or any parameters of systolic function, therefore it was excluded from the multivariable model.

While regression analysis controlled for sex and diabetic status, it is difficult to completely adjust for these differences in baseline measures. No study has reported the prevalence of non-ischaemic LGE in asymptomatic diabetic populations for comparison.

3.4.3 Native myocardial T1 mapping – diffuse fibrosis

Historically, diffuse myocardial fibrosis has been difficult to detect without the inherent risks attached to invasive cardiac biopsy. However, the advent of native myocardial T1 mapping and ECV techniques by CMR now permit the quantification of diffuse fibrosis non-invasively[176] with excellent reproducibility and robust validation against biopsy proven collagen volume fraction and extracellular space reported in explanted hearts[105] and biopsied patients with dilated cardiomyopathy[106]. Two previous studies of anthracycline cardiomyopathy in adults have described abnormal elevation of ECV in cancer survivors three years[108] and seven years[107] after anthracycline treatment. The former established that this elevation occurs independently of other risk factors

and the latter correlated this elevation directly with left atrial volume and negatively with diastolic function, and also described higher ECV in patients with reduced LVEF, compared with those with preserved LVEF. A further paediatric study provided complementary data correlating native myocardial T1 and ECV with exercise capacity, anthracycline dose and subtle ventricular remodelling[167]. This study complements this work by demonstrating significant correlations between absolute native myocardial T1 measurements, ECV and measures of left ventricular volumes and systolic function in anthracycline cardiomyopathy and in this regard, provides novel insight. Patients with increased native myocardial T1 measurements displayed significant adverse left ventricular remodelling and reduced systolic function (as measured by LVEF), compared with those with normal native myocardial T1 measurements. A greater number of hospitalisations occurred in the subgroup with increased native myocardial T1 measurements. In addition, absolute values of native myocardial T1 and ECV correlated significantly with left ventricular volumes and native myocardial T1 also correlated with LVEF, MAPSE and LAVi, all of which would suggest a greater burden of diffuse fibrosis in patients with larger ventricular volumes and worse systolic function. Finally, it is noteworthy that 13 (36%) of the subgroup had diffuse fibrosis judged by an abnormal native myocardial T1 measurement, but only one (8%) of these had coexisting evidence of focal fibrosis when assessed qualitatively by LGE. Therefore, relying solely on LGE would have misclassified 92% of these patients as not having fibrosis. This finding is again in keeping with the histopathological report of focal fibrosis in only 10% of explanted hearts with anthracycline cardiomyopathy[6]. CMR T1 techniques appear to offer additional insight in anthracycline cardiomyopathy, yet the precise role of these sequences as a diagnostic tool, method of monitoring and means of guiding therapy is yet to be defined and large scale, collaborative effort from multiple stakeholders is warranted.

3.4.4 Study limitations

This was a retrospective single centre study of a small and relatively heterogeneous cohort of patients referred for CMR on clinical grounds and, as such, the findings should be interpreted in this context and viewed as hypothesis generating. The small sample size and low number of events mean that firm conclusions, particularly regarding clinical outcomes, cannot be drawn and large, prospectively conducted, multicentre studies are warranted to address this area of important clinical uncertainty. Native myocardial T1 and T2 mapping sequences, and therefore ECV estimation was not available for the entire study population due to the introduction of this sequence to the department in 2015. However, it is felt that this subgroup provides insight into the utility of this sequence in anthracycline cardiomyopathy and the available data were therefore included. It is also important to note that for statistical purposes, we chose a native myocardial T1 cut off value of the median +1 standard deviation (1064ms) to divide groups. This has the potential to include patients with normal range native T1 in the 'elevated' group. Indeed, this is why this group is not described as having 'abnormal' native T1 measurements. Furthermore, we performed correlative analysis of native T1 as a continuous, rather than dichotomised measure. This confirmed significant associations of native myocardial T1 with multiple measures of left ventricular volumes and systolic function. Finally, complementary echocardiographic, electrocardiographic, blood biomarker (Troponin, BNP), and histopathological data were not consistently available and the cross sectional design of the study meant that temporal changes could not be assessed. A prospective study incorporating these measures prior to, during and after cancer therapy could address these limitations.

3.5 Conclusion

Late stage anthracycline cardiomyopathy is characterised by pathological increase in iLVESV, normal or dilated iLVEDV, low (19%) prevalence of focal septal fibrosis identified by LGE, and higher (36%) prevalence of diffuse fibrosis identified by elevated native T1 measurements. Both forms of fibrosis were associated with adverse left ventricular remodelling and reduced LVEF during the follow-up period studied. A higher rate of hospitalisation for heart failure was observed in patients with elevated native myocardial T1 mapping measurements.

3.6 Novel Findings

1. LGE (surrogate CMR marker of focal fibrosis) is not a common finding (19%) in late stage anthracycline cardiomyopathy but its presence is associated with adverse left ventricular remodelling and reduced LVEF.

2. Elevated native myocardial T1 mapping values (surrogate marker of diffuse fibrosis) are more common (36%) in late stage anthracycline cardiomyopathy and are associated with adverse left ventricular remodelling, reduced LVEF and hospitalisation for heart failure.

Chapter 4 CMR Characterisation of Anthracycline Cardiotoxicity in Adults with Normal Left Ventricular Ejection Fraction

4.1 Introduction

Anthracycline chemotherapy is efficacious but confers excess cardiovascular risk, both in the immediate and long term. Current surveillance strategies rely principally on the assessment of LVEF to diagnose cardiotoxicity[2, 19, 158]. However, declines in LVEF may only manifest once tissue level damage is extensive, and its reliance on geometric assumptions and susceptibility to loading conditions are recognised limitations. Consequently, quantification of myocardial deformation and non-invasive characterisation of myocardial tissue using CMR have emerged as important tools that provide complementary mechanistic insight and may facilitate early detection of systolic impairment in patients treated with anthracycline.

CMR studies of childhood cancer survivors have improved our understanding of the long term effects of anthracycline on myocardial deformation and myocardial tissue composition[167, 177, 178], reporting detectable differences in myocardial strain, LV mass, native myocardial T1, and ECV. To date, few long term adult studies have been conducted and most have either included patients with impaired systolic function[107, 108], in whom strain and tissue characteristics are recognised to be impaired, exclusively enrolled female patients with breast cancer[55] or neglected to include a control arm[179].

Accordingly, a cross sectional case control study of anthracycline treated cancer survivors with normal LVEF was conducted, comparing them to a control population of similar age and sex using CMR feature tracking and advanced tissue characterisation techniques. It was hypothesised that structural and functional myocardial abnormalities would be identified.

4.2 Methods

4.2.1 Study design and participants

A retrospective cross sectional study of consecutive patients referred for clinically indicated CMR (1.5T) at the centre (Bristol Heart Institute) between March 2012 and March 2019 was performed. The study enrolled patients age > 18 years with normal LVEF who had been treated with anthracycline. Patients were not eligible if they had co-existing cardiac disease (e.g. myocardial infarction, significant valvular heart disease, cardiomyopathy), contraindications to CMR, or if anthracycline treatment had been administered within the past three months.

Forty-five patient controls of similar age and sex distribution, with normal resting electrocardiogram, and normal CMR findings (normal cardiac volumes and LVEF, absence of myocardial oedema and LGE) served as controls. Indications for clinical CMR in patient controls included: assessment of palpitations when echo was equivocal or not diagnostic (n=18), atypical anginal chest pain (n=15), family history of cardiomyopathy (n=9), and investigation of hypertension (n=3). All participants provided written consent for use of anonymised data for research purposes at the time of CMR. Formal Research Ethics Committee (REC) approval was not obtained. The local institutional research and innovation department (Bristol Royal Infirmary) approved the study as a service evaluation exercise. The research team designed the study, gathered and analysed the data and vouch for data fidelity.

4.2.2 CMR

4.2.2.1 Left ventricular volumes, ejection fraction and strain

Patients underwent CMR on a single 1.5 Tesla system (Avanto, Siemens, Erlangen, Germany). Short axis steady state free precession cines (typical parameters: 8mm slice thickness [no gap], temporal resolution 38.1 ms, echo time 1.07 ms, in plane pixel size 1.5 x 0.8 mm) of the left ventricle were used to measure left ventricular (LV) volumes, ejection fraction (EF) and mass by drawing epicardial and endocardial borders at end systole and end diastole. Measurements were

indexed to body surface area, according to established methods[180, 181]. Global longitudinal (GLS), global circumferential (GCS) and global radial strain (GRS) parameters were calculated from short and long (two, three and four chamber) axis cines using feature tracking post-processing software (CMR42, Circle Cardiovascular Imaging, Calgary, Canada) in two (2D) and three dimensions (3D).

4.2.2.2 Native myocardial T1 mapping, ECV and indexed myocardial cell volume

MOLLI recovery sequences[170] (35° flip angle, 100ms minimum TI, 80 ms TI increment, 150 ms time delay with 5-(3)-3 heartbeat acquisition scheme) provided motion corrected, short axis native myocardial T1 maps of the mid-ventricular septum, on which a region of interest was drawn to determine native myocardial T1 measurements. ECV was calculated using the established formula[153]: ECV = (1-haematocrit) x (ΔR1 myocardium/ΔR1blood) with the 4(1)3(1)2 MOLLI variant. Indexed myocardial volume was calculated by dividing indexed LV mass by myocardial specific gravity 1.05 g/ml. Myocardial cell volume fraction was calculated, as previously described[182], as (1-ECV), and multiplied by indexed myocardial volume to estimate indexed myocardial cell volume. This technique has previously demonstrated excellent reproducibility[183].

4.2.2.3 Reproducibility

Two experienced CMR readers (IH, BB) blinded to all study data, independently analysed 10 anthracycline treated patients in random order to provide interobserver reproducibility data. These 10 anthracycline treated patients were re-analysed in a blinded random order four weeks later by IH to provide intra-observer reproducibility data.

4.2.3 Data analysis

Statistical analysis was performed using SPSS (SPSS version 24, Armonk, United States of America: IBM Corp.). Data are presented as mean ± standard deviation for continuous variables; counts and percentages for categorical variables. Continuous variables were assessed with unpaired student t-test and Mann-

Whitney U test for normally and non-normally distributed data, respectively. Intra- and interobserver reproducibility was assessed using intraclass correlation coefficient (ICC) and coefficients of variation (CoV). The CoV was calculated as the standard deviation of the differences, divided by the mean. ICCs were defined as poor (≤ 0.49), moderate (0.5-0.74), good (0.75-0.89) or excellent (≥ 0.9). A *p* value of < .05 was considered significant.

4.3 Results

4.3.1 Patient characteristics

Baseline demographic data of all participants are displayed in Table 4-1. The mean age (53 ± 16 *vs.* 56 ± 16 years, *p* = .3) and sex distribution (60% female *vs.* 60% female, *p* = 1.0) were similar in each group, as were the body surface area and medical history. The only statistically significant difference between the groups was the haematocrit, which was significantly lower in anthracycline treated patients, compared with controls (36 ± 7 *vs.* 41 ± 3%, *p* < .001). The majority of patients were treated for haematological malignancy (*n* = 33; 73%), or breast cancer (*n* = 10; 22%), received a mean doxorubicin equivalent dose of 237 ± 83 mg/m2, and underwent CMR at a median interval of 11 (range 3-36) months after completion of anthracycline treatment. The interval between completion of anthracycline treatment and CMR did not correlate with cardiac volumes, systolic function or myocardial tissue characteristics. However, after adjusting for age and sex, regression analysis revealed a weak but statistically significant negative association between anthracycline dose and indexed myocardial cell volume (r = -0.31, *p* = .02; Figure 4-1). Radiotherapy was administered to a minority (*n* = 9, 20%) and there were no significant differences in any of the measured parameters between those who did and did not receive radiotherapy (*p* > .05 in all cases).

Table 4-1 Demographic data

	Controls (*n* = 45)	Anthracycline treated patients (*n* = 45)	*p*
Mean age (years)	53 ± 16	56 ± 16	.3
Sex, *n* (%)			
Male	18 (40)	18 (40)	1.0
Female	27 (60)	27 (60)	1.0
Mean body surface area (m^2)	1.92 ± 0.24	1.89 ± 0.24	.62
Mean body mass index (kg/m^2)	27.2 ± 5.7	26.7 ± 5.8	.69
Medical history, *n* (%)			
Hypertension	3 (7)	3 (7)	1.0
Diabetes	1 (2)	3 (7)	.31
Cancer diagnosis, *n* (%)			
Breast	n/a	10 (22)	
Haematological	n/a	33 (73)	
Other	n/a	2 (4)	
Cancer therapy			
Mean DOX (mg/m2)	n/a	237 ± 83	
Median interval (months)	n/a	11 (3 – 36)	
Radiotherapy, *n* (%)	n/a	9 (20)	
Haematocrit (%)	41 ± 3	36 ± 7	< .001
Plus-minus values are means ± SD. - = interquartile range. DOX = doxorubicin equivalent dose, interval = interval between end of chemotherapy and CMR			

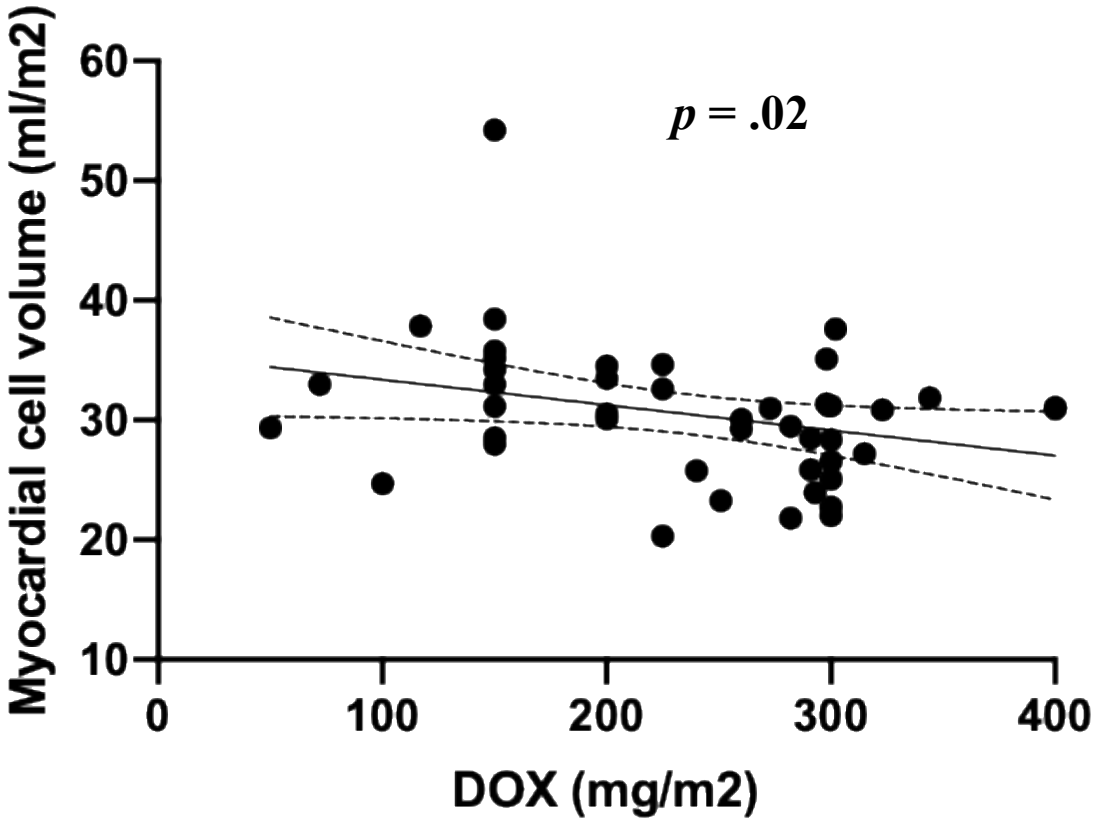

Figure 4-1 Linear regression analysis of myocardial cell volume and doxorubicin dose

Linear regression analysis adjusting for age and sex demonstrating a significant negative association between indexed equivalent doxorubicin dose (DOX; x axis) and indexed myocardial cell volume (y axis).

4.3.2 Ventricular volumes and ejection fraction

Left ventricular end diastolic volume indexed (LVEDVi) (69.2 ± 14.8 *vs.* 72.2 ± 10.3 ml/m^2, *p* = .26) left ventricular end systolic volume indexed (LVESVi) (28.6 ± 8.0 *vs.* 28.3 ± 4.83 ml/m^2), and LVEF (59.5 ± 4.1 *vs.* 60.8 ± 2.4%, *p* = .07) were similar between anthracycline treated patients and controls, respectively. Indexed right ventricular end diastolic volume (66.8 ± 14.6 *vs.* 72.7 ± 14.1ml/m^2, *p* = .06), indexed right ventricular end-systolic volume (27.4 ± 7.0 *vs.* 30.4 ± 9.5 ml/m^2, *p* = .09), and right ventricular ejection fraction (59.0 ± 5.7 *vs.* 59.0 ± 7.0%, *p* = .96) were also similar for anthracycline treated patients and controls, respectively. However, MAPSE was significantly reduced in anthracycline treated patients compared with controls (13.5 ± 3.0 *vs.* 14.7 ± 2.4mm, *p* = .04, Figure 4-2) but tricuspid annular plane systolic excursion (TAPSE) was not (21.7 ± 4.1 *vs.* 22.9 ± 5.6, *p* = .24). All values fell within established normal reference limits for CMR[172].

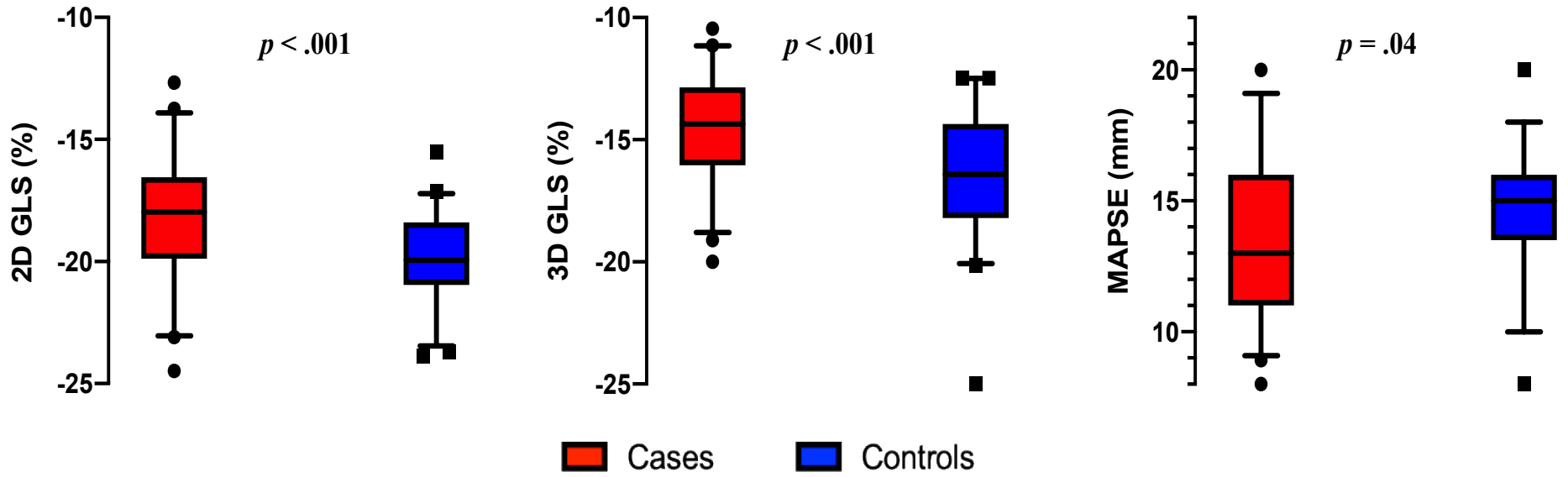

Figure 4-2 Comparison of functional parameters between anthracycline treated patients (cases) and controls

Box and whisker plots comparing functional parameters (2D feature tracking GLS, 3D feature tracking GLS and MAPSE) demonstrating statistically significant differences assessed by unpaired t tests. In each example, cases display statistically significant impairment, compared to controls. Median, interquartile range, 95% confidence intervals and outliers (black dots and squares) are displayed.

4.3.3 Feature tracking strain

2D GCS (-21.1 ± 2.6 *vs.* -21.9 ± 2.4%, p = .18), 2D GRS (42.8 ± 9.5 *vs.* 43.8 ± 10.3%, p = .61), 3D GCS (-17.7 ± 2.8 *vs.* -18.8 ± 2.8%, p = .07), and 3D GRS (42.6 ± 11.4 *vs.* 45.8 ± 10.4 %, p = .16) were similar between anthracycline treated patients and controls, respectively. However, both 2D GLS (-18.3 ± 2.6 *vs.* -20.0 ± 2.0%, p <.001) and 3D GLS (-14.5 ± 2.3 *vs.* -16.4 ± 2.6%, p < .001) were significantly impaired in anthracycline treated patients, compared with controls (Figure 4-2).

4.3.4 Tissue characterisation

Native myocardial T1 was significantly higher in anthracycline treated patients, compared with controls (1021 ± 40 *vs.* 996 ± 35ms, p = .002), and ECV was significantly higher in anthracycline treated patients, compared with controls (29.5 ± 4.5 *vs.* 27.4 ± 2.3%, p = .006) Figure 4-3. No anthracycline treated patients or controls had any segments of LGE. There was no significant difference in native T1 or ECV between males and females (p = .126 and .197, respectively). In addition, there was nod ifference in

4.3.5 Left ventricular mass and myocardial cell volume

Indexed left ventricular mass (LVMi) was significantly smaller in anthracycline treated patients compared with controls (45.6 ± 8.7 *vs.* 50.3 ± 10.1 g/m2, p =.02, Figure 4-3). Furthermore, indexed myocardial cell volume was significantly lower in anthracycline treated patients, compared with controls (30.5 ± 5.7 *vs.* 34.8 ± 7.2 ml/m^2, p = .002, Figure 4-3).

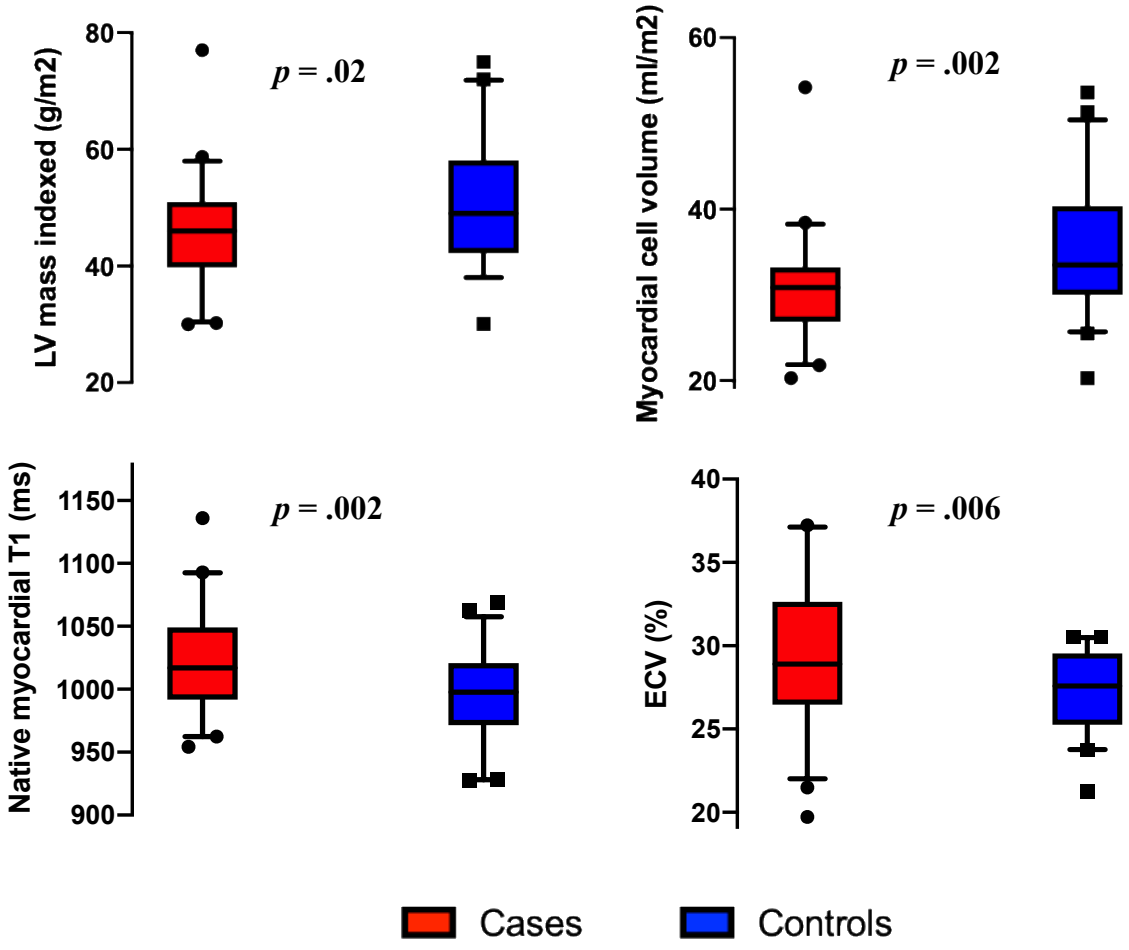

Figure 4-3 Comparison of tissue parameters between anthracycline treated patients (cases) and controls

Box and whisker plots comparing tissue parameters (LV mass indexed, indexed myocardial cell volume, native myocardial T1 and extracellular volume fraction (ECV)), which demonstrate statistically significant differences between anthracycline treated patients (cases) and controls. Median, interquartile range, 95% confidence intervals and outliers (black dots and squares) are displayed.

4.3.6 Reproducibility

Excellent interobserver reproducibility of 2D GLS, 3D GCS, 3D GRS, native T1 and ECV was demonstrated, with 2D GCS and 2D GRS showing good reproducibility, and only 3D GLS showing moderate reproducibility (Table 4-2). Intra-observer reproducibility of all parameters was universally excellent (**Error! Reference source not found.**).

Table 4-2 Interobserver reproducibility of parameters

Parameter	MD	SD	ICC (95%CI)	CoV (%)
2DGLS	0.05	1.29	0.93 (0.72 – 0.98)	-4.2
2DGCS	-0.84	2.00	0.86 (0.42 – 0.96)	-4.4
2DGRS	2.40	5.19	0.88 (0.55 – 0.97)	6.6
3DGLS	0.08	2.1	0.71 (0.18 – 0.93)	-7.6
3DGCS	-0.03	0.51	0.98 (0.94 – 0.99)	-1.7
3DGRS	-0.93	3.06	0.98 (0.93 – 0.99)	3.5
Native T1	5.4	16.1	0.93 (0.71 – 0.98)	0.8
ECV	0.21	1.56	0.92 (0.71 – 0.98)	3.3

MD = mean difference, SD = standard deviation, ICC = intraclass correlation coefficient, CI = 95% confidence interval, CoV = coefficient of variation, 2D = two dimensional, 3D = three dimensional, GLS = global longitudinal strain, GCS = global circumferential strain, GRS = global radial strain, ECV = extracellular volume fraction

Table 4-3 Intra-observer reproducibility of parameters

Parameter	MD	SD	ICC (CI)	CoV (%)
2DGLS	0.27	0.68	0.98 (0.90 – 0.99)	-2.3
2DGCS	0.11	0.93	0.97 (0.90 – 0.99)	-1.9
2DGRS	-0.89	3.9	0.93 (0.72 – 0.98)	2.7
3DGLS	-0.5	0.94	0.95 (0.79 – 0.99)	-4.9
3DGCS	-0.2	0.72	0.97 (0.89 – 0.99)	-2.0
3DGRS	1.2	1.74	0.98 (0.97 – 0.99)	2.4
Native T1	1.9	12.2	0.96 (0.84 – 0.99)	0.6
ECV	0.9	1.1	0.97 (0.87 – 0.99)	2.2

MD = mean difference, SD = standard deviation, ICC = intraclass correlation coefficient, CI = 95% confidence interval, CoV = coefficient of variation, 2D = two dimensional, 3D = three dimensional, GLS = global longitudinal strain, GCS = global circumferential strain, GRS = global radial strain, ECV = extracellular volume fraction

4.5 Discussion

This study investigated structural and functional myocardial changes in anthracycline treated cancer survivors with normal range LVEF at a median follow up interval of 11 months compared with a healthy control population of similar age and sex. Four important findings are reported. First, several measures of longitudinal systolic function (MAPSE, 2D GLS and 3D GLS) are significantly impaired in anthracycline treated cancer survivors, as compared with controls in the presence of normal LVEF. Second, LV mass and myocardial cell volume are significantly reduced in anthracycline treated cancer survivors. Third, surrogate CMR markers of myocardial fibrosis and extracellular expansion (native myocardial T1 and ECV) are significantly elevated in cancer survivors. Fourth, these imaging biomarkers show good to excellent levels of inter- and intra-observer reproducibility.

4.5.1 Systolic function

While LVEF is the most widely used non-invasive parameter to monitor cardiac function in patients receiving cardiotoxic therapy, tissue level damage may already be extensive by the time a change in LVEF is detected, and limitations include reliance on geometric assumptions and the influence of loading conditions. Consequently, semi-automated techniques that track myocardial deformation to quantify strain have emerged as powerful tools to appraise systolic function[49]. Echocardiography derived strain is well established, whereas evidence for CMR derived strain in cardio-oncology populations is comparably limited and furthermore, partitioned by different strain techniques. It is reported that GLS is an early marker of systolic dysfunction and may deteriorate prior to asymptomatic declines in LVEF[49]. Our findings support this hypothesis by demonstrating that CMR feature tracking GLS is impaired in long term anthracycline treated cancer survivors, compared with controls, in the setting of a normal LVEF.

Several prospective adult studies have reported detectable reductions in components of 2D CMR strain using various techniques during the acute phase of anthracycline treatment [82, 84, 102, 118]. This comparatively large study of feature tracking strain in anthracycline treated adult cancer survivors complements this work by confirming persisting long term changes, and replicates similar results from survivors of childhood cancer with normal LVEF[177]. One previous prospective adult study of 10 patients with a mean post-treatment LVEF of 52.8% reported significant reduction of both CMR 2D GCS and 2D GLS at three months, compared with healthy controls. Similar results were found for 2D GLS but the findings for GCS were not replicated, which have also been reported by other groups[84]. These discrepancies are probably accounted for by different populations (mean LVEF was higher at 59.5% in this study), methodologies (strain technique, hardware and software), and follow up intervals. Furthermore, and to the best of our knowledge, no published CMR studies of 3D strain in the context of anthracycline treatment exist. While there are inherent differences between CMR derived and echocardiography derived strain[184], the results are broadly in line with an echocardiographic study reporting the potential utility of 3D based techniques in a breast cancer population[185]. Santoro et al. reported that while 3D strain was potentially superior to 2D methods, it was only feasible in 60% of patients due to the effects of age, BMI, radiotherapy, left mastectomy and breast prostheses on image quality. By contrast, 3D strain was feasible in our entire cohort. However, it should be noted that the only parameter that exhibited a statistically significant reduction in patients (3D GLS) also showed the lowest interobserver reproducibility (ICC 0.71) and has no established normal reference range. Therefore, CMR derived 3D GLS continues to be a research tool. All of which highlights the requirement for large, robust, longitudinal research to delineate the role of CMR based strain techniques, particularly with respect to prognosis and clinical outcomes, which in other cardiovascular populations have been strongly associated with death[81].

4.5.2 Tissue characterisation

The advent of native T1 and ECV techniques furnish the unique ability to quantify interstitial myocardial fibrosis non-invasively with excellent reproducibility and robust validation against biopsy proven collagen volume fraction and extracellular space[105, 106]. Previous studies of anthracycline treated survivors of childhood cancer have reported conflicting results. Some studies reported significant, detectable changes in native T1 and ECV as compared with controls at mean follow up intervals of 7.6[167] and 9.6 years[177], whereas others did not[178], with female sex reported to contribute to elevated values. Long term adult studies have reported elevated native T1 and ECV in the context of LVEF impairment[107, 108] with conflicting data in the presence of normal LVEF[108, 179], though the latter study lacked a control arm and included 26 breast cancer patients treated with trastuzumab, of whom only 62% received anthracycline. Native myocardial T1 and ECV values in this study were comparable to those reported by other groups[107, 108, 186] and further evidence of statistical differences in native T1 and ECV in adult populations with normal LVEF is provided. However, further research is required to determine the precise role and utility of these imaging biomarkers for evaluating patients treated by potentially cardiotoxic therapy, and whereas their relationship with cardiovascular outcomes and ability to risk stratify in other disease states has recently been described[187], this is yet to be replicated in cardio-oncology populations. It is also worth noting that significant baseline differences in haematocrit partly contributed to the difference in ECV, though significant differences in native myocardial T1 measurements argue in favour of a significant difference beyond haematocrit.

4.5.3 Left ventricular mass and myocardial cell volume

LV mass loss following anthracycline treatment has previously been reported by studies generally composed of patients with impaired LVEF [104, 108, 113], and has been associated with worsening heart failure symptomatology[188] and major adverse cardiac events[104]. A recent study of 27 women with normal baseline LVEF treated with anthracycline for breast cancer replicated these findings and

provided additional insight into the underlying composition of the myocardium by prospectively documenting a reduction in LV cardiomyocyte mass, purported to represent cardiomyocyte atrophy[55], which correlated with the degree of myocardial injury quantified by troponin elevation. The results support these findings and extend this observation to adult cancer survivors with normal range LVEF, in whom indexed myocardial cell volume was significantly lower than a control population. Furthermore, the degree of myocardial atrophy (represented by indexed myocardial cell volume) was shown to be inversely associated with anthracycline dose, even using modern dose capped regimens (Figure 1).

4.5.4 Reproducibility

Previous studies of patients receiving chemotherapy have reported high levels of inter- and intra-observer reproducibility for native T1, ECV, and 2D feature tracking strain[186]. However, this is the first study to combine the assessment of tissue characteristics and strain parameters, and the first to report the reproducibility of 3D feature tracking CMR strain in the context of anthracycline treatment. Overall, higher levels of intra-observer, compared with inter-observer reproducibility were noted and therefore, it may be prudent for the same reader to re-measure values from different timepoints for the purpose of serial evaluation. Whilst reproducibility of parameters is high, recent evidence of significant temporal variability and recognised overlap with healthy controls[186] continues to represent a barrier to incorporation in routine clinical practice. Adoption of automated analysis and artificial intelligence may be one method to improve reproducibility, pending confirmatory data.

4.5.5 Study limitations

The cross sectional design of the study precludes assessment of temporal changes in individuals treated with anthracycline, or their relationship with major adverse cardiovascular events, such as heart failure hospitalisation or mortality. The data were obtained at rest and as such, do not provide information on the functional or exercise capacity of patients, compared with controls, which may potentially be of greater interest. A longitudinal case control study including

assessment of exercise capacity could address these limitations. In addition, the study used specific hardware and software to collect and analyse data, which impacts the external validity of the results to other populations and clinical systems, and should be borne in mind when interpreting the results. The addition of another group of patients with impaired LVEF would also be of of additional value and could be a focus for future work.

4.6 Conclusion

Anthracycline treated cancer survivors with a normal LVEF have significant perturbations of CMR derived GLS (2D and 3D), native myocardial T1, ECV, indexed LV mass and indexed myocardial cell volume, compared with a control population of similar age and sex (Figure 4-4). These parameters display good to excellent levels of inter- and intra-observer reproducibility. Therefore, CMR feature tracking strain and advanced tissue characterisation techniques may have a role to play in the evaluation of long term anthracycline cardiotoxicity by providing complementary discriminatory information to traditional LVEF focused techniques.

4.7 Novel findings

1. Anthracycline treated cancer survivors with a normal LVEF have significant perturbations of GLS (2D and 3D), native myocardial T1, ECV, indexed LV mass and indexed myocardial cell volume, compared with a control population of similar age and sex, which are readily detectable by CMR.

2. Indexed myocardial cell volume, which serves as a marker of cardiomyocyte loss, is inversely associated with anthracycline dose, even using modern dose capped therapeutic regimens.

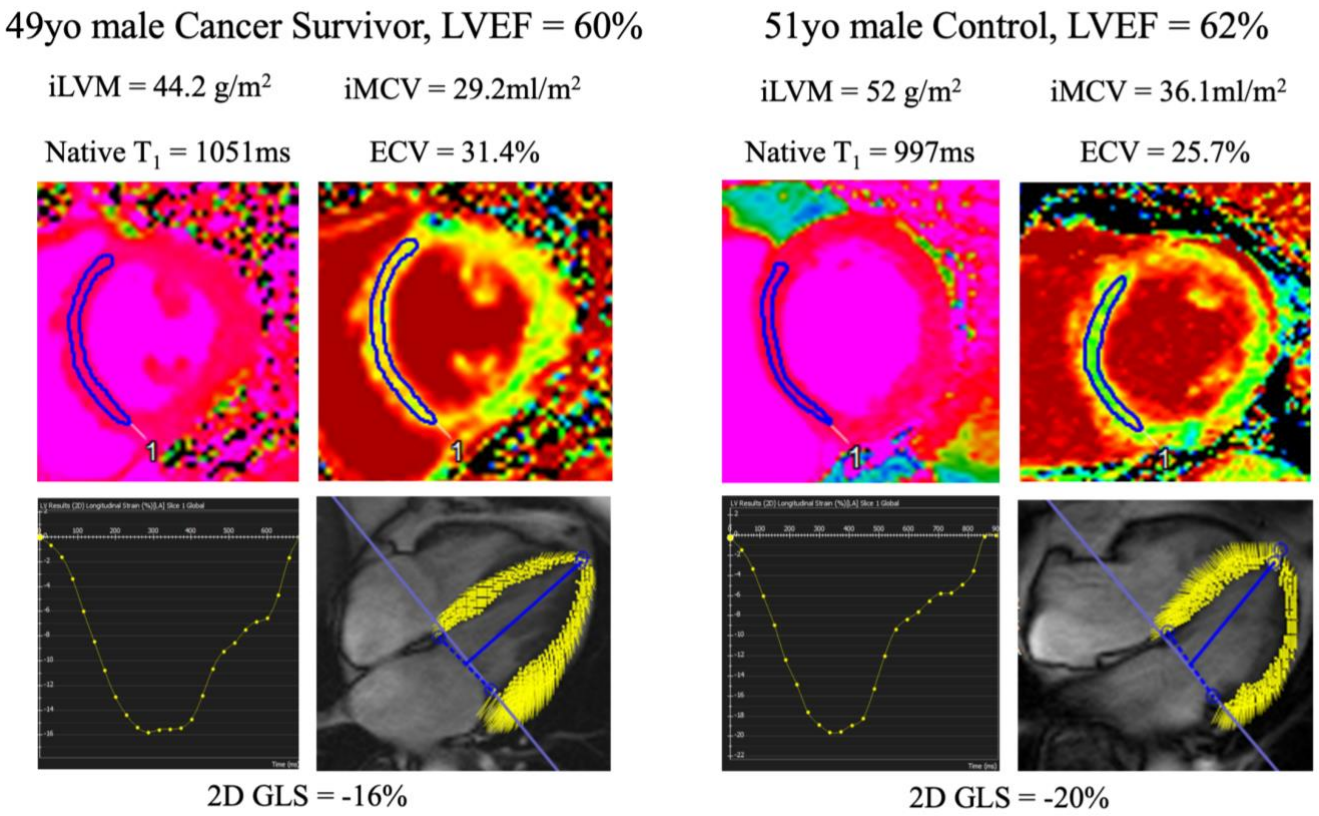

Figure 4-4 Representative case examples

Native myocardial T1 and ECV maps (top row), 2D feature tracking GLS curves and bSSFP cine images at end systole with myocardial strain overlay (bottom row). Regions of interest are drawn on the mid interventricular septum of the maps (blue crescents) to quantify native myocardial T1 (left column) and ECV (right column). LVEF = left ventricular ejection fraction, iLVM = indexed left ventricular mass, iMCV = indexed myocardial cell volume, ECV = extracellular volume fraction, GLS = global longitudinal strain.

Chapter 5 Prospective Multiparametric CMR Characterisation and MicroRNA Profiling of Anthracycline Cardiotoxicity: A Pilot Study

5.1 Introduction

Anthracycline chemotherapy provides effective treatment for several forms of cancer but its use is mitigated by dose dependent cardiotoxicity[189]. Consequently, societal guidelines recommend serial monitoring of LVEF with cardiac imaging in patients receiving these regimens[2, 19, 158].

Mitochondrial oedema and intracardiomyocyte vacuolisation are the histopathological hallmarks of early anthracycline cardiotoxicity[4, 190] but importantly, these changes seem to predate myocardial functional changes[4]. Indeed, tissue level damage may already be extensive by the time a change in LVEF is detected. The later stages of cardiotoxicity are characterised by cell death, replacement fibrosis[6] and an adverse prognosis[7].

Contemporary dose capped regimens mean that cardiotoxicity affects approximately 9% of patients[20]. Furthermore, recovery of LVEF following anthracycline is unpredictable, with only a minority of patients returning to baseline systolic function[20]. Identifying cardiotoxicity early and instituting protective therapy reduces cardiovascular adverse events[36] and facilitates therapeutic efficacy. Notwithstanding the recognition of contributory clinical risk factors such as age, female sex, pre-existing cardiovascular disease[19, 158] and genetic predisposition[191], predicting individual susceptibility to anthracycline cardiotoxicity continues to pose a significant challenge. All of which underscores the need for of an accurate, reproducible, non-invasive biomarker that can identify cardiotoxicity at an early stage.

CMR is the reference standard method to assess cardiac anatomy and function[77], and the advent of multiparametric sequences furnishes the unique ability to

characterise myocardial tissue non-invasively. The ability of CMR to detect myocardial oedema and fibrosis with native T2, and T1 mapping sequences, respectively was recently validated histopathologically in a porcine model of anthracycline cardiotoxicity[5]. However, to date, few adult human studies have used prospective CMR to catalogue the cardiac effects of anthracycline [82, 93, 102, 110, 192] and fewer still have included multiparametric mapping techniques[120, 193].

Circulating microRNAs (miRNA) are short non-coding RNAs that regulate elemental cellular processes and play a critical role in cardiovascular biology[65]. Their relative stability and ease of quantification, combined with high levels of sensitivity and specificity, make them an appealing biomarker in several cardiovascular disease states, with emerging animal[67] and latterly human models[68, 70] confirming temporal dysregulation in the context of anthracycline cardiotoxicity.

Accordingly, the objective of this pilot study was to prospectively characterise anthracycline cardiotoxicity and explore the ability of multiparametric CMR and miRNA to predict recovery of LVEF following completion of anthracycline chemotherapy.

5.2 Methods

From April 2017 to August 2018, 24 patients were prospectively enrolled at two haematology centres in Bristol, United Kingdom: Bristol Haematology and Oncology Centre and Southmead Hospital. The study was approved by the West Midlands - Solihull Research Ethics Committee. All participants provided written informed consent.

The study enrolled consecutive patients age > 18 with a new diagnosis of high grade non-Hodgkin (NHL) and Hodgkin lymphoma (HL) scheduled for > 6 cycles of anthracycline, or acute myeloid leukaemia (AML) scheduled for full dose treatment with curative intent. Patients were not eligible if they had a history of

prior cardiac disease or malignancy, prior chemotherapy, or had contraindications to CMR (ferromagnetic foreign body, claustrophobia, severe renal impairment [eGFR < 30 ml/min/1.73m^2]).

CMR, transthoracic echocardiography, electrocardiogram (ECG), serum biomarkers (including miRNAs and Troponin I [TnI]) and symptom enquiry were performed on the same day at three time points: at baseline, on completion of chemotherapy, and six months after completion of chemotherapy. Three patients were withdrawn between baseline and completion of chemotherapy (two deaths, one lost to follow up) and another four between completion of chemotherapy and six month follow up (three deaths, one lost to follow up; Figure 5-1), giving a total of 17 patients with complete assessments. In secondary analysis, these 17 patients were divided into tertiles according to recovery of LVEF between completion of chemotherapy and six month follow up. Predictors of LVEF recovery were explored from baseline and completion of chemotherapy visits.

The research team designed the study, gathered and analysed the data, prepared the manuscript, and vouch for data fidelity. Blinded, random order analysis of anonymised imaging data was undertaken at the end of the study. Data was collected and managed using Research Electronic Data Capture (REDCap)[194], hosted at Bristol University Hospitals NHS Foundation Trust.

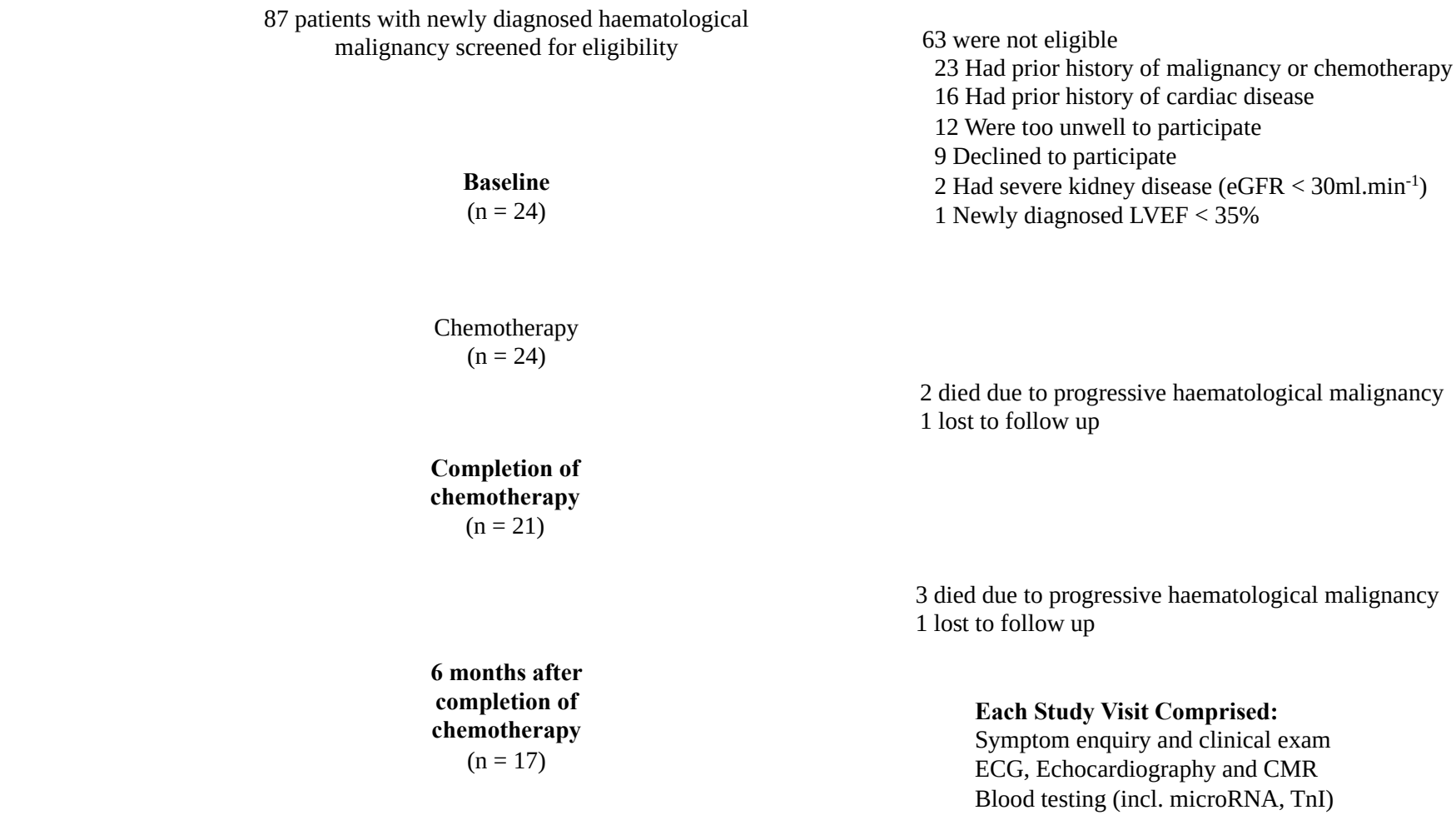

Figure 5-1 Study enrolment and follow up

5.2.1 CMR

5.2.1.1 Left ventricular volumes, ejection fraction and strain

Patients underwent CMR at 1.5 Tesla (Avanto, Siemens, Erlangen, Germany). Short axis steady state free precession cines (typical parameters: 8mm slice thickness [no gap], temporal resolution 38.1 ms, echo time 1.07 ms, in plane pixel size 1.5 x 0.8 mm) of the left ventricle were used to measure left ventricular (LV) volumes, ejection fraction (EF) and mass by drawing epicardial and endocardial borders at end systole and end diastole. Measurements were indexed to body surface area, according to established methods[168]. Average global longitudinal, circumferential, and radial strain parameters were calculated from short and long (two, three and four chamber) axis cines using voxel tracking post-processing software. Lateral MAPSE was measured in the four chamber view (CMR42, Circle Cardiovascular Imaging, Calgary, Canada).

5.2.1.2 Native myocardial T1 mapping, T2 mapping and ECV

MOLLI recovery sequence[170] (35° flip angle, 100 ms minimum TI, 80 ms TI increment, 150 ms time delay with 5-(3)-3 heartbeat acquisition scheme) was used to obtain a motion corrected, short axis native myocardial T1 map of the mid ventricular septum, on which a region of interest was drawn to determine native myocardial T1 measurements. A blood sample was taken immediately prior to CMR to determine Troponin I and haematocrit (Abbott Laboratories, United States). The latter allowed calculation of ECV using the established formula[153]: ECV = (1-haematocrit) x ($\Delta R1_{myocardium}/\Delta R1_{blood}$). Post-contrast T1 maps were obtained with the 4(1)3(1)2 MOLLI variant. Short axis mid ventricular T2 maps were obtained using a T2 prepared steady state free precession sequence (223.77/1.12; 70° flip angle, section thickness 8 mm; field of view, 340-400 mm; matrix 156x192; voxel size 2.3 x 1.9 x 8 mm), on which a region of interest was drawn to determine native myocardial T2 measurements, using Argus software (Siemens Healthineers, Germany).

5.2.2 Echocardiography

Echocardiograms were performed by two fully accredited researchers using a single Epiq machine (Philips, Netherlands). Biplane 2D LVEF was calculated using Simpson's rule from apical four and two chamber images and 3D LVEF using semi-automated software (HeartModel, Philips, Netherlands). GLS was measured by tracing three fiducial landmarks (apex, lateral and medial mitral annulus) on the endocardial border with automated function imaging (Automated Cardiac Motion Quantification [aCMQ], Philips, Netherlands). The fidelity of contours to cardiac motion was tracked through the cardiac cycle and manually adjusted, if necessary. Measures were not recorded if there was inadequate visualisation of $\geq$ 3 contiguous myocardial segments.

5.2.3 Electrocardiography

A 12 lead surface electrocardiogram (ECG) was obtained at each study visit and assessed for abnormalities of rhythm, conduction intervals, ST and T wave morphology.

5.2.4 Serum Troponin I and miRNAs

Blood was drawn at each study visit. Troponin I (TnI) was determined at the bedside (Abbott Laboratories, United States of America). Platelet poor plasma was prepared by drawing blood into a sodium citrate vacutainer and centrifuging at 2240 g for 10 minutes at room temperature. The plasma supernatant was removed to a fresh tube without disturbing the buffy coat and centrifuged again at 2240 g for 10 minutes at room temperature. The supernatant was removed to a fresh Eppendorf LoBind microcentrifuge tube and stored at -80°C[195]. RNA extraction, small RNA sequencing and data QC and alignment were performed on completion of the study by Qiagen (Germany) on samples obtained prior to, and on completion of chemotherapy. Differential expression analysis (likelihood ratio test following false discovery rate (FDR) correction) comparing circulating miRNA expression in patients in tertile 1 (poor LVEF recovery) with tertile 3 (good LVEF recovery) was performed in R using the edgeR package[196, 197]. A miRNA was considered to be significantly altered if FDR was less than 0.05.

5.2.5 Data analysis

Data from the 17 patients who completed all visits were divided into tertiles according to LVEF recovery between completion of chemotherapy and six month follow up. Predictors of LVEF recovery were explored from baseline and completion of chemotherapy visits. Overall data are presented as mean ± standard deviation for continuous variables; counts and percentages for categorical variables. Paired analysis (Wilcoxon signed rank test) was used to assess temporal changes. Following rejection of a Kruskal-Wallis test, post hoc Dunn's pairwise comparison was used in secondary analysis to explore predictors of left ventricular recovery, comparing tertiles, as defined above. Associations between variables were assessed with linear regression. Based on the available literature[82, 93, 102], a target sample size of 14 was calculated to give 90% power at an alpha level of 0.016 to detect a statistically significant change in LVEF of 6.2%. Statistical analysis was performed using Stata (Stat version 13, StatCorp LLC, United States) with a p value of $< .05$ considered significant.

5.3 Results

5.3.1 Patient characteristics

Baseline demographic data of all participants are displayed in

Table 5-1. The mean age was 56 years (range 18 to 75). One patient was newly diagnosed with one segment of subendocardial LGE in keeping with previously unrecognised myocardial infarction at baseline. All patients had normal baseline LVEF and GLS on CMR and echocardiography. Patients received anthracycline at a mean total doxorubicin equivalent dose of 272 mg/m2 (range 112 to 412). Nineteen patients (79%) received a monoclonal antibody, and fourteen (58%) received either prednisolone or methylprednisolone during the course of their treatment.

Table 5-1 Baseline data

	(*n* = 24)
Mean age (range), years	56 (18-75)
Male *n* (%)	14 (68)
Female *n* (%)	10 (42)
Race	
White	22 (92)
Mixed race (Black/White)	1 (4)
Middle Eastern	1 (4)
Mean body surface area, (m^2)	1.91 ± 0.22
Mean body mass index, (kg/m^2)	26.1 ± 4.8
NYHA Class I, *n* (%)	24 (100)
Medical history, *n* (%)	
Hypertension	5 (21)
Diabetes	2 (8)
Dyslipidaemia	5 (21)
Current Smoker	6 (25)
Ex-Smoker	8 (33)
Mean Alcohol intake (range), Units/week	4 (0-20)
Cardiovascular medications, *n* (%)	
Angiotensin converting enzyme inhibitor	1 (4)
Angiotensin II receptor blocker	1 (4)
Beta blocker	1 (4)
Statin	5 (21)
Haematological diagnosis, *n* (%)	
Acute myeloid leukaemia	9 (38)
Non-Hodgkin lymphoma	12 (50)
Hodgkin lymphoma	3 (13)
Chemotherapy, *n* (%)	
Anthracycline	24 (100)
Idarubicin	3 (13)
Daunorubicin	6 (25)
Doxorubicin	15 (63)
Mean Doxorubicin equivalent dose (range), mg/m^2	272 (112 - 412)
Monoclonal antibody	19 (79)
Rituximab	12 (50)
Gemtuzumab	5 (21)
Alemtuzumab	4 (17)
Cyclophosphamide	12 (50)
Prednisolone/methylprednisolone	14 (58)
Radiotherapy, *n* (%)	2
Cumulative radiation dose (Gy)	21 ± 13
Plus-minus values are means ± standard deviation.	

5.3.2 CMR

LVEDVi did not change between any timepoint (Table 5-2). LVESVi increased significantly between baseline (31 ± 11 ml/m^2) and completion of chemotherapy (35 ± 9 ml/m^2, $p < .001$), and decreased significantly between completion of chemotherapy and six month follow up (32 ± 6 ml/m^2, $p = .014$), with no difference between baseline and six month follow up ($p = .89$). LVEF was normal (61 ± 3%) at baseline, decreased significantly on completion of chemotherapy (53 ± 3%, $p < .001$), increased significantly at six month follow up (55 ± 3%, $p = .018$) but remained significantly decreased compared with baseline ($p < .001$). MAPSE decreased significantly from baseline to six month follow up (14.0 ± 2.6 to 12.5 ± 2.4 mm, $p = .048$) and RVEF also decreased significantly between baseline (60 ± 6%) and completion of chemotherapy (56 ± 6, $p = .005$), before partially recovering at 6 months (59 ± 1, p = 0.47) . One patient met the criteria for cardiotoxicity (> 10% absolute percentage drop in LVEF to a value < 53%[19]) on completion of chemotherapy, which recovered by six month follow up. All measures of 2D feature tracking (FT) CMR strain worsened significantly on completion of chemotherapy, compared with baseline ($p < .001$ in all cases; Table 5-2). All measures of 2D FT strain worsened significantly between completion of chemotherapy and six month follow up ($p < .05$), but of all FT CMR strain parameters, only 2D GLS was persistently impaired at six month follow up (-19.0 ± 2.6%), compared with baseline (-21.1 ± 3.1, $p = .002$).

No significant temporal changes in native myocardial T1 measurements or ECV were observed during the study (Table 5-2). Native myocardial T2 measurements increased significantly from baseline to completion of chemotherapy (54.0 ± 4.6 to 57.8 ± 4.9 ms, $p = .001$) but no other significant between visit differences were recorded. Furthermore, neither the absolute native myocardial T2, nor the change in native myocardial T2 between baseline and completion of chemotherapy correlated with any parameters of left ventricular systolic function. No new LGE was observed during the study. Indexed left ventricular mass did not change significantly (Table 5-2)

Table 5-2 Temporal changes in CMR, echocardiographic, serum and physiological metrics

	Visit 1 *n*=24	Δ V1-V2 *p* value	Visit 2 *n*=21	Δ V2-V3 *p* value	Visit 3 *n*=17	Δ V1-3 *p* value
CMR						
LVEDVi (ml/m^2)	77 ± 19	0.24	74 ± 15	0.30	72 ± 14	0.17
LVESVi (ml/m^2)	31 ± 11	**0.02**	35 ± 9	**0.014**	32 ± 6	0.89
LVEF (%)	61 ± 3	**<0.001**	53 ± 3	**0.018**	55 ± 3	**<0.001**
MAPSE (mm)	14.0 ± 2.6	0.17	13.0 ± 3.0	0.89	12.5 ± 2.4	**0.048**
LAVi (ml/m^2)	45 ± 12	0.10	41 ± 10	0.11	43 ± 9	0.47
LVMi	46 ± 9	0.52	47 ± 7	0.16	44 ± 7	0.22
RVEF (%)	60 ± 6	**0.005**	56 ± 6	0.37	59 ± 6	0.28
2D GLS (%)	-21.1 ± 3.1	**<0.001**	-17.8 ± 2.5	**0.02**	-19.0 ± 2.6	**0.002**
2D GCS (%)	-23.7 ± 3.3	**<0.001**	-21.1 ± 3.5	**0.02**	-22.5 ± 2.8	0.255
2D GRS (%)	55.2± 11.6	**<0.001**	44.7 ± 8.8	**0.03**	49.9 ± 9.0	0.09
3D GLS (%)	-12.9 ±3.2	0.528	-12.3 ± 3.4	0.11	-14.1 ± 2.9	**0.043**
3D GCS (%)	-18.9 ± 3.0	**<0.001**	-17.0 ± 3.0	0.94	-16.9 ± 9.3	0.37
3D GRS (%)	51.8 ±16.7	**0.001**	40.2 ±11.5	**0.014**	47.3 ±13.1	0.22
Myocardial T1 (ms)	1048 ± 33	0.348	1055 ± 35	0.91	1055 ± 36	0.61
Myocardial T2 (ms)	54.0 ± 4.6	**0.001**	57.8 ± 4.9	0.14	55.3 ± 3.7	0.13
ECV (%)	29.4 ± 3.4	0.40	30.3 ± 3.9	0.11	28.2 ± 4.7	0.39

T_2 SI ratio (ms)	1.7 ± 0.5	**0.053**	2.1 ± 0.7	**0.03**	1.9 ± 0.5	0.07
Echocardiography						
2D LVEF (%)	62 ± 3	00.18	60 ± 7	0.939	59 ± 4	0.11
3D LVEF (%)	61 ± 4	**0.001**	57 ± 4	**0.035**	60 ± 4	0.81
GLS (%)	-20.6 ± 2.6	0.12	-19.2 ± 3.6	0.56	-18.9 ± 2.1	0.053
Serum						
TnI (pg/ml)	4.3 ± 13.2	**0.02**	22.9 ±29.9	0.06	6.9 ± 6.0	0.57
Hb (g/dL)	11.8 ± 2.7	0.112	11.0 ± 2.5	**0.02**	12.9 ± 2.2	0.10
Haematocrit (%)	35 ± 8	**0.02**	26 ± 15	**0.046**	36 ± 11	0.93
eGFR (ml/min/1.73m^2)	83 ± 27	0.12	94 ± 31	0.321	104 ± 55	0.65
CRP	35 ± 57	0.11	12 ± 14	0.70	17 ± 31	**0.03**
Physiology						
Body mass (kg)	76 ± 15	0.72	74 ± 14	0.55	75 ± 16	0.80
HR (bpm)	79 ± 12	0.55	74 ± 9	0.95	74 ± 8	0.23
SBP (mmHg)	131 ± 15	0.29	128 ± 12	0.12	123 ± 22	0.16
DBP (mmHg)	78 ± 9	0.40	75 ± 12	0.19	72 ± 13	**0.019**

Values are mean ± standard deviation or n (%). 2D = Two dimensional, 3D = Three dimensional, CMR = Cardiovascular magnetic resonance, CRP = C reactive protein, DBP = Diastolic blood pressure, ECV = extracellular volume fraction, GLS = Global longitudinal strain, GCS = Global circumferential strain, GRS = Global radial strain, Hb = Haemoglobin, HR = Heart rate, LAVi = Left atrial volume indexed, LVEDVi = Left ventricular end diastolic volume indexed, LVESVi = Left ventricular end systolic volume indexed, LVEF = Left ventricular ejection fraction, LVMi = LV mass indexed, MAPSE = Mitral annular plane systolic excursion, RVEF = Right ventricular ejection fraction, SBP = systolic blood pressure, T1 = T1 relaxation time, T_2 = T_2 relaxation time, T_2 SI ratio = T_2 signal intensity ratio of myocardium: skeletal muscle, TnI = Troponin I, eGFR = estimated glomerular filtration rate. Statistically significant results highlighted in bold font.

5.3.3 Echocardiography

Echo derived 2D LVEF was not significantly different across study visits. However, 3D LVEF decreased significantly between baseline and completion of chemotherapy (61 ± 4 to 57 ± 4%, p = .001) and increased significantly between completion of chemotherapy and six month follow up (57 ± 4 to 60 ± 4%, p = .035). There was no significant difference between baseline and six month follow up (p = .81). Echo derived GLS did not differ significantly across study visits, though there was a trend towards worsened GLS from baseline to six month follow up (-20.6 ± 2.6 to -18.9 ± 2.1%, p = .053).

5.3.4 Electrocardiography

One patient had ECG evidence of old anteroseptal myocardial infarction at baseline. No changes to rhythm, conduction intervals or ST segments, or T wave morphology were detected during the study.

5.3.5 Troponin

Troponin I increased significantly between baseline and completion of chemotherapy (4.3 ± 13.2 to 22.9 ± 29.9pg/ml, p = .02) but did not otherwise differ. Three patients (14%) exhibited subclinical cardiac injury (TnI of 0.06 to 0.10ng/ml) on completion of chemotherapy.

5.3.6 Cardiovascular physiology and symptoms

The only significant change to physiological metrics during the course of the study was that diastolic blood pressure decreased significantly between baseline and six month follow up (78 ± 9 to 72 ± 13mmHg, p = .019; Table 5-2). At baseline, 91% (22/24) of patients were NYHA class I. This had fallen to 66% (14/21) by completion of chemotherapy and 65% (11/17) by six month follow up. Seven patients (33%) and five patients (33%) reported NYHA class II dyspnea at completion of chemotherapy and six month follow up, respectively. One patient (6%) was NYHA class III at six month follow up.

5.3.7 Recovery of left ventricular ejection fraction

Patients were divided into tertiles according to CMR LVEF recovery between completion of chemotherapy and six month follow up (Figure 5-2). Baseline demographic and study data by tertile are displayed in Table 5-3. Patients in tertile 1 (poor recovery) trended towards being older than those in tertile 2 (partial recovery) and tertile 3 (good recovery), though this difference was not significant (p = .11). Baseline CMR derived MAPSE was significantly different among tertiles of LVEF recovery, measuring 11.7 ± 1.5 mm, 13.7 ± 2.7 mm, 15.7 ± 3.1 mm in tertiles 1, 2 and 3, respectively (Figure 5-2; p = .028). Baseline myocardial T1 was also noted to be significantly different between tertiles, but measures were not ordinal, with tertile 2 exhibiting the highest values at baseline (Table 5-3). No data from completion of chemotherapy, nor change (Δ) between baseline and completion of chemotherapy, were significantly different between tertiles of LVEF recovery, apart from LVEF at 6 month follow-up (Table 5-4). Echocardiography and CMR derived MAPSE correlated significantly (p < .001) but baseline echocardiography derived MAPSE was not significantly associated with LVEF recovery (p = .32).

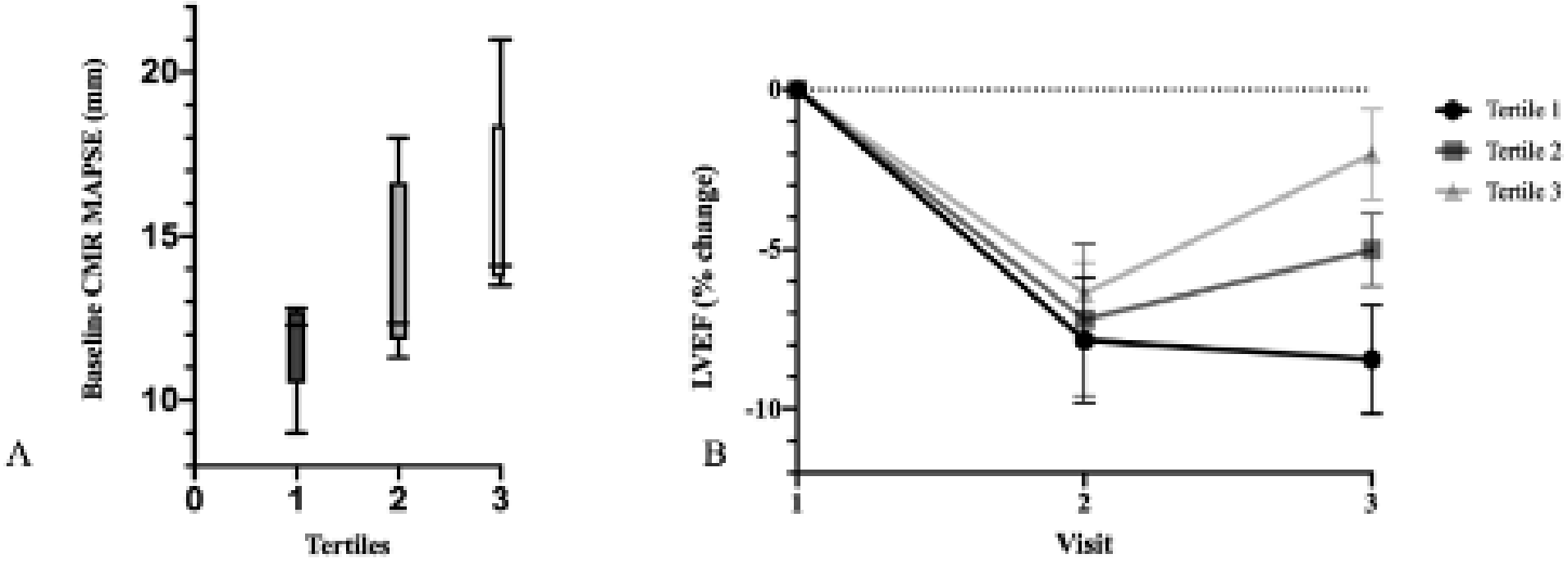

Figure 5-2 Assessment of left ventricular function by tertile

Panel A: Baseline CMR MAPSE by tertile of LVEF recovery. Panel B temporal absolute % change in LVEF by tertile. Visit 1 = baseline, visit 2 = completion of chemotherapy, visit 3 = 6 months after completion of chemotherapy.

Table 5-3 Baseline data by LVEF recovery tertiles

	1 Poor recovery (*n* = 6)	2 Partial recovery (*n* = 6)	3 Good recovery (*n* = 5)	*p* value
Mean age (years)	67 ± 6	51 ± 18	42 ± 24	0.11
Female sex (%)	50	83	40	0.34
BSA (kg/m^2)	2.0 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	0.59
Dox (mg/m2)	296 ± 12	283 ± 58	274 ± 56	0.17
CMR				
LVEF (%)	60 ± 3	61 ± 4	59 ± 4	0.66
MAPSE (mm)	11.7 ± 1.5	13.7 ± 2.7	15.7 ± 3.1	**0.028**
2D FTGLS (%)	-18.3 ± 3.1	-21.7 ± 1.6	-21.7 ± 3.0	0.10
2D FTGCS (%)	-22.2 ± 3.4	-24.4 ± 2.6	-23.3 ± 4.0	0.85
2D FTGRS (%)	49.6 ± 8.3	57.5 ± 12.7	53.5 ± 11.3	0.55
Myocardial T1 (ms)	1045 ± 30	1077 ± 30	1022 ± 27	**0.02**
Myocardial T_2 (ms)	53 .0 ± 5.2	54.6 ± 3.4	52.9 ± 2.8	0.73
Echo				
3D LVEF (%)	60 ± 3	64 ± 6	58 ± 3	0.09
GLS (%)	-20.1 ± 2.5	-20.9 ± 2.5	-20.5 ± 3.0	0.87
MAPSE (mm)	12.8 ± 2.1	14.7 ± 2.5	15.4 ± 2.8	0.32

Values are mean ± standard deviation or n (%). 2D = two dimensional, 3D = three dimensional, CMR = cardiovascular magnetic resonance, Dox = Mean doxorubicin equivalent dose, FT = feature tracking, GLS = global longitudinal strain, GCS = global circumferential strain, GRS = global radial strain, LVEF = left ventricular ejection fraction, MAPSE = mitral annular plane systolic excursion, T1 = T1 relaxation time, T_2 = T_2 relaxation time.

Table 5-4 Data from completion of chemotherapy and 6 month follow up by LVEF recovery tertile

	1 Poor recovery (*n* = 6)	2 Partial recovery (n = 6)	3 Good recovery (n = 5)	*p* value
Completion of chemotherapy				
CMR				
LVEF (%)	54 ± 1	53 ± 3	52 ± 4	0.41
MAPSE (mm)	12.0 ± 5.3	12.3 ± 1.2	13.1 ± 1.0	0.8
2D FTGLS (%)	-16.5 ± 2.7	-18.8 ± 1.7	-18.4 ± 2.9	0.26
2D FTGCS (%)	-21.4 ± 2.0	-22.4 ± 1.6	-19.3 ± 5.9	0.36
2D FTGRS (%)	44 ± 9	45 ± 6	44 ± 13	0.96
Myocardial T1 (ms)	1042 ± 3	1082 ± 28	1045 ± 20	0.06
Myocardial T_2 (ms)	55.2 ± 2.8	59 ± 4	56 ± 3	0.08
Echo				
3D LVEF (%)	55 ± 4	60 ± 3	55 ± 4	0.06
GLS (%)	-18.4 ± 3.8	-21.0 ± 4.2	-18.2 ± 1.8	0.38
6 month follow up				
CMR				
LVEF (%)	53 ± 1	56 ± 2.5	57 ± 3	**0.04**
MAPSE (mm)	12.0 ± 2.6	12.0 ± 2.2	13.8 ± 2.3	0.40
2D FTGLS (%)	-17.5 ± 2.3	-19.3 ± 2.2	-20.7 ± 2.5	0.10
2D FTGCS (%)	-23.0 ± 1.1	-22.6 ± 3.9	-21.9 ± 3.2	0.83
2D FTGRS (%)	48 ± 5	52 ± 9	51 ± 13	0.75
Myocardial T1 (ms)	1072 ± 44	1057 ± 36	1034 ± 10	0.21
Myocardial T_2 (ms)	53 ± 4	56 ± 4	57 ± 3	0.16
Echo				
3D LVEF (%)	56 ± 2	64 ± 2	60 ± 3.3	0.06
GLS (%)	-17.6 ± 1.5	-20.3 ± 1.8	-18.7 ± 2.7	0.12

Values are mean ± standard deviation or n (%). 2D = two dimensional, 3D = three dimensional, CMR = cardiovascular magnetic resonance, Dox = Mean doxorubicin equivalent dose, FT = feature tracking, GLS = global longitudinal strain, GCS = global circumferential strain, GRS = global radial strain, LVEF = left ventricular ejection fraction, MAPSE = mitral annular plane systolic excursion, T1 = T1 relaxation time, T_2 = T_2 relaxation time.

In analysis of baseline MiRNAs according to tertiles of LVEF recovery, two MiRNAs were significantly dysregulated (likelihood ratio test) after false discovery rate correction: MiRNA-181-5p and MiRNA-221-3p (Figure 2-1). Both were expressed to a significantly higher degree in tertile 1, whose LVEF did not recover between completion of chemotherapy and six month follow up. No

significant dysregulation of MiRNAs was detected on completion of chemotherapy according to patient groups defined by tertiles of LVEF recovery.

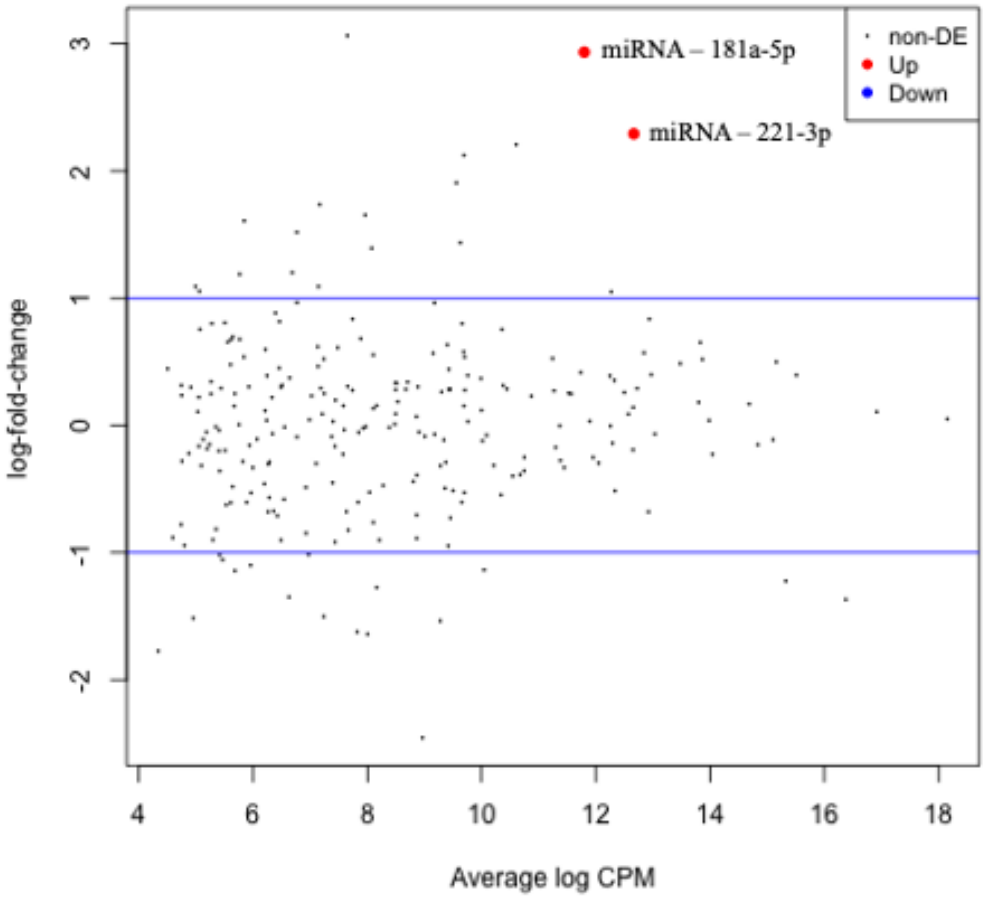

Figure 5-3 Dysregulation of mMiRNAs at baseline by tertile of LVEF recovery

The average log count per million is displayed along the X axis. Log fold change in dysregulation is displayed along the y axis. miRNA 181a-5p and miRNA-221-3p display both elevated counts and upregulation among patients who exhibit poor recovery of LVEF. CPM = count per million, DE = Dysregulated expression.

5.4 Discussion

In this prospective, multiparametric CMR, echocardiography and blood biomarker study of anthracycline cardiotoxicity, five important findings are

reported. First, multiple measures of left ventricular systolic function (CMR LVEF; CMR FT GLS, GCS and GRS; echo 3D LVEF) deteriorate significantly on completion of anthracycline treatment compared with baseline. Second, several measures of systolic function recover over the next six months, with only CMR LVEF, CMR MAPSE, and CMR 2D FTGLS persistently depressed. Third, native myocardial T2 mapping elevation occurred following anthracycline treatment but was not predictive of subsequent LVEF recovery. Fourth, no changes in native myocardial T1, ECV, LGE or LV mass were recorded with the caveat that only one patient met criteria for cardiotoxicity during the course of the study. Fifth, analysis of LVEF recovery by tertile revealed that CMR MAPSE was significantly different at baseline in patients exhibiting poor recovery of LVEF following completion of anthracycline. Furthermore, baseline expression of miRNA-181-5p and miRNA-221-3p was significantly higher in those with poor LVEF recovery.

5.4.1 CMR volumes and systolic function

Commensurate with previous prospective studies of anthracycline cardiomyopathy[82, 120], significant reductions in LVEF were observed early after completion of anthracycline treatment, which were driven by an increase in iLVESV (iLVEDV did not change). In addition, partial recovery of LVEF was observed late after anthracycline treatment and built on previous reports describing similar trends[55, 179] by exploring baseline and early predictors of LVEF recovery by dividing the populations into tertiles of LVEF recovery. CMR derived MAPSE was the only baseline imaging metric significantly associated with LVEF recovery, which is a novel finding. Long axis systolic motion of the mitral annulus is an integral component of cardiac mechanics and the utility of CMR derived MAPSE as a powerful independent predictor of cardiovascular outcomes has recently been reported in the context of hypertension[198] and myocardial infarction[199]. Indeed, it is reported that long axis motion (for which MAPSE is a surrogate) is responsible for approximately 60% of stroke volume[200]. Furthermore, MAPSE can be calculated quickly and easily from standard cine images, without the need for specialised sequences. Therefore, the role of CMR

derived MAPSE as a baseline predictor of LVEF recovery after anthracycline treatment may potentially be of clinical interest in selecting patients for more frequent monitoring or the institution of preventive therapies, though confirmation by larger, powered prospective studies is certainly required. It is also noteworthy that despite the administration of comparatively high doses of anthracycline to a patient cohort with coexisting haematological malignancy and increasing breathlessness (judged by NYHA class), only one patient met the criteria for cardiotoxicity during the course of the study, highlighting the fact that in the vast majority of patients, changes were subclinical.

5.4.2 CMR strain

The study prospectively documented anthracycline related changes in 2D FT strain in all three planes and as such, provides novel insight. All measures of CMR strain were significantly impaired on completion of anthracycline treatment. However, FTGCS and FTGRS were statistically comparable to baseline by the end of the study, whereas FTGLS remained persistently impaired. These data support and replicate previous work by Ong et al.[84] who reported similar finding for FTGLS and FTGCS in a prospectively studied cohort of breast cancer patients treated with trastuzumab, half of whom also received anthracycline. However, measures of strain at baseline and on completion of chemotherapy were not associated with subsequent LVEF recovery.

5.4.3 CMR T1 and T2 mapping

Recent animal data demonstrated that T2 mapping abnormalities are the earliest marker of cardiotoxicity, which manifested histopathologically as intracardiomyocyte vacuolisation[5]. Importantly, cessation of anthracycline at this stage (6 weeks after initiation of 0.45mg/kg intracoronary doxorubicin, administered on alternate weeks) led to resolution of imaging and histological changes, whereas continuation led to progressive abnormalities of imaging, histological and functional markers, thus potentially identifying cardiotoxicity at a reversible stage[5]. In turn, human studies have reported significant [91, 201] and non-significant[120] elevation of T2 mapping early after anthracycline

chemotherapy, though their relationship to subsequent cardiac dysfunction is hampered by short duration of follow up[120, 201] and lack of sequential data collection[91]. This study provides additional evidence of significant elevation of T2 mapping early after chemotherapy, with prospective evaluation confirming resolution of T2 mapping over a longer follow up period. However, early T2 mapping elevation was not associated with subsequent LVEF recovery, either on a grouped or individual level and therefore the translational clinical utility of this finding in human studies is yet to be confirmed.

The potential role of native T1 mapping and ECV in the detection of early cardiotoxicity have been highlighted by animal[67] and human[55, 91, 120, 201] studies describing significant temporal changes. By contrast, no significant changes to T1 mapping or ECV were found during the course of the study. This discrepancy may partly relate to the differing imaging timing and populations studied (this is the only study exclusively of haematological malignancies), the low incidence of cardiotoxicity, as well as the confounding effects of potent anti-inflammatory treatment (58% received concomitant steroid in the study; not reported by others) and cancer itself[108] on native T1 mapping and ECV. In addition, the technical evolution of the MOLLI sequence employed may have conferred reduced sensitivity to detect myocardial inflammation, compared to previous iterations. When taken in combination with a recent report of overlapping temporal variability between patients and healthy controls[186], the precise translational role for these sequences in modern monitoring strategies is yet to be defined.

5.4.4 Echocardiography

Echocardiography is the most accessible and widespread method to monitor cardiac function during anthracycline therapy[2]. Despite changes in multiple CMR metrics, only 3D LVEF changed significantly, in keeping with a previous report highlighting the relative lack of sensitivity of 2D methods to detect temporal changes in LVEF[48]. Furthermore, no baseline or early echocardiographic

predictors of LVEF recovery were identified. It is also noteworthy that while echo-derived GLS trended towards initial impairment and subsequent recovery, these changes did not meet statistical significance. This may be accounted for by the small sample size and recognised intra-observer variability of this technique.

5.4.5 MicroRNAs

Circulating miRNAs regulate target gene expression and are an appealing biomarker because they are readily quantifiable, have a long half life, and remain stable at extremes of temperature and pH. However, few human studies have examined their utility to detect anthracycline cardiotoxicity, and have reported a range of expression profiles[68, 70, 202]. In this study analysing predictors of LVEF recovery after completion of anthracycline, it was identified that miRNA-181a-5p and miRNA-221-3p were significantly increased at baseline in patients whose LVEF showed poor recovery after anthracycline chemotherapy. A recent systematic review and meta-analysis reported that miRNA-221 was associated with a poor overall survival in human carcinoma[203] and miRNA 181a-5p expression has been associated with poor outcomes in patients with colorectal cancer[204] but their role in anthracycline cardiotoxicity has not been reported previously. Therefore, it is uncertain whether increased miRNA levels at baseline signify susceptibility to cardiotoxicity or are a broader marker of more advanced or aggressive malignancy with an adverse prognosis. Indeed subclinical functional and morphological cardiac dysfunction has previously been linked to cancer itself[205] independent of chemotherapy and further scientific exploration of these relationships is warranted.

5.4.6 Study limitations

This study intentionally recruited a homogenous cohort of patients to minimise baseline confounding factors and used specific hardware and software to collect and analyse data, which reduces the external validity of the results to other populations and clinical systems. This was a pilot study, powered to detect temporal changes in LVEF and as such, secondary analysis for baseline and early predictors of LVEF recovery are to be considered exploratory and require further

validation before firm conclusions can be drawn. The low incidence of cardiotoxicity and absence of major adverse cardiovascular events over a relatively short follow up period mean that the impact of the observed subclinical temporal changes on clinical outcomes has not been established.

5.4.7 Conclusions

In this prospective multimodality pilot study of anthracycline cardiotoxicity, several significant temporal changes to cardiac systolic function and tissue characteristics in response to anthracycline treatment have been demonstrated. Importantly, a degree of recovery of LVEF between completion of chemotherapy and six month follow up is confirmed in the majority of patients. Baseline CMR derived MAPSE and baseline circulating miRNA-181-5p, and -221-3p were associated with poor recovery of LVEF six months after completion of anthracycline chemotherapy. Pending validation by larger studies, these potential 'predictive' biomarkers may be a useful tool to inform individualised treatment and monitoring strategies for patients scheduled to receive anthracycline treatment.

5.5 Novel findings

1. Baseline CMR derived MAPSE and baseline circulating levels of miRNA-181-5p and miRNA-221-3p were associated with poor recovery of LVEF six months after completion of anthracycline treatment.

Chapter 6 General Discussion

It is not practical, possible, nor indeed desirable to undertake CMR in all patients receiving potentially cardiotoxic treatment and therefore, its use in this setting must be adjudicated carefully, balancing resource use, clinical need and the latest scientific evidence. In this thesis, important insights that are uniquely provided by multiparametric CMR and may potentially be of interest at several stages of the cancer patient pathway have been described. Below is a summary of the main contributions to the field of CMR in cardio-oncology.

6.1 Summary of major findings

1. Confirmation that late stage anthracycline cardiomyopathy is characterised phenotypically by pathological increase in LVESVi.

2. Confirmation that focal fibrosis (adjudicated by the presence of LGE) is not a common finding (19%) in late stage anthracycline cardiomyopathy

3. Novel finding that the presence of focal fibrosis is associated with adverse left ventricular remodelling and reduced LVEF.

4. Novel findings that elevated native myocardial T1 mapping values (surrogate CMR marker of diffuse fibrosis) are more common (36%) in late stage anthracycline cardiomyopathy and are associated with adverse left ventricular remodelling, reduced LVEF and hospitalisation for heart failure.

5. Novel finding that anthracycline treated cancer survivors with a normal LVEF have significant perturbations of GLS (2D and 3D), native myocardial T1, ECV, indexed LV mass and indexed myocardial cell volume, compared with a control population of similar age and sex.

6. Novel finding that indexed myocardial cell volume, which serves as a surrogate marker of cardiomyocyte loss, is inversely associated with anthracycline dose, using modern dose capped regimens.

7. Confirmation that CMR 2D and 3D feature tracking strain, native T1 and ECV display good to excellent levels of inter- and intra-observer reproducibility.

8. Confirmation that prospective non-invasive evaluation of patients receiving anthracycline treatment with CMR echocardiography and blood biomarkers identifies multiple, significant temporal changes in cardiac structure and function from treatment initiation to 6 months after completion of treatment.

9. Novel finding that baseline CMR derived MAPSE and baseline circulating levels of miRNA-181-5p and miRNA-221-3p were associated with poor recovery of LVEF six months after completion of anthracycline treatment.

6.2 Translational outlook

In chapter 2, the scientific evidence was critically reviewed, CMR imaging sequences discussed, guideline based clinical indications presented and indication specific CMR protocols proposed. This chapter serves as a useful summary of the current role of CMR in evaluating patients receiving potentially cardiotoxic therapy. The findings contribute to this body of evidence and provide further insight into potential applications of CMR in the evaluation and monitoring of patients receiving cancer therapy. Specific features of anthracycline cardiomyopathy not identifiable by other methods have been demonstrated, and potential baseline markers of susceptibility to anthracycline cardiomyopathy, which are of potentially significant interest to inform risk stratification and monitoring strategies have been demonstrated. However, further validation studies are required and the integration of these findings and indeed CMR in general, into clinical monitoring strategies has several barriers to overcome. Despite encouraging data on the utility of tissue characterisation sequences to identify cardiotoxicity early in animal models[5], in line with other human studies[91], a role for these sequences in current clinical practice was not identified. This may partly be explained by the heterogeneity of adult human subjects compared with animal models but concerns regarding the temporal variability and overlap of these parameters with healthy control subjects[186] are legitimate and merit further exploration. Recent work specifically addressing the variability in echocardiographic and CMR assessment of left ventricular function identified 3D echo-LVEF, 2D echo GLS and CMR-LVEF as the most reproducible parameters exhibiting the least overlap with variability in healthy volunteers[206], indeed CMR LVEF had the lowest variability in this study. Therefore, following a baseline CMR study, a shortened protocol where only cine imaging is acquired to permit LVEF assessment could be employed for serial evaluation and could be accomplished efficiently (10-15 minutes) and without the need for contrast. The advent of compressed sensing and rapid acquisition protocols could streamline this process further and could be a focus for future study.

6.3 Limitations

This thesis provides several important insights into the cardiovascular effects of cancer therapy. However, the data presented have several important limitations. The data presented in chapters 3 and 4 were collected retrospectively, and as such, are subject to referral bias. All the data were collected at a single centre using specific hardware and software, which impacts its external validity. Furthermore, the data consist of small sample sizes. Small sample sizes are subject to both type I and type II errors and as such, require further validation by larger studies. That being said, the sample sizes compare favourably with other CMR studies in the field (Table 2-2) and as a body of work, provide important insights into several aspects of anthracycline cardiomyopathy. This also illustrates the difficulty of researching clinical conditions with low incidence and prevalence, where small sample sizes may be all that are available. In chapters 4 and 5, the design, methodology and results of a prospective study that was powered to detect a change in LVEF and based on the anticipated incidence of newly diagnosed haematological malignancy during the study period in the local clinical catchment are reported. Exploratory analysis identified three previously unreported baseline predictors of recovery of LVEF at six months. However, it is important to note that the study was not adequately powered to detect baseline predictors, which in terms of informing treatment and monitoring strategies, would be one of the most clinically useful findings. Pragmatically, the sample size was constrained by the incidence of disease, funding, and time constraints, all of which are difficult to overcome. Therefore, further study is required to validate these baseline predictors in larger cohorts, but these findings serve as a useful starting point.

Chapter 7 Future directions

There are a multitude of unanswered questions regarding the role of CMR in the field of cardio-oncology and the progressive incorporation of this unique tool into clinical guidelines and subsequently, clinical practice, requires a sound evidence base. Building on the work presented in this thesis, further evaluation and validation of non-invasive predictors of cardiotoxicity at baseline is warranted. Baseline predictors of cardiotoxicity may represent the most clinically useful piece of information, allowing clinicians to design individualised risk stratification, monitoring, treatment and preventive strategies for their patients. As discussed in section 6.2, an assessment of CMR in comparison to echocardiography (current practice), ideally in the form of an RCT should be conducted in patients receiving cardiotoxic therapy to determine if one method is superior. If these assessments could be of comparable cost and resource use, which may be achievable with shortened, contrast free CMR protocols, this would be of additional value.

In addition, exercise intolerance is a feature of cancer therapy and indeed, cancer itself[207]. The majority of CMR studies to date have evaluated cardiac function at rest, with only a few exceptions[167]. However, studying cardiac function during exercise CMR may provide important insights, which may be of more clinical relevance to the patient's symptoms and quality of life, the latter of which has also only been evaluated by a small number of studies[82]. Finally and perhaps most importantly, data regarding the prognostic implications of non-invasive biomarker derangement to hard clinical endpoints in cardio-oncology populations are lacking and large scale, multicentre studies are required to obtain sufficient statistical power to address this area of clinical uncertainty. In addition, the role and clinical impact of training and employing dedicated cardio-oncology specialists is uncertain and could be the subject of an informative RCT in the field.

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Chapter 9 Appendix - Prospective Study Protocol

9.1 Introduction

Cancer is a leading cause of illness and death but mortality rates are falling predominantly because of improvements in chemotherapy, radiotherapy and supportive care. However, a wide range of cancer therapies have been associated with cardiotoxicity, leading to significant cardiovascular morbidity and sometimes causing death. Anthracyclines, commonly used to treat haematological malignancy, are the most frequently implicated agents[19].

Agents used in the treatment of cancer have unique cardiotoxic effects that vary in the extent, pattern and timing of cardiac injury. This injury can be fully reversible, partially reversible or irreversible. Individual susceptibility varies and the full effects may not become apparent for months or years later, as evidenced by survivors of childhood cancer. Consequently, the non-invasive echocardiographic evaluation of left ventricular function, commonly by measuring either LVEF or GLS in patients receiving potentially cardiotoxic treatment has become established practice. If a significant deterioration in LVEF occurs, chemotherapy is modified or withdrawn and cardioprotective treatment initiated. However, the expression of cardiac damage by deteriorating left ventricular systolic or diastolic function may not be detectable by echocardiography until a significant amount of cardiac injury has occurred[101].

CMR has emerged as the reference standard for measuring left ventricular volumes, mass and ejection fraction with a greater ability to identify subtle changes to cardiac structure and function than echocardiography[77]. In addition, cardiac MRI has the unique capability to characterise myocardial tissue non-invasively, providing a virtual biopsy of histopathological changes and early identification of subclinical cardiac injury. Early identification of cardiac injury

provides an opportunity to modify or withdraw cancer therapy, implement primary prevention strategies and potentially avoid irreversible cardiac injury.

To date, there are only a limited number of small, mainly retrospective studies using cardiac MRI to evaluate cardiotoxicity due to cancer therapies (Table 2-2) and only one study of ten participants specifically evaluating patients treated for haematological malignancy[102]. However, these initial reports suggest an evolving role for cardiac MRI in evaluating early and late chemotherapy related cardiac injury.

Furthermore, there is increasing interest in the role of non-coding RNAs (ncRNA) e.g. microRNA, long non-coding RNA and circular RNA, as potential biomarkers of cardiovascular disease with reports of identifiable deregulation both in rats[208] and in human induced pluripotent stem cell derived cardiomyocytetes[209] exposed to anthracycline with emerging reports of dysregulation in human studies[68].

The diagnostic and prognostic role of cardiac MRI and novel biomarkers in patients treated with potentially cardiotoxic therapy for haematological malignancies, including those undergoing haematopoietic cell transplantation merits further evaluation.

9.2 Background

9.2.1 Anthracycline cardiomyopathy

Improvements in cancer therapy and supportive care have led to significant improvements in cancer survival. However, survivors are at increased risk of cardiovascular disease, partly because cancer and cardiovascular disease share common risk factors (smoking, age, and obesity) but also because chemotherapy itself can cause cardiac injury. Anthracyclines and related compounds that are often used to treat haematological malignancy are the most frequently implicated agents, leading to significant cardiovascular morbidity and sometimes causing death. Cardiac injury has been attributed to reactive oxygen species

formation, transcriptional changes in intracellular triphosphate production in cardiac myocytes and through interaction with cardiac topoisomerase IIβ[3]. At the tissue level, this manifests as myocardial inflammation[210], oedema[4] and vacuolisation[43], which seem to predate overt impairment of left ventricular function. During the later stages of anthracycline cardiomyopathy, myocardial fibrosis as evidenced by LGE has been described[117], though these findings have not been widely reproduced. In the context of overt heart failure, mortality can be as high as 60% at two years[7], emphasising the importance of early identification of cardiac injury, the modification of therapy, the institution of primary prevention strategies and avoidance of long term cardiovascular complications. No established definition of early cardiovascular injury in this setting exists but a > 5% drop in LVEF in association with symptoms or a > 10% asymptomatic drop in LVEF to a value below the lower limit of normal is commonly used[2]. Therefore, cardiovascular imaging has an important role in detecting early cardiac injury.

9.2.2 Current use of cardiovascular imaging to detect anthracycline cardiomyopathy

9.2.2.1 Echocardiography

In the United Kingdom, 2D transthoracic echocardiography is commonly used to screen and monitor patients receiving potentially cardiotoxic cancer therapy. However, it is relatively insensitive to small reductions in EF[211], though sensitivity may be improved by using 3D echo or intravenous contrast. Myocardial strain imaging, particularly GLS, may be better able to identify subtle changes to left ventricular function with an early reduction in strain predictive of subsequent cardiomyopathy[117]. However, the challenges of an adequate acoustic window remain.

9.2.2.2 CMR

CMR is considered the gold standard for measurement of left ventricular volumes and function[77] and has the unique capability to characterise myocardial tissue

non-invasively, thus providing a virtual biopsy of histopathological changes and an opportunity to identify subclinical cardiac injury prior to deterioration in left ventricular function. Additional strengths include the lack of ionizing radiation and lack of dependence on assumed geometric models or on an adequate acoustic window. However, the lack of widespread availability and higher operational cost mean that it is currently recommended only when the discontinuation of cancer therapy is being considered or when echocardiographic data are thought to be unreliable or conflicting[19].

Contrast enhanced cardiac MRI is a well established non-invasive imaging tool. Gadolinium is an extracellular agent that accumulates in areas of interstitial expansion and permits the quantification of focal myocardial fibrosis and infiltration by assessing LGE[212]. The most common finding in anthracycline induced cardiomyopathy is absence of LGE but lateral wall LGE has been reported in one single centre study[117]. Other specialised sequences allow characterisation of pathological tissue without contrast. A T2 weighted sequence, Short-Tau Inversion Recovery (STIR), identifies increases in tissue free water content e.g. due to myocyte swelling and interstitial oedema. To date, only one small published paediatric study[90] has reported acute myocardial oedema in patients receiving anthracyclines. Furthermore, the introduction of T1 and T2 mapping, a quantitative tool to identify diffuse myocardial processes not detectable by traditional LGE imaging has added a new dimension to the understanding and assessment of various myocardial diseases. T1 mapping promises to detect early disease, objectively quantify disease severity, and may provide prognostic insights into certain conditions. Using this technique, an increased ECV, used as a surrogate marker of myocardial fibrosis, was reported in a cohort of patients receiving anthracyclines, compared with age and sex matched controls[107]. However, the routine use of CMR in clinical practice is limited by its availability and higher operational cost compared with echocardiography.

9.2.2.3 Biomarkers

Considerable investigative effort has been devoted to the role of biomarkers as early markers of cardiac injury. Cardiac troponins are the gold standard biomarker for identifying myocardial injury and have been reported to predict LV dysfunction in patients receiving cardiotoxic chemotherapy[52]. However, the optimal timing of sampling is unclear, the optimal cut off level for determining positivity has not yet been established[2] and those with positive Troponin assays may go on to develop irreversible LV dysfunction. Therefore, their use in this context is yet to enter routine clinical practice.

More recently, non-coding RNAs (including microRNA, long non-coding RNA, and circular RNA) have showed promise in the cardiovascular arena for their potential as novel biomarkers as well as therapeutic targets. For instance, different microRNAs have been implicated in the pathogenesis of a variety of cardiovascular diseases[66] and have consequently become an appealing diagnostic and therapeutic target. This applies both to microRNA circulating in the blood, but also, interestingly, to those carried by extracellular vesicles known as exosomes. A recent study reported that the plasma concentration of exosomes carrying a cargo of cardiac microRNAs is increased following coronary artery bypass graft surgery[213]. The role of microRNAs as early biomarkers of anthracycline induced cardiac injury has been explored in animal models[214] and latterly in human induced pluripotent stem cell derived cardiomyocytes[209]. Interestingly, several microRNAs showed either early or prolonged deregulation in response to anthracycline, the former prior to the emergence of markers of cytotoxicity. However, these findings are yet to be explored in human subjects.

9.2.3 Rationale for the study

Whilst survival of cancer patients is improving, the unintended cardiovascular side effects of cancer treatment carry significant implications for cancer survivors. Subclinical, cancer therapy induced cardiac injury can be difficult to predict and/or detect with transthoracic echocardiography because it is relatively insensitive to subtle changes in left ventricular function. Cardiac MRI may be able

to either predict or identify cardiac injury at an earlier stage, thus limiting the extent of injury and potentially avoiding serious late clinical sequelae. Cardiac biomarkers, particularly miRNAs, also show promise in being able to predict cardiotoxicity or identify it during the early, reversible phase. If cardiotoxicity can be identified or predicted, the opportunity to tailor monitoring strategies, adjust therapy, institute primary prevention strategies and ultimately, avoid significant cardiovascular sequelae may be afforded, thus maximising benefit, minimising risk and improving patient outcomes. Few studies have employed CMR to study anthracycline cardiotoxocity prospectively and none have previously combined this evaluation with simultaneous miRNA analysis.

9.3 Study aims

9.3.1 Primary aim

To prospectively characterise anthracycline cardiotoxicity in patients receiving cancer therapy for haematological malignancy using multiparamtetric CMR and novel biomarkers.

9.3.2 Secondary aims

To explore baseline and early non-invasive parameters that predict subsequent development of, and recovery from anthracycline cardiotoxicity in patients receiving cancer therapy for haematological malignancy.

9.4 Experimental Hypothesis

9.4.1 Hypothesis for primary aim

The null hypothesis states that there will be no significant changes to measured parameters during the course of the study. The alternative hypothesis states that there will be significant temporal changes to study parameters during the course of the study.

9.4.2 Hypothesis for secondary aim

The null hypothesis states that there will be no baseline or early non-invasive parameters that predict the development of or recovery from anthracycline cardiotoxicity. The alternative hypothesis states that there will be baseline or early non-invasive parameters that predict the development of or recovery from anthracycline cardiotoxicity

9.5 Outcome measures

9.5.1 Primary outcomes

Prospective change in LVEF assessed by CMR

9.5.2 Secondary outcomes

Prospective change in T1, T2 mapping and Extracellular Volume (ECV) values, assessed by CMR

Prospective change in 2D global longitudinal, circumferential, and radial feature tracking CMR derived strain.

Prospective change in 2D and 3D LVEF and 2D GLS by echocardiography

Prospective change in biomarkers of cardiac injury (Troponin and miRNA)

9.5.3 Additional outcomes

Baseline and early predictors of LVEF recovery between completion of chemotherapy and six month follow up will be explored.

9.6 Study design

This was a multicentre cohort study to investigate the role of CMR and novel biomarkers (miRNAs, Troponin I) to identify cardiac injury in patients receiving potentially cardiotoxic treatment for haematological malignancy. The study population included patients who were scheduled to receive potentially

cardiotoxic treatment for haematological malignancy, including those undergoing haematopoietic stem cell transplantation.

The study was conducted at two centres; The Bristol Haematology and Oncology Centre, which is part of the Bristol Royal Infirmary, University Hospitals Bristol NHS Trust, and Southmead Hospital, North Bristol NHS Trust. Southmead Hospital was a Participant Identification Centre (PIC). The Bristol Royal Infirmary (including The Bristol Haematology and Oncology centre and The Bristol Heart Institute) was the main research centre, conducting all research activities.

9.6.1 Subjects and recruitment

Participants were identified from those attending the haematology clinic at the Bristol Haematology and Oncology Centre and Southmead Hospital by reviewing their medical records. Participants were initially be approached and provided with verbal and written information by a member of the direct care team, usually a consultant haematologist or haematology specialist registrar. All staff involved in identifying potential participants had substantive or honorary contracts with the relevant trust and were members of the direct care team. If an interest in taking part was expressed, further questions were answered either by a member of the direct care team or by a clinical research fellow. Consent was obtained by a member of the direct care team or a clinical research fellow.

9.6.1.1 Inclusion criteria

Participant may enter study if ALL of the following apply

1. Age ≥ 18 years

2. Newly diagnosed haematological malignancy

   (Either high grade non-Hodgkin lymphoma scheduled for ≥ 6 cycles anthracycline, Hodgkin Lymphoma scheduled for ≥ 6 cycles anthracycline or Acute Myeloid Leukaemia scheduled and fit for full dose treatment with curative intent).

9.6.1.2 Exclusion criteria

Participant may not enter study if ANY of the following apply

1. Contraindications to CMR (Ferromagnetic foreign body, severe claustrophobia, severe renal impairment)

2. Contraindications to Gadolinium contrast (severe renal failure [eGFR < 30], previous allergy, pregnancy, or breastfeeding)

3. Unable to give consent

4. Prisoners

5. Prior cardiac disease (e.g. impaired LVEF, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting or other cardiac surgery or invasive procedure, ≥ moderate valvular heart disease, congenital heart disease, arrhythmia, hypertrophic cardiomyopathy, amyloidosis, sarcoidosis or intracardiac mass)

6. Prior haematological disease or malignancy

7. Prior systemic chemotherapy or thoracic radiotherapy

9.6.2 Study schedule

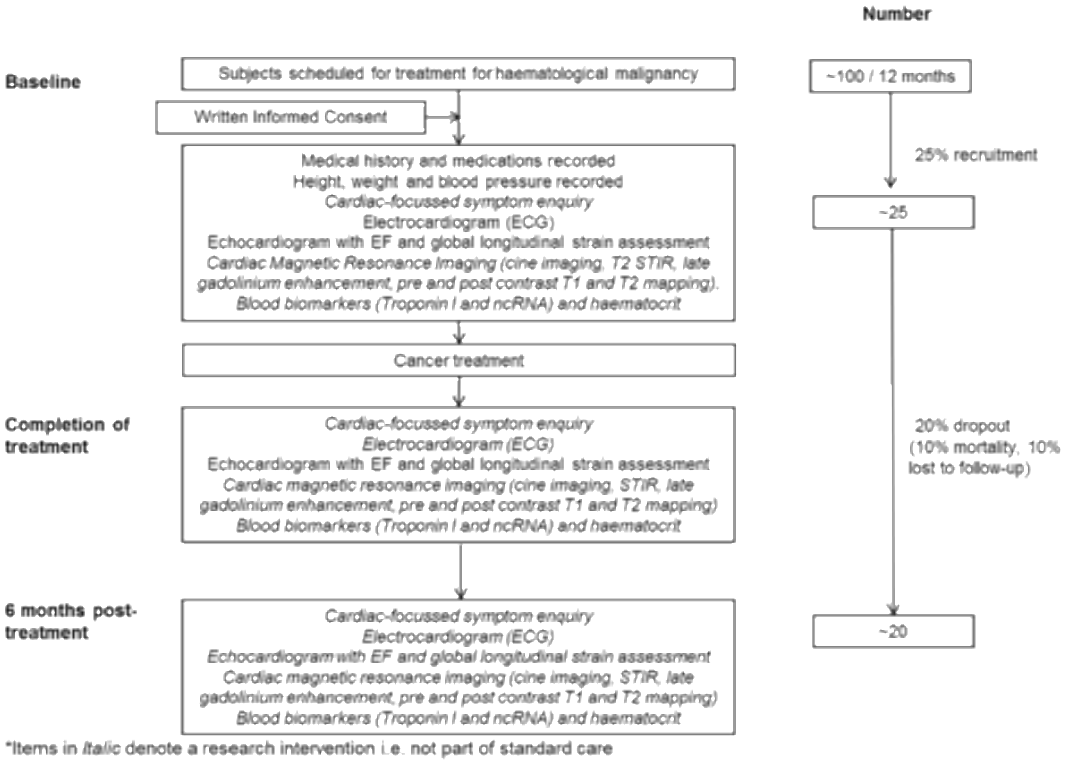

Figure 9-1 Study schedule

9.6.3 Participant pathway

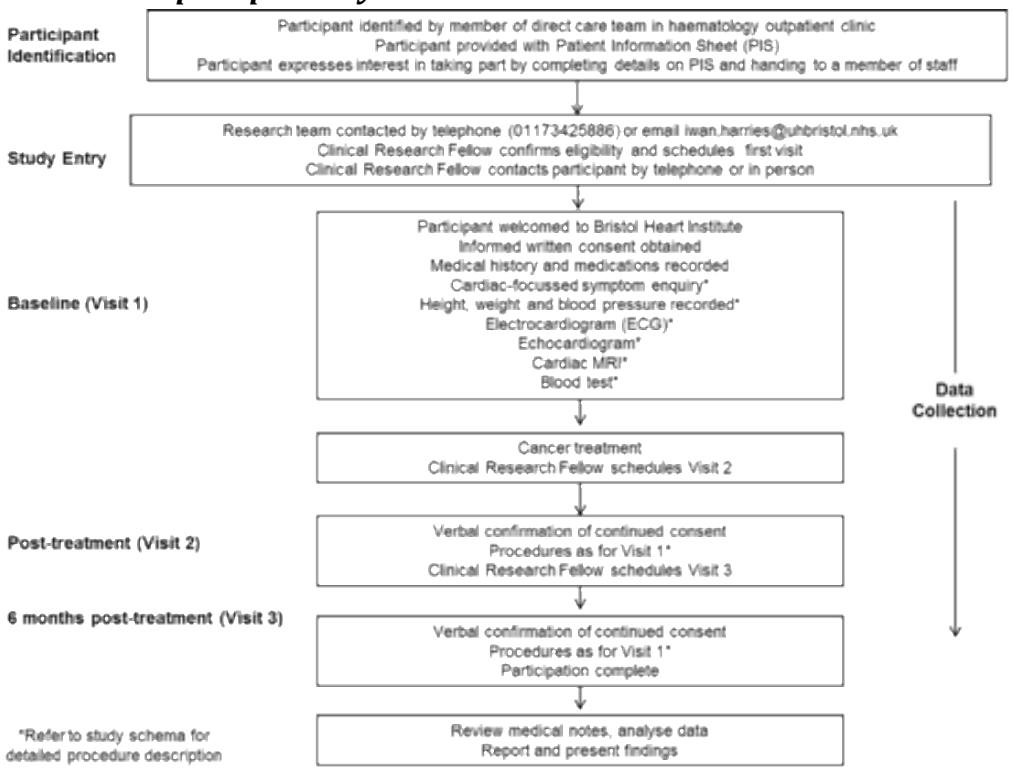

Figure 9-2 Participant pathway

9.7 Study Procedures

9.7.1 Baseline data

Baseline data were collected by the research team and recorded in a case report form (CRF). Data included date of birth, ethnicity, sex, medical history, prescribed medications and smoking and alcohol status.

9.7.2 Symptom enquiry

A cardiovascular focused symptom enquiry was undertaken at each study visit including New York Heart Association (NYHA) dyspnoea class and the presence of orthopnoea or paroxysmal nocturnal dyspnoea.

9.7.3 Physiological data

Weight and height were measured at the beginning of each study visit and recorded in the CRF. Body surface area (Mosteller) was calculated and body mass index (BMI) by dividing the weight in kilogram by the height in metres squared. A

standard 12 lead surface electrocardiogram was recorded in a semi supine position, interpreted by the research team and the result (including heart rate in beats per minute) recorded in the CRF. Blood pressure was recorded with the participant lying supine in the MRI scanner using an appropriately sized automated blood pressure cuff and the result recorded in the CRF.

9.7.4 Blood sampling

Venepuncture was performed according to standard procedures using aseptic technique. Near patient analysis of blood samples was undertaken using an iSTAT (Abbott laboratories, United States of America) testing unit. Readings were printed from the analyser and recorded in the CRF. The iSTAT testing unit was calibrated and control samples analysed with each new box of testing cartridges in accordance with the manufacturer's instructions.

9.7.5 MicroRNA

Following venepuncture, 5 ml of blood was drawn into a sodium citrate vacutainer. This was transported to the laboratory and centrifuged at 2240 g for 10 minutes at room temperature. The plasma supernatant was removed to a fresh Eppendorf tube without disturbing the buffy coat and centrifuged again at 2240 g for 10 minutes at room temperature. The supernatant was then removed to a fresh Eppendorf LoBind microcentrifuge tube, pseudo-anonymised and stored at -80°C in preparation for blinded batch analysis. See Appendix **Error! Reference source not found.** for detailed miRNA processing protocol.

9.7.6 CMR

CMR was performed at each study visit following confirmation of consent. The CMR study protocol is included in Appendix **Error! Reference source not found.** and specific technical information is described in section 5.2.1.

9.7.7 Echocardiography

Echocardiography was performed on the same day as CMR and blood biomarker analysis. All analyses were performed on a single EpiQ machine (Philips, Netherlands) by two fully accredited echocardiographers. The echocardiography

study protocol is included in Appendix **Error! Reference source not found.** and specific technical information is described in section 5.2.2.

9.8 Statistics

9.8.1 Justification of target sample size

Based on a combination of prior publications of change in LVEF, a target sample size of 14 subjects gives 90% power at an alpha level of 0.016 to detect a statistically significant difference of 6.2% in LVEF (61.5 ± 2.9% *vs.* 55.3 ± 3.1%). There are few, if any, previously published data on myocardial oedema, T1 and T2 mapping or microRNA in this setting in human subjects to guide power calculations and, as such, this aspect is considered exploratory. Overall, the aim was to recruit 25 subjects over a period of one year, based on the incidence of disease and an anticipated recruitment rate of 25%.

9.9 Safety reporting

This study did not require participants to undergo additional therapeutic interventions. Therefore, it was felt unlikely that clinical adverse events (AE) could be attributed to study specific procedures. AE's arising from procedures that constitute standard care were not reported. Expected events due to study procedures are listed in **Error! Reference source not found.**; these were not reported.

Table 9-1 Expected adverse events not to be reported

Procedure	Expected outcomes
Insertion of intravenous cannula	Pain, bleeding, bruising, fainting, local skin irritation or inflammation, localised skin reaction to dressing
Electrocardiogram	Local skin irritation to adhesive pads
Echocardiogram	Local skin irritation to adhesive pads
CMR	Nausea, headache, claustrophobia, anxiety.

Any other AE arising during the conduct of the study was reported in accordance with the process for reporting adverse events set out by University Hospitals Bristol and The University of Bristol.

The University has a Service Level Agreement with UH Bristol to ensure that all SAE reporting is managed by UH Bristol on behalf of the University. For that reason, all SAEs must be recorded and reported to UH Bristol, in accordance with UH Bristol Research Safety Reporting Standard Operating Procedure. UH Bristol will regularly inform the University about SAEs. Expedited reporting takes place where necessary to agree corrective / preventative actions.

9.10 Ethical considerations

9.10.1 Risks and anticipated benefits

This was an observational study that did not change the patients' standard care. There were therefore few anticipated risks to patient safety resulting from the study. A list of expected outcomes arising from research procedures is detailed

above; Table 9-1. Clinical reports were not issued for research scans unless there was an explicit request from the clinical team to help inform clinical care. However, if a significant, unexpected abnormality e.g. previously unrecognised myocardial infarction was discovered, the finding was communicated on an urgent basis to the clinical team in the best interests of the patient.

The main benefit of the study was the provision of high quality evidence to address this important area of clinical uncertainty.

9.10.2 Informing potential participants of possible benefits and known risks

Information about possible benefits and risks of participation were described in the patient information sheet (PIS). Participants were provided with the opportunity to ask questions and as much time as they required regarding the benefits and risks of participation prior to enrolment.

9.10.3 Obtaining informed consent

Written, informed consent was obtained from each participant prior to entry into the study. This was obtained either by a trained member of the direct care team or by a trained clinical research fellow in a confidential environment (outpatient clinic of either the Bristol Haematology and Oncology Centre or Southmead Hospital). Eligible participants were provided with written information, allowed to ask questions and as much time as they required to decide whether they wanted to take part. The research fellow or member of the direct care team or PI were responsible for the consent process.

9.11 Research governance

This study was conducted in accordance with:

- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines

- Research Governance Framework for Health and Social Care

9.11.1 Sponsor approval

Any amendments to the study documents were approved by the sponsor prior to submission to the HRA.

9.11.2 Study permissions

The study was performed subject to NHS Research Ethics Committee (REC) and Health Research Authority (HRA) approval, including confirmation of capacity and capability from participating NHS organisations.

Any amendments to the study, after a favourable opinion from the HRA and NHS REC had been given were submitted to the Sponsor for review prior to submission for regulatory approval and implementation.

9.11.3 Investigators' responsibilities

Investigators were required to ensure that all required local research permissions had been obtained and that any contractual agreements required were signed off by all parties before recruiting any participant. Investigators were required to ensure compliance with the protocol and study manual. Investigators were required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or any regulatory authorities.

Investigators were required to read, acknowledge, and inform their study team of any amendments to the study documents approved by the HRA that they receive and ensure that the changes were complied with.

9.11.4 Monitoring by sponsor

The University of Bristol (UoB) has a Service Level Agreement in place with a local NHS Trust (UH Bristol). As part of this, UH Bristol undertake monitoring of research projects where UoB is fulfilling the responsibilities of a research sponsor. A minimum of 10% of UoB Projects are monitored.

9.11.5 Indemnity

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University.

This does not in any way affect an NHS Trust's responsibility for any clinical negligence on the part of its staff (including the Trust's responsibility for University of Bristol employees acting in connection with their NHS honorary appointments).

The NHS indemnity scheme applied for insurance and/or indemnity to meet the potential legal liability of the sponsor or employer for harm to participants arising from the design of the research.

All medical and academic staff conducting the research held honorary contracts with Bristol University Hospitals NHS Foundation Trust giving them the protection of the NHS indemnity scheme.

9.12 Data protection

Data were collected and retained in accordance with the UK Data Protection Act 1998.

9.12.1 Data handling, storage and sharing

9.12.1.1 Data handling

Study data were uploaded to the NHS PACS server hosted at the UH Bristol NHS Trust. Data transmitted from the NHS server to the University of Bristol were pseudo-anonymised by assigning a unique participant identifier and removing all patient identifiable information other than the unique identifier. The "look up" table for the unique participant identifier remained on the NHS server. Standard operating procedures for database use, data validation and data cleaning were available and regularly maintained.

9.12.1.2 Data storage

All the imaging data were stored in the UHB PACS system. The site files, including participant consent forms were kept securely in the NHS CMR Unit of the Bristol Heart Institute. The study data were stored and maintained in a pseudo-anonymised form in a Bristol University RedCap database.