CARDIOVASCULAR MAGNETIC RESONANCE IN CARDIOMYOPATHIES

A thesis submitted to Imperial College London for the degree of Doctor of Philosophy

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Declaration

I hereby certify that all material in this dissertation which is not my own work has been properly acknowledged.

Gillian C Smith
Abstract

**Background:** Cardiomyopathy (CM) frequently leads to heart failure which is associated with a high degree of mortality, morbidity and financial burden on healthcare providers. Confidence in the recognition of risk factors or in early diagnosis allows timely intervention, before heart failure develops. The right ventricle (RV) has historically been overlooked when assessing cardiac function and response to therapy but its importance is increasingly recognised. Cardiovascular magnetic resonance (CMR) is established as the gold standard for assessing the functional consequences of cardiovascular disease, being non-invasive and with high accuracy and reproducibility. It can also provide *in-vivo* tissue information which may be diagnostic before functional changes are apparent. With this backdrop I tested the hypothesis that CMR can provide early disease markers in patients at risk of heart failure in 3 unrelated conditions associated with heart failure including: Emery-Dreifuss muscular dystrophy (EDMD), anthracycline induced cardiomyopathy and cardiac iron overload in transfusion dependent patients.

**Methods and Results:** Patients with 3 different clinical substrates for the development of CM were studied. In group 1, patients with EDMD were studied by CMR and echo. There was a significant reduction in inferior wall contractility using CMR tagging (-0.062±0.02 versus -0.094±0.03 in the control group, p=0.048) and in echo derived early diastolic myocardial posterior wall velocity gradients (4±1.2 vs. 7.1±2.7 s⁻¹, p=0.02). Bi-ventricular ejection fraction (EF) was normal and no late gadolinium enhancement (LGE) was detected. These findings demonstrated the relative insensitivity of EF in the detection of early disease and the need for careful follow up in these patients.

In group 2, anthracycline mediated cardiotoxicity (AMC) was studied. The risk of heart failure rises with cumulative dose but not all individuals are susceptible. In a cohort of patients with early breast cancer an increase in early gadolinium relative enhancement (EGRE) from baseline to day 3 correlated with a reduction in LVEF after a year.
(R=0.34, p=0.01) suggesting a potential clinical role for EGRE in the prediction of late AMC.

In group 3, patients with thalassaemia major (TM) were studied. The coefficient of variance (CV) for a new black blood T2* sequence was found to be significantly lower than the white blood sequence (1.47% vs 4.23%, p<0.001) and this was adopted for clinical use. The RV response to iron chelation therapy was examined in 3 clinical trials. Using deferiprone monotherapy RVEF increased from 69.6±5.2 to 72.2±5.3% (p=0.001) with a reduction in RV end-systolic volume (ESV) from 37.7±11.7 to 34.2±11.3 mL (p=0.009). With deferiprone/deferoxamine combination therapy the RVEF increase from 60.2±7.2 to 63.8±5.9% (p<0.001) and RVESV decreased from 60.8±24.2 to 50.6±17.3 mL (p<0.01). These improvements mirrored the LV response. However, the response to deferasirox monotherapy was different with no change in LVEF, but an increase in RVEF from 66.1±6.1 to 68.8±5.4 (p=0.001) driven by an increase in RV end diastolic volume (EDV) from 69.3±19.8 to 76.1±17.1 (p<0.001) with a reduction in RV mass from 32.8±7.8 to 24.7±5.6 (p<0.001) and LV mass (78.6±16.9 to 66.5±12.9, p<0.001). These results suggest a different pharmacological action to deferiprone and deferoxamine, and indicate a possible role of RV measurements in risk assessment.

**Conclusions:** This thesis has demonstrated that CMR can be used to identify a variety of markers of early CM, namely that: Strain abnormalities in EDMD are not associated with identifiable fibrosis in EDMD, early inflammatory changes post anthracycline exposure can predict late functional changes and that CMR provides sensitive markers of therapeutic efficacy on RV function in iron overloaded patients.

These data improve our understanding of the early effects of CM and demonstrate that novel CMR techniques may play a clinically useful role in earlier detection of ventricular abnormality, and assessment of differential treatment responses.
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Abbreviations

ACE, angiotensin converting enzyme
ALT, alanine aminotransferase
AMC, anthracycline mediated cardiotoxicity
ANOVA, analysis of variance
Arrhythmic cardiomyopathy
ARVC, arrhythmogenic right ventricular cardiomyopathy
ASD, atrial septal defect
ATP, adenosine triphosphate
AV, atrio-ventricular
Better-Care, The Breast Cancer, Early disease: Toxicity from Therapy with adjuvant Epirubicin Regimens- Cardiac Assessment and Risk Evaluation
BCMR, British society of CMR
BMD, Becker's muscular dystrophy
BMI, body mass index
BNP, brain natriuretic peptide
BP, blood pressure
BSA, body surface area
CM, cardiomyopathy
CMR, cardiovascular magnetic resonance
CV, coefficient of variance
CRF, case report form
CRP, c-reactive protein
DCM, dilated cardiomyopathy
DIR, double inversion recovery
DMD, Duchenne muscular dystrophy
DNA, deoxyribonucleic acid
DTPA, diethylenetriaminepentaacetic acid
ECG, electrocardiogram
EDMD, Emery Dreifuss muscular dystrophy
EDTA, ethylenediaminetetraacetic acid
EF, ejection fraction
EGRE, early gadolinium relative enhancement
EPI, echo planar imaging
ESV, end systolic volume
EDV, end diastolic volume
EPIC, Evaluation of Patients Iron Chelation with Exjade
FEC, fluorouracil/epirubicin/cyclophosphamide
FID, free induction decay
FLASH, fast low angle shot
FPLD, familial partial lipodystrophy
Gd, gadolinium
GRE, gradient echo
HARP, harmonic phase
HCM, hypertrophic cardiomyopathy
HASTE, half Fourier acquisition single shot turbo spin echo
HER, human epidermal growth factor receptor
HGPS, Hutchinson-Guilford progeria syndrome
HR, heart rate
ICD, implantable cardioverter defibrillator
IHD, ischaemic heart disease
IOC, iron overload cardiomyopathy
IR, inversion recovery
LA, left atrium/atrial
LAX, long axis
LGE, late gadolinium enhancement
LGMD, limb girdle muscular dystrophy
LV, left ventricle/ventricular
LVEDV, left ventricular end diastolic volume
LVESV, left ventricular end systolic volume
LVSV, left ventricular stroke volume
LVEF, left ventricular ejection fraction
MAD, mandibuloacral dysplasia
MI, myocardial infarction
MOLLI, modified Look Locker inversion recovery
MR, magnetic resonance
mRNA, messenger ribonucleic acid
MVG, myocardial velocity gradient
NCRN, national cancer research network
NEO-TANGO, epirubicin/cyclophosphamide/ paclitaxel/gemcitabine
NFD, nephrogenic fibrosing dermopathy
NMR, nuclear magnetic resonance
NSF, nephrogenic systemic fibrosis
NTBI, non-transferrin bound iron
NYHA, New York Heart Association
PAP, pulmonary artery pressure
PHT, pulmonary hypertension
PRF, pulse repetition frequency
RBH, Royal Brompton Hospital
RF, radio frequency
RMH, Royal Marsden Hospital
ROI, region of interest
ROS, reactive oxygen species
RV, right ventricle/ventricular
RVEDV, right ventricular end diastolic volume
RVESV, right ventricular end systolic volume
RVSV, right ventricular stroke volume
RVEF, right ventricular ejection fraction
RWMA, regional wall motion abnormality
SAX, short axis
SCMR, society for cardiovascular magnetic resonance
SD, standard deviation
SHMD, specific heart muscle disease
S/N signal to noise
SNR signal to noise
SPAMM, spatial modulation of magnetisation
SSFP, steady state free precession
STIR, short TI inversion recovery
T, Tesla
TACT-2, epirubicin/capecitabine
TDI, tissue Doppler imaging
TE, echo time
TI, thalassaemia intermedia
TM, thalassaemia major
TR, repetition time
TSE, turbo spin echo
TrueFISP, true fast imaging with steady state precession
WHO, world health organisation
Chapter 1: Summary and Objectives

Heart failure is a common late manifestation of cardiomyopathy (CM) that is distressing for the patient and associated with high morbidity and mortality. The recognition of causative factors and early disease may provide opportunities to slow or prevent the onset of symptomatic disease, however ejection fraction (EF), the most commonly cited index of cardiac function, often remains within normal limits early in the disease process. It is possible that other cardiac parameters may reveal abnormality. This thesis explores the use of cardiovascular magnetic resonance (CMR) in 3 groups of patients with known risk of CM with disparate causes for ventricular abnormality. Particular questions addressed are:

- Can CMR can provide early disease markers in Emery-Dreifuss muscular dystrophy (EDMD). The leading hypothesis being that fibrosis plays a role in cardiac involvement?

- Is there a link between anthracycline induced early inflammatory changes (CMR and urinary markers) and a late reduction in cardiac function?

- Can the T2* sequence for tissue iron assessment in current clinical use be optimised to improve reproducibility and therefore reliability?

- How does iron chelation therapy affect the right ventricle (RV)?

The word cardiomyopathy is derived from Greek, and means heart muscle disease. The term was previously restricted to heart muscle disease of unknown aetiology, which was a reflection of the lack of knowledge of disease pathophysiology at the time. Diseases affecting the myocardium associated with inflammatory or metabolic disorders etc were termed specific heart muscle disease (SHMD). Our understanding of disease pathophysiology has been enhanced by recent advances in imaging technology, molecular science and genetics which has blurred this distinction and brought SHMD under the CM umbrella. Cardiomyopathies are now classified by their aetiology or dominant pathophysiology, although full consensus on terminology has yet to be achieved.
Non-invasive imaging modalities are well established for assisting in the management of patients with CM. Cardiovascular Magnetic Resonance (CMR) was first attempted in humans in 1983-4, and since then improvement in hardware and acquisition software has resulted in applications for functional assessment and tissue characterisation. The development of Steady State Free Precession (SSFP) sequences in which a non-zero state of magnetisation is maintained, has improved signal to noise coupled with reduced motion sensitivity and faster scan duration. With its superior intrinsic contrast and spatial resolution, and lack of ionising radiation, SSFP cine imaging is now considered the reference technique for cardiac functional assessment. CMR can also characterise myocardium with 2 techniques: a) using a gadolinium MR contrast agent, changes at a tissue level such as fibrosis and oedema can be identified before global abnormality occurs and b) intrinsic contrast from differential tissue relaxation properties such as occurs in iron deposition.

The patient cohorts studied for this thesis have specific or acquired CM with well recognised aetiologies. In all these patient groups, previous reports have tended to refer to functional changes of the left ventricle (LV). The RV has been largely ignored however its importance is being increasingly recognised as a prognostic factor in CM. To address this, emphasis has been placed on changes in RV function in this work.

EDMD is a rare neuromuscular disorder with cardiac involvement that usually requires pacemaker insertion. This has to date limited CMR investigation in later disease. Taking a well characterised cohort of patients from a specialist referral centre chapter 5 first explores the hypothesis that fibrosis is a substrate for myocardial dysfunction as found in other muscular dystrophies. In this group in whom close follow-up using conventional echocardiography had uncovered no functional abnormality, atrial volumes CMR tagging and tissue Doppler echocardiography were added to ventricular volumetric analysis in an attempt to reveal early changes.

Anthracycline CM and iron overload CM are examples of toxic CM. In anthracycline cardiotoxicity, population risk increases with dosage but individual reactions are idiosyncratic. Even at a low dose some patients develop CM, but other patients may be denied optimal treatment. The prediction of late complications may help tailor therapy.
Chapter 6 describes a 1 year follow-up study of patients receiving adjuvant chemotherapy for early breast cancer using CMR and inflammatory markers measured at three days post chemotherapy showing that CMR can predict late myocardial dysfunction.

Iron overload CM occurs in patients requiring life-long transfusions, such as beta-thalassaemia major, and can be reversed if appropriate iron chelation therapy is administered, but heart failure is still the commonest cause of death worldwide. Unfortunately liver iron concentration (LIC) and ferritin levels do not predict cardiac iron burden. CMR T2* can accurately show cardiac iron levels and therefore indicate patients at risk of heart failure and subsequent death. There are however technical challenges associated with the established white blood technique which may reduce diagnostic accuracy. Chapter 7 describes the validation of a new T2* sequence that has increased the diagnostic robustness of CMR iron overload assessment and has now been adopted into routine clinical use.

A relationship between myocardial T2* and RV function has been established but there is no data on the response of the RV to chelation therapy. Deferiprone is known to improve LV function in patients with cardiac iron overload, both as a monotherapy improves and in combination with deferoxamine. Deferasirox does not improve LV function. Chapters 8 and 9 examine the effect on the RV of deferiprone and combination therapy respectively, the results as expected showing an improvement in RVEF associated with a reduction in RV end systolic volume (ESV). Unexpectedly the cardiac functional response to deferasirox monotherapy (chapter 10) showed an increase in RVEF associated with an increase in RV end diastolic volume (EDV) with a reduction in RV and LV mass but no improvement in LVEF. These results show differential effects on the RV of the iron-chelators, which have potential clinical impact.

In summary, this work for this thesis has shown CMR to be a versatile imaging modality for exploring phenotypic changes in CM. Its strengths in characterising the myocardium have been used to define diagnosis, evaluate treatment efficacy and contribute to risk assessment for clinical outcomes, for both the left and right ventricles.
In particular strain abnormalities in EDMD identified using CMR and echo were not associated with macroscopic myocardial fibrosis. Early CMR inflammatory changes in AMC were related to early changes in urinary isoprostanes and late changes in cardiac function. RV functional improvement with deferiprone mono and combination therapy is associated with, but not dependent upon, improved LV function. The results for the deferasirox trial however suggest a different therapeutic effect with interesting ramifications.
Chapter 2: Cardiovascular Magnetic Resonance

2.1 Basic principles of magnet resonance

2.1.1 Nuclear spin

Atomic nuclei are usually made up of positively charged protons and uncharged neutrons. Both possess quantum spin characteristics (angular momentum) that can cancel each other out resulting in a zero net nuclear spin. Isotopes with an uneven number of protons and neutrons have a net spin and are useful for MR if in sufficient abundance. This is due to the fact that a rotating charge (dipole) produces an electrical current which in turn generates a magnetic field in the direction of the axis of rotation, termed the magnetic moment. The magnetic moment is a directional force, the characteristics of which determine the effect of an external magnetic field.

![Proton Spin Diagram]

Figure 2-1 Proton spin velocity is constant however the angle of spin is random outside a magnetic field.

Although nuclear rotational or spin velocity is constant the angle of the spin or axis is variable and the sum effect of the individual nuclei can be expressed as a vector. Out of a magnetic field nuclear spin orientations are random therefore net magnetisation is
effectively zero. The utilisation and manipulation of these spin vectors is the basis of magnetic resonance imaging.

Commonly used nuclei in MR are 1H, 31P, 23Na, 14N, 13C and 19F. Hydrogen is the most abundant element in the body being the major component of water and fat. In addition the nucleus of the hydrogen atom, consisting of one positively charged proton, gives the strongest MR signal when compared with other elements.

Although an analogy can be drawn between a magnetic dipole and a bar magnet or electrostatics a magnetic dipole has a particular feature that is exploited for MR imaging, it precesses when placed in an external magnetic field. In other words the spin direction rotates.

2.1.2 Spin behaviour in a magnetic field

When placed in a magnetic field, B0, the spin vectors of the nuclei align with the external field (designated the Z axis) in a direction dependant on their energy state. The low energy state is where the poles are aligned (parallel or spin up) and the high energy state (anti-parallel or spin down). There is a small excess of nuclei in the more stable lower energy state dependent upon external field strength, temperature and proton density. It is approximately 9 parts per million at body temperature and field strength of 1.5T which leads to a very weak net magnetisation or paramagnetism. Paramagnetism is the term used for substances that exhibit no magnetic properties outside a magnetic field but show a slight positive interaction within an applied field. It can also be called positive magnetic susceptibility. The result of this is a net magnetisation in the Z axis, termed Mz. The torque exerted by the external field affects the spin angular momentum causing a precessional motion, of the spin axis. This can be likened to the effect of gravity, acting as an off-centre force, on the spin of a gyroscope.
Spin vectors align with an external magnetic field and the torque exerted causes the spin vectors to precess.

Precessional speed is proportional to the external field strength (but is much lower than the proton’s rotational velocity). The stronger the external magnetic field, the higher the precession or Larmor frequency expressed by the equation $\omega = \gamma B_0$ where $\omega$= Larmor frequency, $\gamma$= gyromagnetic ratio of the nucleus and $B_0$ = field strength. The gyromagnetic ratio is a constant that relates frequency and external magnetic field strength and is the ratio of the magnetic moment to the angular momentum. It is different for each nucleus. For $^1\text{H}$ it is 42.58 MHz/T. The Earth’s magnetic field is only about 50 $\mu$T and nuclei precess at approximately 2 kHz. In MR scanners field strength is high, usually 0.5 to 7 Tesla (T). Nuclei therefore precess at radio frequency (RF) in MR systems. For example the precessional frequency for $^1\text{H}$ at 1.5 T is approximately 63MHz (which is why shielding against extraneous RF waves is necessary).

2.2 Excitation and energy level transition

Nuclei in the lower and higher energy states are in equilibrium within the constant external field. Nuclei can undergo a transition from high to low or low to high by the absorption of a photon with exactly the same energy as the difference between the two states. This energy is related to its frequency by Planck’s constant designated $h$, (where
h=6.626x10^-34 Js) by the equation \( \Delta E = hf \) where \( f \) is the precessional frequency. This in turn is related to the Larmor frequency \( \omega \) by the equation \( \omega = 2\pi f \). The fact that each isotope has a unique resonant or Larmor frequency which in turn is directly proportional to the external field can be utilised for imaging. The term nuclear magnetic resonance refers to the process by which the nuclei within a magnetic field absorb and emit electromagnetic energy. Applying an alternating voltage of the correct frequency to a radio-frequency (RF) coil creates a second, much weaker, alternating field \( (B_1) \) which, in an MR system, is aligned perpendicular to the external field \( (B_0) \). This is termed the RF pulse. Before commencing a MR examination the system will calculate the precessional frequency (centre frequency) of the subject within the magnet bore to determine the frequency of the RF pulse to be utilised. The oscillating field is rotated at the Larmor frequency in the XY plane, perpendicular to \( B_0 \). This will change the net magnetisation vector which spirals from alignment with the \( B_0 \) field into a rotational motion in the XY plane. (Ridgway 2010) The strength (amplitude) and duration of the RF pulse will determine the angle of the rotation, which is termed flip angle in MR imaging. This process is known as excitation.

![Figure 2-3 The RF pulse rotates net magnetisation from the Z axis into the XY axis. The angle of rotation is dependent upon the duration and amplitude of the pulse.](image)

Although within an external magnetic field the precessional frequency of individual nuclei will be the same their precession is out of phase such that transverse magnetic
vectors cancel. The alternating, or rotating, RF pulse however not only changes magnetisation along the Z axis. As the applied field is rotating at the Larmor frequency of the spins it creates phase coherence, i.e. they start to spin in phase thus generating net magnetisation transverse to the Z axis (designated Mxy). This happens simultaneously with the loss in magnetisation in the Z axis. The rotating transverse magnetisation induces a voltage which can be detected by a receiver antenna which is used to provide the raw data for the MR image.

2.3 Longitudinal relaxation and T1

When the RF field is turned off the spins revert back to their lower state or equilibrium spontaneously over a period of time emitting photons of energy at the Larmor frequency. Thus the net magnetisation realigns with the Z axis, the speed of which is determined by the surrounding protons (or tissue in the case of clinical MR imaging). This transition is termed longitudinal relaxation and is due to the exchange of energy with the environment or ‘lattice’. Accordingly this process can be referred to as spin-lattice relaxation. The process is exponential and the time constant describing this return to equilibrium of the longitudinal magnetization along the Z axis is termed T1. The value of the magnetisation is given by 

\[ M_z = M_0(1 - \frac{1}{e}) \]

where \( M_0 \) is the equilibrium value. At the time T1 63% of the original longitudinal magnetisation has recovered. Protons in large molecules such as lipids or proteins have fairly short T1 values as they are tightly bound to adjacent molecules and more sensitive to field fluctuations (due to induced fields from neighbouring nuclei and unpaired electrons). This means they can exchange energy to their surroundings and change their spin states rapidly. In contrast, protons in water are more mobile and less affected by field fluctuation and therefore change their spin state less readily. The fact that different tissues have a unique T1 is utilised in MR imaging and provides intrinsic contrast. For example the T1 for normal myocardium has been measured to be between 1000ms and 1100ms at 1.5T and between 1200ms and 1500ms at 3T. For blood it is between 1400ms and 1600ms at 1.5T and between 1600ms and 2100ms at 3T. (Stanisz 2005, Sharma 2006, Sibley 2011)
2.4 Transverse relaxation and T2

After the RF pulse has ceased the spins also rapidly de-phase causing the transverse magnetization, and therefore the signal, to decay. This is termed free induction decay (FID) and is caused by the interaction between neighbouring spins (spin-spin relaxation). As with longitudinal relaxation the process is exponential and the decay time is defined by the time constant T2 where transverse magnetisation has reduced to 37% of the maximal value (Mxy(t) = MxyMax e^{-t/T2}). The decay is dependent upon how the proton is bound, protons in fat and other solid tissues will generally dephase much more quickly than those in liquids. The loss in phase coherence thus loss in signal is accelerated by the proximal fluctuating magnetic fields, generally due to protons in large molecules, frequently encountered in biological systems. These localised non-uniformities cause a slight change in the precessional frequency reducing phase coherence and transverse magnetisation. The combination of T2 and other dephasing factors is termed T2* substituting T2* for T2 in the previous equation. T2* is always shorter than T2 which is always shorter that T1. Different scan acquisition sequences utilise the difference in these relaxation parameters for different tissues to provide image contrast.
Figure 2-4 The 90° RF pulse rotates net magnetisation from the Z axis into the XY plane. Longitudinal net magnetisation returns to equilibrium slowly, the time constant T1 represents when 63% of the original magnetisation has recovered. Transverse magnetisation returns rapidly to baseline through a process termed free induction decay and is represented by the time constant T2* when it has reduced to 37% of the maximal value.
2.5 The MR scanner

The MR system is composed of four basic functional units. These are:

• The magnet, which for modern medical systems is a superconducting electro-magnet.

• The RF system which consists of RF transmit and receive antennae and amplifiers

• A gradient system to induce field gradients in all three spatial directions for spatial encoding.

• A computer system for acquisition and image processing.

![Diagrammatic representation of a MR system](image)

Figure 2-5 Diagrammatic representation of a MR system

2.5.1 Main magnet

MR systems can be in the form of a tunnel or an open system and there are specialised systems for scanning extremities or for animal work. Tunnel based systems are used for CMR as the magnetic field tends to be stronger and more homogeneous.
A clinical MR system consists of a super-conducting magnet (electromagnet) which induces a static homogeneous magnetic field. It is made from coils of conducting wires (niobium/titanium alloy in copper). It is cooled by liquid helium to almost absolute zero at which point the wires lose electrical resistance thus once the required field strength is attained no further electric input is necessary (hence the term super-conducting). Homogeneity of the field is of paramount importance as inhomogeneities cause distortion in spatial encoding. The system must be shielded from extraneous magnetic or RF activity which will interfere with scanner performance. In addition shielding will prevent magnetic and RF interference from the scanner affecting nearby equipment. The system is therefore placed in Faraday cage which blocks radio waves.

Newer scanners also have active magnetic shielding integral to the scanner. These use additional coils at each end of the magnet to reduce the fringe fields for safety reasons.

### 2.5.1.1 Shimming

As the perfect magnet does not exist homogeneity is improved by a process termed shimming. Passive shimming involves placing ferromagnetic material around the main magnet, usually during scanner set-up. Active shimming uses shim coils to induce small currents to compensate for small induced inhomogeneities during scanner use.

### 2.5.2 Gradient Coils

Spatial localisation is determined by using the gradient coils. These coils are placed in each spatial direction and superimpose linear variation to the main magnetic field. This in turn alters the resonance frequency and spin phase in proportion to the local field allowing spatial differentiation. The performance of the gradient coil is very important as it affects image quality and the ability to perform rapid imaging. Gradient performance is determined by the peak gradient and slew rate (the gradient switching speed measured in mT/m). Generally amplitude and slew rate increase with increasing main magnetic field strength but the gradient system itself is a determinant factor. Gradient systems can be upgraded without changing the main magnet. It is the vibration of the gradient coils and their surroundings during switching that causes the characteristic noise of the MR scanner.
The rapid gradient changes can induce electrical currents in nearby conducting materials (Eddy currents) which in turn can affect the gradient fields and hence the image quality. This can be countered by active coil shielding or manipulating the coil currents to offset Eddy currents.

2.5.3 RF Coils

The RF pulses that energise the protons in the magnetic field are produced by the body coil within the bore of the magnet. The body coil is a transmit-receive coil (i.e. comprises transmit and receive antennae) which produces the excitation pulse as well as receiving the MR signal (from net spin in the XY direction). The RF system also incorporates transmit and receive amplifiers to ramp up the signals. The body coil has a large measurement field but low signal to noise ratio (SNR). A stronger signal is detected by placing a coil closer to the area of interest. In addition smaller volume samples give a better SNR. For this reason smaller surface or volume coils, either transmit-receive or receive only, tend to be used for focused examinations. Volume coils encompass the body part to be scanned whereas surface coils are placed directly over the area of interest. The trade-off for smaller coils is that signal uniformity decrease rapidly away from the coil itself thus limiting the scan field. Array coils, which are made up of small coil elements which can be summated, combine a higher SNR with a larger measurement field. For CMR the receiver coil is usually an array of four or more coils placed directly on the chest.

2.5.4 Image formatting

The received analog signal needs to be digitised by an analog to digital converter for processing. The digital data are then stored, according to phase and frequency, in a temporary spatial domain termed k-space. K-space is filled with the raw data during the scan, usually one line per TR and with the same number of rows and columns as the final image. 2D Fourier transformation of the raw data in k-space produces the image. The centre data in k-space contribute to the rough shape of the structure and contrast. The more marginal k-space points determine resolution. Other than linear filling, k-space can be filled centrically, radially, spirally and in numerous other ways.
Figure 2-6, k-space is a matrix which is filled by the raw frequency and phase information. This is then Fourier transformed to produce the image.

2.6 Sequences used in CMR

2.6.1 The fundamentals of image acquisition

Simplistically a magnetic resonance image can be thought of as a proton map. Image formation relies on the correlation of signal intensity and spatial information derived from proton density coupled with spin manipulation. Once the sample (or patient) has been placed within the main magnetic field a complex interaction between operator, computer system, gradient fields and RF pulses produces a bounty of imaging possibilities. By manipulating the acquisition protocols the intrinsic contrast potential of the modality can be exploited to show gross anatomy, function and tissue characteristics.

2.6.1.1 Slice selection

If a patient is in a uniform magnetic field all proton spins would be similarly excited by the applied RF pulse and image formation impossible. Similar to most other medical imaging modalities MR acquires tomographic images. The first step in obtaining an image is to select the focal plane. This is achieved by activating the slice encoding, or select, gradient. As previously stated the precessional frequency is proportional to the
applied external field strength. By applying a gradient perpendicular to the desired image plane, protons in every plane will precess at a different frequency determined by the local field strength. A slice select RF pulse is applied at the same time as the gradient. Only the protons precessing at the same frequency as the applied RF pulse will absorb energy and therefore a signal is only generated within that selected slice. The thickness of the slice is determined by the range of frequencies within the RF pulse, the bandwidth, and the slope of the slice select gradient (a steeper gradient excites a thinner slice). Slice orientation is defined by changing the centre frequency of the RF pulse to correspond to that of the desired location.

Figure 2-7 Slice selection: The gradient coils induce a magnetic gradient along the Z axis. Precessional frequencies will vary along the direction of the gradient. Only the protons precessing at the same frequency as the applied RF coil will be excited.
2.6.1.2 Phase encoding

The tomographic section or slice has now been isolated but it is not yet possible to
determine from where within the slice a particular signal comes from. The next step is
to sort the signals according to their origin within the slice. While the spins are still in
the transverse plane after the RF pulse and slice select gradient a gradient field is
applied briefly in the chosen phase encoding direction, usually designated the y axis.
The phase change along the column depends on the polarity and amplitude of the
gradient. After each phase encoding a frequency encoding is acquired. A series of
phase encoding gradients are applied sequentially with differing gradients. The symbol
used in sequence diagrams to represent phase encoding is a diagrammatic
representation of the change in gradient magnitude and polarity (figure 2-8). The
number of steps is dependent on the field of view (FOV) and desired resolution (which
is proportional to the number of phase encoding levels used). The phase encoding for
each column along the y axis is performed separately, therefore to complete the slice
acquisition the complete process of slice select, phase encoding and frequency
encoding needs to be repeated. These phase encoding steps are termed repetitions and
there are usually between 128 and 256 for cardiac imaging. The duration is termed the
repetition time (TR). The resultant difference in the phase of the magnetisation vector
along the y axis is used during Fourier transformation.
2.6.1.3 Frequency encoding

A frequency encoding gradient is applied to spatially encode in the other perpendicular direction to phase encoding. The frequency encoding gradient (or readout gradient) is applied during signal acquisition. It imparts a changing precessional frequency along the direction of the gradient thus providing spatial information in for this example the x axis. If the final image has 256 pixels across in the frequency encode direction then 256 frequencies are differentiated within the signal, i.e. one frequency per pixel is defined.

Figure 2-8 A gradient in the Y axis produces incremental phase encoding steps. A compressed representation of the above diagram is used to depict the phase encoding steps in a sequence diagram.
The phase and frequency encode gradients are always applied perpendicular to each other and the slice selection gradient direction. However, the three gradients can be rotated to any angle to allow any slice plane to be acquired. This constitutes an advantage of gradient based MR technology over other imaging modalities. The sequence of pulses is repeated, usually 128 or 256 times, with different phase encode amplitudes, to generate an image. The timing between repetitions is termed the repetition time (TR).

![Gradient induced phase encoding along the Y axis and frequency along the x axis provides spatial information.](image)

2.6.1.4 Contrast manipulation

Image contrast is largely dependent upon T1 and T2 and how much relaxation of each has occurred before readout and subsequently applying the next excitation. If the TE is very short and very little dephasing has occurred T2 will have little effect on tissue contrast. If in addition the TR is short tissue with a longer T1 will not have regained much longitudinal magnetism to be used for the next excitation. This will result in low
signal in the final image. The contrast in this case will be determined by the T1 of the constituent tissues and the acquisition is described as T1 weighted. Lengthening the TE allows T2 to cause dephasing for longer which will reduce signal from tissues with a shorter T2. If the TR is also long longitudinal relaxation will have recovered for most tissues and T1 plays little part in the image contrast. The image contrast is now dependent upon the TE and therefore T2 and is said to be T2 weighted. Proton density weighted contrast is achieved by having a short TE and long TR eliminating most of the effects of T1 and T2 and signal strength is dependent upon the number of proton present. The type of weighting selected depends on what is to be demonstrated by the image. Further contrast manipulation can be obtained by using a contrast medium such as Gd-DTPA which reduces the T1 and gives a bright signal on T1 weighted images.

2.6.1.5 Cardiac gating

Motion artefacts due to respiratory motion can be eliminated by breath-holding if the acquisition is short enough. There is still a problem with cardiac motion and unless the acquisition time is extremely fast blurring of the image will occur. Cardiac gating synchronises the beginning of the acquisition with the R wave of the electrocardiogram (ECG).

2.6.2 Basic sequence steps

An example of a very simple imaging sequence is shown below. Using the three perpendicular gradients the imaging slice is selected and spatially encoded. Firstly a slice select gradient is applied along with a RF pulse of sufficient amplitude and duration to rotate the net magnetisation through 90° into the XY plane. Spatial encoding is achieved by the application of a phase encoding followed by a frequency encoding gradient in the other two perpendicular planes. The resultant FID signal can be detected using a RF receiver coil.
Figure 2-10 A schematic diagram of a basic sequence showing the order of steps to produce the image. The FID decay signal is sampled by a receiver coil. The graphic depicting the phase encoding represents the multiple phase encoding steps.

Although the FID can be utilised as a signal for imaging, the problem with this basic scan sequence is that signal decay, especially in a biological system, is very rapid due to dephasing caused by field inhomogeneities. In addition the magnetic field gradients used to create the signal further disrupt the FID. (Ridgway 2010) The time in which a signal can be detected after the RF pulse is therefore limited to just the latter part of the signal, resulting in a relatively poor SNR. This can be compensated for by generating an echo by applying field gradients or a 180° refocusing RF pulse.

2.6.2.1 Gradient echo sequences

Gradient echo (GRE) sequences are produced by the application of bipolar gradient pulses. The first gradient causes protons to lose phase coherence along the direction of
the gradient which causes the FID signal to drop. Applying a second gradient of the same amplitude and in the same direction but of opposite polarity will re-phase the spins with a resultant reappearance of the signal (echo). If the gradient continues to be applied spins will once again lose coherence and the signal will drop again. In a standard gradient echo acquisition the basic pulse sequence is repeated with different phase encoding gradients until k-space is filled. For gradient echo imaging the slice select pulse is usually between 5° and 60° and this factor, along with the lack of a 180° refocusing pulse, permits a much shorter TR. Contrast and signal strength are determined by the flip angle and longitudinal magnetisation. A larger flip angle gives more T1 weighting. Spoiler gradients can be applied at the end of the sequence to dephase remaining spins and ensure only longitudinal magnetisation is present when the next RF pulse is applied. Alternatively RF phase cycling (RF spoiling) can be used to minimise the transverse magnetisation.

Figure 2-11 The gradient echo sequence uses gradients of opposite polarity to dephase then rephase the spins to produce a gradient echo.
Gradient mediated refocusing is fast and is therefore suitable for dynamic imaging. As the TR is very short the low flip angle is necessary. Basically the images become T2* weighted but as the TE is also very short this is not a problem. The trade off is loss in SNR and an increase in signal loss and magnetic susceptibility artefacts induced by magnetic field inhomogeneity compared with spin echo sequences. This factor however plays a positive role in CMR based tissue iron overload evaluation. FLASH imaging (fast low angle shot) is an example of this type of sequence and was used for CMR functional imaging before balanced steady state free precession (SSFP) acquisition methods became available.

2.6.2.2 SSFP imaging

SSFP is used to describe a gradient echo sequence whereby a non-zero steady state is achieved for both longitudinal and transverse magnetisation. The flip angle tends to be higher than other GRE sequences. The TR is shorter than T1 and T2. Transverse magnetisation is not lost or spoiled but rephased at the time of the next excitation. This is added to the transverse magnetisation induced by the following RF pulse. Repeating this process results in a decreasing Mz component until a steady state is achieved for both longitudinal and transverse components. The steady state is maintained by the relationship between flip angle and TR. T2* and T1 properties determine contrast. This is influenced by the TR which is selected to optimise contrast in the selected slice. TrueFISP (true fast imaging with steady state precession) is an example of a SSFP sequence and is available on Siemens scanners. SSFP sequences are rapid with excellent blood to myocardial contrast due to the relatively low myocardial signal compared with high signal from blood and fat. They are therefore ideal for cardiac imaging. There are problems with susceptibility artefacts although these can usually be counteracted by shim and RF frequency adjustments.
2.6.2.3 Spin echo sequences

Spin echoes are generated by applying a RF pulse that rotates the magnetisation through $180^\circ$ after the run time $\tau$ following the $90^\circ$ pulse.

Figure 2-13 Diagrammatic representation of a spin echo sequence.
This results in a recovery of phase coherence lost by magnetic field inhomogeneities and therefore transverse magnetisation which reaches its maximum at a time $2\tau$ producing what is termed a spin echo. In other words it removes the $T2^*$ effects therefore relaxation is dependent upon the $T2$ of the tissue. This time to maximal signal is termed the spin echo time and this is normally arranged to coincide with the imaging echo time (TE). The frequency encoding gradient is applied once again during the spin echo and the signal from the echo is read out.

Figure 2-14 The $180^\circ$ pulse re-phases the protons thus prolonging transverse relaxation and therefore signal.

The disadvantage is the increase in scan time due to the additional step and the requirement to wait for $M_z$ to recover prior to repeating the sequence.

**2.6.2.4 Multi-slice spin echo sequences**

Spin echo sequences are routinely used for anatomical imaging. Most investigations involve the exploration of entire body parts or organs requiring multiple tomographic sections. If each slice was acquired separately the time burden would be huge as $T1$ needs to recover to ensure sufficient longitudinal magnetisation for the next excitation. To get around this the next slice is selected and excited while the first recovers and so on through the stack of planes to be imaged. This process is repeated until $k$-space is filled for each slice.
2.6.2.5 Turbo spin echo

Scan time can be further reduced by inducing a chain of 180° pulses after the 90° pulse in a multi-echo sequence. This produces a series of echoes of reducing amplitude while T2 relaxation continues allowing several lines of k-space to be filled per excitation. The T2 is generally long enough for a relatively large number of echoes to be collected. For an echo train length (or turbo factor) of 5 the scan will take 1/5 of the time which is useful for cardiac work as it generally can be acquired during a breath-hold.

2.6.2.6 HASTE sequences

These are rapid single shot acquisitions in which an image slice can be obtained in less than one second. The acronym stands for Half Fourier Acquisition single Shot Turbo spin Echo (HASTE). A single 90° pulse is followed by as many 180° as there are lines of k-space to be filled. Further sequence acceleration is attained by partial k-space filling. If just over half of k-space is acquired, based on a couple of reasonable assumptions, it is possible to calculate the remaining k-space at the expense of signal to noise. An image can be acquired every cardiac cycle meaning that the sequence is insensitive to respiratory motion. This acquisition is useful for obtaining an overview of gross anatomy and for subsequent scan plane set up.

2.6.3 Prepulses

2.6.3.1 Inversion recovery sequences

Inversion recovery (IR) sequences are sequences in which the 90° pulse is preceded by a 180° inversion pulse. The initial pulse flips the magnetisation through 180° in the Z axis. Ensuing relaxation is thus only T1 recovery as there is no x-y magnetisation. This recovery proceeds for a time known as the inversion time (TI). This is in the order of T1 after which the imaging sequence is applied. IR sequences therefore usually have a long TR but short TE such that image contrast depends mainly upon the TI. The prolonged T1 time separates the recovery curves of different tissues thereby creating very good T1 contrast. Manipulation of the inversion time is utilised for assessment of tissue viability post contrast injection.
2.6.3.2 STIR

Short TI (sometimes called short Tau or inter-pulse time) inversion recovery sequences suppress the signal from fat by selecting a TI where the magnetisation of fat is zero so none is available to flip into the XY plane. On the resultant image fat will appear dark but fluids that tend to have a long T1 and T2, will be bright. This makes the sequence sensitive for the detection of fluid which is useful for the detection of inflammation.

2.6.4 T2* sequences

2.6.4.1 Bright blood

2.6.4.1.1 Multiple breath hold single echo

Gradient echo are faster than spin echo sequences consequently motion artefacts are reduced. They are also very sensitive to local field inhomogeneities which are increased by the presence of iron particles. Based on these factors a gradient echo sequence was developed in this unit.(Anderson 2001) For the pilot study a 10mm thick single mid ventricular short axis slice was imaged at nine different echo times (TE 5.6-17.6) during nine separate breath-holds. The longer the echo time the less transverse magnetism there is left to give a signal. With increasing iron levels protons dephase earlier due to the increased local field inhomogeneity so that signal is lost even with shorter echo times for the most iron overloaded tissue. The acquisition is gated to the R-wave so that images are acquired during diastole where cardiac motion should be minimal. Although the reproducibility of this technique is generally good there can be problems with miss-registration due to breath-hold position inconsistencies. It is also time consuming as each scan lasts about 10-15 seconds and needs to be repeated for each TE.

2.6.4.1.2 Single breath hold multi echo

Improved gradient performance has lead to the subsequent development of a single breath-hold multi-echo sequence. This has addressed the issues associated with the multiple breath-hold sequence allowing all the images to be acquired during one breath-hold. (Westwood 2003) Instead of having one echo time per acquisition a series of
gradients are applied resulting in a train of echoes with increasing TEs. This means that the T2* decay and therefore iron loading can be assessed in one short acquisition.

![Diagram of multi-echo sequence](image)

**Figure 2-15** The multi-echo sequence uses a train of different echo times that are acquired during a single breath-hold.

### 2.6.4.2 Black blood T2*

The reproducibility of the single breath-hold sequence is very good (Westwood 2003) but contrast between the myocardium and blood pool may be sub-optimal and despite flow compensation, artefacts from blood and motion may compromise accuracy. A black blood sequence which suppresses the blood signal has been developed to improve image quality. (He 2007) To achieve blood signal nulling a double inversion recovery (DIR) preparation is used. A non selective 180° pulse is applied on the R wave which inverts the spins within the RF coil volume. The inversion time is set to null the blood signal. The second selective 180° pulse restores magnetisation within the selected slice. This means spins outside the slice are inverted but magnetisation is restored within selected slice. Blood motion within the heart is rapid. In the time between inversion and
acquisition the blood within the selected sliced is replaced by that previously inverted and is nulled at the time of imaging and appears black on the image.

### 2.6.5 Myocardial tagging

The use of spin tagging to produce saturated bands within the myocardium was first described in 1988. (Zerhouni 1988) This concept was further developed to produce a series of parallel saturation bands throughout the image using spatial modulation of magnetisation (SPAMM). (Axel 1989)

### 2.6.6 Imaging using a contrast agent

Gadolinium is a lanthanide element. It is the most commonly used intravenous MR contrast agent. This is due to the affect on the relaxation time in the tissues permeated. The T1 relaxation time is reduced which increases signal intensity (SI) on T1 weighted images. Although highly toxic, the Gd3+ ion can be chelated with diethyleneetriaminepentaacetic acid (DTPA) to form Gd-DTPA. This is a very stable compound with retained paramagnetic properties. It is generally safe however there have been reports of nephrogenic systemic fibrosis (NSF) and nephrogenic fibrosing dermopathy (NFD) occurring in patients with severely impaired renal function. This is thought to be due to the prolonged time the compound is in the body and the subsequent breakdown of the chelate. Gadolinium containing contrast agents have been classified as high, medium and low risk for the development of NSF according to current evidence. As a consequence risk minimising measures have been advocated. Renal function should be assessed prior to receiving a high risk compound and is advisable with medium and low risk agent, particularly in patients of ≥ 65 years. The use of a high risk agent is contra-indicated in patients with severe renal impairment, if it is necessary to use a medium/low risk agent the minimal dose should be used and not repeated within 7 days. There is no supporting evidence that haemodialysis plays a role in the prevention of NSF in patients not already undergoing this treatment. A full list of risk minimisation measures can be found on the MHRA website. (MHRA 2010) First pass imaging is used for CMR angiography and myocardial perfusion. Late Gd-DTPA enhancement highlights areas of tissue damage and fibrosis.
2.6.7 Acquisition acceleration

2.6.7.1 Echo planar imaging

Echo planar imaging (EPI) is an ultra fast imaging technique that can use a single excitation pulse to fill k-space and therefore acquire the entire image. Alternatively, hybrid EPI can be applied to acquire a number of echoes or k-space lines after each excitation. For both, a bi-polar gradient induces a train of gradient echoes, the number of which is termed the EPI factor. The T2* is relatively short limiting the readout to a maximum of between 64 and 128 echoes. Data rows in k-space are filled in alternating directions. Applying the phase encoding gradient for a short “blip” prepares for the filling of the next line in k-space. Image acquisition can be as short as 50 ms which generally makes the acquisition relatively motion insensitive. As EPI is the read-out part of the sequence contrast can be obtained by combination with selected preparation pulses. EPI is used frequently for myocardial perfusion imaging which requires the collection of a number of complete image slices every cardiac cycle.

2.6.7.2 Parallel imaging

The duration of a MR acquisition is largely dependent upon the phase encoding step which in turn depends on the gradient switching rate. The speed of image generation can be accelerated by using a parallel acquisition technique using an array coil. This is achieved by reducing the phase encoding steps and filling missed data rows by using individual coil information or interpolated data. There is some loss of SNR with parallel imaging but this is offset by the ability to obtain more data in a limited time span, for example a breath-hold.

2.7 Clinical applications

2.7.1 History

The phenomenon of nuclear magnetic resonance (NMR) was first described by Rabi in the 1930s and further explored in the 1940s by Bloch and Purcell. (Rabi II 1938, Bloch 1946, Purcell 1946)The combination of a magnetic field and RF waves were observed to cause atoms to emit a radio signal. In 1971 Damadian noted that the signal from ex-
vivo cancerous tissue differed from that of normal tissue and later in the 1970s the first in vivo scan was performed. (Damadian 1971, Damadian 1977) Since the early 1980s the development and use of magnetic resonance imaging for clinical purposes has exploded (the word nuclear was dropped because of negative associations). Attempts at cardiac imaging were originally disappointing due to cardiac motion and respiratory artefacts. The development of better hardware and gradient performance led to faster scanning with the possibility of the data being acquired within a breath-hold. ECG triggering meant cardiac motion was no longer such a problem. MR imaging of the heart became a sub-specialty akin to echocardiography and is now referred to as cardiovascular magnetic resonance (CMR). The society for cardiovascular magnetic resonance (SCMR), set up in 1996, has its own academic journal and the British society of CMR (BSCMR) was established in 2004 with 62 active centres within the UK (BSCMR newsletter June 2011).

2.7.2 Overview

CMR does not use ionising radiation which is a major benefit over radionuclide or fluoroscopy based imaging modalities. This is an important consideration when sequential studies are required, particularly in children. Additionally spatial orientation is not limited and there is not the tissue attenuation problem associated with radionuclide or echo imaging. Any part of the heart can be imaged in any plane due to the use of gradient technology. The 3D contiguous data sets provided by CMR are of particular importance in the evaluation of complex anatomy in congenital heart disease. CMR is less operator dependant than echo but does require a thorough knowledge of anatomy. Intrinsic contrast can be manipulated to highlight disease pathology. Injecting a contrast agent gives even more possibilities. Negative aspects include the relatively long duration of most imaging protocols and therefore time needed to acquire an image. Temporal resolution for cine imaging is not as good as echocardiography and is generally not real-time which means it may not be the investigation of choice for looking at valve motion for example. Patients need to be within the tunnel bore for the duration of the examination which can be problematic for patients who are claustrophobic or have difficulty lying flat due to breathlessness or musculoskeletal discomfort. In addition the magnetic field is a potentially dangerous environment. A
strong field can turn ferrous objects into projectiles and although extremely infrequent, this has on occasion resulted in death. There are certain contra-indications to having a scan such as the presence of ferrous objects in areas of the body that may be damaged by field induced movement. These include cerebral clips or ocular metal fragments. Having an implanted non MR compatible pacemaker or defibrillator has been regarded as an absolute contra-indication however MR compatible pacemakers are now available. Generally well seated metallic implanted devices are considered safe. Scanning critically ill patients can cause problems as monitoring may be challenging and the patient must be removed from the scan room to administer cardio-pulmonary resuscitation.

Despite the perceived risks and challenges associated with CMR its use has become established in clinical cardiology and its use is increasing continually. It is considered the investigation of choice for cardiac functional assessment and myocardial viability. In fact CMR is now the gold standard for cardiac volumetric and mass measurement and the assessment of systolic functional parameters. Spin echo imaging plays a relatively small role in CMR compared with imaging of other body regions. This is due to the images being degraded by cardiac and respiratory motion. Manipulation of spin echo sequences, as described previously, can shorten acquisition times sufficiently to provide useful information, for example TSE, however most sequences used to visualise the heart use gradient echo technology. Apart from the description of congenital malformations most information required from CMR pertains to function.

2.7.3 CMR for functional assessment

This is where SSFP sequences play centre stage for the accurate depiction of volumetric parameters and regional abnormalities. The availability of 3-D data cine sets allows assessment of global function of both left and right ventricles. The analysis of tagged data gives information on the myocardial velocity derived strain parameters which are analogous to those derived using tissue Doppler imaging. Initially analysis was largely visual or by performing time consuming manual tag tracking. Harmonic phase MRI (HARP) uses the fact that image phase is related to motion and spatial derivatives are calculated for each pixel to produce displacement maps. Regional cardiac motion is
thus decoded during Fourier transformation and information on global function and regional strain derived. When superimposed on LAX and SAX cine images in plane motion can be characterised as longitudinal, radial or circumferential. Resultant systolic and diastolic strain patterns are sensitive to early functional change as the sub-endocardial region is more prone to deformational abnormality than the sub-epicardium.

2.7.4 Tissue characterization

Turbo spin-echo sequences with T1 and T2 weighting can be used with and without fat suppression to visualise fibrous or fatty infiltration within the myocardium. The fat will appear bright on the image. Fibro-fatty replacement of the myocardium is one of the features of arrhythmogenic right ventricular cardiomyopathy but it also occurs with ischaemic heart disease, Chagas’ disease and neuro-muscular disorders and may represent the end point for myocyte injury. (Vatta 2007) STIR sequences highlight areas of increased fluid identifying regions of inflammation.

2.7.4.1 Late enhancement

The use of Gd-DTPA in acute ischaemia was first described in 1984 using an animal model. (McNamara 1984) The ischaemic zone showed a well defined segment of high intensity after Gd-DTPA injection compared with normal tissue. In a later clinical study of acute MI an increased signal was noted in the infarcted area 5-10 days post event but not in chronically infarcted tissue however the group used spin-echo sequences at 0.35 or 0.5T and each scan took at least 2 hours. (Eichstaedt 1989) With the improvement in scanner hardware and acquisition software late enhancement with Gd-DTPA has become the investigation of choice for the identification and sizing of myocardial infarcts. Gd-DTPA based contrast agents pass through the normal myocardium fairly rapidly, not crossing normal cell membranes. It accumulates however where the interstitial space is increased due to cell damage, loss of cell membrane integrity and fibrosis. Segmented inversion recovery GRE sequences show small areas of damage not detectable by other imaging techniques. (Mahrholdt 2002) The high spatial resolution permits differentiation between viable and non-viable tissue and delineates transmurality. (Kim 2000, Simonetti 2001) This has been shown to identify patients
who will benefit from revascularisation therapy. Standardisation of protocols has provided a robust and reproducible technique. (Wagner 2006) Late enhancement with Gd-DTPA has also been reported in patients with necrosis and fibrosis associated with a myocarditic process. The pattern of distribution can distinguish the injury from that associated with ischaemic changes. (Friedrich 2009) A wide range of cardiomyopathic aetiologies have shown characteristic patterns of LGE. In dilated cardiomyopathy (DCM) the majority of patients have no LGE but those that do, tend to have a mid-wall distribution unlike IHD and these patients have a worse outcome. (McCrohon 2003, Assomull 2006) Mid-wall LGE has also been reported in patients who have received anthracycline treatment for cancer. (Perel 2006) In hypertrophic cardiomyopathy (HCM) the extent of LGE is also associated with outcome. (Moon 2003) These findings potentiate appropriate intervention, for example the implantation of a defibrillator. LGE has been reported in patients with muscular dystrophies. (Varghese 2004, Silva 2007, Mavrogeni 2009) Cardiac involvement in the myopathic process leads to fibre necrosis and fibro-fatty replacement. Pre-clinical detection of cardiac fibrosis can determine the need for therapeutic intervention.

2.7.4.2 Iron overload assessment

Loss of signal on MR images due to tissue iron was first noted in the 1980s (Stark 1983, Brasch 1984). This is due to the fact that iron in the form of ferritin and haemosiderin shortens proton relaxation times, in particular T2. This is termed susceptibility induced relaxation. The relaxation time is inversely proportional to iron concentration. Initial studies used spin-echo sequences and iron burden was assessed by either measuring T2 or comparing signal intensity with skeletal muscle which does not accumulate iron to give a signal intensity ratio. (Chezmar 1990, Mavrogeni 1998, Jensen 2001) Liver imaging and iron assessment was reliable with good correlation with biopsy results. It was less successful for the heart as the spin echo sequences used required long acquisition times and were motion sensitive causing image degradation by respiratory and cardiac motion artefacts. Gradient echo CMR can measure the relaxation parameter T2* decay over a range of echo times. The first T2* sequence described was a multi breath-hold mid-ventricular short axis acquisition with zero delay R-wave triggering resulting in end-diastolic imaging. (Anderson 2001) A single breath-
hold sequence was later developed which reduced the scan time, improved image registration between images, and had good reproducibility. (Westwood 2003) Signal decay caused by field inhomogeneities induced by tissue iron can be plotted, a decay time constant of <20ms is considered to be indicative of iron overload. (Anderson 2001) This technique has become the mainstay of clinical evaluation and follow-up of thalassaemia major (TM) patients.

Indeed, over the last decade, there has been a marked reduction in deaths attributable to tissue iron overload within the UK which coincides directly with the introduction of T2* CMR as a diagnostic tool. (Modell 2008) Prior to CMR use, efficacy of chelation therapy and dose adjustment of was based on LIC or ferritin levels. Despite apparently optimal therapy patients still developed heart failure and with the use of CMR it became clear that these parameters do not reflect myocardial iron burden. Life expectancy for patients in high income countries with good compliance to therapy is now over 50 years, although survival in low income areas is still poor. (Telfer 2009). The protocol has been installed onto CMR scanners from different vendors at multiple sites throughout the world with reproducible results, (Kirk 2010) and has been used to investigate the cardiac efficacy of chelating agents in multi-centre trials. (Pennell 2006, Tanner 2007, Pennell 2010, Pennell 2011) A more recently developed DIR black blood sequence with mid diastolic timing showed good reproducibility in a pilot study as blood signal is suppressed thus reducing artefacts, a systematic review of comparative reproducibility forms part of this thesis.

The involvement of iron in anthracycline cardiotoxicity has been reported in several in vivo and in vitro studies. The suggested mechanism is the impairment of iron metabolism pathways leading to the accumulation of iron in Cardiomyocytes and the reaction of anthracyclines with iron producing free radicals. (Minotti 1998, Minotti 2001, Kwok 2003, 2004, Xu 2005, Cardinale 2008) Reports of the efficacy of iron chelators in preventing anthracycline induced heart disease are conflicting. (Hershko 1996, Kwok 2000, Bryant 2007a, Bryant 2007b, Kaiserova 2007, Popelova 2008, Popelova 2009) There is a consensus however that a better understanding of the pathogenesis of anthracycline induced cardiomyopathy and the role of iron is needed
and to date no study has reported the follow-up of patients using CMR assessed iron loading.
Chapter 3: Classification of Cardiomyopathy

3.1 Background

The classification of heart muscle disease is complicated and often contradictory, because there are a large number of heterogeneous conditions, with a lack of consistency in inclusion. (Richardson 1996, Maron 2006) In 1980 the World Health Organisation (WHO) and ISFC reported that the term cardiomyopathy (CM) should be restricted to diseases involving heart muscle of unknown aetiology, (WHO 1980) and other diseases affecting the myocardium, for example those associated with inflammatory or metabolic disorders, should be termed "specific heart muscle disease". Over the past 30 years however our understanding of the interaction between genotype and other factors has lead to a blurring of this distinction and this definition is now considered obsolete. Causative mechanisms have been implicated for diseases hitherto of unknown aetiology, and most workers in the field now consider CM to be "disease of the myocardium", but with the notable exception of myocardial dysfunction related to ischaemia (although the term ischaemic cardiomyopathy is remarkably persistent especially in the USA).

Improved phenotypic description due to improvements in imaging technology over the past few decades can take a large proportion of credit for this partial solution to the conundrum, as the sensitivity for identifying clinically silent abnormalities has greatly improved. Advances in genetic linkage and molecular studies have also taken our understanding forward. For example, channelopathies are due to ion channel protein mutations causing life threatening arrhythmias. The cellular substrate for the disease cannot be made by biopsy or autopsy, but they are now considered cardiomyopathies by some expert panels. (Maron 2006)

Hypertrophic cardiomyopathy (HCM) was first described over 100 years ago but was largely ignored until the late 1950s when Brock and Teare made surgical and pathological reports. (Brock 1957, Teare 1958) The later discovery of more than one affected individual within a family led to genetic linkage studies and screening within families. Many genes are now implicated and we now know that patients carrying a
mutated gene can be phenotypically normal suggesting incomplete penetration, late age of expression or an extraneous catalyst mechanism.

Until fairly recently dilated cardiomyopathy (DCM) was considered ‘idiopathic’ with no identifiable aetiology. Again it was noted that there tended to be more than one affected individual within a family which instigated family screening programs. A large study in London (Keeling 1995) showed that 29% of asymptomatic relatives of DCM patients had abnormal echocardiograms and 20% had left ventricular enlargement as defined by echocardiographic criteria. Of these, 27% became symptomatic over the following 3 years. Events included 1 sudden death and one relative requiring cardiac transplantation. In another study anti-heart antibodies were found to be an independent predictor of progression to disease in asymptomatic relatives with normal echocardiographic examinations. (Caforio 2007) It is now known that approximately 25% of DCM patients have a genetic causation (about 40 genes implicated) with new genes regularly being discovered. (Michels 1993, Keeling 1994, Goerss 1995, Baig 1998)

Overall, a pragmatic approach is to divide the cardiomyopathies that are driven by primary genetic defects into main groups according to the dominant clinical manifestations, for example hypertrophic (HCM), dilated (DCM), restrictive and arrhythmic (ACM) and in 2008 the European working group on myocardial and pericardial diseases proposed a new classification outlined in the table below.(Elliott 2008)
3.2 Specific cardiomyopathy

Although there is still not complete consensus regarding classification, in the light of the discovery that CM appears to be the product of gene mutation with or without a trigger mechanism, cardiac disorders previously termed specific heart muscle disease have now been accepted under the CM umbrella and given either the label specific CM (Richardson 1996) or mixed/acquired CM. (Maron 2006) These include CM due to general systemic disease including connective tissue and infiltrative processes, peripartum CM and sensitivity and toxicity reactions. In this thesis, I have concentrated my research into patients with 3"specific" CMs of very different aetiology, namely Emery-Dreifuss muscular dystrophy (EDMD), β-thalassaemia major (TM) and patients receiving adjuvant anthracycline therapy for breast cancer. In all these CMs there are significant research questions.
3.2.1 Emery-Dreifuss muscular dystrophy

3.2.1.1 The muscular dystrophies

The muscular dystrophies (MDs) comprise a heterogeneous group of genetically transmitted diseases characterised by progressive muscular weakness and atrophy. (Emery 1998, 2002b, a) They are classified (as outlined in table 3-2) according to their clinical presentation, distribution of muscle weakness, age of onset, disease progression, protein mutation and inheritance pattern. Recent advances in genetic mapping have shown links to numerous loci and additional genetic information is being accumulated rapidly. Cardiac involvement is common in the inherited MDs. The X-linked Duchenne MD (DMD) is the commonest (affecting 1 in 3,500 male births) and one of the most severe forms. It is due to a mutation in the dystrophin gene and dystrophin is usually absent in DMD patients. Onset is in early childhood and some degree of cognitive impairment is usual. Disease progression is rapid, affected individuals usually die in their early 20s. Becker muscular dystrophy (BMD) is also due to a dystrophin gene mutation but here dystrophin is reduced. Muscle weakness is similar to DMD but progression is slower and less severe. The incidence of BMD is approximately 1 in 18,450 males. Myocardial hypertrophy and DCM are common in both forms. EDMD, caused by mutations in genes encoding for the nuclear membrane and laminar proteins emerin and lamin a/c, is much less common and can be X-linked (EDMD1), autosomal dominant (EDMD-2) or rarely autosomal recessive. (Hermans 2010) The incidence of EDMD1 is approximately 1 in 100,000 males, the incidence of EDMD 2 is unknown. EDMD is characterised by relatively mild muscular weakness (humero-peroneal distribution) with early joint contractures and spine rigidity. Although skeletal involvement is less severe than in the dystrophinopathies, EDMD is associated with DCM and there is a high risk of sudden cardiac death, especially in EDMD2. For this reason, and the lack of prospective studies in the literature, a cohort of patients with EDMD2 were systematically reviewed using CMR and echocardiography.
### Table 3-2 Classification of the Muscular Dystrophies

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutation</th>
<th>Symptoms</th>
<th>Cardiac complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duchenne (DMD)</td>
<td>Dystrophin</td>
<td>Early onset, severe muscle weakness</td>
<td>DCM, often masked by severity of skeletal weakness</td>
</tr>
<tr>
<td>Becker (BMD)</td>
<td>Dystrophin</td>
<td>Less severe and slower progression than DMD</td>
<td>DCM</td>
</tr>
<tr>
<td>Emery-Dreifuss (EDMD)</td>
<td>Lamin, Emerin</td>
<td>Proximal muscle weakness, contractures</td>
<td>DCM, arrhythmias</td>
</tr>
<tr>
<td>Fascioscapulohumeral (FSHD)</td>
<td>Unknown</td>
<td>Face, proximal upper</td>
<td>arrhythmias</td>
</tr>
<tr>
<td>Myotonic (DM)</td>
<td>Myotonic protein kinase</td>
<td>Distal than proximal</td>
<td>n/a</td>
</tr>
<tr>
<td>Epidermolysis bullosa</td>
<td>Plectin</td>
<td>Generalised weakness, skin blistering, contractures</td>
<td>n/a</td>
</tr>
<tr>
<td>Oculopharyngeal (OPMD)</td>
<td>Poly-A-binding protein 2</td>
<td>Throat, eyelids</td>
<td>n/a</td>
</tr>
<tr>
<td>LGMD1A-2J (Limb girdle)</td>
<td>Various</td>
<td>Proximal muscle weakness</td>
<td>Cardiac symptoms may occur</td>
</tr>
<tr>
<td>Miyoshi (distal)</td>
<td>Dysferlin</td>
<td>Posterior leg, can eventually affect upper extremities</td>
<td>n/a</td>
</tr>
<tr>
<td>Laing (distal)</td>
<td>Unknown</td>
<td>Anterior leg, possible proximal extension, neck flexors</td>
<td>n/a</td>
</tr>
<tr>
<td>Tibial (distal)</td>
<td>Titin</td>
<td>Anterior leg, can eventually affect upper extremities</td>
<td>Cardiac symptoms may occur</td>
</tr>
<tr>
<td>Welander</td>
<td>Unknown</td>
<td>Distal upper, eventually lower</td>
<td>n/a</td>
</tr>
<tr>
<td>Nonakel (distal)</td>
<td>Acetylglucosamine epimerase</td>
<td>Anterior leg, possible proximal extension</td>
<td>n/a</td>
</tr>
<tr>
<td>Myofibrillar/Desmin (distal)</td>
<td>Desmin</td>
<td>Distal then proximal lower extremities</td>
<td>DCM</td>
</tr>
<tr>
<td>MDC1A-C (Congenital)</td>
<td>Various</td>
<td>Proximal limb, cognitive, seizures</td>
<td>n/a</td>
</tr>
<tr>
<td>Muscle-eye-brain (Congenital)</td>
<td>POMGnT1 glycotransferase</td>
<td>Mild, generalised weakness, cognitive</td>
<td>n/a</td>
</tr>
<tr>
<td>Bethlem (Ulrich syndrome) (Congenital)</td>
<td>Type VI collagen</td>
<td>Proximal, early contractures</td>
<td>n/a</td>
</tr>
<tr>
<td>Rigid spine (Congenital)</td>
<td>Selenoprotein N1</td>
<td>Spinal extensor contractures, spine rigidity, restrictive lung disease</td>
<td>n/a</td>
</tr>
<tr>
<td>Fukuyama (Congenital)</td>
<td>Fukutin</td>
<td>Proximal upper, distal lower, face, neck</td>
<td>n/a</td>
</tr>
<tr>
<td>A7 intergrin (Congenital)</td>
<td>A7 intergrin</td>
<td>Mild generalised weakness</td>
<td>n/a</td>
</tr>
</tbody>
</table>

#### 3.2.1.2 The nuclear envelope

The nuclear envelope comprises a double layer of membranes which separate the genetic material in the nucleus from the cytoplasm. The nuclear envelope pores allow communication between the nucleus and cytoplasm. Each membrane is composed of a
lipid bilayer. The outer membrane is covered with ribosomes and is in continuity with the rough endoplasmic reticulum, reforming it after cell division. The inner membrane is connected to the dense fibrillar nuclear lamina which plays an important role in maintaining the architecture of the nucleus. (Verhaert 2011) The protein emerin is an integral component of the vertebrate inner nuclear membrane and may play a role in anchoring the cytoskeleton to the membrane. The intermediate filament protein lamins A and C closely interact with emerin to form the nuclear lamina. A deficiency of either emerin or lamin A/C produces the characteristic slowly progressive distal skeletal muscle wasting and contractures of EDMD.

3.2.1.3 Genetics of Emery-Dreifuss muscular dystrophy

EDMD is a genetically heterogeneous form of muscular dystrophy. (Bonne 2000) First described in the early 1900s, it was initially considered to be a milder form of DMD. Evaluation of a large family with X linked EDMD in 1966 led to its distinction as a separate disease (EDMD 1), and in the 1980s an autosomal dominant form was described (EDMD 2), and a 3rd form has also been described. (Emery 1966, Miller 1985, Bonne 2000, Raffaele Di Barletta 2000)

The first gene responsible for EDMD to be identified was the emerin gene, located on chromosome Xq28 and encoding for the ubiquitous nuclear envelope protein emerin. It is found in most cell types however it is most highly expressed in skeletal muscle. EDMD1 is a result of mutations in genes coding for emerin. More recent studies identified defects in the LMNA gene encoding for two other nuclear envelope proteins, Lamins A and C, responsible for both the autosomal dominant and autosomal recessive forms EDMD 2 and 3. (Bonne 1999, Raffaele Di Barletta 2000)

Since the discovery of the LMNA gene mutations it has become clear that a number of mutations within this gene are responsible for a large series of allelic disorders. (Wehnert 2002, Mercuri 2004) These include limb girdle muscular dystrophy 1B (LGMD1B) and a form of dilated cardiomyopathy with conduction defects (DCM-CD) (Muchir 2000) the Dunnigan-type familial partial lipodystrophy (FPLD) (Shackleton 2000), a form of autosomal recessive axonal neuropathy (Charcot-Marie-Tooth disease, AR-CMT2) (De Sandre-Giovannoli 2002), mandibuloacral dysplasia (MAD) (Novelli
EDMD types 1, 2 and 3 can be distinguished clinically and by their inheritance pattern. Although the clinical course is similar, cardiac involvement is an invariable finding in EDMD2, which usually occurs by the second to third decade of life. This includes atrial and ventricular arrhythmias and conduction defects, followed by complete heart block or atrial paralysis but also affecting the left ventricle directly in the form of dilated cardiomyopathy. (Morris 1999, Becane 2000) Recently, it has become apparent that cardiac involvement in EDMD2 may be more severe that in other forms of muscular dystrophy. (Funakoshi 1999, Sanna 2003, van Berlo 2004) There are only a few previous studies (Zacharias 1999) reporting pathological endomyocardial autopsy findings in EDMD2 with unique atrial pathology and adipose tissue infiltration with deposition of antihuman IgG. The investigation of cardiac involvement in EDMD2 has mainly been based on two-dimensional echocardiography (2DE) and 24 hour ambulatory ECG (Sanna 2003) and it has been postulated that rhythm or conduction disturbances are the primary cardiac involvement in these patients.

3.2.1.4 Clinical features

Skeletal involvement in EDMD is less severe than in DMD or BMD, which are caused by a mutation in the gene coding for the protein dystrophin, found in the muscle and brain sarcolemma. Muscle weakness in EDMD is slowly progressive and the distribution is scapulo-humero-peroneal. Muscle contractures are typical of the syndrome and these can occur before muscle weakness. The major cause of mortality and morbidity is however cardiac involvement, which is nearly always present. Whereas skeletal muscle symptoms are often mild in EDMD, cardiac involvement may be profound, usually manifesting by the second or third decade. Cardiac involvement in autosomal dominant EDMD2 is an invariable finding and may be more severe than in other muscular dystrophies with a very high percentage of pacemaker dependence and sudden death. (Funakoshi 1999, Sanna 2003, van Berlo 2004) These abnormalities range from ventricular arrhythmias and conduction defects to complete heart block, atrial standstill and cardiomyopathy. (Morris 1999, Becane 2000) Cardiac disease
usually presents after the onset of skeletal muscle weakness. Patients often present with syncopal episodes in the second/third decades. The fact that so many require pacing has made CMR based long term follow-up or investigating patients with established cardiac disease virtually impossible. Later findings include atrial and ventricular cardiomyopathy. In EDMD1, 10-20% of female carriers have conduction defects and are at risk of sudden death.

In DMD and BMD cardiac abnormalities are characterised by systolic dysfunction with regional wall motion abnormalities (RWMA) which are usually infero-lateral. These RWMA's are associated with myocardial fibrosis which can be seen with late gadolinium enhancement (LGE) using CMR. Based on the findings and with the paucity of in-vivo studies of cardiac function in EDMD in the literature, we hypothesised that myocardial fibrosis could play a role in this patient cohort and examined this hypothesis with a CMR study.

3.2.2 Anthracycline cardiotoxicity

The anthracyclines doxorubicin and epirubicin remain the mainstay of anti-tumour therapy for many malignancies including soft tissue tumours, lymphomas and leukaemias. They are also used for both advanced breast cancer and as adjuvant therapy in early disease. For advanced disease, the long term effects of therapy are less important than palliation being compromised by early toxicity. For adjuvant therapy however, where the aim of therapy is a cure or long term remission, long term effects are an important factor.

Breast cancer is the most common cancer in the UK accounting for 31% of all cancers. The lifetime risk of developing breast cancer is 1 in 8 for women in the UK and is it the second most common cancer in women under 35. Survival rates have been improving over the last 30 years. For women in England and Wales diagnosed between 1996 and 2000 the 5 year survival is now over 80% and it is 73% for 10 years. (Cancer Research UK on line) This improved survival means the long term sequelae of anti-cancer therapy are a major concern. Aside from the toxicity of the anthracycline therapy itself, with an aging population the incidence of co-morbidity such as hypertension or coronary artery disease is more likely. Additional radiotherapy can augment
cardiotoxicity and the synergistic anti-cancer effects of newer targeted agents such as trastuzumab also exacerbate cardiomyocyte damage.

3.2.2.1 Anthracycline pharmacology

Anthracyclines are a group of antibiotics originally derived from a soil bacterium, Streptomyces. They are have been used for anti-cancer chemotherapy for about 40 years and were first termed anti-tumour antibiotics. They act by causing cell damage and inhibiting cell replication. Anthracyclines consist of a planar, hydrophobic tetracycline ring with a glycosidic link to daunosamine sugar. They are positively charged and intercalate with unravelled strands of DNA during the process of cell division. Topoisomerases are enzymes that control the winding of DNA to permit the synthesis of proteins and DNA replication by creating transient breaks in the strands (i.e. they change the topology of the DNA molecule). Topoisomerase I and III are classified as type I enzymes as they cleave a single DNA strand. Topoisomerase II enzymes are classified as type II enzymes as they cut both DNA strands and are believed to play a role in the separation of daughter DNA strands and structural remodelling. Topoisomerase poisons which include anthracyclines, block this catalysis by binding to the DNA, the topoisomerase itself, or both molecules. Anthracyclines are believed to inhibit topoisomerase II by intercalation. This structural modification of the DNA thus causes functional changes which block transcription. Anthracyclines also bind to mitochondrial DNA inhibiting basic cellular function resulting in ATP depletion. Sensitivity to topoisomerase poisons occur in cells where the repair of DNA double strand breaks is defective. This property gives anthracyclines their cancer cytotoxicity but may be implicated in cardiotoxicity.

Anthracycline molecules also possess quinone moieties which through electron transfer reactions generate oxygen free radicals which cause intra and extra-cellular damage. It is believed that ferritin is converted into an oxidative form producing oxygen superoxides and hydrogen peroxide resulting in oxidative stress damage to DNA, mRNA proteins and lipids. Oxidative stress can also affect mitochondrial calcium transport causing tissue damage and impaired contractility. Increased lipid peroxidation and free radical generation has been documented after anthracycline administration.
The cytotoxic actions of anthracyclines have the potential to affect all cells, especially rapidly dividing cells hence their antineoplastic effect. Cardiomyocytes are particularly susceptible to oxidative stress, possible due to the relatively low levels of anti-oxidant enzymes in the heart. Catalase activity is low in the heart therefore the myocardium relies on glutathione peroxidase to detoxify hydrogen peroxide. Anthracyclines however deplete glutathione. Concomitant iron chelation with dexrazoxane has been shown to reduce cardiotoxicity, possibly by blocking the free radical producing redox reaction. (Takemura 2007) Its use is associated with myelosuppression which further potentiates cardiotoxicity and trial results have been contradictory. (Basser 1994, Swain 1997, Swain 2004) Chelation therapy with dexrazoxane has been reported to reduce anti-cancer efficacy although again published findings are conflicting, so it does not tend to be used in adjuvant therapy where anthracycline dosage is low. The role of dexrazoxane as an anti-oxidant has also been challenged recently. (Simunek 2008, Simunek 2009) What is clear is that cardiotoxicity is cumulative and dose dependent.

3.2.2.2 Mechanisms of anthracycline cardiotoxicity

Cardiomyocytes are believed to have a limited mitotic capacity after the embryonic phase of proliferation. Cardiomyocyte replication virtually ceases during the early post-natal period and renewal only occurs if mediated by stem cells. In other words they are usually considered terminally differentiated. Young adults have approximately 8.2 billion cardiomyocyte nuclei but on average 0.6% are lost per annum. (Olivetti 1991) Ventricular wall thickness is maintained by cell volume increase, but overall ventricular mass decreases with cell death. Response to cell injury therefore is limited to remodelling and or cellular hypertrophy, thus their regeneration capacity is limited with an increase in vulnerability throughout the normal aging process. The incidence of cancer increases with population age. In addition long term survival rates are improving due in part to adjuvant chemotherapy in early disease. This has lead to a cohort of long term cancer ‘survivors’, with the added corollary of how to deal with long term treatment toxicity. In addition to dose dependency risk of cardiotoxicity is influenced by patient age (advanced or young age) duration of survival and the presence of cardiovascular comorbidity. Treatment with anthracyclines carries the greatest risk of
acute and late cardiotoxicity. Rarely cardiac toxicity can be acute, occurring during or immediately after exposure and is more frequent if administered is bolus or rapid infusion. Arrhythmias are common during the first 24 hours post dosing, usually in the form of increased ventricular ectopy. According to reports this is not associated with long term sequelae. (Lefrak 1973, Steinberg 1987) These studies however did not use sensitive markers of ventricular function to follow long term outcome. Other symptoms include vasodilatation, hypotension. A rare but more serious complication is acute myocarditis/pericarditis, which was more common in early trials using high single doses. This syndrome tends to occur 1-3 days post administration and can result in ventricular dysfunction and sudden death. (Bristow 1978b, Ettinghausen 1986, Towns 2008, Pfeffer 2009) Heart failure is very rare in patients with no risk factors and at low dosages. Damage is now believed to occur during exposure, particularly as troponin 1 is released post administration. (Eschenhagen 2011) Sub-acute cardiotoxicity tends to occur up to eight months after the last cycle, typically peaking at three months. Chronic AMC is considered “early” if onset of ventricular dysfunction and heart failure is within a year of exposure, otherwise it is termed “late onset”, typically developing after a prolonged asymptomatic period. It is the late onset form of AMC that is becoming an increasing problem, with an ageing population and the improvement in the long term survival of cancer patients. It is known to be related to cumulative dosage and culminates with a dilated cardiomyopathy and heart failure which tends to develop by 2-5 years after the last dose. As the risk of heart failure is known to increase with cumulative dosage LVEF is measured prior to each cycle. The pathogenesis of anthracycline induced CM is not fully understood but pathological appearances include a dilated heart with endomyocardial fibrosis and hypertrophy of surviving myocytes. (Mortensen 1986) Endomyocardial biopsies show sarcoplasmic reticulum dilatation, vacuole formation, myofibril loss and necrosis. (Floyd 2005) At lower dosages (<400mg/m²) the incidence of heart failure is low but increases rapidly at doses >550mg/m² with a 50% risk at doses ≥1g/m². The risk increase is non-linear and suggesting there is no ‘safe-dose’ for anthracyclines. Some patients appear to be able to tolerate very high dosages whereas myocardial dysfunction has been reported at low cumulative doses implying individual sensitivity. Sub-clinical abnormalities have been
identified using non-invasive imaging modalities in asymptomatic patients. Once heart failure is establishes prognosis is poor.

Doxorubicin was the first anthracycline drug to be widely used as an anti-cancer drug. Its association with cardiotoxicity however lead to the search for an analogue with an improved therapeutic index as long term survival depends not only on anti-cancer efficacy but also treatment induced irreversible side effects. Epirubicin is an analogue of doxorubicin and has a similar mode of action in binding to DNA thus inhibiting replication and protein synthesis. It does have a more favourable therapeutic index being associated with a significantly lower incidence of cardiotoxicity than Doxorubicin and is as effective as doxorubicin in the adjuvant setting.

3.2.2.3 Trastuzumab cardiotoxicity

The growth factor receptor gene, human epidermal growth factor receptor (HER2) is over-expressed in 25-30% of breast cancer cases in whom abnormally high levels of the encoded protein is found in the malignant cells. The disease is more aggressive in these patients who subsequently have a poorer prognosis. (Slamon 1987, Slamon 1989, Slamon 2001) Trastuzumab (Herceptin) is a recombinant humanised monoclonal antibody specific to the HER2 protein. It has been shown to inhibit tumour growth and enhances the anti-tumour effect of anthracycline therapy. (Baselga 1998, Popat 2008) Trials have concluded that adding trastuzumab to adjuvant therapy improves survival of HER2 positive women by approximately 33%, the risk of disease recurrence being reduced by about 50%. (Piccart-Gebhart 2005, Romond 2005, Smith 2007) Trastuzumab is usually well tolerated however it is associated with cardiotoxicity, a side effect not discovered until late in clinical trials, especially when combined with anthracycline therapy. (Schneider 2002) Cardiotoxicity with anthracyclines is estimated to be about 10% however this rises to 28% with the addition of trastuzumab with a concomitant rise in the incidence of NYHA class III/IV heart failure from 3 to 16%. (Suter 2004) The mechanism of cardiotoxicity appears different to those of anthracycline injury and is not dose dependent. Cardiac dysfunction is not associated with myocyte loss or troponin release. Clinical trials have reported cardiac dysfunction persisting for 20-25 days (the half life of trastuzumab) but then returning to normal in
most patients and recent data suggests there should be no long term adverse effects. (Eschenhagen 2011) There is however a paucity of long term follow-up studies. The likely pathogenesis is due to the blockage of HER2, which is expressed in low levels in most body tissue including the heart and is important for myocyte repair and adaption to stress. (Crone 2002, Cardinale 2010a, Ewer 2010) Gene deletion of HER2 or HE4 has lead to CM in mice. (Crone 2002) This is more marked following anthracycline therapy, possibly due to anthracyclines altering the configuration of surface HER2 receptors or interfering with cell signalling pathways. (Schneider 2002) There is less evidence of trastuzumab mediated cardiotoxicity when combined with regimens other than anthracyclines. In essence the synergistic anti-cancer properties are reflected in the effect on the heart therefore the issue of cardiotoxicity in this group of patients is likely to be an increasing problem.

3.2.2.4 Early biomarkers and functional assessment

Anthracycline mediated cardiomyocyte damage is now believed to occur mainly during exposure, this hypothesis has been supported by the release of troponin I after administration. (Eschenhagen 2011) Presentation is usually much later, maybe due to a lack of compensatory mechanisms, with heart failure occurring late in disease development. A normal LVEF does not necessarily mean the absence of a toxicity reaction. (Ewer 2008) The possibility of a genetic predisposition to cardiotoxicity is being increasingly explored. (Wojnowski 2005) What is clear is that there is a need for formalised follow-up studies looking at biomarkers for the prediction of toxicity and more sensitive indicators of functional change to better characterise myocardial damage. In this thesis, I examined this issue using CMR, and allowed for the complicating factor of HER2 treatment.

3.2.3 Iron overload cardiomyopathy

3.2.3.1 Iron metabolism

Iron plays a pivotal role in many biological processes. Free iron is however toxic to cells. Damage is related to the production of reactive oxygen species (ROS) formed as levels of labile iron rise, which cause oxidative damage to membranes and
mitochondrial respiratory chain enzyme dysfunction. As mentioned previously, cardiomyocytes are possibly more susceptible to oxidative stress due to relatively low anti-oxidant and catalase activity. In the healthy state iron within the organism is conserved and recycled by a complex series of interactions. Ferritin is a protein found in all cells which acts as an iron buffer by binding iron in a safe format for storage and transportation. Iron is stored in the core of the ferritin molecule as Fe3+ in a crystalline solid. Each ferritin molecule can hold about 4500 Fe3+ ions. To remove the iron from ferritin, the Fe3+ is reduced to Fe2+ and hydrated thus allowing the iron to pass from the ferritin lattice. Haemosiderin is an intra-cellular iron storage complex composed of ferritin, denatured ferritin and other material. It is usually found in phagocytes and is abundant following haemorrhage and in the presence of iron overload. Iron from haemosiderin is not readily released. Transferrin is an iron transporter. Its major role is the carriage of iron from the duodenum and macrophages to all, and particularly erythropoietic, tissue.

3.2.3.2 Iron overload and toxicity

As iron levels accumulate due to erythrocyte catabolism this recycling network is saturated. With no other means of iron excretion, iron accumulates as toxic non-transferrin bound iron (NTBI) in the plasma. This then accumulates mainly within parenchymal tissue and the reticulo-endothelial system. In the pituitary gland this can cause a wide range of endocrine disorders including growth retardation, delayed sexual maturity and infertility. Excess iron in the thyroid may lead to hypothyroidism and musculoskeletal problems, and diabetes is common due to excess iron in the pancreas. Hepatic iron overload can cause fibrosis and cirrhosis and hepatocellular cancer. However the major cause of death worldwide in chronically transfused patients is cardiac iron overload. (Olivieri 1994) This has been increasing as haemoglobinopathy patients live longer. Iron overload cardiomyopathy (IOC) is the result of the accumulation of iron within the myocardium and is characterised by the presence of systolic and diastolic cardiac dysfunction. This cardiomyopathy has been shown to be reversible with timely and adequate medical intervention with chelating agents. (Hahalis 2005)
3.2.3.3 The thalassaemias

In the early 20th century, investigators including Cooley described a severe anaemia with associated splenomegaly and bony changes. The disease was first termed Cooley’s anaemia and later thalassic anaemia as the first cases identified were of Mediterranean origin. The name was ultimately shortened to thalassaemia and incorporates the Greek words for sea and blood (thalassa = sea, haema = blood). (Olivieri 1999, Weatherall 2004) This has turned out to be a misnomer as the thalassaemias are now known to include a heterogeneous group of inherited haemoglobinopathies in patients of different ethnicities with the majority of cases in South East Asia. (Cohen 2004) Although folklore attributes its spread eastward to the travels of Alexander the Great and his army, the fact that there are many mutations makes this unlikely. More plausible is that the heterozygote offers some protection against the malarial parasite Plasmodium falciparum and de novo mutations have occurred in different regions. (Weatherall 2004, 2005) Recent migratory patterns have expanded disease distribution to north Europe, North and South America and Australasia.

Figure 3-1 The orange shaded area highlights the endemic distribution of thalassaemia. It follows a wide band through southern Europe, North Africa and the Nile delta, the Middle East, the Indian sub-continent and south-east Asia. These areas are, or were in the past, malarial zones.
Haemoglobin is a tetramer comprising four sub-units. Each sub-unit contains haem, a porphyrin ring that holds a ferrous ion (Fe2+) at its centre by four covalent bonds. This group reversibly bonds with oxygen and gives the molecule its characteristic colour. In human adults the haem groups are held within four globular protein units designated the α and β chains. The three dimensional folding of these chains produces steric hindrance preventing the oxygenation of the Fe2+ iron to its ferric (Fe3+) state.

![Diagram](image)

**Figure 3-2 Diagrammatic representation of a normal haemoglobin molecule.** The Fe2+ ion to which oxygen reversibly binds is at the centre of the haem group which is held within the globular protein chains.

There are more than 200 mutations of the β-globin genes, found on chromosome 11, which cause the β-thalassaemia phenotype and more than 80 mutations and deletions of α-globin genes on chromosome 16 causing α-thalassaemia. If the synthesis of these proteins is imbalanced, normal erythrocyte function is disrupted resulting in anaemia. The α-globin mutation is more common in South East Asia and Africa whereas the β-globin mutation is prevalent in Southern Europe and the Middle East.

The thalassaemias are the commonest single gene disorders worldwide. It is estimated that about 7% of the worldwide population have the mutation. B-thalassaemia is endemic to Southern Europe and is now prevalent to the UK via migratory patterns. Approximately 60,000 children are born every year with the homozygous β-
thalassaemia major (TM). (Weatherall 1996, Weatherall 2005) and is consequently of medico-economic importance within the European Union.

The β-thalassaemias are classified according to phenotype and genetic profile. Fetal haemoglobin consists of two α and two γ globin chains. During the first year of life the synthesis of fetal haemoglobin declines with a switch to adult haemoglobin production. The mutation of both β-globin genes in TM leads to a marked reduction or absence of β-chain synthesis which in turn leads to a relative excess of unbound α-chains causing ineffective erythropoiesis and damage to the cell membrane and haemolysis. The resultant microcytic anaemia induces bone marrow expansion (causing the characteristic bony abnormalities) and splenomegaly. The increased plasma volume caused by the shunting through the expanded marrow space exacerbates the anaemia. Further adaptive mechanisms include extra-medullary haematopoiesis and erythroid hyperplasia. Without regular blood transfusions patients usually die during early childhood. Thalassaemia intermedia (TI) patients are also homozygotes but they present later and many do not require transfusion therapy as the β-chain mutations are less severe, however pulmonary hypertension is a frequent complication due to chronic anaemia. Thalassaemia minor (often referred to as thalassaemia trait) is the heterozygous state which results in mild anaemia which is usually asymptomatic.

Blood transfusion therapy became standard therapy for patients with TM during the 1960s. (Modell 1983) If the haemoglobin level is kept above 9g/dL the bone marrow is suppressed with a reduction in hepato-splenomegaly, extra-medullary haematopoiesis and bony abnormalities. (Olivieri 1999) This improves the short term clinical status and outlook for these patients, however this therapy comes with a longer term problem of tissue iron overload, which is the most important complication of TM worldwide. Even in patients not receiving transfusional therapy, iron overload has been described in patients with severe anaemia and erythroid expansion. (Pootrakul 1988, Olivieri 1999) This is due to reduced hepcidin production caused by the increased ineffective erythropoiesis. Hepcidin is produced by the liver and regulates iron absorption and recycling. It internalises and degrades the iron export protein ferroportin, found on the surface of duodenal enterocytes, hepatocytes and macrophages. The iron is then excreted at the end of the cell life span. A reduction in hepcidin leads to increased iron
absorption and its subsequent deposition in parenchymal tissue. (Ganz 2005) In TM patients regular transfusions exacerbates this iron accumulation as each unit of blood contains about 200-250mg of iron.

Iron chelation therapy can reduce tissue iron levels and cardiac complications. Prior to iron chelation therapy, patients died from the complications of iron overload, usually by the second or third decades of life. The hexadentate iron chelating agent deferoxamine was the first used clinically and it became standard therapy in the 1970s. It has a high affinity for iron but is a large positively charged lipophobic molecule and is poorly absorbed by the digestive system. It also has a short plasma half life. (Kushner 2001, Porter 2001) These factors make it ineffective via oral transmission and administration needs to be intra-venous or sub-cutaneous. It is advised that patients with dangerously high levels of iron or those who develop important symptoms receive 24hour continuous intra-venous chelation therapy. For longer term therapy administration is sub-cutaneous, typically overnight five times per week. (Porter 2001) This optimises therapeutic efficacy but at a financial cost (the pumps and attachments are expensive). The demanding regime associated with parenteral therapy has inherent psychosocial and practical issues which modulate compliance and therefore efficacy, especially in adolescents and young adults. Poor compliance should be suspected if indices of iron overload increase unexpectedly. There are also drug related toxic effects including sensory neural loss leading to deafness and sight impairment. (Olivieri 1986, Porter 1989)

Use of the orally active bidentate iron chelator deferiprone (L1) in humans was first reported in 1987. (Kontoghiorghes 1987b) It has a much smaller neutrally charged lipophilic molecule which allows good gastrointestinal absorption and cellular access. (Kushner 2001, Porter 2001) The plasma half life is longer allowing oral administration with three doses per day (Kontoghiorghes 1987a) It is cheap to produce, does not require infusion equipment and has shown superior efficacy to deferoxamine in the removal of cardiac iron and improvement in LV systolic function. (Anderson 2002, Pennell 2006, Maggio 2009, Pepe 2011) Although deferoxamine is a very efficient chelator with a high iron affinity, its large molecular size limits its potential for intracellular chelation. Deferiprone is a small lipophilic molecule and therefore has
better access to intra-cellular iron. The cause of the enhanced improvement in LV function with deferiprone over deferoxamine therapy is not fully understood, however superior antioxidant potential has been demonstrated in a number of in vitro and in vivo studies. (Kontogiorghes 2009) Other possible explanations are the reduction of reactive oxygen species and the restoration of normal mitochondrial function. (Kakhlon 2008, Kontogiorghes 2009) Agranulocytosis and neutropenia have been reported to be associated with deferiprone therapy but are usually reversible with discontinuation. Previously reported findings of hepatic fibrosis (Olivieri 1998) was not reproduced in a later large study and was probably due to the presence of iron and chronic hepatitis C. (Wanless 2002) The drug is still however not licensed in North America. Some patients do not achieve optimal iron balance with deferiprone monotherapy and there are tolerance issues. The combination of deferoxamine with deferiprone improves iron balance in a significant proportion of patients compared with monotherapy with either drug. (Anderson 2002, Daar 2006, Tanner 2007, Porter 2009) This can be either by simultaneous administration or by alternating the drugs. If both chelators are present a synergistic ‘shuttle’ effect may be achieved by combining the strong iron affinity of deferoxamine with the ability of deferiprone, the weaker chelator, to penetrate cells. (Link 2001)

Deferasirox is a relatively newly developed orally active tridentate ligand which has a half life of 8-16 hours permitting once daily (or sometimes twice daily) administration. Unlike deferiprone it is licensed in North America, is well tolerated and has shown efficacy in reducing iron burden. (Nisbet-Brown 2003, Cappellini 2006, Cappellini 2007) The recent large multicentre EPIC (Evaluation of Patients Iron Chelation with Exjade) cardiac sub-study has shown its efficacy in removing iron from the heart over one and two years although LVEF remained unchanged. (Pennell 2010, Pennell 2011) A study by Wood et al (Wood 2010) showed a significant reduction in cardiac T2* over 18 months in 48% of patients however there was a clear demarcation between responders and non-responders. Deferasirox was effective in patients with a moderate cardiac and liver iron burden but not in those with severe overload which would indicate more aggressive therapy for these patients.
3.2.4 The right ventricle in cardiomyopathy

The role of the right ventricle (RV) has been relatively under-investigated in the study of the pathophysiology of cardiac disease, being often regarded as a passive conduit. (Voelkel 2006) The relative success of the Fontan procedure and tricuspid valvullectomy for endocarditis supported this view to some extent. With many imaging modalities the description of RV structure and function is limited by its complex geometry, proximity to the chest wall and being obscured by the lungs and sternum although the use of CMR has overcome these limitations and normal ranges for functional indices have now been established. (Maceira 2006b)

The RV consists of an inlet section which includes the tricuspid valve, chordae tendineae and papillary muscles, the trabeculated distal myocardial segment, and the infundibulum section (or outflow tract). Although biventricular function is closely interlinked, it is apparent that the left and right ventricles are inherently different. (Strniskova 2006, Wang 2006) Morphogenesis studies have shown that the left and right ventricles have different progenitor cells and arise from different regions during early development. The mammalian heart develops from the primary heart tube from which the LV arises. Right sided myocardial cells are derived from myocardial precursors anterior to the primary heart tube known as the anterior heart field. (Thomas 1998, Cai 2003, Zaffran 2004, McFadden 2005, Verzi 2005)

The ventricles are linked by sharing the septum, epicardial fibres and the pericardial space. The epicardial layers are arranged circumferentially and are in continuity with those of the LV. The deeper fibres are arranged longitudinally from base to apex in continuity with the septum. Under normal conditions the stroke volume of the RV is the same as the LV however as pulmonary vascular resistance is low the RV has approximately 25% less stroke work and is consequently more compliant with much thinner walls. The RV has considerably less contractile reserve than the LV and is therefore more sensitive to afterload due to reduced muscle mass and chamber geometry. A change in chamber geometry alters the normal orientation of muscle fibres which impairs contraction. (Bronicki 2010)
Right ventricular dysfunction may occur as a result of numerous mechanisms. Primary lung disease and LV dysfunction may lead to increased pulmonary vascular resistance thus increasing RV afterload. Pulmonary hypertension (PHT) plays a large role in RV pathology. The initial response to pressure overload is hypertrophy followed by functional impairment and compensatory dilatation. This is further exacerbated by tricuspid regurgitation due to annular dilatation which in turn can produce RV dysfunction. The precise mechanism of RV failure secondary to PHT is not fully understood although ischaemia, microvascular endothelial dysfunction and myocyte apoptosis may be implicated. (Voelkel 2006)

Myocardial ischaemia and myopathic processes can affect both ventricles. In addition LV dysfunction may cause reduced RV perfusion. Right ventricular dysfunction can further exacerbate LV dysfunction by reducing LV preload. Reports on RV involvement in myocarditis, MI and DCM all cite RV dysfunction as both a complementary and independent predictor of outcome stressing the importance of assessing RV function in cardiac disease. (Mendes 1994, Juilliere 1997, de Groote 1998, Larose 2007, Miszalski-Jamka 2010) Patients with severe heart failure but maintained RV function have better survival and exercise capacity. (Di Salvo 1995, Karatasakis 1998) RVMI occurs in approximately 50% of patients with inferior MI and 10% of patients with anterior MI. (Cabin 1987, Zehender 1993) I therefore assessed the effect of chelation therapy on the RV in thalassemia in this thesis, which has not previously been explored.
Chapter 4: CMR Methods for Imaging Cardiomyopathy

4.1 Determination of ventricular volumes and mass

Ventricular volumes, mass and ejection fraction are the most commonly cited parameters for cardiac functional assessment and follow-up. Normal ranges indexed to age, gender and BSA have been established for the left ventricle (Maceira 2006a) and the right ventricle. (Maceira 2006b) This aids the identification of early disease as functional changes frequently precede clinical symptoms in cardiomyopathic conditions. (Keeling 1995, Baig 1998) Early detection permits early therapeutic intervention and potentially an improved outcome. Ventricular volumetric data are also common surrogate end points for pharmacological trials. The accuracy and reproducibility of CMR are superior to other imaging modalities, which makes CMR the technique of choice for longitudinal follow-up and pharmacological trials as study sample sizes can be reduced. (Bellenger 2000) In direct comparison trials, the inter-study reproducibility of CMR for dilated, hypertrophied and remodelled hearts is superior to 2D echocardiography. (Grothues 2002) Right ventricular anatomy and function can be assessed with confidence which is a challenge for other modalities. (Grothues 2004, Maceira 2006b) This is particularly important as the role of the right ventricle as a predictor of outcome in heart disease is being increasingly recognised. (Mendes 1994, Juilliere 1997, de Groote 1998, Larose 2007, Miszalski-Jamka 2010) It is also possible to assess diastolic function with CMR. The scan time is long however in order to acquire images from sufficient cardiac phases for adequate temporal resolution. Echocardiography with its higher temporal resolution is still preferred by many centres for looking at diastolic physiology. Myocardial tagging is used for systolic strain analysis to investigate regional abnormality. The only major challenges to obtaining good quality CMR cine images are arrhythmias (as cine images are averaged over a number of cardiac cycles) and inadequate breath-holding. Real time acquisition where the whole image is acquired within a single heart beat can compensate for acquisition problems, but the frame rate and spatial resolution is poor. Navigator sequences monitoring the diaphragm allow free breathing acquisitions, and
arrhythmia rejection sequences are available, but both these techniques prolong the acquisition. In practice however, CMR is readily able to provide a set of geometrically correct long axis and contiguous short axis cines with excellent boundary definition using SSFP sequences, which with their improved blood to myocardial contrast are almost universally utilised. The resultant data set provides a three dimensional representation of cardiac chambers from which cardiac boundaries can be delineated and blood and myocardial volumes calculated.

4.1.1 Volume and mass calculation by manual planimetry

Cardiac volumetric and mass data can be derived by the manual planimetry of endocardial and epicardial boundaries. Particular care must be taken with the placement of the basal slice during acquisition if using planimetry. During endocardial contouring, the blood pool below the AV valves should be included and basal regions with thin, non trabeculated muscle should be considered atrial and excluded from ventricular assessment. Papillary muscles are excluded from the blood pool and included as mass. (Hudsmith 2005) The blood pool and myocardial volume are calculated by using Simpson’s rule. Myocardial mass is derived by multiplying myocardial volume by its specific gravity (1.05g/mL$^3$). Stroke volume and ejection fraction are derived from the calculated diastolic and systolic volumes.
Figure 4-1 Ventricular contours can be manually planimetered and the blood pool and myocardial volumes calculated by using Simpson’s rule.

4.1.2 Volumes measured by CMRtools

Although manual delineation of cardiac parameters is an important technique, it suffers from being slow to perform and the treatment of blood pool in relation to the AV valves can be operator dependent. Therefore semi-automated software is preferred. For the purpose of this thesis, I used software called "CMRtools" (Cardiovascular imaging solutions, London, UK), a commercially available multi-function DICOM viewing/analysis package which carries a class 1 CE mark and has FDA clearance. "LVtools" is the plug-in of this package which allows the calculation of functional indices. This semi-automated analysis package has been shown to have very high inter and intra observer reproducibility when compared with other contouring methods. (Miller 2010) A multi-dimensional Catmull-Rohm spline interpolation
algorithm is interactively applied to provide an approximation for surface temporal and spatial definition. Points of attachment are applied to the insertion points of AV and VA valves for diastole and systole to allow valve tracking throughout the cardiac cycle which provides a three dimensional model of valve plane motion. The valve planes are utilised to represent the interfaces between the ventricles, atria and great arteries ensuring that only blood volume associated with the ventricles is used for analysis. Data reformatting is used to identify the motion of the surface in long axis and temporal views. An interactive thresholding tool is then applied to define solid structures.

![LVtools](image)

**Figure 4-2** Using LVtools cardiac borders are roughly contoured. Fine detail is achieved using thresholding. The result is a 3-D ventricular model with displayed phase-volume curves and derived indices.

The first step is to identify and load the cine images needed to create the model. This comprises a stack of SAX cines covering both ventricles from base to apex and four long axis projections depicting the valves. The centre of the LV blood pool is identified by the operator and the endocardial surface roughly delineated for the selected diastolic and systolic cardiac phases. Using the appropriate long axis images, the valve plane is
identified for diastole and systole, and more reference points can be set if diastolic filling curves are desired. To finely differentiate between solid structures and blood pool a mid-ventricular short axis slice is selected and the thresholding tool is utilised to highlight operator defined solid tissue. The first selected threshold is automatically applied to all SAX slices but subsequent fine-tuning of separate slices is possible. To obtain mass measurements the LV epicardial surface needs to be defined accurately as this step does not utilise thresholding. This process is repeated to derive right ventricular parameters but this time approximating the centre of the blood pool to the septum as the program subtracts all previously defined LV structures. A separate window displays the volume/phase curves for systole and diastole with the calculated functional indices.

One advantage of the 3-D model over manual planimetry is that the valve plane tracking removes uncertainty about inclusion of the most basal slice which may be partially within the atrium due to incorrect piloting.

4.2 Assessment of inflammation (non-contrast).

Inflammatory or myocarditic changes often precede myopathic changes. (Feldman 2000) Myocarditis has been described in up to 12% of young adults dying suddenly. (Virmani 2001, Doolan 2004, Puranik 2005, Fabre 2006) Between 5-10% of patients with myocarditis have been reported to progress to a dilated cardiomyopathy. (Fuster 1981, Kawai 1999) Early identification could permit early therapeutic intervention with the potential for reducing long term sequelae. The identification of myocarditis can be problematic and is often reached only by exclusion. Patients may present with chest pain, ECG changes resembling infarction and a troponin rise. Ventricular function may appear normal if inflammation is focal. (Mahrholdt 2004, Mahrholdt 2006) Myocardial biopsy is invasive and localised and areas of infiltration may be missed. It would be advantageous to be able to predict possible long term myocardial damage by identifying early inflammatory changes in the scenario of known myocardial insult. An example of this is for planned anthracycline chemotherapy which is known to be cardiotoxic and is associated with long term cardiac functional impairment. CMR is ideally suited for looking for inflammatory changes by virtue of its intrinsic contrast
properties and by the utilisation of an injected contrast agent which can be used to track hyperaemia and fibrosis. The fact that the entire myocardium is visualised means that focal abnormalities can be identified. Transient increases in myocardial wall thickness and volumes have been described using echocardiography and CMR. (Hiramitsu 2007, Friedrich 2009) SSFP cine imaging is ideally suited for this purpose to obtain baseline and follow-up data from patients who will receive known cardiotoxic substances. STIR sequences highlight areas with increased fluid which would be expected in areas of inflammation with myocardial hyperaemia, increased capillary permeability and loss of cell membrane integrity. An increase in signal intensity (SI) has been noted using a contrast enhanced T1 weighted sequence in the setting of acute myocarditis. (Friedrich 1998) A small study of patients prior to and also 3 days post the first cycle of anthracycline chemotherapy showed increased contrast enhancement of the myocardium compared with skeletal muscle using a T1 weighted fast spin echo sequence, and this correlated with a later reduction in LVEF, with the enhancement returning to normal by 6 months. (Wassmuth 2001) As myocardial hyperaemia is a feature of myocardial inflammation it is likely that imaging the early wash in kinetics of gadolinium contrast agent would exhibit an accelerated signal increase. For myocardial perfusion imaging a rest-stress protocol is utilised where the baseline status is compared with a pharmacologically induced hyperaemic state. For this thesis a perfusion acquisition was performed at baseline and 3 days post first cycle in patients undergoing adjuvant chemotherapy for early breast cancer.

4.3 Myocardial perfusion, hyperaemia and fibrosis with gadolinium contrast.

For CMR, gadolinium contrast dynamics can be divided into 3 phases: 1) perfusion; 2) early enhancement at 1-2 minutes; and 3) late enhancement at 10-15 minutes. First pass imaging is performed as a bolus of gadolinium contrast is injected. Image data is acquired before the bolus (baseline) and throughout delivery. Resultant processed data can give information about tissue perfusion. Early enhancement allows assessment of hyperaemia, and late enhancement highlights damaged tissue as the gadolinium contrast agent has a slower wash-out than in normal areas.
Figure 4-3 The contrast arrives rapidly into the blood pool and then the normal myocardium when first pass image acquisition is performed. After approximately 10 minutes the contrast has largely washed out of normal tissue but remains in fibrosed tissue giving delayed contrast.

4.3.1 Early enhancement

Myocardial perfusion CMR is performed during the first pass of the contrast agent. During this time blood T1 is reduced approximately 50 fold. It is used to detect regional impairment of myocardial blood flow during rest and stress. Most commonly hyperaemia is induced by an intravenous infusion of adenosine at a rate of 0.14 mg/kg/minute over three minutes. In the normally perfused myocardium, blood flow will increase several fold (usually with an associated increase in heart rate) which is termed the myocardial perfusion reserve. However, haemodynamically significant lesions restrict this and a comparison between rest and stress perfusion represents the myocardial perfusion reserve. A rapid acquisition sequence (particularly as the heart rate can be high at peak stress) is necessary to obtain a series of T1 weighted images per cardiac cycle to image the contrast agent wash in to the myocardium. With such a
demanding imaging protocol, there is a trade-off between image quality and spatial and temporal resolution, as well as how many slices of the heart can be visualised. Depending on the heart rate, usually 3 short axis slices are visualised which adequately represents all coronary territories. The acquisition sequence used is usually turboFLASH, TrueFISP or hybrid echo planar imaging (EPI). The FLASH sequence has a short TR with a spoiler gradient and therefore a low flip angle which limits SI and SNR. The TrueFISP sequence has an improved signal intensity and S/N but artefacts are problematic. As the EPI sequence collects multiple lines after each RF pulse data acquisition is faster potentially reducing artefacts.

The myocardial signal intensity curve (signal intensity/time) representing the change in SI can be obtained at rest and stress. The ratio of stress to rest perfusion reflects the myocardial perfusion reserve index. There are quantitative methods available which involve the measurement of multiple variables and the accurate description of arterial input, usually derived from the LV cavity or aortic root. (Christian 2004) Perfusion Tools is a plug-in of CMRtools and uses a model-based approach for first pass perfusion assessment with Fermi deconvolution. The software features include the definition of arterial input and provide multi-slice perfusion quantification at rest and stress giving a segmental assessment of perfusion indices.

For early enhancement imaging at 1-2 minutes after gadolinium injection, which shows myocardial hyperaemia and capillary leak in inflammation, T1 weighted inversion recovery imaging is used. The increased blood volume in the affected area causes an increase in contrast uptake in the early vascular phase. (Friedrich 2009)

4.3.2 Late enhancement

Late gadolinium enhancement (LGE) reflects the delayed washout of the contrast agent from the myocardium where the interstitial space is increased due to fibrosis or protein deposition, or due to loss of myocyte membrane integrity from myocardial damage and inflammatory changes. Myocyte damage along with lymphocyte infiltration and oedema are characteristic of acute myocarditis. The contrast washes through normal myocardium fairly rapidly (as it does not cross the normal cell membrane) but accumulates in damaged areas. Using a segmented gradient echo inversion recovery
sequence, the TI can be set to show optimal contrast between normal myocardium, blood pool and the contrast enhanced abnormal tissue. In effect the signal from normal myocardium is nulled. This is usually best achieved between about 15 and 30 minutes after the contrast injection when the concentration of contrast in damaged tissue is still high but has reduced significantly in the blood pool and normal myocardium. (Kwong 2006) This is particularly important in the case of spatially heterogeneous areas of damage, for example sub-endocardial defects or the infarct periphery. (Schmidt 2007) The TI needs to be constantly adjusted to compensate for the normal washout kinetics of viable tissue.

4.4  T2* assessment of myocardial iron

Myocardial T2* is measured using a well described gradient echo sequence, with a constant TR (to avoid T1 effects), and progressively incremental TE. (Anderson 2001, Westwood 2003) To measure myocardial T2*, a mid-ventricular septal region of interest (ROI) is selected avoiding blood pool and proximal vessels. Using the septum avoids susceptibility artefacts from tissue interfaces. Myocardial SI is inversely proportional to TE which are plotted against each other and fitted to a mono-exponential curve (SI=P0•e^{-TE/T2*}). The rate of decay increases with iron burden. In heavily iron overloaded patients, the SI for higher TEs may fall below background noise causing the curve to plateau and underestimating T2*. Curve fitting can be improved by adding a constant offset or truncating later echo times. The latter method is more reproducible and gives values closer to those obtained by the DIR sequence. (He 2008) ROIs can be drawn and plotted manually or an off-line analysis package utilised. All analysis for this thesis was performed using Thalassaemia tools, a plug in of CMR tools. This program allows rapid ROI delineation which is propagated throughout all TEs. The curve is fitted automatically and truncated by the operator if necessary.
Figure 4-4 Thalassaemia tools is an off-line automated analysis program that allows rapid isolation and propagation of ROIs through all echo times and fits points to a mono-exponential decay curve. This patient has a T2* of 17.85 which indicates mild myocardial iron overload.
5.1 Introduction

Due to the high incidence of cardiac involvement and sudden death in EDMD2 early detection of cardiac abnormalities is important in conditions associated with CM to select the best therapeutic strategy with the aim to slow any cardiac remodelling and the onset of cardiac related symptoms. Timely intervention with steroid and ACE inhibitor therapy has been reported to be effective in patients with DMD. (Duboc 2005, Ramaciotti 2006, Kwon 2012) The hypothesis that fibrosis may play a role in the development of cardiomyopathy (CM) in EDMD2 was tested by using late gadolinium enhancement (LGE). Functional assessment was by CMR cine imaging and echocardiography. CMR myocardial tagging and echo myocardial velocity gradients (MVG) were used to image early changes in strain patterns that may occur before global dysfunction.

5.2 Methods

Eight consecutive genetically proven EDMD2 patients were enrolled from patients attending the regular outpatient clinics of the Dubowitz Neuromuscular Centre and invited to participate in the study. Patients with a contraindication to CMR such as cerebral clips, implantable devices and in particular cardiac pacemakers were not included. As the disease incidence is low and many patients do have implanted pacemakers, the potential study population was correspondingly low. Six patients were male, the age range from 7-42 years (mean 18.5). The table below summarises the clinical profiles of the patient group. All patients had muscle weakness associated with mild to severe mobility impairment. Of the patients studied, one was in atrial flutter and one had right bundle branch block. Patients were asked to undergo detailed echocardiographic imaging and 24 hour ECG at the Hammersmith Hospital and CMR imaging at the Royal Brompton Hospital on separate days for their convenience. The time between scans ranged from two weeks to three months.
An age matched control group was obtained from 8 healthy volunteers. It was not possible to match the controls for body surface area as the patient group had lower body weights than could be found in healthy volunteers. The study was approved by HH and RBH ethics committees and informed written consent was obtained from each subject or subject’s guardian before entering the study.

5.2.1 **Standard echocardiography**

A comprehensive standard echocardiographic examination was performed using a commercially available diagnostic ultrasound system (HDI 5000, Philips Medical Systems, Crawley, UK) with a 4-2 MHz multifrequency transducer and second harmonic imaging capability for better endocardial border detection. Left atrial (LA) size and left ventricular (LV) dimensions at end-diastole and end-systole were measured to provide an overall assessment of LV function. LV end-diastolic wall thickness was measured and used to calculate left ventricular mass using the Devereux formula. (Devereux 1986) Cardiac dimensions were corrected for body surface area. Valves and great vessels were subjectively assessed for structural abnormalities. Doppler blood pool velocities were measured across the mitral valve in diastole to assess diastolic filling from early (E-wave) and late (A-wave) transmitral velocities. (Ommen 2003)

5.2.2 **Tissue Doppler echocardiography (TDI)**

From the apical projection, pulsed wave TDI velocities were measured in systole and diastole at the atrio-ventricular ring level. Samples were taken from the lateral, septal, anterior and posterior walls using apical four and two chamber projections. These velocities reflect the longitudinal vector of shortening of the myofibrils and provide an estimate of the ventricular function along the longitudinal axis. From the parasternal long axis view, an M-mode trace was obtained depicting the endo and epicardial boundaries. Onto this trace, a colour coded tissue velocity map was superimposed showing spatial and temporal velocity differences during systole and early and late diastole from which the MVG between subendocardium and subepicardium could be calculated and thus, the radial LV function estimated. The angle of the M-mode beam was aligned to be perpendicular to the myocardium (≤5 degrees). Aliasing was
eliminated by appropriate PRF settings and grey scale gains were adjusted to provide clear delineation of the endocardial and epicardial borders. The ECG was continuously recorded in all cases.

Data captured in freeze frame were downloaded onto a PC with specially designed software (HDI Lab, Philips Medical Systems, Best, The Netherlands). Myocardial borders (endocardial and epicardial) of the left ventricular posterior wall were defined and traced by an experienced echocardiographer. The MVG was definable as the slope of linear regression of myocardial velocity along each M-mode scan line throughout the myocardium. (Palka 2000) This may be considered equivalent to radial strain rate of the myocardium.

5.2.3 Cardiovascular magnetic resonance

The CMR was performed using a 1.5 T scanner (Magnetom Sonata, Siemens AG Medical Engineering, Erlangen, Germany) using front and back surface coils and prospective ECG triggering. Images for all but one patient were acquired during breath-hold. All anatomical imaging was performed using a prospectively gated steady state free precession (SSFP) sequence, typically flip angle 60, echo time 1.55 and slice thickness 7mm. The acquisition window and TR were adjusted according to the R-R interval. Segmentation was adjusted to provide ≥ 20 phases. From the scout images ventricular long axis views were obtained to depict valve motion. Using the long axis cines as scouts a full set of SAX slices, fully covering the ventricles and atria, were obtained. Accurate assessment of ventricular diastolic and systolic volumes and left ventricular mass was facilitated using semi-automated segmentation (CMRtools, Cardiovascular Imaging Solutions, London, UK). Papillary muscles were included in LV mass and subtracted from LV blood pool. Atrial size was similarly measured during full atrial diastole. All cardiac dimensions were corrected for body surface area. Turbo spin-echo imaging with T1 and T2 weighting with and without fat suppression was used to show the presence of fibro-fatty replacement. Turbo spin-echo is a sequence used for tissue characterisation and can detect fibrous of fatty infiltration within the myocardium. Fat appears brighter due to increased contrast. Axial cuts optimally showing right ventricular myocardium and short axis cuts through the base, mid-
ventricle and apex were obtained to exclude the presence of myocardial fat. Typical T1 weighted parameters included slice thickness 8mm, TE 6.7, TR according to R-R interval, flip angle 180° and for T2 weighting with fat suppression slice thickness 8mm, TR twice R-R (triggering on alternate pulses) TE 60, TI 170. Gadolinium-DTPA was given as a peripheral intravenous bolus (0.1mmol/kg bodyweight) to test whether fibrosis plays a role in cardiac involvement in EDMD. Contrast enhanced images were acquired using a segmented inversion recovery sequence after 10 minutes. The slice thickness was 8mm, TE 4, flip angle 20°, trigger pulse 2. The TR was adjusted for the R-R interval and the TI was constantly adjusted from 300 to 440 to compensate for normal contrast washout dynamics. Areas of late gadolinium enhancement, if seen, were to be expressed as a percentage of normal tissue. (Kim 2000, Moon 2003)

Magnetic resonance tagging sequences spatially modulate longitudinal magnetisation at end-diastole thus creating a grid of tag patterns that when analysed revealed local and global cardiac motion patterns. The harmonic phase method (HARP) uses the fact that image phase is related to myocardial motion and spatial derivatives are calculated for each pixel to produce displacement maps. (Kuijer 2001, Sampath 2003) This analysis method overcomes the time constraints of conventional processing and tracking of tags. The tag sequence produces sinusoidal tag patterns at particular spatial frequencies. Myocardial deformation produces localised frequency variations which are used to calculate components of Eulerian and velocity fields. Fourier transformation of the tagged image produces central spectral and harmonic peaks, the location of which are determined by the tag pattern. Band-pass filtering is applied to eliminate phase cancellation and isolate the selected spectral peak, typically the lowest harmonic frequency. This filter is then applied to all the images. The resultant image consists of a harmonic magnitude image which is used to isolate the myocardium and a harmonic phase image which describes myocardial motion. The derived ‘angle’ image is used track the phase of the tags by subtracting the baseline tag. (Osman 1999, Axel 2005, Ibrahim 2011) Two long-axis and 3 short-axis projections were acquired to give good coverage of all myocardial segments and depicting longitudinal, radial and circumferential motion. From this data, we calculated minimal principal strain for controls and study patients. Minimal principal strain is defined as maximal shortening parallel to the fibre direction and correlates with circumferential strain patterns.
5.2.4 Statistics

Descriptive statistics are presented as mean ±1 standard deviation. The relationships between sets of continuous variables were examined using unpaired student t-test. All tests were two-tailed and p<0.05 was considered statistically significant.

5.3 Results

5.3.1 Clinical characteristics

The table below summarises clinical findings including cardiac and respiratory data for the time of the study. All patients carried a heterozygous LMNA gene mutation. The average patient age was 18.5 years±12 (range 7-42). There were 2 females and 6 males. They were all asymptomatic from the cardiac viewpoint and none was on any medication. Six patients had normal sinus rhythm with no conduction abnormalities on 24 hour Holter monitoring. One patient was in atrial flutter, while another had first-degree atrio-ventricular block. Musculoskeletal symptoms started at an average age of 2.75±1.25 (range 1-4) years. All patients were ambulant with mild to marked limitation of functional capacity. One patient could only walk with crutches and another was able to walk short distances independently with difficulty.
Patient demographics including clinical and functional characteristics.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at study (years)</th>
<th>Sex (M/F)</th>
<th>CK (x increased)</th>
<th>Onset of symptoms (years)</th>
<th>Course</th>
<th>Maximum functional ability</th>
<th>Severity</th>
<th>Wasting</th>
<th>Weakness</th>
<th>Contractures</th>
<th>Spine</th>
<th>ECG</th>
<th>24h Holter</th>
<th>Echocardiogram</th>
<th>FVC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.6</td>
<td>M</td>
<td>2.5</td>
<td>1.5</td>
<td>Stationary</td>
<td>I</td>
<td>++</td>
<td>Medi al aspect of calves</td>
<td>TF</td>
<td>TA, TA</td>
<td>Mild rigidity</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:30%</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>M</td>
<td>4</td>
<td>3</td>
<td>Stationary</td>
<td>I</td>
<td>++</td>
<td>Dorsal thinning of both calves</td>
<td>UL&gt;LL</td>
<td>E, H, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>M</td>
<td>4</td>
<td>1</td>
<td>Progressive</td>
<td>I</td>
<td>++</td>
<td>Scapulo-peroneal at base of calves</td>
<td>UL&gt;LL</td>
<td>E, H, TA, HSS</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:33%</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>M</td>
<td>5</td>
<td>4</td>
<td>Slowly progressive</td>
<td>I</td>
<td>++</td>
<td>Scapulo-peroneal at base of calves</td>
<td>UL&gt;LL</td>
<td>E, H, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>F</td>
<td>5</td>
<td>1.5</td>
<td>Slowly progressive</td>
<td>I</td>
<td>++</td>
<td>Scapulo-peroneal at base of calves</td>
<td>E, TA</td>
<td>E, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>F</td>
<td>15</td>
<td>3</td>
<td>Slowly progressive</td>
<td>I</td>
<td>++</td>
<td>Scapulo-peroneal at base of calves</td>
<td>E, TA</td>
<td>E, H, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>M</td>
<td>15</td>
<td>4</td>
<td>Slowly progressive</td>
<td>I</td>
<td>++</td>
<td>Scapulo-peroneal at base of calves</td>
<td>E, TA</td>
<td>E, H, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>M</td>
<td>15</td>
<td>4</td>
<td>Slowly progressive but rapid after 30 yrs</td>
<td>WD</td>
<td>++</td>
<td>Medi proximal</td>
<td>UL&gt;LL</td>
<td>E, H, TA</td>
<td>Rigid</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal, FS:32%</td>
</tr>
</tbody>
</table>


The body mass index (BMI, kg/m²) of the patient group was lower than in controls (15.2±3.43 g/m² vs. 18.1±2.4 kg/m², p=0.076). This was due mainly to the fact that the adults and older children in the patient group had very low body weights. In the control group only the younger children had low BMIs. The normal adult range for BMI is 19-25 kg/m². This low body weight was accredited largely to muscle wasting. There was no difference between the BSA of the two groups, although the BSA of the patients was below the expected values for age. (Mosteller 1987)
5.3.2 Echocardiographic findings

Echocardiographic imaging was optimal in all 8 patients with clear endo and epicardial border delineation. Patients had normal left ventricular function at the time of examination with a fractional shortening ranging from 33-37% (mean 35%). There was no difference between patients and controls for LA size, left ventricular size and fractional shortening. LV mass was similar between patients and controls. There were no differences in LV mass as calculated by echo or CMR (63.3±12 g/m² for echo and 60±18 g/m² for CMR, p=0.95). Mitral E and A wave diastolic velocities were similar for both groups as were the annular E to A wave ratios. TDI velocities were normal in all mitral annular regions and were similar between patients and controls. The mitral E to annular E’ wave ratio was also normal.

Table 5-2 Echo derived parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Mean± SD</th>
<th>Controls Mean± SD</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>18.5±12</td>
<td>15.1±11</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>BSA (m²)</strong></td>
<td>1.2±0.3</td>
<td>1.4±0.3</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>LA/BSA (mm/ m²)</strong></td>
<td>24±6</td>
<td>28±4</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>LV fractional shortening (%)</strong></td>
<td>35±2</td>
<td>38±8</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>LV mass/BSA (g/m²)</strong></td>
<td>63.3±12</td>
<td>63±19</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>Mitril E/A</strong></td>
<td>1.73±0.3</td>
<td>1.69±0.5</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Pulsed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall systole (cm/s)</td>
<td>0.13±0.02</td>
<td>0.11±0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall E (cm/s)</td>
<td>0.18±0.05</td>
<td>0.19±0.06</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Doppler</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall A (cm/s)</td>
<td>0.05±0.02</td>
<td>0.08±0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>E/E’</td>
<td>5±0.67</td>
<td>5±1.7</td>
<td>0.58</td>
</tr>
<tr>
<td>E’/A’</td>
<td>4.6±2.7</td>
<td>2.4±0.64</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Colour</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradient early diastole (s⁻¹)</td>
<td>4±1.2</td>
<td>7.13±2.7</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>TDI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradient systole (s⁻¹)</td>
<td>3.4±1.14</td>
<td>3.5±1.07</td>
<td>0.88</td>
</tr>
</tbody>
</table>
MVGs were similar between patients and controls during systole. In early diastole however, during the rapid filling time, the early diastolic gradient was lower in patients than in controls suggesting early myocardial dysfunction (4 ± 1.2 s-1 vs. 7.1 ± 2.7 s-1, p=0.02).

5.3.3 Cardiovascular magnetic resonance

All patients tolerated the CMR scan well. The youngest patient (age 5 years) could not be cannulated via superficial access and he therefore could not be given Gd-DPTA. This patient also could not breath-hold adequately therefore a TrueFISP real-time acquisition sequence was utilised. Resolution was not as good as the breath-hold sequences but adequate for boundary definition and planimetry. Good quality breath-hold images were obtained for all but this one patient, slice thickness 5-7mm according to BSA. Another patient had severe spinal contractions, which made breath-holding difficult and had to be brought out of the scanner during the examination due to discomfort. This prolonged overall scanning time but adequate images were obtained with minimal patient stress.

Table 5-3 CMR findings

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p=</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LV end-diastolic volume/BSA (mls/m²)</strong></td>
<td>73±15</td>
<td>72±7</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>LV ejection fraction (%)</strong></td>
<td>0.72±5</td>
<td>0.72±4</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>LV mass/BSA (g/m²)</strong></td>
<td>60±18</td>
<td>61±14</td>
<td>1</td>
</tr>
<tr>
<td><strong>RV end diastolic volume/BSA (mls/m²)</strong></td>
<td>62±18</td>
<td>69±10</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>RV ejection fraction (%)</strong></td>
<td>58±11</td>
<td>63±8</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Left atrial volume/BSA (mls/m²)</strong></td>
<td>41±25</td>
<td>38±6</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Right atrial volume/BSA (mls/m²)</strong></td>
<td>60±28</td>
<td>55±13</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Inferior wall minimal principal strain (%)</strong></td>
<td>-0.062±0.024</td>
<td>-0.094±0.032</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Left and right ventricular (RV) ejection fractions were within normal limits for both patient and control groups. LV and RV volumes corrected for BSA were also within normal limits. There was no significant difference between the corrected ventricular
volumes between the two groups. Mean LV mass when corrected for body surface area was below the published normal value of 87±12 g/m². (Lorenz 1999) The corrected mass for the control group was also below the normal range. Some of these differences may be explained by the relative youth of both patient and control samples when compared with the age range of the previously described group (8-55 yrs, mean 28±9) versus 7-42 yrs mean 18.5 and 6-30 yrs mean 15. The EDMD2 group did however have a particularly low corrected LV mass, which may be further explained by their relative physical inactivity due to mobility limitations. LV mass was similar between the two groups. RV mass was not measured due to difficulties with reproducibility in children.

The LA volume CMR was greater in both patient group and controls compared with previously published data. (Poutanen 2000) This group did not however include the atrial appendages in their volume measurements and used FLASH sequences for their acquisitions. No patient showed areas of late gadolinium enhancement. There was however a significant reduction in inferior wall strain as determined by HARP tagging at -0.062 + 0.024 versus -0.094 + 0.032 in the control group (p=0.048).
Figure 5-1 Minimal principal strain pattern in the inferior segment of the mid-cavity LV determined by CMR tagging. The dashed line is the minimum principal strain for EDMD patients, while the solid line is the average strain for the age-matched normal volunteers.

5.3.4 Follow-up

Patients were re-assessed clinically two years after completion of the study. In brief, LV function remained normal as defined by conventional echocardiographic criteria, but one patient had developed atrial fibrillation.

5.4 Discussion

This is the first study to identify subclinical myocardial dysfunction in consecutive patients with the autosomal dominant form of EDMD. The small series of patient reports available, selected on the basis of specific cardiac abnormalities, suggest that the evolution of AV block in the X-linked form seems to evolve slowly, culminating in
complete heart block or atrial paralysis for which pacing is indicated to prevent sudden death. (Dreifuss 1961, Emery 1966, Wyse 1987) The severity of conduction disturbances appears greater in the dominant form of the disease and ventricular fibrillation may occur. This might explain the relative high frequency of sudden death in this group of patients despite pacing or ICD insertion. LV dysfunction may occur in the form of apparently isolated cardiomyopathy, especially in EDMD2. One study described 54 living members of one family with a lamin A/C mutation of whom 17 presented with cardiomyopathy and cardiac conduction system disease. (Becane 2000) It is also possible that some individuals undergoing cardiac transplantation for idiopathic cardiomyopathy may in fact have undiagnosed EDMD. (Merchut 1990) It is therefore important in this group of patients to risk stratify for pacing/ICD insertion and to predict progressive myocardial disease allowing early pharmacological intervention.

Only one study to date (Sanna 2003) has described the range of cardiac abnormalities in unselected population and found that only 3 of their 10 patients had evidence of ventricular dysfunction on the basis of conventional 2 dimensional echocardiography. Whether EDMD leads primarily to conduction abnormalities as opposed to myocardial fibrosis and dysfunction has remained unknown. In the current study we hypothesised that patients may develop primary myocardial dysfunction, which will subsequently lead to conduction abnormalities.

TDI is a relatively new echocardiographic modality, which has been used to describe global and regional ventricular function. It has been used to describe early changes of myocardial function when conventional echocardiography remains normal. (Dutka 2000) TDI requires modification in signal processing of the returned Doppler signals during which the higher velocities obtained from the blood pool are filtered out. The great advantage of TDI is the high frame rate which increases the sensitivity of this technique. (Nagueu 1997, Rambaldi 1998, Sutherland 1999, Price 2000, Waggoner 2001). In addition, information on myocardial deformation using the MVG from subendocardium to subepicardium can be obtained, furthering the assessment of myocardial function. Several groups have reported myocardial abnormalities in sub-clinical conditions leading to cardiomyopathies. (Dutka 2000, Meune 2004) In this study, early diastolic MVGs were abnormal in a homogeneous group of autosomal
dominant EDMD patients while conventional echocardiography failed to identify any abnormalities.

The most often quoted indices of ventricular function, stroke volume and ejection fraction, are unavoidably load and heart rate dependant; therefore, the measurement of ventricular volumes in a non-controlled setting is arguably open to error. While LA dimensions can be measured by echocardiography, CMR can accurately define atrial volumes and function. Interestingly, these were normal by both echocardiography and CMR. Atrial volumes were not increased in comparison to the controls for this young patient cohort. This perhaps would refute any primary atrial dysfunction in these patients. Few reports of cardiac muscle pathology in EDMD are available but myocardial changes with focal degeneration and fatty fibrous replacement have been described. (Yoshioka 1989)

CMR has been used to depict fatty and fibrous infiltration in patients with myotonic dystrophy but not using newer technology including TSE, late enhancement imaging or tagging. (Vignaux 2002) There was no evidence of late enhancement of the myocardium with gadolinium and no evidence of fibro-fatty replacement using the TSE sequences. There does however need to be significant loss of cellular integrity over a macroscopically visible area to detect this. Nevertheless, there was a significant difference in inferior wall strain as determined by HARP tagging suggesting early abnormalities of ventricular function. Although our patient numbers were limited by the rarity of the disease, these findings support those of other studies of patients with systemic degenerative disease. Studies on patients with Friedreich’s ataxia and restrictive cardiomyopathy have shown that the MVG at early diastole is the most sensitive marker of early changes in myocardial function. (Dutka 2000, Palka 2000)

The relationship between physical immobility and measurements of cardiac function, including MVGs is not established, but this should have no more affect that the opposite phenomenon, athletic training. A recent study has demonstrated a relationship between long-term athletic training and the diminution of age related changes in early diastolic function. (Palka 1999)
These results suggest that cardiac dysfunction precedes significant fibrosis and that the pathogenesis for the cardiac involvement in EDMD2 is different from that observed in DMD and BMD. These findings are in keeping with recent observations on the cardiac involvement in the animal model with targeted inactivation of the LMNA gene. (Nikolova 2004) Both CMR tagging and MVG by tissue Doppler consistently showed myocardial dysfunction in the infero-posterior LV wall. Relevant findings in this animal model were early (by 2 weeks of life) cardiac dysfunction with thin-walled, dilated and globular-shaped LV, with a lower mass than in control mice. In similarly aged mice, no evidence of myofibril hypertrophy, necrosis or interstitial fibrosis was found. Despite the lack of significant histological changes, the contractile properties of single LV myocytes were studied and significantly reduced shortening was demonstrated. At least part of the observed abnormality was related to disruption of the physiological links between desmin at sarcomeres and lamin. Desmin filaments are directly linked between the nuclear surface and the cytoskeleton, and it is possible that lamin contributes to this linkage. Our data and the experimental animal work favour therefore a model in which intracellular cardiac defect are an early finding in the process of the disease. This is followed only at a later stage by myocyte loss mostly by apoptosis and eventually fibrosis. (Nikolova 2004) This observation has important implication for the understanding of the pathogenesis of the cardiac involvement in EDMD.

5.5 Clinical implications

Early detection of myocardial dysfunction in patients with EDMD may potentiate early introduction of ACE inhibition or other treatment in advance of permanent pacemaker implantation. This could delay the latter while reducing or delaying LV remodelling.

5.6 Study limitations

Although our study involved a relatively small number of patients, this study is unique in that patients were carefully characterized, genotyped and were selected because they had no evidence of underlying cardiomyopathy and/or pacemakers, while all previous studies involved patients who already had an established cardiomyopathy or conduction system disease. In addition, the fact that so many patients have a permanent pacemaker
inserted precluded many from our study, as this currently constitutes a contraindication to CMR. This meant that we were unable to study older patients in whom myocardial abnormality may have been more readily detectable. CMR compatible pacemakers are now available and therefore in the future such patients would not be precluded from CMR. Myocardial dysfunction was assessed in the anterior septum by CMR tagging and posterior wall using tissue Doppler. This was due to data optimization for both imaging techniques. Nevertheless, abnormalities were found by both, CMR and tissue Doppler suggesting perhaps a diffuse process of myocardial involvement. T1 mapping (MOLLI) is an emerging acquisition protocol for the quantification of diffuse fibrosis. This technique was not available at the time of this study.

5.7 Conclusions

This study suggests that, unlike DMD or BMD, cardiac involvement in EDMD does not appear to involve myocardial fibrosis in the early stage of the disease. The frequent implantation of pacemakers into patients with EDMD has unavoidably restricted the study of a larger patient population in the past, including those with cardiac symptoms. While global systolic function may be normal using conventional echocardiography, there appears to be subtle myocardial abnormalities manifested as a reduction in early diastolic myocardial gradients by echo and systolic strain patterns by CMR. Routine measurement of these may be useful in the early detection of cardiac involvement in EDMD. CMR studies with paced patients (Martin 2004) and the increased availability of MR friendly pacing systems opens the possibility of ongoing assessment of these patients even after the implantation of permanent pacing systems.
Chapter 6: Anthracycline cardiotoxicity

6.1 Introduction

As detailed in chapter 3, cardiotoxicity due to anthracycline exposure can occur at any time during or post treatment. Although there is no consensus regarding the classification of anthracycline mediated cardiotoxicity (AMC), it is generally divided into 2 forms, with an acute or a chronic onset which is further sub-categorised into early and late onset. The increasing incidence of late onset AMC highlights a need for cardiac assessment prior to and during chemotherapy and in addition long term follow-up with more prospective longitudinal trials. Oncologists are now avoiding high doses of anthracyclines but as not all patients are susceptible to long term cardiac damage some patients are potentially being denied optimal anti-cancer therapy which flags the need for an “early warning system” to identify the patients at risk. (Cardinale 2004)

6.1.1 Pharmacogenetics of anthracycline cardiotoxicity

There are a number of studies reporting inter-individual differences in cardiac response to a uniform anthracycline challenge. (Lefrak 1973, Von Hoff 1979, Grenier 1998, Ciotti 2001, Wassmuth 2001, Kremer 2002, Nousiainen 2002, Sorensen 2003) It has been postulated that the inter-individual response differences to an extrinsic stimulus result from genetic differences. (Montgomery 1997, Brull 2001, Jamshidi 2002, Dhamrait 2003) The fact that susceptibility has been found to differ according to gender and race adds support to these findings. (Trachtenberg 2011) The identification of a relationship between genotype and phenotypic response using accurate characterisation would help the identification of patients at risk of AMC and allow timely intervention and optimal individual therapy tailoring. There are CMR techniques which could potentially help with this characterisation. The basic measurement of left ventricular volumes, mass and function are essential, but in addition there are 2 specialised CMR techniques of interest.
6.1.2 CMR in anthracycline cardiotoxicity

The first CMR technique has potential to detect the acute cardiotoxic effects of anthracyclines including myocardial inflammation and dysfunction. (Bristow 1978b) CMR is widely used in the assessment of acute myocardial injury, and changes in tissue signal characteristics have been shown to reflect events associated with cellular jeopardy. (Friedrich 1998) Increased early gadolinium relative enhancement (EGRE), which compares cardiac with skeletal contrast uptake, is associated with myocardial hyperaemia, oedema and capillary leakiness. (Friedrich 2009) It has been shown to increase in acute infective myocarditis and is associated with a long-term decrease in LVEF. (Friedrich 1998, Wagner 2003) A pilot study by Friedrich’s group of 22 patients undergoing anthracycline chemotherapy showed that an increase in EGRE on the 3rd day after commencing treatment was related to a decrease in EF at 28 days post onset of treatment, although there was only a modest correlation with LVEF at 6 months. (Wassmuth 2001) Nineteen of the patients had received a second course of therapy before the 28 day scan. The number of chemotherapy cycles was not stated but is usually 6 and therefore the results did not really reflect late onset AMC. The study did however suggest a use for EGRE in the prediction of late onset AMC. We hypothesised that an increase in EGRE early in treatment could predict long term outcome in patients receiving anthracyclines. We also explored the use of STIR imaging in the study, because of its use in identifying myocardial oedema.

A second possible CMR technique of interest is the assessment of myocardial iron. Increased intracellular iron loading has been described in cell and rat models of AMC. (Kotamraju 2002, Kwok 2003, Miranda 2003) The iron chelator dexrazoxane has been shown to ameliorate AMC although deferasirox has been reported to afford no cardioprotection against AMC, (Xu 2005) and mechanisms other than iron scavenging have also been proposed. (Lyu 2007) Although we would not expect to see iron levels in the concentration found in iron overloaded transfusion-dependent patients such as thalassaemia major, we hypothesized that CMR T2* sequences might detect a rise in myocardial iron in patients who go on to develop AMC.
6.1.3 Other biomarkers of cardiotoxicity

Finally, markers of cardiac damage and dysfunction, troponins and natriuretic peptides rise after anthracycline administration. (Cardinale 2010b) As much of the literature supports a major role for oxidative stress it would be expected that markers such as F2-Isoprostanes, which are formed by the peroxidation of fatty acids by free radicals, would also be elevated. We hypothesised therefore that the early CMR markers would correlate with biomarkers of cardiac damage and oxidative stress which could be used as a predictor of late AMC instead of the more complex cardiac imaging.

6.1.4 Study objectives

In order to test these hypotheses, we participated in the Breast Cancer, Early disease: Toxicity from Therapy with adjuvant Epirubicin Regimens Cardiac Assessment and Risk Evaluation (BETTER-CARE) sub-study B performing CMR before chemotherapy, at 3 days post-chemotherapy and at 12 months follow-up.

6.2 Methods

6.2.1 Patient selection into the main BETTER-CARE study

Patient recruitment for the main BETTER-CARE study was mediated via the NCRN. Approximately 1700 UK women per year are newly diagnosed with breast cancer and seen in the local cancer networks, 45 to 60% of whom require adjuvant chemotherapy. Based upon the experience of the oncologist principal investigators, it was predicted that approximately 18% of patients would fulfil study recruitment eligibility and be willing to participate. It was initially calculated that 276 patients were required to detect a 2% change in LVEF with treatment. Allowing for dropouts the initial target number for the main study was 296. The original age range was 30-60 years and estimated recruitment was around 200 patients per annum and that the target number would be achieved within 18 months. As it became apparent that recruitment was significantly slower that predicted (see limitations) the age range was changed to ≥ 18 years and the sample size reduced to 189 subjects. A target of 247 recruited study subjects was therefore set which would allow for patient withdrawal and the introduction of Trastuzumab for HER2 positive patients. Final eligibility criteria therefore included:
female sex, early breast cancer, > 18 years old, epirubicin based chemotherapy and no previous cardiovascular disease as outlined in table 6.1.

Table 6-1 Eligibility criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tbody>
<tr>
<td>1 Gender: Female</td>
<td>1 Previous chemotherapy or radiotherapy</td>
</tr>
<tr>
<td>2 Age: &gt;18 years</td>
<td>2 Bilateral Breast surgery with bilateral axillary node clearance or planned bilateral radiotherapy</td>
</tr>
<tr>
<td>3 Race: White-European ethnicity</td>
<td>3 Planned external beam radiotherapy resulting in high dose-volume cardiac irradiation; internal mammary node irradiation; patients with a pectus excavatum; bilateral radiotherapy</td>
</tr>
<tr>
<td>4 Diagnosis: Histological-proven breast cancer</td>
<td>4 Biochemical Liver Pathology (Bilirubin &gt; 17μMol/L, AST or ALT &gt;45 iu/L)</td>
</tr>
<tr>
<td>5 Adjuvant chemotherapy: Epirubicin cumulative dose 300mg/m² to 450mg/m² BSA</td>
<td>5 Any pre-existing cardiovascular disease</td>
</tr>
<tr>
<td></td>
<td>6 Family history of familial cardiomyopathy</td>
</tr>
<tr>
<td></td>
<td>7 Conditions associated with increased risk of heart disease</td>
</tr>
<tr>
<td></td>
<td>8 Current thyroid disease</td>
</tr>
<tr>
<td></td>
<td>9 Anaemia with Hb &lt;10.0 g/dl</td>
</tr>
<tr>
<td></td>
<td>10 Drugs with cardiovascular effects</td>
</tr>
</tbody>
</table>

6.2.2 Patient selection into the CMR substudy

Initially patients were referred to the RBH CMR unit from the oncology clinics of 10 London hospitals. Due to slow recruitment, provisions were later made for patients to also be recruited and scanned locally at the Mount Vernon, Glasgow and Oxford centres. This included visiting the sites and training them in the desired methodology. All subjects scanned at RBH were invited to participate in the day-3 CMR sub-study. The primary endpoint of the CMR substudy was the change in EGRE in patients who developed chronic AMC. The EGRE study involved returning on the 3rd day following cycle 1 of epirubicin if well enough for an additional CMR scan, and we included blood and urine sample collection at this time.

Patients were approached during their outpatient appointment to discuss chemotherapy by their oncologist. Interested patients were given a copy of the patient information sheet and signed consent was obtained for all participants. A patient information DVD was also available. Eligibility was checked against the study criteria by the oncologist.
or NCRN research nurse and subsequently recorded in a case report form (CRF). Patients were eligible to take part in BETTER-CARE if they were enrolled in other studies provided they would receive 300-450mg/m² of epirubicin. Information about the patient at enrolment and their subsequent course of treatment was noted in the oncology CRF. The NCRN nurse then contacted the study coordinator or the CMR unit directly to schedule the scans. All study participants were given an appointment for a baseline CMR assessment which was usually four to ten days prior to commencing chemotherapy. Patients went on to have their course of treatment as prescribed by the oncologist. This could include radiotherapy and/or trastuzumab. Follow-up was arranged for 52 weeks after the final dose of epirubicin and 3 months after the final dose of trastuzumab if given.

### 6.2.3 CMR acquisition

CMR, scans were acquired using 1.5 T Sonata and Avanto systems (Siemens Medical Systems, Erlangen, Germany). Using long and short axis localisers, left and right ventricular LAX inflow and outflow cines were acquired ensuring accurate depiction of each AV and VA valve motion from two views. Using the long axis cines to define the SAX plane, contiguous SAX cines were acquired with a 10mm offset from base to apex (slice thickness 7mm with a 3mm gap). All cines were acquired using a SSFP (TrueFISP) sequence. Retrospective ECG gating with 50 calculated phases ensured coverage of the entire cardiac cycle. A mid ventricular STIR image, slice thickness 10 mm, TR adjusted for HR was acquired to test whether changes in SI at day 3 predicted long term change in EF. A T2* acquisition with 16 echo incremental times but constant TR was performed to measure myocardial iron, using both the white blood and the DIR preparations, (chapter 7) prior to contrast administration. To compare relative contrast enhancement between the myocardium and skeletal muscle a TSE sequence with TR=RR, TE 44, base resolution 192, phase resolution 100, echo train length 17 and slice thickness 20mm was used. A mid ventricular SAX slice, adjusted to optimally visualise both myocardium and left latissimus dorsi, was imaged with the chest coil turned off (to compensate for posterior and lateral signal loss when using the chest coil) prior to and after contrast injection. A 0.1mmol/Kg bolus of Gd-DTPA (Magnevist, Schering) was given via a peripheral vein using a power injector at a rate of 7 mL/Sec
followed by a bolus of normal saline. To test for myocardial hyperaemia an interleaved segmented hybrid EPI perfusion sequence was applied to measure myocardial contrast enhanced signal intensity upslope however technical difficulties lead us to abandon this approach. (See limitations) The relative enhancement sequence was applied at 30 second intervals from 60 to 180 seconds post injection to ensure optimal imaging of tissue contrast dynamics. For the tissue characterisation images, care was taken to image the same mid papillary region for all examinations at all time points. At baseline and end of study, long and short axis late gadolinium enhancement (LGE) images were acquired using a segmented IR turbo flash sequence between 5 and 20 minutes post contrast injection with a slice thickness of 8mm, offset 2mm and pixel size 1.8 x 1.8mm. All CMR image analysis was performed using plug-ins of the viewing/analysis package CMRtools described in chapter 4. Changes in myocardial and skeletal signal intensity post contrast EGRE images were processed according to the methodology described by Wassmuth et al. (Wassmuth 2001) CMR derived markers of inflammation were measured blinded to volumetric changes. Measurement of volumetric parameters was performed by Dr P Kotwinski, UCL, London as part of the main study blinded to time point and changes in CMR derived inflammatory markers.

6.2.4 Adjustments for trastuzumab therapy

During the course of the study significant advances were made in adjuvant therapeutic regimens for patients with early breast cancer. The most important was the introduction of trastuzumab for HER2 positive patients. Trastuzumab was licensed in the UK for early breast cancer in 2006. Synergistic cardiotoxicity complicates therapy although the mechanism is different to anthracycline cardiotoxicity and it is usually reversible. Clinical trials have shown that cardiac dysfunction persists for 20-25 days (the half-life of trastuzumab) with cardiac function returning to normal in the majority of patients. (Ewer 2005, Guarneri 2006) The original study design was therefore modified to allow recovery time after treatment.

6.2.5 Serum and urine biomarkers

Plasma, serum and urine were collected at each time point for analysis of F2 isoprostanes, troponin T, hsCRP and NT-pro BNP. The ratio between urinary F2
isoprostanes and creatinine was measured by Professor Kevin Moore, UCL, London using high pressure liquid chromatography. Contractual issues have delayed the CRP, troponin T and BNP assays.

6.2.6 Statistics

Power calculations showed that a change of 0.35 in EGRE with a SD of 0.9 (estimated from the literature) would yield a power of 80% at p=0.05 to detect a difference between the cardiotoxicity and non-cardiotoxicity groups with a sample size of 51 patients. Data are presented as mean ± standard deviation (SD). Urinary F2-isoprostane data were log transformed to give a normal distribution. Changes in log values were then transformed back and presented as the geometric mean. Initial analysis of CMR data used a random effects model adjusted for age, BMI, systolic and diastolic blood pressure, radiotherapy and time from end of therapy. These factors did not change the results significantly therefore difference between values were analysed using a paired t-test with a threshold 0.05 for statistical significance. Correlations were using Pearson correlation coefficients.

6.2.7 Ethics

The study was performed with full ethics approval from the South East Multi-Regional Ethics Committee. Study Reference: 04/MRE01/79. Site specific approval was obtained from the local ethics committees at all participating sites. Written consent was obtained from all study subjects.

6.2.8 National Cancer Research Network (NCRN) Support

The study was accepted into the NCRN local portfolio. Study Name: The Breast Cancer, Early disease: Toxicity from Therapy with adjuvant Epirubicin Regimens – Cardiac Assessment and Risk Evaluation (BETTER-CARE), NCRN Trial ID 1516.

6.3 Results

The first patient was scanned in November 2005 and the last baseline scan was April 2009. Recruitment was closed in May 2009 due to time and financial restrictions. A
total of 191 patients had a baseline scan at RBH. All patients received epirubicin based chemotherapy treatment regimes. The majority received fluorouracil/epirubicin/cyclophosphamide (FEC) or FEC/docetaxel (82%). Other regimes were FEC/methotrexate, epirubicin/cyclophosphamide/ paclitaxel/gemcitabine (NEO-TANGO) and epirubicin/capecitabine (TACT-2). All patients included in the analysis received between 300 and 450 mg/m² epirubicin (cumulative dose). No patient received dexrazoxane or other cardioprotective medication. Other chemotherapy agents administered have not been reported to be associated with cardiotoxicity. The majority of patients had radiotherapy (87.3%) and 19.3% received trastuzumab. Two patients attended for CMR, but did not complete the scan due to claustrophobia. The final 1 year follow-up scan took place in June 2011. Of the patients approached for a day three scan for the CMR substudy, 61 agreed and were well enough to return. Six did not return for a one year follow-up scan including one patient receiving long term trastuzumab therapy for metastatic disease and there was one non cardiovascular death. One patient was discovered to have sustained a MI during chemotherapy and was subsequently excluded from the analysis. In 3 patients the EGRE data for either baseline or day-3 was technically inadequate for analysis. We therefore report data on the 51 patients who completed the CMR substudy protocol with acceptable data quality.

Table 1.2 shows the mean values and changes between time points for all parameters measured at baseline, day 3 and one year follow-up for the patients taking part in the day 3 study who returned for follow-up. No significant differences were found between the patients receiving trastuzumab and those not (p>0.1) therefore the patient results were combined. ERGE mean values and changes are for baseline and day 3 only.
Table 6-2 EF, LV mass and inflammatory imaging changes in the 51 patients who successfully completed the protocol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Change (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline – Day 3</td>
</tr>
<tr>
<td>LVEDV mL</td>
<td>115.2 (17.6)</td>
<td>117.2 (15.8)</td>
</tr>
<tr>
<td>LVESV mL</td>
<td>32.5 (7.6)</td>
<td>33.6 (6.9)</td>
</tr>
<tr>
<td>LVEF %</td>
<td>72.7 (4.7)</td>
<td>71.6 (4.2)</td>
</tr>
<tr>
<td>LVMass g/m²</td>
<td>105.6 (12.3)</td>
<td>109.1 (12.9)</td>
</tr>
<tr>
<td>RVEDV mL</td>
<td>130.3 (19.8)</td>
<td>132.8 (20.0)</td>
</tr>
<tr>
<td>RVESV mL</td>
<td>41.5 (8.8)</td>
<td>43.4 (9.3)</td>
</tr>
<tr>
<td>RVEF %</td>
<td>67.9 (5.4)</td>
<td>67.5 (5.8)</td>
</tr>
<tr>
<td>EGRE</td>
<td>2.36 (0.8)</td>
<td>2.74 (1.1)</td>
</tr>
<tr>
<td>STIR</td>
<td>63.3 (19.8)</td>
<td>67.1 (20.0)</td>
</tr>
</tbody>
</table>

For the whole group of 51 patients, there was a significant reduction in mean LVEF (-2.6% p=0.0002) from baseline (72.7 ±4.7%) to 1 year follow up (70.1 ±5.2%). Mean RVEF fell from 67.9 ±5.4% at baseline, to 66.5 ±5.3% at follow up (-1.4%, p<0.006). LV mass showed a significant increase from baseline (105.6 ±12.3 g/m²) to 109.1 ±12.9 g/m² at day 3 (+3.5g/m², p= 0.00004) but returned to baseline by the one year follow up. RV mass was not measured due to poor reproducibility. The mean increase in EGRE was significant (+0.38 ±0.9, p=0.003) from 2.36 ±0.8 at baseline to 2.74 ±1.1 at day 3. Mean STIR signal intensity showed a trend towards increase (p=0.07) from 63.3 ±19.8 at baseline to 67.1 ± 0 at day 3.

There was a significant correlation between changes in EGRE from baseline to day 3 and a reduction in LVEF at follow up (R=0.34, p=0.01). There was also a weaker correlation between the increase in STIR SI between baseline and day 3 and reduction in LVEF at follow up (R=0.27, p=0.05) as shown in figure 1.1.
Figure 6-1 Graph showing the correlations between Changes in EGRE and STIR from baseline to day 3 and the change in LVEF from baseline to follow up.

For patients with a ≥5% reduction in LVEF from baseline to follow up (n=22, mean reduction 7.8% ±3.6, p<0.00001) mean ERGE increased from 2.23 ±0.7 at baseline to 2.95 ± .1 at day 3 (p=0.002) and STIR SI increased from 63.8 ±20.8 to 73.6 ±24.5 (p=0.003). In the remaining patients (n= 29), there was a mean increase in LVEF from baseline to follow up of 1.7% ±3.2 (p=0.01), and the mean EGRE and STIR showed no significant change, (2.46 ±0.95 to 2.58 ±1.20 at day 3, p=0.39; and 63.2 ±19.5 to 63.3 ±16.2, p=0.98 respectively).

No relationship was found between the changes in EGRE or STIR and change in RVEF at follow up. There was no correlation between increase in LV mass at day 3 and reduction in LVEF at follow up (R=0.05, p=0.77). There was a significant increase in cardiac T2* between baseline (31.7 ± 3.6ms) and day 3 (33.0 ± 4.4ms) p= 0.02. There was no significant between the baseline value and follow-up (30.8 ±5.0, p=0.3)

Urinary F2-isoprostane/ creatinine mean values showed a trend towards decrease from 0.106 ±0.063 at baseline to 0.096 ±0.064 at day 3 (p=0.17). There was a negative correlation between the value at day 3 and both EGRE at day 3 (R=0.39, p=0.005) and an increase in EGRE from baseline to day 3 (R=0.32, p=0.03). There was a negative correlation between F2-isoprostane levels at day 3 and RVEF at day 3 (R=0.32, p=0.03) There was also a weak negative relationship between F2-isoprostanes at day 3 and a reduction in LVEF from baseline to follow up (R=0.26, p=0.06)
6.4 Discussion

AMC has long been recognised as an unwanted corollary of successful cancer treatment. With ongoing improvements in anti-cancer therapy and an increasingly ageing population we can only expect the incidence to increase. Early diagnosis can either guide modulation of the therapy regime or flag the need for cardiological intervention, however the identification of patients at risk before commencing anti-cancer treatment would be the optimal approach. This would permit different individual therapeutic strategies to give the best chance of cancer cure or long remittance but at the same time avoiding the risk of cardiac pathology, in other words tailoring treatment.

The results of this study have shown a significant relation between EGRE, which is a CMR marker of myocardial hyperaemia and a late deterioration in left ventricular function. The relation is however only moderate and further work will be needed to establish whether such a test could be used to predict chronic ACM. Of interest is that the CMR oedema marker of STIR imaging also predicted the reduction in left ventricular function, but with lower power.

Wassmuth reported that an increase in EGRE at day 3 post first chemotherapy cycle related to a later decrease in LVEF in a pilot study of 22 patients with diverse oncological diagnoses. (Wassmuth 2001) In this study patients were scanned prior to and then 3 days and 28 days post first cycle, and 18 patients were also scanned 6 months after the start of treatment. The group found that an increase in EGRE by a factor of >5 at day 3 correlated strongly with a decrease in LVEF at 28 days and weakly with the change at 6 months. As most chemotherapy courses last for 6 months, the changes described cannot be considered chronic but the findings led us to believe there may be a use in this methodology for predicting late events, and we therefore designed our trial to have greater power to show an effect, to have a more homogeneous patient cohort, and to have longer follow-up which is clinically relevant to the development time of ACM after chemotherapy. Treatment regimens were carefully monitored and markers of inflammation and cardiac damage assessed. We also collected samples to measure markers of inflammation and cardiac damage to be subsequently related to genetic markers although the DNA and serum biomarker data is not available to date. The changes in EGRE in our patients were much lower than those
described by Wassmuth. This could be due to the anthracycline regime used, as anthracycline dosage tends to be lower in adjuvant therapy. They stated that there was no correlation between chemotherapy dose and myocardial signal intensity or functional changes in their patient group (data not included in the publication) however their cohort included patients with cancers with a poorer prognosis and the majority received doxorubicin therapy which is associated with a higher incidence of AMC. (Hortobagyi 1989, Pfeffer 2009) They used a 1.0 T (Siemens Impact Expert) MR scanner, but the scan sequence used was similar and we applied the same formula for calculating EGRE as in their study.

It was expected that the urinary F2-isoprostane to creatinine ratio, as a marker of oxidative stress, would increase at day 3 but in fact there was a reduction. Furthermore this reduction related to changes in EGRE between baseline and day 3. Il’yasova et al found that oxidative stress biomarker mean values, including F2-isoprostanes, were increased one hour after treatment administration in a group of women with early breast cancer but that they had normalised 24 hours later. (Il’yasova 2010) A more recent study by this group however noted two distinct patterns of response. One group showed an increase in biomarkers one hour post treatment with a subsequent decrease at 24 hours and the other showed a mixed response with a reduction in some biomarkers one hour post administration and this group had higher baseline biomarker levels. (Il’yasova 2011) In both studies they did not measure biomarker levels after 24 hours post anthracycline exposure. The authors postulated that differing mechanisms including antioxidant defence and homeostatic redox control may be implicated and relate to an individual’s susceptibility to AMC. The decreased levels of F2-isopostanes at day 3 in our study may be a reflection of overshoot of the defence mechanism that is more marked in patients who are more susceptible to AMC. The fact that the decrease correlates with a subsequent increase in EGRE may indicate a longer equilibration process for macroscopic changes.

Although it was hypothesised that a rise in myocardial iron might be identified by a reduction in T2* at day 3, in fact there was an unexpected increase in T2*. This may have resulted from the confounding effect of an increase in tissue oedema, prolonging spin-spin relaxation and therefore T2*. Tissue oedema would increase myocardial
volume and the increase in LV mass at day 3 would support this proposition, as does the increased STIR signal which we found at day 3. Zagrosek et al reported a parallel change in LV mass in myocarditis patients showing a transient increase in myocardial/skeletal signal intensity ratio. (Zagrosek 2008) We found a significant increase in LV mass at day 3, but somewhat surprisingly this did not correlate with a reduction in LVEF at follow up indicating that oedema might be generic response to anthracycline treatment, rather than being indicative of specific damage likely to ultimately result in loss of ventricular function. The patients were scanned a year post completion of treatment yet there is an increasing risk of heart failure for at least 5 years. (Eschenhagen 2011) Prolonging the follow up period may identify a relationship.

6.5 Limitations

It was originally intended to restrict recruitment to women aged 30-60, of white-European ethnicity, receiving 360-450mg/m$^2$ epirubicin, receiving either no radiotherapy or only right-sided radiotherapy; who had who had a booking BP <140/90, BMI <30 and no history of cardiovascular disease or diabetes. However we found only 7.5% of patients with planned adjuvant chemotherapy fulfilled these criteria and they were modified in June 2005. It was subsequently decided that all patients over 18 years could be included plus those with planned left-sided radiotherapy as around 80% of patients with early breast cancer receive radiotherapy therefore exclusion would have significantly hampered enrolment. Moreover modern radiation therapy is much less likely to be associated with cardiac damage. (Barrett-Lee 2009) Indeed a random effects model of volumetric changes with adjustment for variables including age, trastuzumab and radiotherapy showed no significant difference from the unadjusted changes.

Other modifications included increasing the booking BP (<160/100) and BMI (<35) and reducing the epirubicin dose to 300-450mg/m$^2$ to better reflect the range of regimens used at the participating centres. Due to the extended length of the study, several scanner upgrades occurred which meant non standard sequences needed to be modified. There is no evidence that this impacted on the CMR measures, but we cannot exclude this possibility. Due to the prolonged duration of the study, contracts which
were in place for performing the biochemical assays expired, therefore this data will only become available in the future and cannot currently be reported.

6.6 Conclusions

This study has shown a relationship between early tissue changes detected by CMR after anthracycline exposure and a long term reduction in left ventricular ejection fraction. Furthermore the CMR derived tissue changes were related to changes in markers of oxidative stress which supports the role of oxidative stress in the development of AMC which may help the characterisation of patients susceptible to AMC. It would be interesting to follow these well characterised patients for a longer period to better understand the pathophysiology of AMC and the significance of early biomarkers and mid-term phenotypic changes in long term outcome. Further work is needed to establish whether these promising results with EGRE could have a clinical role in predicting late ACM.
Chapter 7: Optimisation of CMR Sequences for assessment of Iron Overload

7.1 Introduction

Although transfusions improve short term health and survival in TM patients, the consequent tissue iron deposition leads to organ damage in the long term. Those treated only with transfusions usually die early from cardiac failure secondary to myocardial siderosis. (Modell 1982, Borgna-Pignatti 2004) Myocardial dysfunction is directly linked with cardiac iron burden, (Kirk 2009) but often occurs late and can be hard to reverse once established. Direct, accurate assessment and early treatment of iron loading is pivotal in the management of these patients, along with tailored cardiac chelation therapy which may reverse or prevent myocardial dysfunction. (Wolfe 1985, Brittenham 1994) Over the last decade, there has been a marked reduction in deaths attributable to tissue iron overload within the UK which coincides with the introduction of T2* cardiovascular magnetic resonance (CMR) as a diagnostic tool. (Modell 2008)

The first T2* sequence developed was a multi breath-hold, white blood, end-diastolic acquisition. (Anderson 2001) This technique was time consuming and was compromised by miss-registration due to inconsistent breath-holding. A single breath-hold sequence was subsequently developed to reduce scan time and improve image registration between images, and this improved reproducibility. (Westwood 2003) This technique became the standard for evaluation and follow-up and has been installed on CMR scanners from different vendors at multiple sites throughout the world (Kirk 2010) and is still being used to investigate the cardiac efficacy of chelating agents. (Pennell 2006, Tanner 2007, Tanner 2008, Pennell 2010, Pennell 2011) Despite its success however, the white blood technique has some undesirable characteristics: the contrast between blood pool and myocardium may be suboptimal, and despite flow compensation, artefacts from motion and blood flow may compromise measurement accuracy.
More recently, a double inversion recovery ‘black blood’ sequence has been reported which suppresses the blood signal, and a preliminary comparison with white blood T2* imaging suggested good reproducibility. (He 2007) The aim of study A was therefore to compare inter and intra-observer reproducibility between the established white blood sequence and the newer black blood sequence in a large group of patients, and to evaluate the interstudy reproducibility and artefacts when using the black blood sequence. For study B, we examined the possible role of performing T2* imaging in systole instead of diastole.

7.2 Methods for study A: black blood imaging

100 TM patients were studied to compare the black blood and white blood acquisitions. The cohort included 50 consecutive patients with cardiac siderosis (T2* <20ms) and 50 consecutive patients with no cardiac iron (T2* >20ms) as measured using the conventional white blood T2* acquisition. Cardiac T2* values ranged from 4.5 to 43.8ms across all patients. The age range was 11–50 years (mean 27) and 51% were female. All patients underwent both the conventional white blood and the new black blood T2* scan to test intra and inter-observer reproducibility. To test inter-study reproducibility, 23 patients with T2* values from 6.9 to 46.1 ms had a second scan for both black and white blood acquisitions on the same day.

All patients were scanned using a 1.5 T scanner (Siemens Sonata, Erlangen, Germany) with a 6 channel phased array cardiac receiver coil and ECG gating. A mid-ventricular short axis slice was obtained for all scans. The white blood acquisition used a multi-echo gradient-echo sequence (flip angle 20°, matrix 128x256 pixels, sample bandwidth 810 Hz/pixel, slice thickness 10mm and field of view 40cm). The short axis images were acquired within a single breath-hold at 8 echo times from 2.54 to 17.90 ms at approximately 2 ms increments. For white blood diastolic imaging, the images were acquired immediately after the R-wave. For the black blood acquisitions, a double inversion recovery (DIR) pulse was applied on the R-wave and the inversion time extended into diastole, generating a similar set of 8 images at increasing echo times.

Image analysis was performed using Thalassaemia tools (a plug in of CMRtools, Cardiovascular Imaging Solutions, London, UK). A full thickness ROI incorporating
the ventricular septum was selected avoiding blood pool and cardiac vessels. (Reeder 1998, Ghugre 2006) T2* was derived by using an exponential curve fit, \[ SI = SI_0 \cdot \exp(-\frac{TE}{T2^*}) \], where SI is signal intensity, TE is the echo time and SI0 is a constant representing signal intensity at time zero. A truncation method was used to account for background noise at the longer echo times and improve curve fit, (He 2008) although this was not generally necessary for the black blood acquisitions.

Figure 7-1 Rapid signal decay in heavily iron loaded hearts causes the curve to plateau. Later points that fall below background noise level are removed to improve curve fit in frame a (red crosses). For the black blood acquisition (b) background noise is reduced thus the curve fit is good for the full range of echo times without truncation (R² = 0.9995) which reduces the likelihood of analysis errors.

Analyses for all sequences were performed by two experienced investigators. Each data-set was anonymised and presented to observers in random order. Intra-observer reproducibility was assessed using 2 sets of measurements derived by observer 1. To
test inter-observer reproducibility, the first read results were compared with those of observer 2. Inter-study reproducibility was assessed by observer 1 using data from the repeated studies. Analysis of image quality was conducted by 2 observers using the following 5 point scale:

0 - Very poor image quality with unusable images;
1 - Poor image quality, just able to make out the heart but a lot of artefact;
2 - Average image quality, not all of septum clearly seen and a lot of artefact;
3 - Good image quality with moderate septal artefact;
4 - Very good quality, with minimal septal artefacts;
5 - Excellent image quality with no significant septal artefact.

To quantify the reproducibility, the coefficient of variance (CV) was calculated from the standard deviation of the differences between analyses divided by their mean. A paired, two-tailed Student’s t-test was performed on the natural log transformation of squared differences between methods to assess differences in measurement variability between methods. (Moon 2002) Friedman’s test was used to analyze the image quality scores. A p value of < 0.05 was considered significant. The local research ethics committee approved the study and patients gave informed consent.

7.3 Results for Study A

There was no significant difference between the mean cardiac T2* values for all 100 patients for the black blood acquisition and the conventional white blood sequence with diastolic imaging (20.5ms vs 21.6ms; p=0.26). Differences between black and white blood acquisitions were also not significant for the iron loaded (n=50, p=0.31) and non-iron loaded (n=50, p=0.081) subgroups. The scatter plot of white blood vs black blood T2* showed the values lay close to the line of identity (fig 7-2)
Figure 7-2 Bland-Altman plot (a) and scatter plot showing the line of identity (b) of myocardial T2* values obtained from white blood and black blood acquisitions. Agreement was good for patients with T2* ≤ 20 (iron overloaded) but discrepancy increased with increasing T2.

### 7.3.1 Inter and intra observer agreement

The intra-observer CV across the full range of T2* values (n=100) was significantly lower using the black blood compared with the white blood sequence (1.47% vs 4.23%, p<0.001).
Figure 7-3 Bland-Altman plot (a) and scatter plot (b) shows blinded intra-observer T2* measurement reproducibility using the white blood acquisition. The improvement in agreement using the black blood sequence can be seen from plots c and d.

In patients with iron overload (T2* <20 ms) the CV was significantly lower for black blood than for white blood acquisition (1.68% vs 2.81%, p<0.001). In patients with no iron overload (T2* >20ms), the CV was also significantly lower for black blood than white blood acquisition (1.28% vs 3.88%, p<0.001).
Figure 7-4 Bland-Altman plot (a) and scatter plot (b) shows agreement between 2 blinded observers T2* for the white blood acquisition compared with the black blood images (c and d).

The inter-observer agreement findings were similarly superior for the black blood acquisition, with a CV of 2.54% (black blood) compared with 4.50% (white blood) for all patients (p<0.001), 2.91% vs. 4.28% for iron overloaded patients (p=0.003) and 2.16% vs. 4.02% for patients with no iron loading (p=0.03)
7.3.2 Inter study reproducibility

For all patients (n=23), the CV for inter-study reproducibility was superior for black blood compared with white blood acquisition (4.07% vs 8.42%, p=0.001). In patients with a T2* <20ms (n=12) the CV was 2.21% for black blood and 7.04% for white blood (p=0.003). In the patients with no iron loading (n=11), the CV was 3.93% and 7.87% respectively (p=0.15).

Figure 7-5 Plots a and b show interstudy reproducibility for the white blood sequence and c and d show the improved reproducibility using the black blood preparation.
7.3.3 Artefact scoring

The mean artefact score was superior for the black blood acquisition compared with white blood (4.57 vs 4.25, p<0.001).

7.4 Discussion for study A

In this study, we have shown improvement in intra and inter-observer variability together with inter-study reproducibility for black blood T2* imaging over the conventional white blood sequence in a large patient sample. For the conventional white blood sequence with no trigger delay, low signal contrast and the consequent difficulty in defining the myocardial/blood boundary may cause error in T2* measurement. This may result in white signal from the blood pool being incorporated into the decay curve for analysis. The errors may also be exacerbated by misregistered, high signal blood pool artefacts which can be superimposed on the ROI. Adding the double inversion recovery pulse to generate the black blood images removes most of the blood signal (and hence artefacts) from the myocardium, making the measurement of T2* more robust. Importantly, the greatest disparity between white blood and black blood T2* values was seen when testing inter-study reproducibility in iron overloaded patients where we found a three-fold difference in the coefficient of variation. This discrepancy may be due to different levels of artefact in the myocardial septum resulting in changes in the overall signal intensity in the region of interest between scans. Indeed analysis of artefact scoring showed significantly poorer artefact scoring for the white blood acquisition which could potentially impart significant errors in longitudinal evaluation.

The improved analysis with reduced requirement for truncation of data points in patients with short T2* values, is an additional advantage in reducing analysis errors, particularly in less experienced centres. These findings combined with the improved reproducibility, suggest that the black blood technique should be used for routine clinical practice, providing centres can install a robust sequence as implemented by the scanner manufacturers.
7.5 Conclusions for study A

In conclusion, this study shows improved reproducibility and reduced artefacts for the black blood sequence in comparison with the previously validated white blood sequence, and therefore the black blood sequence should be considered the imaging technique of choice.

7.6 Study B: Systolic T2* imaging

Although reproducibility is improved there are however some disadvantages to the black blood acquisition. Because of the time taken to achieve blood signal nulling, it is usually necessary to gate on alternate cardiac cycles, unless the RR interval is very long. This can make the acquisition and therefore the breath-hold long which may be challenging for the patient leading to respiratory artefact on the image. Also border recognition can be difficult in the presence of heavy iron loading. Introducing a trigger delay to acquire data in late diastole has been shown to improve inter-observer variability (He 2007) however, in patients with a short R-R interval, the early and late ventricular filling phases tend to merge giving no period of stasis. During late systole, before the AV valves open, wall thickness is maximal, the ventricles are relatively static and surface trabeculations more compacted, reducing partial volume effects. As a result, we hypothesised that acquiring data during this phase of the cardiac cycle (systolic imaging) would provide an easily definable ROI.

7.7 Methods for study B

25 patients with myocardial iron overload underwent examinations with the conventional bright blood sequence with diastolic image acquisition, the black blood sequence and a modified bright blood sequence with systolic data acquisition. The timing of the trigger delay for systolic imaging was derived from the corresponding cine images. Data analysis was performed as described above.

7.8 Results for study B

Taking black blood imaging as the reference, systolic imaging (with the addition of a trigger delay to the bright blood acquisition) showed a better agreement than the
conventional bright blood sequence with no delay (CV 5.82% vs 9.12%). The CV also improved for both intra-observer (from 3.93% to 1.73%) and inter-observer reproducibility (from 5.43% to 3.19%).

Figure 7-6 Frames a and b show T2* values using diastolic white blood vs black blood imaging and frames c and d show the improves agreement between systolic and black blood imaging.
Figure 7-7  Systolic bright blood (a) and black blood imaging (b) show good agreement whereas flow artefacts in the diastolic bright blood image (c) cause overestimation of T2*.

7.9 Discussion for study B

Adding a trigger delay to the bright blood sequence to produce systolic images improved reproducibility of analyses by a factor of two with no scan time penalties.
Although we believe that the black blood DIR sequence is the examination of choice for the measurement of cardiac T2*, the major compromise is scan duration and in addition, it has not yet been possible to transfer this sequence to all makes of scanner. Bright blood systolic imaging with a trigger delay to allow data acquisition which coincides with peak systole appears to be a significant improvement on the conventional sequence acquired immediately after the R-wave. The septal ROI thickness is maximal at this phase of the cardiac cycle with minimal motion and there is a high degree of agreement with the black blood T2* results. Therefore, systolic imaging may be a good alternative where black blood imaging is not available.

7.10 Conclusion for study B

In conclusion, although we have found improved reproducibility across all T2* values for the black blood sequence in comparison with the previously validated bright blood sequence there are time penalties and some problems with boundary definition with a heavy iron burden. Adding a trigger delay to the bright blood acquisition gives systolic T2* images with a thicker ROI which improves reproducibility resulting in a better agreement with the black blood value suggesting that this technique is a valid alternative if black blood imaging is unavailable or in patients with severe iron overload.
Chapter 8: Right ventricular response to deferiprone and deferoxamine

8.1 Introduction

Deferoxamine was the first iron chelating agent licensed for clinical use. Deferoxamine is a large positively charged lipophobic molecule, is poorly absorbed by the digestive system and it has a short plasma half life. (Kushner 2001, Porter 2001) This means the drug must administered subcutaneously or intravenously. This can lead to poor compliance which combined with other factors results in inadequate chelation. (Tanner 2006) Deferiprone is a much smaller neutrally charged lipophilic molecule which allows good gastrointestinal absorption and cellular access and therefore oral administration. (Kushner 2001, Porter 2001) Direct comparison trials show that deferiprone has greater efficacy than deferoxamine for reducing myocardial iron loading and improving LV systolic function. (Anderson 2002, Pennell 2006) However, there is a paucity of data related to the effects of these chelators on the RV, which is known to be an important independent predictor of outcome in dilated cardiomyopathy, (Juiliere 1997) and ischaemic heart disease. (de Groote 1998, Larose 2007, Miszalski-Jamka 2010) Recent papers have established the normal ranges for RV volumetric parameters for non-iron loaded TM patients and shown a significant relation between T2* and RVEF, including a small percentage of patients with impaired RVEF but normal LVEF. (Alpendurada 2010, Carpenter 2010) In order to identify and compare the effects of deferiprone and deferoxamine, we reanalysed the CMR images for the LA16 trial, which was a randomized controlled trial (RCT) comparing the 2 drugs. (Pennell 2006) Our hypothesis was that deferiprone would improve RV function more than deferoxamine.

8.2 Methods

The LA16 RCT consisted of 61 regularly transfused patients with TM from 4 centres in Greece and Italy. (Pennell 2006) All patients were previously treated with subcutaneous deferoxamine monotherapy. Inclusion criteria included a T2* between 8 and 20ms and
LVEF greater than 56% based on the lower normal limit for non-anaemic subjects from previously published data. (Lorenz 1999) No patient had heart failure symptoms. Deferiprone was allocated to 29 patients (actual dose 92mg/kg daily) whilst 32 patients were allocated to continue with deferoxamine therapy (dose of 43mg/kg/day overnight for an average of 5.7 days per week). Written informed consent was obtained according to local ethics committee approval.

Iron loading and cardiac function was assessed using CMR. The T2* sequences were installed at the local CMR facilities, Athens (GE CVi) and Cagliari (GE Signa). The technique was validated by scanning phantoms of known T2* and testing intra-site reproducibility by scanning 5 patients twice at the local centre. The same patients were scanned at the reference site in London (Siemens Sonata) for inter-site reproducibility. A coefficient of variation (CV) ≤15% was defined as acceptable. Site inter-study variability was 2.4% for Cagliari and 3.5% for Athens. Comparison with the reference site yielded CVs of 1.6% and 9.7% respectively. Volumetric data were acquired using a steady state free precession sequence (FIESTA). A set of contiguous short axis cines were acquired to give complete coverage of both ventricles. Care was taken to place the basal slice parallel to the atrioventricular groove. Slice thickness was 8mm with a spacing of 10mm. No patient had a history of, and no CMR scan showed any features of pulmonary hypertension (normal pulmonary artery size, no right ventricular hypertrophy, no systolic septal flattening). Patients were scanned between 3 and 10 days post transfusion at baseline, 6 months and 12 months after entering the trial. T2* values and LV volumetric data were assessed previously using a CMR viewing and analysis software package CMRtools (Cardiovascular imaging solutions, London, UK). (Pennell 2006) LV function was assessed in the RCT using an early version of the analysis package in which LV volumes are quantified manually using direct surface planimetry, and therefore for consistency we elected to use the same version of the software to analyze the RV thus eliminating the need for right sided valve tracking. Care was taken to include blood volume below the pulmonary valve. Basal regions with thin, non trabeculated muscle were considered atrial and were excluded. Papillary muscles were also excluded from the blood pool. (Hudsmith 2005) Although local blinding to the treatment arm was not possible due to the nature of drug administration (oral for deferiprone and subcutaneous for deferoxamine), all remote scan analysis
performed at the core-lab in London was fully blinded to treatment. Study treatment was unblinded on completion of LV and T2* analysis. For the analysis of the RV volumetric data, all data sets were anonymised and analysed in random order using the same analysis package by experienced operators blinded to treatment arm and LV response. To assess reproducibility, data-sets with an improvement in RVEF $\geq 5\%$ at 12 months were reprocessed blindly.

8.3 Statistical analysis

Continuous variables were compared using a paired t-test. T2* values were log transformed and changes expressed as geometric mean with coefficient of variation. Between groups comparison of drug effects were assessed using a repeated measurement ANOVA. Statistical significance was set at $p<0.05$. To quantify reproducibility the coefficient of variance (CV) was calculated.

8.4 Results

Full data on the LA16 study have been published including the patient demographics, (Pennell 2006) but the important trial summary findings are repeated here. In the deferiprone group 27 patients completed the study; 2 patients withdrew due to adverse events (elevated hepatic enzymes, in one case probably due to cytomegalovirus). In the deferoxamine group, 29 patients completed; 1 patient withdrew secondary to a reduction in LV function and 2 for personal reasons. The patient groups were well matched at baseline for cardiac T2*, LV volumes and function and RV volumes and function (table 8-1 below).
Baseline RVEF was within the normal reference range for thalassaemia patients, (Carpenter 2010) except for one patient in the deferoxamine group (RVEF 1% below the normal range). Patient compliance was similar for both groups. Myocardial T2* improved by 18% at 6 months (p<0.001) and 27% at 12 months (p<0.001) in the deferiprone arm. The LVEF rose by 2.0% from 69.7% at baseline to 71.7% at 6 months (p<0.001) and by 3.1% to 72.7 at 12 months (p<0.001). With deferoxamine therapy, T2* improved by 9% at 6 months (p=0.003) and by 13% at 12 months (p<0.001) but LVEF was unchanged being 68.4% at baseline and 68.7 at 6 months (+0.52%, p=0.42) and 68.5 at 12 months (+0.32%, p=0.66).

In the current analysis, the RV mean volumetric and T2* values are detailed below (table 8-2)
Table 8-2 RV volumetric parameters at baseline, 6 and 12 months (mean ±SD) in the 2 treatment arms. The $T2^*$ values show the geometric mean and CV. The p value reflects the change from baseline to 12 months.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 Months</th>
<th>12 Months</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deferoxamine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T2^*$, ms</td>
<td>13.0 (32)</td>
<td>15.4 (38)</td>
<td>16.5 (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RVEDV, mL</td>
<td>122.5 ±24.9</td>
<td>123.2 ±26.0</td>
<td>121.3 ±24.9</td>
<td>0.61</td>
</tr>
<tr>
<td>RVESV, mL</td>
<td>37.7 ±11.7</td>
<td>35.9 ±11.7</td>
<td>34.2 ±11.3</td>
<td>0.009</td>
</tr>
<tr>
<td>RVSV, mL</td>
<td>84.7 ±16.5</td>
<td>87.3 ±16.5</td>
<td>87.1 ±17.0</td>
<td>0.16</td>
</tr>
<tr>
<td>RVIF, %</td>
<td>69.6 ±5.2</td>
<td>71.4 ±4.7</td>
<td>72.2 ±5.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Deferiprone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T2^*$, ms</td>
<td>13.3 (30)</td>
<td>14.4 (37)</td>
<td>15 (39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RVEDV, mL</td>
<td>124.7 ±27.7</td>
<td>124.4 ±26.2</td>
<td>128 ±32.1</td>
<td>0.17</td>
</tr>
<tr>
<td>RVESV, mL</td>
<td>38.1 ±12.6</td>
<td>37.2 ±12.5</td>
<td>39.1 ±13.0</td>
<td>0.38</td>
</tr>
<tr>
<td>RVSV, mL</td>
<td>86.7 ±18.0</td>
<td>87.0 ±15.5</td>
<td>88.9 ±21.3</td>
<td>0.25</td>
</tr>
<tr>
<td>RVIF, %</td>
<td>70.0 ±5.8</td>
<td>70.8 ±5.2</td>
<td>69.9 ±4.6</td>
<td>0.93</td>
</tr>
</tbody>
</table>

To summarise, in the deferiprone arm RV end-diastolic volume (EDV) was stable, RV end-systolic volume (ESV) decreased significantly from 37.7 to 34.2mL at 12 months (p=0.009); and RVEF increased from 69.6% to 72.2% (p=0.001). For the patients on deferoxamine therapy, the changes in RV parameters from baseline to 12 months showed no significant difference. Analysis of between drugs treatment effects was expressed using a repeated measurement ANOVA (table 8-3 below)

Table 8-3 Between drug effect on RV volumetric parameters showing a significant difference in RV ESV and RVEF favouring deferiprone.
There were significant differences favouring deferiprone for the reduction of RV ESV (p=0.014 at 12 months, and improvement in RVEF (p=0.009 at 12 months, (figure 8-1 below).

**Figure 8-1** The response in the 2 treatment arms for RVESV (left pane) and RVEF (right pane) showed a significant improvement for patients treated with deferiprone, which was not seen with deferoxamine (p=0.014 and 0.008 respectively for 12 month difference between drugs)

Non significant differences between drugs were found for RV EDV. With regression analysis, the change in RVEF was found to be inversely related to the baseline EF (p<0.001) with a significant difference between drugs favouring deferiprone by a mean of 3.5% (95% CI 0.8 to 6.3%; p=0.012). The reduction in RVESV over 12 months was also related to the baseline ESV value with borderline significance (p=0.051), and there was a significant difference between drugs favouring deferiprone by a mean of 4.5mL more than patients on deferoxamine (95% CI 1.2 to 7.8mL; p=0.009). Therefore the patients benefitting most from deferiprone treatment are those with the lower baseline values of RVEF. The CV for intra-observer study RVEF measurement was 2.4% at baseline and 2.0% at 12 months. There was no relation between change in RVEF and change in LVEF (r=0.3, p=0.9).

### 8.5 Discussion

RV volumetric and functional parameters have been difficult to measure using conventional imaging techniques due to the irregular geometry of the RV chamber, the
size and quantity of the RV trabeculae, and the proximity of the RV to the chest wall which impairs echocardiographic assessment. CMR suffers less from these drawbacks because of its inherent 3D nature and high blood to myocardium contrast and is therefore considered to be the most accurate and reproducible technique for assessing RV volumes and EF. (Grothues 2002, Grothues 2004) Attention to correct definition of the basal slice during acquisition and subsequent analysis is however pivotal. The improved confidence of measuring RV volumes and function from CMR and other techniques has assisted the understanding the importance of the RV in cardiac disease.

RVEF is an important predictor of outcome in dilated cardiomyopathy, which is both independent of and incremental to LV EF. (Juilliere 1997) The predictive value of RV function has also been shown in congenital heart disease, (Gatzoulis 1995, Graham 2000, Roos-Hesselink 2004) chronic systolic dysfunction, (Meyer 2010) and ischemic heart failure, (Di Salvo 1995, de Groote 1998, Ghio 2001, Larose 2007) with RVEF being shown to be an independent predictor of outcome. Accordingly, the effects of myocardial iron loading on RV function may be important in thalassaemia patients.

In the current study, we found a significant improvement in RVEF (increase) and RVESV (reduction) with deferiprone therapy. These improvements parallel the previously reported LV response. (Pennell 2006) There was no significant increase in RVEDV suggesting loading conditions did not play an important role. A flat RV response was seen in the deferoxamine group, which again mirrors LV behaviour. The between groups analysis showed superiority for deferiprone over deferoxamine for both the reduction in RVESV and the increase in RVEF. The magnitude of improvement in RVEF and reduction in RVESV were greater for patients with a higher ESV and lower EF at baseline. Interestingly neither LVEF nor RVEF improved significantly in the deferoxamine group despite the improvement in T2*. The cause for this difference in functional response is not fully understood, but the explanation may lie in the additional effects of deferiprone on restoring normal cardiac mitochondrial function, (Kakhlon 2008) possibly through effects on reducing reactive oxygen species. (Kontoghiorghes 2009)
There is little other data relating RV function changes with the iron chelators, but a recently published abstract relating to a longitudinal trial of the efficacy of deferasirox in myocardial siderosis, (Smith 2009) showed a significant improvement in myocardial iron levels with an improvement in RVEF at 1 year, but there was no change in LV function at 1, 2, and 3 years of follow up (year 3 paper under peer review). (Pennell 2010, Pennell 2011) The significance of this discrepancy between RV and LV response to deferasirox is not currently clear, though it is possible that the RV response is an early signal of myocardial iron clearance as LV compliance and filling pressure improves. The 3 year RV response to deferasirox therapy is describes in chapter 10.

8.6 Limitations

Data acquisition for this study was originally designed to assess the change in T2* and LV functional parameters in response to therapy. Therefore no RV long axis images were obtained to construct 3-dimentional models for volumetric analysis, but the requirement for this was removed by using direct manual planimetry for quantitative analysis of RV volumes. Pulmonary arterial pressure was not systematically measured using echocardiography of the tricuspid regurgitant jet, but here was no CMR evidence of raised pulmonary artery pressure in our patients, and pulmonary hypertension is rare in well treated thalassaemia major. (Aessopos 2004) Direct RV measurement of T2* would have been interesting in this population to compare with changes in RV volumes and function, however, it is challenging to measure T2* in the thin wall of the RV and this was not attempted in the randomized controlled trial.

8.7 Conclusions

This study has shown that RVESV decreased and RVEF improved with deferiprone monotherapy and this beneficial response was superior to deferoxamine. RV volumetric and function parameters have in the past been neglected when reporting the efficacy of iron chelators for myocardial iron overload, and may have independent prognostic importance, as they do in other cardiac conditions with impaired cardiac function.
Chapter 9: Right ventricular response to deferiprone/deferoxamine combination therapy

9.1 Introduction

Deferoxamine has good iron binding efficacy but its beneficial effects are tempered by poor bio-availability which requires cumbersome treatment regimes which may be a contributory factor to the frequently observed long term complications of heart failure and cardiac death. (Borgna-Pignatti 2004) Deferiprone is both hydrophilic and lipophilic enabling it to readily penetrate myocardial cells and has been shown to be superior to deferoxamine in removing iron from the myocardium with improved cardiac outcomes. (Modell 2000, Anderson 2002, Piga 2003, Borgna-Pignatti 2006, Pennell 2006) It has less efficient iron binding than deferoxamine however and some patients fail to achieve iron balance with monotherapy. By combining deferoxamine and deferiprone more patients achieve iron balance than with either chelator alone. (Wonke 1998, Daar 2006, Kolnagou 2006) Due to differences in their access to body iron pools, the use of a combination of the two chelators seems to have a synergistic effect on the removal of excess iron. (Wonke 1998) If both chelators are available in the body simultaneously they may interact by iron being “shuttled” from deferiprone onto the deferoxamine molecule. (Porter 2009)

A recent randomised controlled trial comparing combination therapy with subcutaneous deferoxamine and oral deferiprone against deferoxamine monotherapy showed combination treatment to be superior in removing cardiac iron and improving LVEF. (Tanner 2007) The beneficial effects of combination therapy on LVEF have also been confirmed in patients with TM and severe iron loading. (Tanner 2008) However, despite this success for LV function, the importance of combination therapy on RV function has not been reported, even though the RV can be affected by the toxic effects of myocardial iron. (Hahalis 2002, Alpendurada 2010) We therefore compared the effects of combination treatment (deferoxamine and deferiprone) with deferoxamine monotherapy on RV function in TM patients with cardiac iron overload.
9.2 Methods

In order to examine the effects of combination treatment on the RV, we reanalyzed imaging data from 2 previously reported trials. The first was a randomized, double-blind, placebo RCT comparing combined therapy of deferoxamine with deferiprone against deferoxamine with placebo in mild-moderate myocardial siderosis. (Tanner 2007) The second trial was a longitudinal open-label study of combination treatment (no comparison arm) in patients with severe cardiac siderosis and impaired LV function. (Tanner 2008) Both trials were run simultaneously in Cagliari Italy, with ethical permissions driving the separation of the patients into the 2 groups, as previously described. (Tanner 2007, Tanner 2008) To summarise briefly; 167 adult TM patients (75 males, mean age 30 ± 5.3 years) were screened for quantification of myocardial iron loading using myocardial T2*. Inclusion criteria for patient screening were: diagnosis of TM currently maintained on subcutaneous deferoxamine monotherapy; age >18 years; and maintaining pre-transfusion haemoglobin > 9 g/dL. Exclusion criteria were: patients who had received deferiprone for a total of >6 months over the last 5 years; patients with previous reaction to deferiprone; neutropenia (absolute neutrophil count <1.5 × 10⁹/L) at screening; thrombocytopenia (<50×10⁹/L) at screening; liver enzymes > 3 times upper limit of normal; any condition making CMR impossible or inadvisable.

Of the 167 patients screened, 108 had significant myocardial siderosis (T2* < 20 ms), of whom 22 (13%) had severe myocardial loading (T2* < 8 ms). Patients with mild to moderate cardiac iron loading who satisfied the trial entry criteria (myocardial T2* 8-20 ms, n=86) were invited for further detailed assessment by CMR. Of these, 65 were subsequently randomized to receive either deferoxamine plus deferiprone (combined group; n=32) or deferoxamine plus placebo (deferoxamine group; n=33), and were followed-up for 12 months. Patients with severe cardiac siderosis (T2* < 8 ms) were excluded from the RCT and it was at the treating clinician's discretion to determine best clinical practice for chelation therapy. Of the 22 patients with severe myocardial siderosis, 15 (9 females, 28.9 ± 4.8 years) received open-label combination therapy according to locally developed protocols, and were followed prospectively over one
year. These patients were used in a secondary comparative analysis against patients from the randomised trial who were on combination therapy.

9.2.1 Cardiovascular magnetic resonance

A mobile 1.5 Tesla CMR scanner (Sonata, Siemens Medical Systems, Erlangen, Germany) was transported from the RBH to Cagliari, Italy for this research. Myocardial and hepatic T2* were assessed using the white-blood single breath-hold multi-echo technique as previously described. (Westwood 2003) T2* analysis was performed using Thalassaemia-Tools with curve truncation to correct for background noise. (He 2008) Right ventricular volumes were determined at baseline and at 12 months from long axis and contiguous short-axis SSFP cine images. (Maceira 2006b) LVtools was used to produce 3-D models for RV volume analysis. These measurements were performed by observers blinded to the patient’s clinical details and chelation regime.

9.2.2 Echocardiography

Doppler echocardiography studies were performed at baseline and at 12 months to look for pulmonary hypertension. Pulmonary artery systolic pressures (PAP) were determined by peak velocity of the tricuspid regurgitation jet plus estimation of right atrial pressures using standard methodology. Pulmonary hypertension was defined as PAP > 40 mmHg.

9.2.3 Biochemistry

Laboratory measures included weekly full blood count (due to the risk of agranulocytosis with deferiprone), serum ferritin (Abbott AXSYM System), B-type natriuretic peptide (BNP-Biosite Diagnostics Inc, San Diego, California), and liver function tests (alanine aminotransferase - ALT).

9.2.4 Statistical analysis

Categorical data are presented as frequency and percentage (%). Continuous variables are presented as mean ± standard deviation (SD), except for BNP, which is displayed in median and interquartile range; and for T2* and ferritin, which use the geometric mean.
(anti-log of the mean of the log data) ± coefficient of variation (CV). Baseline characteristics of both treatment groups were compared using an unpaired two-tailed t-test for continuous variables (except for BNP, which was compared by a non-parametric test) and a chi-squared test for categorical variables. Analysis of variance (ANOVA) was used to compare changes in T2* and RVEF over 12 months with treatment and baseline measures entered as covariates. Changes in RVEF over 12 months within individual groups were compared with a paired t-test. Correlations of myocardial T2* with ventricular function were performed using the Spearman’s rank test. Subgroup analysis was performed according to severity of myocardial iron loading with cut-offs of 8ms and 12ms used to define patients with mild (T2* 12-20 ms), moderate (T2* 8-12ms) and severe (T2* < 8ms) iron loading. Statistical significance was set at p <0.05. All statistical analysis was performed using Stata 10.1 software (StataCorp, Texas, USA).

9.3 Results

The baseline findings and the results of the RCT comparing combination treatment against deferoxamine alone for changes in myocardial T2* and LV EF have been previously published, (Tanner 2007) and are briefly summarized here. The patients randomized to combination therapy or deferoxamine alone were evenly matched at baseline (table 9-1 below).
Table 9-1 Baseline characteristics of the randomized controlled trial population according to treatment arm. Values are presented as n (%), mean ± SD, geometric mean (coefficient of variation), or as median (25th – 75th percentile). RV = right ventricular; EDV = end-diastolic volume; ESV = end-systolic volume; PAP = pulmonary artery systolic pressure; BNP = B-type natriuretic peptide

<table>
<thead>
<tr>
<th></th>
<th>Combined</th>
<th>Deferoxamine</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.8 ± 4.2</td>
<td>28.7 ± 5.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>14 (44%)</td>
<td>13 (39%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Body surface area</td>
<td>1.53 ± 0.15</td>
<td>1.56 ± 0.16</td>
<td>0.5</td>
</tr>
<tr>
<td>Deferoxamine dose (mg/kg/day)</td>
<td>(5 days/week)</td>
<td>40.6 ± 13.2</td>
<td>40.5 ± 14.0</td>
</tr>
<tr>
<td>CMR measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial T2*</td>
<td>11.7 (0.08)</td>
<td>12.4 (0.11)</td>
<td>0.3</td>
</tr>
<tr>
<td>Liver T2*</td>
<td>4.9 (0.52)</td>
<td>4.2 (0.62)</td>
<td>0.5</td>
</tr>
<tr>
<td>RVEDV (mL)</td>
<td>131.2 ± 33.9</td>
<td>132.9 ± 36.7</td>
<td>0.8</td>
</tr>
<tr>
<td>RVESV (mL)</td>
<td>53.3 ± 19.6</td>
<td>53.0 ± 19.6</td>
<td>0.9</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>60.2 ± 7.2</td>
<td>60.8 ± 6.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Echo measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>22.3 ± 5.0</td>
<td>20.9 ± 5.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Blood measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusional red blood cell input (mL/kg/year)</td>
<td>133.4 ± 34.9</td>
<td>130.2 ± 38.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>106 ± 9.6</td>
<td>102 ± 9.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Hepatitis C positive</td>
<td>23 (72%)</td>
<td>26 (79%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>1574 (11)</td>
<td>1379 (10)</td>
<td>0.5</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>13.6 (5.6, 30.1)</td>
<td>15.2 (7.3, 26.2)</td>
<td>0.7</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.77 ± 0.21</td>
<td>0.74 ± 0.23</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The prescribed dose of deferiprone in the combination arm was 75 mg/kg/day. The average dose of deferoxamine in the deferoxamine alone arm (43.4 mg/kg/day for 5 days/week) was significantly higher than the combination arm (34.9 mg/kg/day for 5 days/week, p=0.02). Four patients in the combination arm withdrew from the study (3 due to adverse events), and 3 patients in the deferoxamine arm withdrew (1 due to an adverse event). Thus, 28 patients in the combination arm and 30 patients in the deferoxamine monotherapy arm completed the study.
Over 12 months, the combination treatment group showed superior improvement in myocardial T2* compared with the deferoxamine group (ratio of change in geometric means 1.50 vs. 1.24, p=0.02).

In the combination group, RVEF increased from 60.2 ± 7.2% at baseline to 63.8 ± 5.9% at 12 months (p<0.001) whereas in the deferoxamine group, RVEF did not change (61.0 ± 7.1% at baseline vs. 61.7 ± 6.4% at 12 months, p=0.49). There was a significant difference in the RVEF response between groups favouring combination therapy (3.6 vs. 0.7%, p=0.02)

![Figure 9-1 Change in myocardial T2* (left panel) and in RVEF (right panel) over 12 months according to treatment arm. Vertical lines represent standard error.](image)

The improvement in RVEF in the combined group was mainly driven by a decrease in RV end-systolic volumes (60.8 ± 24.2 ml to 50.6 ± 17.3 ml, p<0.01) rather than a change in RV end-diastolic volumes (135.5 ± 33.0 ml to 132.0 ± 29.5 ml, p=0.10). There was no significant change in PAP between the combination vs. the deferoxamine arm from baseline to one year (-1.8 mmHg vs. +0.3 mmHg, p=0.19).

The median myocardial T2* in the RCT at baseline was 12.0 ms. This value was used as a cut-off to define patients with mild (myocardial T2* 12-20 ms) and moderate iron loading (myocardial T2* 8-12 ms), and this was in accord with the cut-offs used in the original trials. Both subgroups were then analysed according to the chelation regime. Comparing the individual treatment arms, we observed a significant improvement in
RVEF in patients with T2* between 8 and 12 ms on combination therapy (58.5 ±6.9% at baseline vs 63.3 ±6.0% at 12 months, p<0.01), and borderline significant improvement in patients with T2* between 12 and 20 ms (62.1 ±7.3% at baseline vs. 64.3 ±6.0% at 12 months, p=0.08). Conversely, patients on deferoxamine alone had no significant improvement in RVEF, whether the baseline T2* was 8-12ms (59.3 ±6.8% at baseline vs. 59.8 ±6.4% at 12 months, p=0.70) or 12-20ms (62.5 ±7.2% at baseline vs. 63.3 ±6.1% at 12 months, p=0.58). Comparison of the between groups effects showed that combination therapy was superior to deferoxamine alone in improving RVEF in patients with baseline T2* below 12ms (4.7% vs. 0.5%, p=0.01) but not in those with T2* above 12ms (2.2% vs. 0.8%, p=0.47. (figure 9-2).

Figure 9-2 Change in RVEF over 12 months according to treatment arm and myocardial T2* at baseline (T2* 8-12 ms on left panel, T2* 12-20 ms on right panel). Vertical lines represent standard error.

9.3.1 Longitudinal open label study in severe cardiac siderosis

The baseline findings and the results of the longitudinal study in the 15 patients with severe iron loading (myocardial T2* < 8ms) evaluating combination treatment for changes in myocardial T2* and LVEF have been previously published, (Tanner 2008) and are briefly summarized in table 9-2 below.
Table 9-2 Baseline characteristics of the randomized controlled trial population (mild and moderate myocardial iron loading) vs. open-label combination therapy population (severe myocardial iron loading). Values and abbreviations presented as in the previous table.

<table>
<thead>
<tr>
<th>Mild-moderate siderosis</th>
<th>Severe siderosis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>RCT</td>
<td>Longitudinal trial</td>
</tr>
<tr>
<td>65</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.8 ± 4.7</td>
<td>27.8 ± 4.8</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>27 (41.5%)</td>
<td>6 (40.0%)</td>
</tr>
<tr>
<td>Body surface area</td>
<td>1.55 ± 0.15</td>
<td>1.56 ± 0.13</td>
</tr>
<tr>
<td>Deferoxamine (mg/kg/day)</td>
<td>40.6 ± 13.5</td>
<td>40.7 ± 12.0</td>
</tr>
<tr>
<td>(5 days/week)</td>
<td>(5 days/week)</td>
<td></td>
</tr>
</tbody>
</table>

**CMR measures**
- Myocardial T2*: 12.0 (0.13) vs. 6.0 (0.09), N/A
- Liver T2*: 4.5 (0.57) vs. 2.9 (0.67), 0.06
- RVEDV (mL): 132.1 ± 34.6 vs. 146.7 ± 35.1, 0.2
- RVESV (mL): 53.1 ± 19.4 vs. 76.5 ± 27.6, <0.01
- RVEF (%): 60.5 ± 7.0 vs. 49.0 ± 9.4, <0.01

**Echo measures**
- PAP (mmHg): 21.6 ± 5.2 vs. 22.0 ± 5.9, 0.8

**Blood measures**
- Hepatitis C positive: 49 (75%) vs. 11 (73%), 0.9
- Serum ferritin (μg/L): 1472 (0.11) vs. 2057 (0.08), 0.1
- BNP (pmol/L): 15.0 (7.1, 28.8) vs. 26.0 (15.6, 40.5), 0.01

Two patients were in clinical heart failure, and both had BNP levels >100 pmol/l. The mean prescribed doses of deferoxamine and deferiprone at baseline were 38.0 mg/kg for 5.3 days/week (equivalent to 40.7 mg/kg for 5 days/week) and 73.9 mg/kg/day, respectively. During the trial, doses were reduced to 20.3 mg/kg for 4.5 days/week and 65.7 mg/kg/day respectively primarily due to reductions in ferritin. (Tanner 2008) All 15 patients received unblinded combination therapy with deferoxamine and deferiprone throughout the study period. Over 12 months, there was a significant improvement in myocardial T2* (ratio of change in geometric means 1.31, p<0.01).

In 12 of the 15 patients (80%), the baseline RVEF was low compared to a reference TM population with a normal T2*. (Carpenter 2010) The baseline RVESV was significantly raised and there was a trend for a higher RVEDV, resulting in a significantly reduced
mean RVEF (49.0 ± 9.4%) when compared to the population with less severe iron loading. Pulmonary artery systolic pressures were similar in both groups (22.0 ± 5.9 mmHg vs 21.6 ± 5.2 mmHg, p=0.82). Over 12 months, there was a significant improvement in RVEF (10.5 ±5.6%, p<0.01), with no significant change in PAP (22.0 mmHg vs 24.4 mmHg at 12 months, p=0.16). The two patients with heart failure at baseline were asymptomatic at the end of the study.

When grouping the patients on combination therapy (the 32 patients from the combination arm in the RCT plus the 15 unblinded patients on open-label combined therapy), we found an inverse relation between myocardial T2* at baseline and improvement in RVEF over one year (figure 9-3 below).

![Figure 9-3 Breakdown of improvement in RVEF (%) for different groups according to chelation therapy and myocardial T2* baseline. Vertical lines represent standard error.](image)

Patients with severe iron loading had a greater improvement in RVEF than patients with moderate iron loading (10.5% vs. 4.7%, p<0.01).
9.3.2 Entire study cohort

In the cohort of 80 patients with myocardial iron loading, a significant correlation was observed between baseline myocardial T2* and baseline RVEF ($r=0.46$, $p<0.01$) and baseline LVEF ($r=0.50$, $p<0.01$). Accordingly, there was a strong correlation between RVEF and LVEF at baseline ($r=0.82$, $p<0.01$). The improvement in RVEF during the study period also correlated with the improvement in LVEF ($r=0.79$, $p<0.01$); figure 9-4.

![Figure 9-4 Correlation of change in RVEF with change in LVEF over 12 months.](image)

We also analyzed the change in RVEF with the change in cardiac iron as derived from recent human cardiac iron calibration data from Carpenter et al. (Carpenter 2011) There was a significant inverse correlation between the improvement in myocardial iron concentration and the improvement in RVEF ($r=-0.42$, $p<0.01$) .
9.4 Discussion

In the first T2* publication by Anderson et al, normal T2* levels were associated with normal LVEF, but when T2* fell below 20ms, there was a progressive fall in LVEF, showing that increasing iron loading is associated with worsening of LV function. (Anderson 2001) Similar observations have recently been made for the RV, (Alpendurada 2010) suggesting RV dysfunction may be a contributor to heart failure and cardiac mortality in TM patients, as has been found in other cardiac conditions. (Di Salvo 1995, Juilliere 1997, Ghio 2001, Larose 2007, Meyer 2010) However, to date, there has been little published data on the response of the right ventricle to chelation therapy, and no data at all on the most appropriate chelation regime in the presence of right ventricular dysfunction.

Chelation with deferoxamine has been one of the cornerstones for the treatment of TM. It has been extensively studied over the past decades and has shown to decrease the total body iron burden, prevent complications of iron overload and improve survival in TM. (Zurlo 1989, Brittenham 1994, Olivieri 1994) However, long-term deferoxamine monotherapy has been hampered by poor compliance and failure of long term prevention of myocardial iron deposition, heart failure and cardiac deaths. (Modell 2000, Anderson 2002) Deferiprone appears better able to penetrate cells and organelles than deferoxamine. This may in part explain why deferiprone is superior to deferoxamine for removing iron from the heart. (Pennell 2006) The combined use of these two chelating agents which exploits the relative merits of each drug, has been supported in animal models, and has become an attractive therapeutic option in severe cardiac iron loading or when negative iron balance has not been achieved by other methods. (Link 2003, Origa 2005, Tanner 2007, Tanner 2008) Observational, prospective and randomised controlled studies have demonstrated the efficacy of combined therapy in removing iron from the liver and heart, improving endothelial function and left ventricular function, (Tanner 2007, Tanner 2008) as well as endocrine function. (Farmaki 2010) Our current study compared the effects of combination therapy on RV function in TM patients with myocardial iron loading. Our findings from the RCT show combination therapy to be superior to subcutaneous deferoxamine alone in improving RV function in patients with mild and moderate iron loading. In
addition, our data from the longitudinal open-label study show that RV dysfunction is reversible even in patients with severe iron overload. It is of interest that the recovery in RV function was greatest in patients with a more severe degree of myocardial siderosis and RV dysfunction.

It is well recognized that RV performance depends not only on intrinsic contractility but also on RV afterload, which is the resistance that the RV has to overcome during ejection. Increased pulmonary artery pressures reflecting increased pulmonary vascular resistance may thus impair RV function. (Shekerdemian 1997) However, none of the participants in this study had pulmonary hypertension, in line with previous studies suggesting a low prevalence of pulmonary hypertension in regularly transfused TM patients. (Derchi 1999, Aessopos 2004) Whilst an improvement in myocardial T2* was associated with an improvement in RVEF, no significant changes in pulmonary artery pressures were observed throughout the study. Therefore, the improvement in RV function seen in this population was independent of pulmonary artery pressure thus eliminating a potential confounding factor for the evaluation of RV function.

As in the previous study (chapter 8) the magnitude of recovery in RV function parallels the recovery in LV function, and this correlates with the response to chelation therapy. This is in keeping with the findings of other studies in non-ischaemic cardiomyopathies, where it has been observed that LV and RV function is usually affected in a similar way and to a similar extent. (Juilliere 1997, La Vecchia 2001) The close association seen between RVEF and LVEF and the increase of these parameters over time following successful myocardial iron chelation supports the concept that intrinsic RV myocardial contractility is predominantly affected by intracellular iron and plays a key role in RV performance in this toxic-induced cardiomyopathy model. (Alpendurada 2010) Finally, as right ventricular ejection fraction has incremental prognostic value which is additional to LVEF, (Di Salvo 1995, Juilliere 1997, Ghio 2001, Larose 2007) it is reasonable to suggest that the improvement in RVEF seen in this study with the combined use of deferiprone and deferoxamine may contribute to improved outcomes.
9.5 Limitations

This is a retrospective analysis of 2 trials designed to assess the change on myocardial T2* with iron chelation regimes in which RV parameters were not planned as primary end-points. Nevertheless, all data was prospectively collected and the current RV analysis was blinded to the patients’ details and chelation regimes.

9.6 Conclusions

In the RCT of mild to moderate cardiac iron loading, combination treatment improved RV function significantly more than deferoxamine alone. Combination treatment also improved RV function in severe cardiac siderosis. Therefore adding deferiprone to deferoxamine has beneficial effects on both RV and LV function in cardiac siderosis, which may be relevant to the improved mortality reported with deferiprone.
Chapter 10: Response of the right ventricle to deferasirox

10.1 Introduction

Siderotic cardiomyopathy accounts for the majority of deaths worldwide in transfused TM patients. Deferoxamine is an efficient chelator but the route of administration by subcutaneous infusion often leads to non-compliance. Deferiprone is orally active which is more acceptable to patients thus improving compliance. It is associated with an improvement in LV and RV function probably due to its ability to access labile intra-cellular iron (LCI). It does not have approval for use in the USA however due to idiosyncratic agranulocytosis, and unfounded reports of liver fibrosis. (Olivieri 1995) There has therefore been a need for another orally available chelating agent. Deferasirox (Exjade®) is an orally active tridentate ligand with a long half life allowing once daily dosing. It had been in clinical development since 1998. Treatment with deferasirox has been shown to reduce body iron burden in patients with transfusion-dependent anaemias. The removal of myocardial iron has been demonstrated in pre-clinical studies and several clinical trials. (Cappellini 2006, Vichinsky 2007, Porter 2008, Pennell 2010, Pennell 2011) In models using cultured cell preparations, including cardiomyocytes, Glickstein et al demonstrated that deferiprone and deferasirox, but not deferoxamine, gained cellular access and bound labile iron. (Glickstein 2005, Glickstein 2006) The production of reactive oxygen species (ROS) was subsequently reduced, excess cellular iron removed and myocyte contractility restored. The presence of ROS reduces nitric oxide availability leading to impaired endothelium dependent vasorelaxation. In addition, to these favourable cardiac effects, vascular effects have also been seen with iron chelators. Tanner et al demonstrated an improvement in brachial artery flow mediated dilatation in cardiac iron overloaded TM patients with deferiprone/deferoxamine combination therapy, (Tanner 2007) and Cheung et al reported similar findings with an associated decrease in carotid artery stiffness index in with deferasirox therapy. (Cheung 2008) These studies suggest that deferasirox may be effective in improving RV function.
The Evaluation of Patients Iron Chelation with Exjade (EPIC) cardiac sub-study was designed to assess the effects of long term iron chelation therapy with deferasirox, as prospective data on iron chelation for more than 1 year is limited. The objectives included the assessment of drug efficacy, safety and cardiac function using CMR T2* and cine imaging. The primary endpoint was change in cardiac iron burden, with LVEF as the secondary endpoint. One and two year data have already been published showing a continued significant reduction in myocardial iron loading in patients with iron overload, but no improvement in LVEF was seen. (Pennell 2010, Pennell 2011) Chapters 8 and 9 describe the improvement in RVEF with chelation therapy in patients treated with deferiprone monotherapy and also in combination with deferoxamine. In both studies, the improvement in RVEF was associated with an increase in LVEF and driven by an improvement in RV contractility as reflected by the reduction in RVESV. Based on the results for these trials an improvement in RV function with deferasirox would be unexpected however RV volumetric parameters were measured along with the LV and this chapter reports the effects over three years on RV volume, function and mass parameters with reference to the LV response.

10.2 Methods

10.2.1 Patients and dosing

Study participants were a subgroup of patients with β-thalassemia major who were enrolled in the larger EPIC trial (Cappellini 2010) assessing deferasirox across various underlying anemias. The EPIC cardiac substudy was a prospective, open-label, single-arm, multicenter study conducted initially over a core period of 1 year. The study was then extended for an additional 2 years, allowing continuous treatment with deferasirox for up to 3 years in patients who completed the 1-year core study.

The methods and inclusion/exclusion criteria for the EPIC cardiac substudy have been reported in detail in the year 1 (Pennell 2010) and year 2 (Pennell 2011) cardiac substudy findings. Briefly, patients aged ≥10 years with TM who had CMR myocardial T2* >5 to <20ms, LVEF ≥56% (as assessed by CMR), serum ferritin concentrations >2500ng/mL, MRI (R2) liver iron concentration (LIC) of >10mg Fe/g dry weight (dw), and a lifetime minimum of 50 packed red blood cell transfusions were eligible for
inclusion in the cardiac substudy. In patients participating in the cardiac substudy, deferasirox was initiated at a dose of 30mg/kg per day for the first 4 weeks of treatment and this could be increased to 40mg/kg per day. Doses could be adjusted (by 510mg/kg per day) in the case of serum ferritin changes, increased serum creatinine concentrations, increased serum transaminases or adverse events (AEs). Dose increases up to 45mg/kg per day were considered in patients with minimal worsening of cardiac T2* (≤33% from baseline) or no improvement in T2* (≤25% from baseline) provided that LIC was ≥3mg Fe/g dw at the 6-monthly assessments and that there were no safety concerns. However, any dose increases beyond 40mg/kg per day had to be approved by the Study Monitoring Committee and study sponsor. Patients were withdrawn from the cardiac substudy if cardiac T2* decreased by >33% from baseline, or LVEF decreased to 50 <56%, with no improvement to ≥56% at a repeat assessment 3 months after the initial assessment. Patients completing the 1-year core study continued to receive the same dose of deferasirox when entering the extension study. Patients who completed the second year could enter the third year of the study, continuing to receive deferasirox at the dose prescribed at the end of year 2.

10.2.2 Assessments

The primary efficacy endpoint of the cardiac substudy was the change in cardiac T2* from baseline, the 1 and 2 year results of which are reported elsewhere (Pennell 2010, Pennell 2011) The 3 year report is pending. This chapter describes the changes over 3 years in RV function and mass, and includes the changes in LV parameters which were measured as secondary efficacy endpoints during the study. The following parameters were assessed using CMR for both RV and LV function: EF, end-systolic volume (ESV), and end-diastolic volume (EDV) and mass (M). All parameters were assessed at baseline and at 6-monthly intervals following initiation of deferasirox treatment, for a total period of 36 months.

10.2.3 Statistical methods

The patient population reported here are those patients who completed the 3-year extension study and had baseline and 36-month ventricular parameter measurements. Data from the intention-to-treat analysis are not included. The absolute changes from
baseline to 36 months in RVEF, LVEF, RVESV, LVESV, RVEDV, LVEDV, RVM and LVM values were summarized using descriptive statistics. Values for ventricular volumes and mass were corrected for body surface area (BSA; calculated from individual patients’ body weight and height). All data are presented as mean ± standard deviation (SD). Statistical significance was examined using a paired Student’s t-test at a two-sided α level of 0.05.

10.3 Results

10.3.1 Patient characteristics

Of the 114 patients initially enrolled into the 1-year core cardiac substudy (Pennell 2010), 105 completed this period and 101 entered the extension study. A total of 86 (85.1%) patients completed year 1 of the extension. Seventy-one patients elected to continue into the second year of the extension, of which 66 (93.0%) completed the overall 3-year study. Of these patients who completed the 3-year study, 27 were male and mean age at baseline was 20 years (Table 10-1). In patients with baseline cardiac T2* >5 to <10ms, there was a lower proportion of patients aged 10 to <16 years, greater percentages of Caucasian patients and patients who had hepatitis B and/or C, and fewer who had previously received deferoxamine monotherapy, as compared with patients who had baseline T2* 10 to <20ms (Table 10-1). However, transfusion characteristics, duration of chelation therapy and mean iron intake were similar for these groups.
Table 10-1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>T2* &gt;5 to &lt;10ms (n=22)</th>
<th>T2* 10 to &lt;20ms (n=44)</th>
<th>All patients (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>8 (36.4)</td>
<td>19 (43.2)</td>
<td>27 (40.9)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>14 (63.6)</td>
<td>25 (56.8)</td>
<td>39 (59.1)</td>
</tr>
<tr>
<td><strong>Mean ± SD age at baseline, years</strong></td>
<td>21.1 ± 6.5</td>
<td>19.4 ± 7.51</td>
<td>20.0 ± 7.2</td>
</tr>
<tr>
<td><strong>Age group, years, n (%)</strong></td>
<td>5 (22.7)</td>
<td>15 (34.1)</td>
<td>20 (30.3)</td>
</tr>
<tr>
<td>10–16</td>
<td>17 (77.3)</td>
<td>29 (65.9)</td>
<td>46 (69.7)</td>
</tr>
<tr>
<td><strong>Race, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>8 (36.4)</td>
<td>12 (27.3)</td>
<td>20 (30.3)</td>
</tr>
<tr>
<td>Oriental</td>
<td>13 (59.1)</td>
<td>30 (68.2)</td>
<td>43 (65.2)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (4.6)</td>
<td>2 (4.6)</td>
<td>3 (4.6)</td>
</tr>
<tr>
<td><strong>History of hepatitis B/C, n (%)</strong></td>
<td>7 (31.8)</td>
<td>8 (18.2)</td>
<td>15 (22.7)</td>
</tr>
<tr>
<td><strong>Mean ± SD duration of transfusion therapy, years</strong></td>
<td>20.0 ± 6.6</td>
<td>18.2 ± 8.0</td>
<td>18.8 ± 7.6</td>
</tr>
<tr>
<td><strong>Previous chelation therapy, n (%)</strong></td>
<td>12 (54.6)</td>
<td>33 (75.0)</td>
<td>45 (68.2)</td>
</tr>
<tr>
<td>Deferoxamine monotherapy</td>
<td>1 (4.6)</td>
<td>0</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Deferiprone monotherapy</td>
<td>9 (40.9)</td>
<td>11 (25.0)</td>
<td>20 (30.3)</td>
</tr>
<tr>
<td>Deferoxamine + deferiprone‡</td>
<td>13.5 ± 8.7</td>
<td>13.0 ± 7.2</td>
<td>13.2 ± 7.7</td>
</tr>
<tr>
<td><strong>Mean ± SD systemic blood pressure, systolic/diastolic, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1-year core study baseline</td>
<td>105.1 ± 11.0, 67.1 ± 10.0</td>
<td>107.0 ± 11.6, 66.0 ± 7.7</td>
<td>106.4 ± 11.3, 66.3 ± 8.5</td>
</tr>
<tr>
<td>At start of 1-year extension</td>
<td>105.1 ± 11.2, 66.6 ± 7.6</td>
<td>105.4 ± 13.3, 63.1 ± 8.5</td>
<td>105.3 ± 12.6, 64.3 ± 8.3</td>
</tr>
<tr>
<td>At start of 2-year extension</td>
<td>100.9 ± 9.8, 65.3 ± 9.7</td>
<td>107.7 ± 11.0, 63.4 ± 6.7</td>
<td>105.4 ± 11.0, 64.1 ± 7.8</td>
</tr>
<tr>
<td>At end of 2-year extension</td>
<td>105.6 ± 12.9, 67.5 ± 8.0</td>
<td>107.0 ± 11.3, 65.5 ± 7.3</td>
<td>106.5 ± 11.8, 66.2 ± 7.5</td>
</tr>
<tr>
<td><strong>Mean ± SD RBC intake‡, mL/kg/day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1-year core study baseline</td>
<td>0.45 ± 0.72</td>
<td>0.23 ± 0.17</td>
<td>0.31 ± 0.44</td>
</tr>
<tr>
<td>(mean for previous year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At start of 1-year extension</td>
<td>0.38 ± 0.14</td>
<td>0.30 ± 0.11</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>At start of 2-year extension</td>
<td>0.36 ± 0.14</td>
<td>0.29 ± 0.10</td>
<td>0.31 ± 0.12</td>
</tr>
<tr>
<td>At end of 2-year extension</td>
<td>0.35 ± 0.12</td>
<td>0.29 ± 0.10</td>
<td>0.31 ± 0.11</td>
</tr>
<tr>
<td><strong>Mean ± SD iron intake, mg/kg per day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 1-year core study</td>
<td>0.41 ± 0.19</td>
<td>0.33 ± 0.12</td>
<td>0.35 ± 0.15</td>
</tr>
<tr>
<td>During 1-year extension period</td>
<td>0.38 ± 0.15</td>
<td>0.31 ± 0.11</td>
<td>0.34 ± 0.13</td>
</tr>
<tr>
<td>During 2-year extension period</td>
<td>0.37 ± 0.13</td>
<td>0.31 ± 0.11</td>
<td>0.33 ± 0.12</td>
</tr>
</tbody>
</table>
10.3.2 Iron intake and blood pressure

Red blood cell (RBC) iron intake significantly increased from baseline in patients with baseline cardiac T2* 10 to <20ms at the end of the first year (month 7 to 12, +0.07mL/kg/day, P=0.012), but did not change significantly throughout the rest of the study. There were no significant changes in iron intake in patients with baseline cardiac T2* >5 to <10ms. Diastolic blood pressure significantly decreased from baseline in the cardiac T2* 10 to <20ms group after 1 year (−2.9mmHg, P=0.024) and after 2 years (−2.6mmHg, P=0.033), however, by year 3 there was no significant difference compared to baseline. Systolic blood pressure did not change significantly throughout the study. In contrast, systolic blood pressure significantly decreased from baseline after 2 years in patients with baseline cardiac T2* >5 to <10ms (−4.3mmHg, P=0.043), but was not significantly different after 3 years. Diastolic blood pressure did not change significantly.

10.3.3 Deferasirox dosing

For the 66 patients completing the 3-year study, mean overall deferasirox exposure (±SD) during the 3-year treatment period was 157.4 ± 2.2 weeks; 157.4 ± 1.9 for patients with baseline cardiac T2* >5 to <10ms and 157.4 ± 2.4 for patients with baseline cardiac T2* 10 to <20ms. The average actual dose received over the 3-year treatment period was 34.5 ± 5.9mg/kg per day; 35.9 ± 4.8mg/kg per day for patients with baseline cardiac T2* >5 to <10ms and 33.9 ± 6.3mg/kg per day for patients with baseline cardiac T2* 10 to <20ms. The majority of patients received average actual doses between 35 and 40mg/kg per day (n=33) and seven patients received doses >40 to <45mg/kg per day. Final deferasirox dose at the end of the 3-year study was 30 to 40mg/kg per day in 44 (66.7%) patients, 45mg/kg per day in six (9.1%) patients, while the remaining had <30mg/kg per day. All 66 patients who completed 3 years of the study received at least one dose adjustment. All patients had at least one dose increase, with a total of 246 dose increases, most commonly as per protocol (n=142, 57.7%) or due to lack of efficacy (n=85, 34.6%). Eighty-three dose decreases were recorded in 33 patients (50.0%) most commonly due to laboratory test abnormalities (n=32, 38.6%) and adverse events (n=22, 26.5%). A total of 89 interruptions occurred in 49 patients (74.2%) mostly due to adverse events (n=37, 41.6%).
10.3.4 Effect of long term deferasirox therapy on ventricular function

At baseline of the core 1-year study, mean RVEF significantly increased versus baseline in patients with baseline cardiac T2* 10 to <20ms at 1 year until the end of the study (P<0.001 at year 1, p<0.001 at year 2, p=0.0079 at year 3, Figure 10-1.A. Mean ± SD LVEF was 67.4 ± 5.9% (Figure 10-1,B). There were no significant changes in mean LVEF from baseline at years 1, 2 or 3. Mean ± SD baseline RVEF was 66.0 ± 5.9% for all patients.

Figure 10-1 Mean ± SD ejection fraction of the right (A) and left (B) ventricle, according to baseline T2*
Overall mean ± SD baseline RVESV/BSA at the core study baseline was 24.2 ±9.6 mL/m² (Figure 10-2-A). This remained stable over the total study period, with the exception of a significant decrease from baseline to 1 year in patients with baseline cardiac T2* 10 to <20 ms (2.6 ±7.9 mL/m²; p=0.037). Mean ± SD LVESV/BSA was 24.2 ±9.5 mL/m² at baseline (Figure 10-2-B). There was a significant increase from baseline to 1 year in patients with baseline cardiac T2* >5 to <10 ms (+2.9 ±6.0 mL/m²; p=0.037). LVESV then remained stable over the following 2 years. There were no significant changes in LVESV in patients with baseline cardiac T2* 10 to <20 ms.

Figure 10-2 Mean ± SD right (A) and left (B) ventricular end systolic volume (indexed for body surface area), according to baseline T2*
Mean ± SD RVEDV/BSA at core study baseline was 66.0 ±18.3 and 72.2 ±20.7mL/m² in patients with baseline cardiac T2* >5 to <10 ms and baseline cardiac T2* 10 to <20ms, respectively (Figure 10-3,A). Significant increases were observed in both groups at 2 years and these were maintained at the end of 3 years. Mean ± SD LVEDV/BSA for all patients was 75.5 ±19.1mL/m² at baseline and was similar for both baseline T2* subgroups (Figure 10-3,B).

Figure 10-3 Mean ± SD right (A) and left (B) ventricular end diastolic volume (indexed for body surface area), according to baseline T2*

At the end of the core 1-year study, there were no significant changes from baseline in mean LVEDV/BSA. However, at year 2, mean LVEDV/BSA was significantly increased in both subgroups relative to baseline, with an overall mean ±SD change of 5.6 ±11.6mL/m² in all patients (p<0.001). At year 3, this increase was maintained in
patients with baseline T2* 10 to <20ms but not in patients with baseline cardiac T2* >5 to <10ms.

Mean ±SD RVM/BSA at baseline was 33.2 ±7.8g/m2 in all patients (Figure 10-4,A). Significant reductions from baseline in mean RVM/BSA were observed after 2 years, and the downward trend continued up to 3 years of deferasirox therapy. LVM/BSA at baseline was 79.1 ±16.8g/m2 for all patients. At the end of 1 year, mean LVM/BSA had decreased significantly from baseline in both groups of patients with baseline cardiac T2* >5 to <10ms and baseline cardiac T2* 10 to <20ms. This decrease relative to baseline became greater over time in both sub groups, until the end of the 3-year study (Figure 10-4,B).

Figure 10-4 Mean right (A) and left (B) ventricular mass (indexed for body surface area), according to baseline T2
10.4 Discussion

This study reports a significant improvement in RVEF associated with a decrease in RV mass after 3 years of deferasirox therapy in iron overloaded TM patients. It is the first prospective study to assess the RV functional response to iron chelation with deferasirox. Previous reports of deferasirox treatment have described changes in cardiac T2* with LVEF as a primary endpoint. (Pennell 2010, Pennell 2011) Chapters 8 and 9 describe the RV response to chelation therapy over the course of one year where an improvement in RVEF was seen with deferiprone monotherapy, and in combination with deferoxamine. In both cases however the changes were driven by a reduction in RVESV and there was an associated improvement in LVEF. With deferasirox the increase in RVEF was related to an increase in RVEDV and there was a reduction in RV mass with no reduction in RVESV. The mean baseline RVEF (66.0 ±5.9% for all patients) was very similar to that in a study by Carpenter et al (66.2% ±4.1). (Carpenter 2010) The mean indexed RVEDV at baseline was 66.0 ± 18.3 mL/m2 in patients with a baseline cardiac T2* >5 to <10 ms and 72.2 ±20.7 mL/m2 in those with T2* 10 to <20ms. This is despite most of the patients in the severe group being in the older age category. These values are 33% and 26% less than the reference value (98.1 ± 17.3 mL/m2) for non iron loaded TM patients. LVEDV also increased and there was a reduction in mass but there was no decrease in LVESV or increase in LVEF. Diastolic blood pressure decreased significantly in the mild to moderate group after 1 and 2 years of treatment and systolic blood pressure decreased after 2 years in the severe group. No significant differences were noted between baseline and 3 years however. These are very interesting findings suggesting a different pharmacological effect of deferasirox to that of deferiprone or deferoxamine. A recently published study by our group of ex-vivo iron overloaded hearts, showed the RV myocardium had up to 21.9% less iron than the LV. (Carpenter 2011) The RV is however particularly susceptible to iron induced functional impairment, (Walker 2010) and has less contractile reserve. (Bronicki 2010) This does not explain why, with deferasirox therapy, the LVEDV and RVEDV both increased with mass reduction, yet systolic volumes remained unchanged. The changes may be reflecting an improvement in diastolic function, and restriction is considered a feature of myocardial iron accumulation. An echo TDI study showed diastolic functional impairment in a high proportion of iron overloaded patients.
with a normal EF, (Vogel 2003) although a CMR based study by our group showed a weak correlation between LV diastolic filling parameters and T2* in a group of 67 TM patients of whom 46 had iron overload and 5 had an abnormal LVEF. (Westwood 2005)

Another possible mechanism for improvement in RV function might arise from relief of vascular dysfunction caused by iron overload. Haemolysis can cause depletion of the potent vasodilator nitric oxide (NO) and its precursor arginine. It also increases platelet activation and stimulates the release of the vasoconstrictor endothelin-1. (Gladwin 2004, Gladwin 2005, 2008, Kato 2009, Machado 2010) These factors negatively impact upon vascular homeostasis and can lead to endothelial dysfunction, vasoconstriction and hypercoagulability. This can ultimately cause vascular disease, and indeed endothelial dysfunction is associated with the development of atherosclerosis. (Davignon 2004) Interestingly Duffy et al (Duffy 2001) reported an improvement in NO mediated endothelium dependant vasodilatation in patients with CAD when given intra-arterial deferoxamine. Arterial stiffness or loss of compliance is also important as it increases impedance to flow which in turn increases afterload. Using ultrasound Cheung et al showed an abnormal carotid artery stiffness index and brachial artery flow mediated dilatation (FMD) in patients with TM. These patients had a higher indexed LV mass compared with the control group but there was no difference in fractional shortening and no patient showed abnormal filling patterns. (Cheung 2002) Cheung also demonstrated an increase in carotid intima-media thickness compared with controls in a group of TM patients without diabetes mellitus or heart failure. (Cheung 2006) Similar findings were reported by Hahalis et al who also found carotid plaques in three TM patients but none in the well matched control group. (Hahalis 2008) Endothelial dysfunction in TM patients, as measured by brachial artery FMD, has been shown to improve with deferiprone/deferoxamine combination therapy compared with deferoxamine monotherapy. (Tanner 2007) In a recent publication Cheung et al demonstrated an improvement in brachial artery FMD and carotid artery stiffness with deferasirox therapy. (Cheung 2008) There were no changes in echo derived LV functional indices. Changes in BP, LV mass and right sided parameters were not reported. They hypothesised that these findings may reflect the ability of deferasirox to bind labile iron in the vascular walls with subsequent improvement in endothelial
function. The changes were seen by 6 months therefore the authors suggested the observations may be due to a direct almost immediate effect on the arterial walls rather than the long term reduction of iron burden. Our findings would support this hypothesis. An improvement in endothelial function with deferasirox in TM patients would have an important clinical implication. The presence of the β-thalassaemia trait (heterozygote) has been associated with a reduced risk of developing coronary artery disease (CAD) due the protective effect of their lipoprotein and blood rheology profiles. (Tassiopoulos 2005) Thalassaemia trait however only causes a mild anaemia that does not require transfusion therapy. Life expectancy for TM patients is increasing. In 1970 the average life expectancy was only 17 years but by 2000 over 80% of patients had a life expectancy of > 40 years. (Modell 2008) The risk of developing CAD increases with age. Hahalis et al reported premature atherosclerosis in 20 young TI patients but there have been no reports to date on the development of CAD in thalassaemia patients. (Hahalis 2008) The longer surviving TM patients are now entering the age of increased risk therefore we may see the incidence increase as patients survive longer. As endothelial dysfunction is an important factor in the development of vascular disease it would be interesting to see whether the different chelating agents influence its development.

A further possible mechanism for improvement in RV function, might be through improvement in pulmonary artery pressure, and this might fit the finding of reduction in RV mass which we found in our study. The mean baseline RV mass in our patient cohort (33.2 ±7.8g/m²) was less than that quoted by Carpenter et al (38.8 ±7.9g/m2) in their study of TM patients without iron overload. (Carpenter 2010) They only included patients over 18 years whereas a significant number of our patients were children. Interestingly, RV mass in their TM patients tended to be higher than for normal controls. An increase in RV afterload, due to increase pulmonary vascular resistance would cause an increase in RV mass. Pulmonary hypertension (PHT) is a common finding in patients with haemoglobinopathies. (Vichinsky 2004, Aessopos 2005, Farmakis 2011) The pathogenesis of PHT in haemoglobinopathies is likely to be multifactorial but as with changes in the systemic vasculature haemoglobin released by chronic haemolysis can lead to vasoconstriction. Tissue hypoxia is compensated by a high output state, further exacerbated by bone marrow expansion, which in the presence
of increased pulmonary vascular resistance contributes to elevated pulmonary pressures. Iron overload is associated with interstitial pulmonary fibrosis and myocyte dysfunction which are additional contributory factors associated with increased pulmonary vascular resistance. Unfortunately pulmonary pressures were not recorded in our patient cohort. Although common in TI patients who usually do not require regular transfusions for survival, reports on the prevalence of PHT in TM are extremely polarized. (Grisaru 1990, Du 1997, Derchi 1999, Aessopos 2004) A large study of well treated Greek TM patients showed a very low incidence of elevated pulmonary pressures, however the authors did not state cardiac iron levels and patient with poor chelation compliance were excluded from the study. (Aessopos 2004) The two reports of very high incidences were in patient who were poorly treated and with a high prevalence of LV dysfunction, but the patient populations studies were small. (Grisaru 1990, Du 1997) Pulmonary hypertension has been noted to be more frequent in haemoglobinopathy patients who have undergone splenectomy. (Hoepner 1999) It has been reported that splenectomy in patients with haemolytic disorders increases the likelihood of thromboembolism. (Cappellini 2000) This is possibly due to the loss of the filter function of the spleen resulting reduction in the clearance of abnormal red cells as this problem is not seen in patients splenectomised for other reasons. (Peacock 2005) Only 19% of the patients in the study by Aessopos had been splenectomised. (Aessopos 2004) In our patient cohort 50% (54.6% in the severe group) had undergone previous splenectomy. In addition 22.7% had a history of hepatitis which may cause liver fibrosis which could play a contributory role. Liver fibrosis can lead to portal hypertension which is associated with pulmonary hypertension.

10.5 Limitations

Pulmonary artery pressures were not measured in this patient group as the primary endpoint was a change in myocardial iron content as measured by CMR T2*. It would have been interesting to see if pulmonary pressures were increased at baseline and to document any changes associated with therapy, and whether they correlated with changes in RV volume and mass. However, previous experience from our group suggests that pulmonary hypertension is very uncommon in well treated TM patients. CMR is not the modality of choice for the assessment of diastolic function. We did not
therefore assess diastolic function in these patients which would have provided a more sensitive marker of myocyte function.

10.6 Conclusions

This study has shown a reduction in RV mass in iron overloaded patients treated with deferasirox over a 3 year period, and an increase in RV EDV and RVEF. The findings suggest a different pharmacological effect of the chelator deferasirox to that of deferiprone in the possible direct improvement of endothelial function and reduction in pulmonary pressures.
Chapter 11: General discussion and conclusions

11.1 Introduction

Symptomatic heart failure is a common late manifestation of CM that is distressing for the patient and associated with high morbidity and mortality. Identification and accurate assessment of risk factors and early disease can guide therapy and improve outcome. Non invasive imaging plays a pivotal role in the identification and classification of CM. CMR in particular provides a comprehensive phenotypic description in patients with myocardial dysfunction. It is sensitive to early functional changes and often identifies the underlying pathology which is critical for rational treatment that is specific to the disease process. It also provides an accurate assesses of therapeutic efficacy. With this backdrop, the use of CMR in the accurate assessment of early disease features and the recognition of causative factors has been evaluated testing the hypotheses that myocardial fibrosis and strain abnormalities in EDMD precede systolic dysfunction and that inflammatory markers post anthracycline exposure predicts late cardiac functional changes. In addition the value of optimising acquisition sequences to improve reproducibility was addressed and the evaluation of chelation therapy with particular reference to the RV was explored.

11.1.1 Emery-Dreifuss Muscular Dystrophy

Though EDMD is rare, with relatively mild musculoskeletal manifestations, the high risk of cardiac involvement and sudden death has primed the interest of neurologists and cardiologists in trying to establish why this is the case and whether there are early signs of cardiac involvement that might guide therapy. Due to its rarity and the frequency of pacemaker insertion there were very few comprehensive reports in the literature, particularly using CMR, at the time of starting the work for chapter 5. Based on reports from other MD studies it was hypothesised that fibrosis would be implicated in the disease process. As changes in systolic function often occur late in disease process, strain imaging was performed using echo and CMR in an attempt to uncover early functional changes. Unfortunately sudden death is frequently the presenting
symptom in EDMD therefore careful cardiac evaluation and follow up is mandatory. Cardiac family screening has been advocated, including female heterozygotes with X-linked EDMD who are also at risk of cardiac complications. (Fishbein 1993, Bouhouch 2008). Differences between echo derived myocardial velocity gradients and CMR strain patterns compared with the control group were identified although statistical significance was weak, the rarity of the condition and high incidence of pacemaker implantation limiting cohort size. The LVEF derived by both techniques was within normal limits drawing attention to the fact that LVEF is not a sensitive marker of early functional change and that these patients require careful monitoring even if conventional markers of function appear normal. The original hypothesis that fibrosis would play a part in pathogenesis was not supported by our results. However the IR turbo flash LGE acquisition protocol available at the time would only depict macroscopic changes. Very small areas of fibrosis would be difficult to recognise however carefully the images were viewed and a more diffuse process would be missed. Using more recently developed T1 mapping protocols, with and without the use of a contrast medium might have detected diffuse myocardial changes and yielded a different result. Given the high risk of atrial and ventricular arrhythmias and sudden death in these patients, it would be worth pursuing further studies to look for diffuse ventricular and atrial fibrosis. The availability of CMR compatible pacemakers means long term evaluation of these patients who were previously lost to CMR follow-up may also be possible in the future.

11.1.2 Anthracycline Cardiotoxicity

Chapter 6 describes for the first time the relationship between an increase in EGRE detected shortly after initial anthracycline exposure, a change in urinary F2-isoprostanes and a long term reduction in LVEF.

Improvements in chemotherapy regimens have lead to an impressive reduction in morbidity and mortality in cancer patients. The mode of action of many cancer treatments however, can also lead to cardiovascular injury culminating in heart failure. Long term cardiotoxicity is less of a concern for patients with metastatic disease for whom the treatment is aimed at short term improvement in survival and quality of life.
For patients with a good chance of long term survival undergoing adjuvant therapy to reduce the risk of disease recurrence, it is a major factor. Rarely heart failure can occur acutely but more commonly the onset is delayed. Cell disruption and loss have been reported in patients undergoing endomyocardial biopsies early post anthracycline administration suggesting early myocyte damage. (Bristow 1978a) Elevation of biomarkers including BNP and troponins, which would reflect myocyte damage during treatment, correlates with an increased risk of developing late anthracycline mediated cardiotoxicity (AMC) but early changes in LVEF do not, which implies an early compensatory mechanism. (Ewer 2008) The heart has considerable contractile reserve making the detection of early sub-clinical damage difficult. Why some patients go on to develop heart failure but others do not, despite similar drug regimens and cumulative dosages, is one of the most intriguing conundrums faced by oncologists and cardiologists.

In our study, the patients with a reduction in LVEF ≥ 5% at follow up showed a mean increase in EGRE of 46% compared with only 7% for other patients. A significant change in urinary F2-isoprostanes 3 days after anthracycline administration correlated with EGRE at the same time point and showed a relationship with a late reduction in LVEF. The negative correlation between EGRE and the urinary F2-isoprostane to creatinine ratio may be due to the fact that biomarkers of oxidative stress peak 1 hour post anthracycline administration. Future studies should therefore take this into account when collecting urine samples. There was also a relationship between STIR SI and late LVEF. There was no significant change in LVEF or RVEF at day 3 underlining the fact that functional changes do not parallel inflammatory markers. These findings should be useful in the further understanding of AMC. Interestingly, the mean reduction in LVEF one year after completion of adjuvant chemotherapy in patients with early breast cancer was approximately 2% irrespective of whether the patients also received trastuzumab. The fact that trastuzumab did not exacerbate long term change in ventricular function supports previous findings that its cardiotoxicity is generally reversible (Ewer 2005, Guarneri 2006) although data on cardiac function in our patient group during treatment was not available. RVEF also decreased but by a lesser degree (1.2%) after one year which is surprising given the lower cardiac reserve. I initially attempted to look at more sensitive functional parameters by measuring diastolic filling and myocardial tagging
patterns. Interim analysis of ventricular filling parameters in 30 patients proved not worthy of pursuit due to the high level of inter-group variability probably due to the wide age range and differences in body habitus throughout the cohort. Also diastolic parameters derived by evaluating volume changes during filling are highly preload dependent and the temporal resolution of CMR derived data is low compared with echocardiography. As with the EDMD group no LGE was seen at follow-up. This again could reflect a more diffuse process that may be detected using a different acquisition protocol. In addition the follow-up at approximately 1 year post completion of therapy is quite early. Failure to obtain suitable analysis software to perform the myocardial tagging analysis within the time restraints and the scanner upgrade issues led to this not being included in this thesis. This data may provide useful data when analysis software becomes available.

11.1.3 Improving T2* Imaging

CMR has become established as the technique of choice for the management of patients with myocardial iron loading and there has been a concomitant reduction in mortality from siderotic CM within the UK. (Modell 2008) Although the white blood technique developed in our unit has proved successful, with our group disseminating the acquisition sequence and training sites worldwide to perform diagnostic scans in iron overloaded TM patients, there are some reproducibility issues. Using a DIR sequence to null the blood signal chapter 7 described further improvements in the T2* CMR technique. The use of black blood T2* CMR was shown to improve the reliability of iron overload assessment, and this validation has lead to this technique being adopted for routine use at the Royal Brompton Hospital. It is also now being disseminated around the world.

11.1.4 The Right Ventricular Response to Chelation Therapy

Chapters 8, 9 and 10 are the first reports to describe the RV response to chelation therapy. Apart from ARVC, the assessment of RV function has historically not featured prominently in the follow up of patients with CM. There are a few reports in the literature citing the important role of RV function as a predictor of outcome in ischaemic heart disease and other cardiomyopathies (Juilliere 1997, de Groote 1998,
and this caused us to examine the RV in thalassemia major, in which congestive heart failure is the commonest cause of death. There was a significant improvement in RVEF associated with the reduction in myocardial iron burden with both deferiprone monotherapy and deferiprone/deferoxamine combination therapy. No improvement was found with deferoxamine monotherapy in either study. In both trials there was a concomitant improvement in LVEF. In the post mortem study by Carpenter et al, it was noted that the RV wall iron concentration was up to 21.9% less than the global LV level. (Carpenter 2011) As a similar improvement in EF was found for the LV and RV with deferiprone or combination therapy, this may reflect greater sensitivity to iron toxicity or lower reserve in the RV. In this context, the cardiac response to deferasirox therapy described in chapter 10 was anomalous and of particular interest. No significant change in LVEF was seen with the reduction in cardiac iron, but the RVEF increased, and the LV and RV mass reduced. Furthermore, unlike the responses described with deferiprone, this was caused by an increase in RVEDV. Systemic blood pressure decreased in the first 2 years of treatment however there were no significant differences at year 3, perhaps reflecting a drug tolerance effect. The RV response might be explained by a reduction in pulmonary pressures or an improvement in RV compliance, which would suggest an interesting therapeutic potential worth exploring. Pulmonary hypertension has been reported to have a low incidence in TM in studies of well treated European patients. (Derchi 1999, Aessopos 2004) Of note is the fact that the deferiprone and combination trial patients were Italian or Greek patients and all were treated at expert centres. Patient recruitment for the deferasirox study was worldwide including patients of different ethnicities. The suggestion of pulmonary arterial pressure elevation at baseline may reflect either sub-optimal transfusional therapy before commencing the trial or genetic differences in response to iron overload which again has interesting implications.

11.2 Limitations of this research

The rarity of EDMD meant our patient study group was very small, despite collaborating with the Dubowitz Neuromuscular centre at Hammersmith Hospital. Although we found a significant difference in echo MVGs and CMR tagging strain
patterns between patients and controls a larger group of patients would have made the study more robust. Chapters 6, 8, 9 and 10 all report the results of longitudinal studies in which patient recruitment provided an initial challenge. For the anthracycline study (chapter 6) the number of eligible patients was greatly overestimated by the oncologists, as was patient willingness to travel to a separate site to be scanned. This had a negative impact on patient recruitment which took nearly four years rather than the predicted 18 months. This meant that there were a number of scanner upgrades during the study period, which affected the running of the sequences developed on site, and these had to be surmounted. Early breast cancer patients were chosen as the disease is common and usually carries a good prognosis. Adjuvant chemotherapy however, uses relatively low anthracycline doses and the mean decrease in LVEF found was only ~2%, moreover no patient had an abnormal EF at follow up. Changes in EF would be expected to be greater in patients prescribed higher anthracycline doses (thus increasing study power) but this tends to associated with more aggressive malignancy that has a poorer prognosis and there would also be a higher likelihood of the need for heart failure medication. Children are more susceptible to AMC (Von Hoff 1977, Grenier 1998, Lipshultz 2008) however growth would confound long term follow up, and therefore these were not studied.

The data for Chapters 8, 9 and 10 were obtained from international trials. The patients for the deferiprone/deferoxamine combination trial were scanned by our group, taking a mobile scanner from RBH to Italy. This meant all data quality was of high standard and as we were on site we had good access to clinical information. The deferiprone vs deferoxamine monotherapy trial used data obtained by the local site personnel. Our team travelled to the sites for training prior to the trial, but data sets missing adequate LAX cines were received for analysis which prevented 3D modelling as the preferred mode of volumetric analysis. Manual planimetry was used instead, which was slower and less objective. For the deferasirox trial the RBH thalassaemia team travelled to each participating site for training and strict acceptance criteria were applied to incoming data sets. Those judged incomplete or sub-standard were rejected. Consequently all included data was of good quality. We did not have the foresight however to measure baseline pulmonary artery pressure which could have derived from
the TR jet obtained by echocardiography and would have added useful clinical information.

11.3 Conclusions

To summarise, the original findings emerging from this work are as follows:

- There was no evidence of macroscopic myocardial fibrosis in EDMD however strain abnormalities were detected using CMR tagging and echo derived MVGs.

- EGRE correlates with changes in urinary F2 isoprostanes and a late decrease in ventricular function after anthracycline exposure.

- Nulling the blood signal or adding a trigger delay improves the reproducibility of cardiac iron assessment.

- Deferiprone monotherapy and in combination with deferoxamine shows superior improvement in RV function to deferoxamine monotherapy which may have prognostic significance.

- Deferasirox monotherapy improves RVEF with an associated reduction in RV and LV mass but no improvement in LVEF suggesting a different pharmacological effect to deferiprone and the possible direct improvement of endothelial function.

These novel observations have the potential to improve diagnosis in cardiomyopathy and provide ideas for new treatment approaches.

Further work in these areas is described in chapter 12 (Future Directions).
Chapter 12: Future directions

12.1 Technical advances

During the course of acquiring data for this thesis there have been a number of advances in CMR technology and sequence design which can lead to improved phenotype description. An example would be the identification of patients for whom diffuse interstitial myocardial fibrosis might be a substrate for myocardial dysfunction (EDMD and AMC) but no areas of late gadolinium enhancement are demonstrable. The availability of T1 mapping during equilibrium gadolinium infusion has created the potential to test this. (Flett 2010) We are now developing such sequences and validating their use for work with CM patients.

The CMR unit at Royal Brompton Hospital (RBH) is continually working to optimise T2* sequences. Dr Taigang He is exploring the use of parallel imaging to reduce scan time for the inversion recovery sequence as the relatively long breath hold can be a limiting factor for some patients. As we now have the potential to scan at 3T (Siemens Skyra), future diagnostic protocols, including iron overload assessment, are being designed for high field work. Our group are currently adapting and writing sequences and validating measurements against previous work at 1.5T. We are also currently collaborating with Prof John Porter’s team at UCH to improve the measurement and calibration of hepatic iron loading.

12.2 Future collaborative work at RBH

The NIHR Biomedical Research Unit at RBH opened in November last year. Available diagnostic technology includes 3T CMR equipment (Siemens Skyra), Phillips IE33 echo equipment, a catheter lab, and genetics with next generation sequencing. The improved research potential of having this under one roof is significant. The anthracycline cardiotoxicity study (chapter 6) provided a lesson in the difficulty of collaboration between geographically remote units. A joint cardio-oncology clinic between RBH and the adjacent Royal Marsden hospital has recently been established. The primary objective is to provide optimal cardiac care for patients undergoing chemotherapy but lends the added potential for collaborative research between
oncology and cardiology into the mechanisms of cardiac toxicity associated with chemotherapy treatment. A full work-up for patients is planned including CMR, echocardiography and bloods for the genotyping of consenting patients. This provides a multi-modality phenotype description which can be related to the genetic drivers of LV dysfunction. The CMR protocol has been drafted taking into account our experiences with the Better-care study and will include bi-ventricular volumetric data, T1 and T2 mapping and LGE imaging.

Chapters 8, 9 and 10 described how chelation therapy not only improves LV systolic function but also RV and possibly endothelial function. Research into the RV response to cardiac iron overload is ongoing in our unit. In particular ways in which RV myocardial iron can be quantified are being explored using 1.5 and 3T field strengths lead by Dr M H Alam as part of his PhD thesis. The possibility of inter-individual response to iron burden is also being explored in a genetic study lead by Dr Alam into the drivers for cardiac iron loading. The availability of echo equipment opens the potential to measure parameters of diastolic function and assess PA pressures on site.

12.3 International collaboration

The thalassaemia team is currently participating in a worldwide training program sponsored by the Thalassaemia International Federation, Novartis and local health authorities. This involves travelling to sites to install T2* CMR sequences and train personnel in image acquisition and analysis leading to collaborative research opportunities.

12.4 Ongoing prospective trials

Research from our unit, including the improvements described in chapter 7, has helped establish CMR as the diagnostic tool of choice for the diagnosis and follow up of siderotic CM. Our unit is presently concluding a Novartis sponsored CMR driven randomized controlled trial comparing the efficacy of deferasirox and deferroxamine monotherapy in reducing cardiac iron burden in TM patients. We have also just commenced a 2 year trial evaluating the efficacy and safety of
deferasirox/deferoxamine combination therapy in severely iron overloaded TM patients looking at changes in T2* and bi-ventricular volume and mass changes.

Chapters 6, 8, 9 and 10 describe how CMR can show small changes in ventricular function with either therapy or exposure to a cardiotoxic agent. The importance of assessing RV function is also emphasized and we are commencing a new ResMed sponsored trial investigating the bi-ventricular response to adaptive servo-ventilation in patients with chronic heart failure and central sleep apnoea.
Chapter 13: References


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Chapter 14: Appendices

14.1 Personal contribution to the research

For the EDMD study, I worked in collaboration with Professors Petros Nihoyannopoulos (Echocardiology) and Francesco Muntoni (Neuromuscular centre) from the Hammersmith Hospital. I participated in the design of the study and performed all the echo examinations and analyses including the myocardial velocity gradients. I scheduled the patient appointments at RBH and performed all the CMR scans working with Dr Sanjay Prasad. I performed all the CMR analysis with the exception of the myocardial tagging which was carried out by Dr Sharmeen Masood.

For the Better-Care CMR protocol, I participated in the design of the study. I performed the scans for the main study and the day 3 sub-study either alone or with Dr Paul Kotwinski. I analysed all the EGRE, STIR and T2* data and participated in the volumetric analysis for the main study. The day 3 patient left ventricular (LV) volumetric assessment was performed by Dr Kotwinski to eliminate possible bias.

For the black blood T2* study, I acquired all the CMR scans and performed the analysis.

In the right ventricular response to deferiprone vs deferoxamine study I performed the original LV functional data analysis. The subsequent right ventricular (RV) analysis and interpretation was performed by myself and Dr Francisco Alpendurada.

For the combination deferiprone plus deferoxamine trial, I travelled to Italy for all three time points and participated in data collection. I performed the original LV analysis with Dr Mark Tanner, and again I performed the RV analysis and interpretation in concert with Dr Alpendurada.

The deferasirox study involved travelling to participating sites with Dr Taigang He and Professor Raad Mohiaddin to optimize the T2* sequence and train the imaging staff in CMR scanning techniques. Under the supervision of Professor Pennell, I performed all
T2* and volumetric analysis for both LV and RV and was responsible for the interpretation of the ventricular functional response.

### 14.2 Funding

I was supported in this work by the Royal Brompton and Harefield (RBH) charitable funds, and the NIHR cardiovascular Biomedical Research Unit of RBH and Imperial College. The RBH Joint Research Committee funded course fees.

### 14.3 Publications arising from this work

#### 14.3.1 Peer reviewed, published research papers arising from this thesis


#### 14.3.2 Research papers in preparation for submission arising from this thesis

Alpendurada F,* Smith GC,* Carpenter JP, Nair S, Tanner MA, Banya W, Dessi C, Galanello R, Walker JM, Pennell DJ. Effects of combined deferiprone with deferoxamine on right ventricular function in thalassaemia major. *Both authors contributed equally to this study


14.3.3 Other peer reviewed, research papers published during my research period


14.4 Abstracts arising from this thesis


Smith GC, Tanner MA, Cannell TJ, Galanello R, Pennell DJ, CMR assessment of diastolic function in myocardial iron loading, J Cardiovasc Magn Reson 2006; 8: 217
14.5 Other abstracts published during my research period


He T, Gatehouse PD, Smith GC, Mohiaddin RH, Pennell DJ, Firmin DN Correlation of myocardial T2 and T2 measurements in thalassemia patients J Cardiovasc Magn Reson 2008; 10: 64


14.6 Invited lectures

CMR image acquisition, Novartis CiCL670A2409 investigator meeting Cairo January 2007


14.7 Invited peer review

I have been an invited reviewer for Heart and Journal of Cardiovascular Magnetic Resonance.