Novel Biomarkers in Paediatric Dilated Cardiomyopathy

Dilveer Panesar

Submitted for the degree of Doctor of Medicine (Research)

University College London

London, May 2019
I, Dilveer Kaur Panesar confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: Date:
Declaration of Originality

The contents of this Thesis are original work. Where reference sources have been used for illustrations and background material, these are indicated and cited.

There was collaboration with others, in particular:

Data collection concerning patients with dilated cardiomyopathy was done in collaboration with Dr Jakob Hauser. I am also a co-author on the paper ‘Diagnostic performance and reference values of novel biomarkers of paediatric heart failure’, the data for which was collected while I was away from the project on maternity leave.

Data for some control subjects was from the SOCRATES study on obesity and were collected by Dr. Jakob Hauser and Dr. Alexander Jones. Dr Alexander Jones assisted with statistical analysis of the biomarker data. Deborah Ridout assisted with statistical analysis of the biomarker data.
Abstract

Introduction:

The pathophysiology of cardiomyopathy (CMP) is not fully understood in children. In this project, I analysed blood and imaging biomarkers to ascertain the importance of various processes in this condition. Remodelling, fibrosis, inflammation and vaso-reactivity were examined using circulating biomarkers. Diffuse fibrosis was quantified using T1 mapping and the calculation of extracellular volume (ECV) on magnetic resonance imaging (MRI).

Methods:

76 children with CMP and a history of impaired left ventricular (LV) function underwent clinical assessment, 34 had blood biomarkers and 30 had cardiac MRI scans. 88 control subjects (7 adults) had an MRI performed and 25 had novel blood biomarkers measured.

The biomarker panel consisted of: N-terminal pro-brain-natriuretic peptide (NT-proBNP), mid-regional atrial natriuretic peptide (MR-proANP), mid-regional adrenomedullin (MR-proADM), c-terminal endothelin-1 (CT-proET1), soluble suppression of tumorigenicity-2 ligand (sST2), growth differentiation factor-15 (GDF-15), high-sensitivity troponin I (hsTnI) and high-sensitivity C-reactive protein (hsCRP).

29 patients with CMP had T1$_{\text{native}}$ (T1 time pre-contrast, ms) and 26 had ECV (%) measured. 14 control subjects had T1$_{\text{native}}$ and ECV measured (7 adults
and 7 children). Circulating levels of blood biomarkers were measured in the 7 children.

**Results:**

NT-proBNP, MR-proANP, GDF-15, hsCRP and sST2 were significantly higher in patients with CMP versus control subjects (paediatric).

Both $T1_{\text{native}}$ and ECV were significantly higher in patients with CMP and those with DCM versus control subjects (adult controls). Septal regions of the myocardium had significantly higher levels of $T1_{\text{native}}$ in patients compared to control subjects.

**Discussion:**

Blood biomarkers implicating fibrosis, inflammation, endothelial activation and apoptosis are elevated in paediatric heart failure, particularly idiopathic dilated cardiomyopathy (DCM) compared to control subjects.

$T1_{\text{native}}$ and ECV values are higher in patients with CMP and DCM than control subjects. Septal values are especially high.
Impact Statement

This Thesis was made possible by the MD-Paedigree project. The objective of which was to “capture the main features of the cardiovascular system, including the heart, arteries and peripheral circulation, to predict cardiomyopathy progression and plan therapies like heart transplant and ventricular assist devices”. The eventual goal being to “provide cardiologists the tools to deliver patients the best possible medical care.”

This study constitutes the first measurement of these biomarkers in children with and without heart disease. The markers we used need to be studied in larger groups of patients, including in acute heart failure, to allow studies into outcome. Normal values should also be established.

In terms of normal values, T1 maps of normal myocardium in children would be of great use in future studies. We found significant differences in T1\textsubscript{native} between CMP patients and control subjects, meaning that future studies may not require gadolinium contrast.

Patients with Duchenne Muscular Dystrophy (DMD) and high troponin are being offered early echocardiographic testing following these findings. The timing of troponin rise in the disease process remains to be discovered, but the importance of this biomarker in DMD is of immediate clinical interest.

\footnote{1 http://www.md-paedigree.eu}
We addressed and overcame many difficulties in the recruitment and scanning of children with and without CMP. During the course of the project, cardiac magnetic resonance (CMR) scanning, became faster and more widely used. The patients in this study served as early adopters of this rapid scanning and this has been more widely rolled out in the heart failure service at Great Ormond Street Hospital, with scans being reported and available the same day in clinic. This has led to the results of CMR being used for decision making in real time.

The patient data collected allowed our partners to assess the accuracy and precision of the automatic segmentation pipeline. We provided datasets for the engineers to use in the formulation of this process. The reporting of CMR scans is currently labour intensive and expensive, this technology could be used to speed up reporting of scans.
Acknowledgments

This research has been made possible by the hard work and dedication of many people. I would like to extend a special thank you to all of the patients and their families who agreed to take part in the research despite the inconvenience this caused them. I want to express my thanks to the neurology team and the brave patients who had longer scans to allow the first measurements of T1 in the hearts of children without cardiac disease.

This work would not have been possible without the belief and dedication of Professor Michael Burch and Professor Andrew Taylor. Both of them shared their time and experience with me and motivated me to continue with this project.

I would like to thank members of the MRI team, especially Wendy Norman and Rod Jones, who were unerringly patient and shared their knowledge with me whenever I asked. Thanks are also due to Gaby Captur for providing me with the phantom and Jennifer Steeden and Vivek Muthurungu for their specialist knowledge of sequences and T1 mapping. Thanks also to Eric Ogbogbo for taking bloods from the patients for the study and finally, Jessica Cooper and Abbas Kushnood for their help with locating control patients for the T1 mapping study.

I am indebted to the MD-Paedigree project for funding my research and introducing me to a varied and enthusiastic group of researchers. I am especially grateful to Tobias Heimann (Siemens), Marcello Chinali (OPBG) and Marcus Kelm (Berlin Heart Centre) who were very helpful at different
points during the research and analysis. Thank you to Adnane Bachir for helping with genetics questions.

I extend thanks to the research team at Great Ormond Street Hospital including Catriona Baker for her knowledge of everything, Silvia Schievano for her mentorship and friendship, Claudio Capelli for coffee and laughter, Benedetta, Georgia, Emilie, Stephane and Jan for their humour and friendship. Miranda France is owed much gratitude for editing and proofreading my thesis in her role as Literary Fellow at Great Ormond Street Hospital.

Finally, I would like to thank my husband, Mark Jones without whom this project would never have been possible. A special mention to all of the people who helped look after my son Alexander, allowing me to complete this work; Sam, Mum, Cathy, Glyn, Tanveer and Keith. Thank you.
Publications arising from this work

- **Diagnostic Performance and Reference Values of Novel Biomarkers of Paediatric Heart Failure**

- **Understanding and Treating Heart Failure in Children**

- **Book Chapter: ‘Support and Transplant’**

- **Assessment of Diastolic Function in Congenital Heart Disease**
  Panesar, D.K.; Burch M. Frontiers in Cardiovascular Medicine, 2017; 4 (5).

- **Longitudinal analysis using personalised 3D cardiac models with population-based priors: application to paediatric cardiomyopathies.**
Introduction

Currently, prognosis in paediatric CMP is problematic. The possible outcomes for a newly diagnosed patient include death, transplantation, full/partial recovery or chronic functional impairment. In those with a dilated phenotype, some features of the patient and their presentation are known to be prognostic, for example, prognosis is worse if presentation is after 1 year of age, with a lower fractional shortening (FS) Z-score, or if the aetiology is unknown\(^1\). However, although these features are helpful markers on a population level, the long-term outcome for a particular patient is still difficult to predict.

The current blood biomarker most commonly used in paediatric CMP is brain natriuretic peptide (BNP), but despite its widespread use, it has its limitations. Additionally, the process of heart failure (HF) is known to encompass many different pathological processes including inflammatory and neurohormonal pathways, which BNP is unaffected by\(^2\). BNP is released in response to ventricular stretch and does not increase in response to other stimuli known to be involved in the pathophysiology of heart failure, such as inflammation. We have identified a panel of biomarkers involved in novel pathophysiological pathways in heart failure to better understand the process, as well as to attempt to identify better prognostic tools in this disease.

Remodelling is central to this project. Although remodelling is an attempt to heal the damaged myocardium, the inability of the heart to regenerate myocytes results in replacement fibrosis and an energetically inefficient structure of the cellular architecture. Additionally, pressure and volume loading
following the initial insult lead to a diffuse, interstitial fibrosis, which is difficult to quantify due to the risks and inaccuracy of endomyocardial biopsies. This pattern of fibrosis has been well described in post-mortem studies. A novel imaging technique (T1 mapping) seeks to measure the extracellular component of the myocardium and therefore quantify interstitial fibrosis. This study is the first to apply this technique to a paediatric population including control subjects. The process of reverse remodelling (functional improvement) as well as deterioration (adverse remodelling) will be investigated in terms of prognostic factors and markers.

The overall aim of this Thesis is to investigate novel circulating and imaging biomarkers in paediatric CMP. These can then be considered in terms of diagnostic or prognostic benefit. Understanding the role of these biomarkers in the disease process could uncover therapeutic targets and allow us to understand the reasons for deterioration more fully in our patients.
Hypotheses

This study aimed to investigate circulating and imaging biomarkers in children with heart muscle disease.

The hypothesis of the study was that there were different levels of circulating and imaging biomarkers between children with CMP and control subjects. The hope is that as more information is gathered, these biomarkers may be used to help determine disease processes, functional status and even prognosis.

We hypothesised that novel circulating biomarkers are not only significantly higher in children patients with CMP than control subjects, but they also correlate with LV function (ejection fraction), vary with aetiology and reflect remodelling processes.

A further hypothesis was that patients with CMP of all aetiologies have significantly higher levels of $T_1^{\text{native}}$ and ECV than control subjects, MRI assessment of fibrosis will correlate with both severity of disease (ejection fraction), functional class and will vary with aetiology.

We also hypothesised that those blood biomarkers that reflect fibrosis and remodelling will correlate with the MRI assessments of fibrosis/remodelling.
Aims and Objectives

- To characterise paediatric cardiomyopathy in terms of cellular processes and pathophysiology using circulating and imaging biomarkers;
- To test the diagnostic utility of circulating and imaging biomarkers; and
- To discover the normal values for circulating and imaging biomarkers (in children)
Thesis Outline

This thesis consists of an overview of CMP in children alongside various experiments to develop an understanding of the disease. Specifically:

**Chapter 1** is an introduction to CMP including incidence, aetiology and prognosis.

**Chapter 2** details the methods used in the project, including biomarker measurement and T1 mapping techniques.

**Chapter 3** describes the results of the study measuring biomarkers in children with CMP and healthy controls subjects.

**Chapter 4** T1 mapping is used to quantify diffuse fibrosis in paediatric patients with CMP and adult controls.

**Chapter 5** Discussion of both studies including limitations and future directions.
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACEi</td>
<td>Angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ADM</td>
<td>Adrenomedullin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>ASD</td>
<td>Atrial septal defect</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CHD</td>
<td>Congenital heart disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CK</td>
<td>Creatinine kinase</td>
</tr>
<tr>
<td>CMP</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiac magnetic resonance imaging</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>CT-proET-1</td>
<td>C-terminal endothelin 1</td>
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</tbody>
</table>
LVAD: Left ventricular assist device
LVEDVi: Left ventricular end diastolic volume indexed to body surface area
LVEF: Left ventricular ejection fraction
LVESVi: Left ventricular end systolic volume indexed to body surface area
LVH: Left ventricular hypertrophy
LVNC: Left ventricular non-compaction
MHC: Major histocompatibility complex
MR: Mitral regurgitation
MRI: Magnetic resonance imaging
MR-proADM: Mid-regional pro-adrenomedullin
MR-proANP: Mid-regional pro-atrial natriuretic peptide
NYHA: New York Heart Association (classification of HF)
NT-proBNP: N-terminal pro-brain natriuretic peptide
PCWP: Pulmonary capillary wedge pressure
R (r): Pearson’s correlation coefficient
Rc: Lin correlation coefficient
RCM: Restrictive cardiomyopathy
Rho (ρ): Spearman’s rank correlation coefficient
ROI: Region of interest
SD: Standard deviation
sST2: Soluble suppression of tumourgenicity-2 ligand
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Tissue inhibitor of metalloproteinase-1</td>
</tr>
<tr>
<td>TnC</td>
<td>Troponin C</td>
</tr>
<tr>
<td>TnI</td>
<td>Troponin I</td>
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<tr>
<td>TnT</td>
<td>Troponin T</td>
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1 Introduction

1.1 Dilated Cardiomyopathy

This section will detail the current state of knowledge on paediatric dilated cardiomyopathy (DCM). This is because although patients included in this study had various aetiologies, their common feature was a dilated phenotype.

DCM is a rare disorder of the heart muscle with a number of known causes including genetic, metabolic, infective, inflammatory, toxic and endocrine. The common end-point of these insults is a dilated and poorly functioning left ventricle. DCM remains the commonest cause of CMP and heart transplantation in children with a peak incidence in the first year of life. Only around 30-40% of children have a known aetiology, with the remainder termed 'idiopathic'.

1.1.1 Incidence

DCM is the commonest cause of heart muscle disease and transplantation in children. The incidence is between 0.34-0.73 per 100 000 per year\textsuperscript{1, 4}; with differences, attributable to inclusion/exclusion criteria of studies and regional variations. It is more common in boys and in non-white populations\textsuperscript{1}. The sex difference is probably due to X-linked conditions such as DMD and Becker muscular dystrophy (BMD), which are known causes of this condition. The median age of onset in a multi-centre UK study of children was 1 year\textsuperscript{4}. Prognosis was worse if onset occurs after the age of 1, in boys and if the cause
is unknown. The aetiology is known in only 30-40% of cases and, where an infective cause has been identified, the prognosis is better.

At the macroscopic level, DCM results in a heart with dilated ventricles, but the overall myocardial mass may be increased\(^5\). As myocytes are terminally differentiated, the myocardium can only respond by replacing dead cells with fibrosis. This leads to a decrease in the ability of the myocardium to generate sufficient contractile force, thus increasing ventricular volume. The globular ventricle has unfavourable haemodynamics due to increased end-diastolic volume as described by the Frank-Starling law\(^6\). This leads to a decreased ejection fraction (EF) and causes a cycle of progressive dilatation. Mitral and tricuspid regurgitation may then ensue, further compounding the problem.

### 1.1.2 Aetiology

#### 1.1.2.1 Infective

The commonest cause of DCM in developed countries is a viral infection, specifically enteroviruses of the Coxsackie B serotypes\(^7\). Worldwide, aetiologies differ from region to region, with Chagas disease the most prevalent\(^8\).

Most children with mild left ventricular (LV) dysfunction recover fully. Of those with more severe dysfunction (left ventricular ejection fraction, LVEF <35%), 25% progress to death or transplantation, 50% develop chronic DCM and the rest recover fully\(^9\). Presentation varies from asymptomatic ECG changes to
cardiogenic shock and fulminant myocarditis. A prodrome of fever, coryza or gastroenteritis is not always present.

The cardiac damage occurs via three pathways: initially there is direct invasion by cardiotropic viruses via specific receptor-mediated endocytosis. This leads to immunological activation followed by CD4+ activation and clonal proliferation of B-cells. Eventually, circulating anti-heart antibodies are produced, resulting in infection-triggered autoimmune cardiac damage. The coxsackie protease cleaves dystrophin and disrupts the cytoskeletal integrity of the cardiac myocyte\textsuperscript{10}. These processes interact and cause cell damage throughout the myocardium.

Immunotherapy would seem a sensible treatment given the secondary damage done by the immune response itself. However, prednisolone\textsuperscript{11} and intravenous immunoglobulin\textsuperscript{12} have not been found to improve outcome compared to placebo in randomised, double-blind clinical trials.

\textbf{1.1.2.2 Toxicity}

It has long been known that anthracycline antibiotics can cause CMP. The effects can be acute or chronic in onset. Subclinical cardiac dysfunction occurs in all patients, with 2-5% progressing to acute heart failure\textsuperscript{13}. Pathogenesis involves increased fibrosis and cell death.

The risk of developing heart failure is dependent on cumulative dose of the drug- 350mg/m\textsuperscript{2} in infants, 450mg/m\textsuperscript{2} in children and 550mg/m\textsuperscript{2} in adults.
There is some evidence that dexrazoxane, an intracellular iron chelator and free iron scavenger reduces anthracycline-related CMP\textsuperscript{14}.

1.1.2.3 Hereditary

DCM is usually transmitted as an autosomal dominant trait (25%) with lower frequencies of autosomal recessive, X-linked and mitochondrial transmission. The mutations that cause DCM, although resulting in a similar phenotype, affect a wide range of genes with diverse functions.

Two major forms of autosomal dominant DCM have been identified: isolated, and that associated with disease of the conduction system. The underlying gene mutations can include disruption of the myocyte architecture, including mutations in cardiac Lim protein (CLP\textsuperscript{15}), cypher/ZASP (LBD3\textsuperscript{16}), δ-sarcoglycan (SGCD\textsuperscript{17}), desmoplakin (DSP\textsuperscript{18}), desmin (DES\textsuperscript{19}), dystrophin (DMD\textsuperscript{20}), telethonin (TCAP) and vinculin (VCL\textsuperscript{21}).

Mutations of the many proteins in the dystrophin-associated complex that cause skeletal muscular dystrophies frequently also cause DCM. Some patients present with a cardiac-only phenotype. Mutations in the desmin gene may cause DCM in more complex ways, resulting in the formation of electron-dense bodies. Mutations in Lamin A/C also cause disruption of the myocyte cytoskeleton\textsuperscript{22}. This protein, present in all somatic cells, is integral to the process of mitosis (the relevance in terminally differentiated myocytes is unclear).
Sarcomeric mutations can cause DCM or hypertrophic cardiomyopathy (HCM). The affected genes include cardiac actin (ACTC), titin (TTN), tropomyosin (TPM1) and myosin heavy chain (MYH7). Some mutations in Troponin T (TNNT2) appear to affect the sarcomeric response to calcium. Mitochondrial syndromes may affect the myocyte’s ability to regulate calcium uptake, with KATP channel mutations predicted to cause cellular calcium overload.

### 1.1.2.4 Myopathy

A noteworthy subset of hereditary CMP includes the X-linked muscular dystrophies including DMD and BMD, which together account for 5% of familial DCM. These result from mutations in the dystrophin gene and cause skeletal muscle weakness and progressive CMP. Symptoms of skeletal muscle weakness start in childhood, with most affected patients becoming non-ambulatory by the second decade of life. The CMP occurs as a result of fibro-fatty replacement of the myocardium by the third decade in almost all boys. With increased survival, due to supported ventilation and steroid use, cardiac disease is becoming the main cause of death.

The cardiac disease develops as DCM with a thinning LV wall, cardiac fibrosis and decreased systolic function. The onset of cardiac disease may be masked by decreased physical activity, so regular echocardiograms for asymptomatic children are important. Steroid treatment decreases the risk of developing HF by 4% per year of treatment. Also, steroids delay the onset of CMP from 15.2 years in the untreated population to 13.2 years in the treated population.
ACE inhibitors (ACEi) have been used in this group and have been found to be useful in slowing the decline in EF over time\textsuperscript{26}. Studies have been done to compare angiotensin receptor inhibitors to ACEi\textsuperscript{27} and combination therapy with beta-blockers\textsuperscript{28} to monotherapy. Neither has shown any difference. BMD has a more benign course, with less skeletal muscle weakness and a better long-term prognosis. Cardiac transplantation can be considered to prolong life in this subset of patients.

X-linked CMP involves the cardiac muscle only and can be cured by heart transplantation. Female carriers of the DMD gene mutation may develop DCM later in life (fifth decade). Lamin A/C gene mutations, result in atrial arrhythmia and progressive AV disease, which often precede DCM. Some mutations result in juvenile-onset muscular dystrophies including Emery-Dreifuss muscular dystrophy (EDMD) and familial partial lipodystrophy with insulin-resistant diabetes\textsuperscript{29}.

\textbf{1.1.2.4.1 Magnetic Resonance Imaging findings in Dilated Cardiomyopathy Associated with Myopathy}

Late Gadolinium enhancement (LGE) is a technique using MRI to distinguish areas of normal myocardium from focal fibrosis (see section 1.6). Given the mechanism of disease in DMD, this is unsurprising. Silva et al. found LGE in 7/10 boys with DMD aged 7-18 years. There was also a correlation between reduced LVEF and the presence of LGE\textsuperscript{30}. It has also been shown that those with LGE positive segments have a greater decline in EF over time\textsuperscript{31}. The location of fibrosis and hypokinesia in patients with DMD appears to follow a
similar pattern in most patients, starting in the postero-basal region and spreading to the inferior and lateral free wall of the LV\textsuperscript{32}.

T1 mapping is a new technique which allows quantification of diffuse fibrosis (see section 1.7). This may help to identify patients earlier in the disease process, allowing for earlier and more aggressive treatment of disease. Indeed, Soslow et al. showed patients with DMD had higher $T_1$\textsubscript{native} (pre-contrast) and ECV than control subjects, even with preserved LVEF and negative LGE\textsuperscript{33}. ECV was also found to be greater in patients with DMD than published normal values in a series of 47 adults with DMD\textsuperscript{34}.

1.1.2.5 Inborn Errors of Metabolism

Inborn errors of metabolism account for less than 5% of cases; mitochondrial disease is the most common (around 50%), then Barth syndrome (25%). Primary or systemic carnitine deficiency accounts for 10\%\textsuperscript{1}.

Barth syndrome is an X-linked disorder also known as 3-Methylglutaconic aciduria type II. Most patients are male and present with hypotonia, poor growth, neutropenia and CMP in infancy. While the cardiac manifestation can be life-threatening, it is mostly resolved by puberty\textsuperscript{35}.

Other causes include mucopolysaccharidosis type I (Hurler Syndrome) and type VI (Maroteaux–Lamy syndrome), glycogen storage disorder type IV (Anderson disease), long-chain 3-hydroxacyl-CoA dehydrogenase deficiency, and mitochondrial disorders such as MERFF (myoclonic epilepsy with ragged-red fibres).
1.1.2.6 Other

Hypocalcaemic rickets can present as isolated CMP an important and treatable differential, with therapy often resulting in complete remission\(^{36}\). Thyroid hormone dysregulation has also been shown to affect cardiac function\(^{37}\).

1.1.3 Prognosis

Older age at presentation and reduced systolic function have been found to negatively affect prognosis in a UK-based, multicentre trial\(^{4}\). In another trial involving long-term follow up of 175 patients, age at presentation <4m or >5y, familial CMP and lower baseline LV FS Z-score at presentation negatively affected survival\(^{38}\). A similar follow up study confirmed a poor prognosis for patients with familial DCM, showing that they are more likely to be on medication at follow-up with a higher mortality\(^{39}\). Similar results have been noted in other studies\(^{40, \, 41}\). Those with left ventricular hypertrophy (LVH) on ECG, depressed systolic function persisting after 3 months and greater LV dilatation, were more likely to develop chronic cardiac dysfunction\(^{42}\). In a study of ambulant outpatients with DCM, only peak VO\(^{2}\) was associated with the study end-points of urgent heart transplantation or death\(^{43}\).

Recent advances in the field of genetics and prophylactic studies have resulted in a ‘grey area’ with patients with a strong family history or other risk for developing the condition treated earlier in the disease process before systolic dysfunction occurs. The effectiveness of early treatment in asymptomatic patients remains unknown.
52 genes were measured as part of this study (see section 2.3.1). Various circulating and imaging biomarkers have also been found to have prognostic utility in adults with DCM. These will be discussed in section 1.3.

1.1.4 Difference between Adults and Children with DCM

The question of whether paediatric and adult DCM are similar entities is partly driven by therapeutic considerations. The fact remains that children show a disappointing effect of neurohormonal therapies which have been shown to be successful in treating adults. The Pediatric Carvedilol Trial failed to show a benefit of treatment with beta-blockers (commonly used in adults) in children with DCM\textsuperscript{44}. Registry data failed to show an improvement in outcomes since the commencement of ACEi and beta-blockers in children compared to the era of digoxin and diuretics\textsuperscript{45}.

This has led to theories about the differences between the two groups. Patel et al. hypothesised that adult and paediatric DCM differ due to the fact that only adults undergo adverse remodelling in the form of cardiomyocyte hypertrophy, myocardial fibrosis, inflammation and capillary loss\textsuperscript{46}. This study involved analysing explanted hearts and core-samples from VAD-implantation in both adults and children, with HF and healthy controls. Children with DCM showed minimal fibrosis and hypertrophy compared to both adults and age-matched controls.

A potential explanation to link the two findings- that children do not respond to neurohormonal manipulation and medications supposed to cause remodelling, and the failure to demonstrate adverse remodelling in paediatric
specimens, is that children do not undergo adverse remodelling. Rather, they may undergo reverse remodelling (or recovery), which is a rare outcome in adults\textsuperscript{47}.

1.2 Diastolic Dysfunction

The following is taken from my own published paper ‘Assessment of diastolic function in congenital heart disease’\textsuperscript{48}: “Diastole denotes the filling phase of the cardiac cycle. Filling is determined by myocardial relaxation as well as atrial contraction and atrial and ventricular compliance. Myocardial relaxation begins when the myofibrils return to an unstressed state and this precedes mitral valve (MV) opening (isovolumic relaxation). Adenosine triphosphate is used to actively uncouple calcium from the contractile apparatus and return it to the sarcoplasmic reticulum. Active relaxation is only responsible for early diastolic filling, whereas compliance is important throughout filling and especially during atrial contraction.

The early part of diastole is active relaxation, which is an energy-consuming process. The latter part is due to compliance or stiffness of the ventricle. Isovolumic relaxation time (IVRT) can be measured by invasive catheterization measurements. The index used in its measurement is the time constant of isovolumic pressure decline (\(\tau\)). In non-invasive measurement, IVRT is the closest measurement to assess this value. However, as with all indices of diastolic function, the loading conditions must be considered.
The stiffness of the myocardium also plays an important role in diastolic function. The mass of the LV affects the stiffness as do the viscoelastic properties of the myocardium (cellular and extracellular components). Attempts are made to measure this increase in myocardial stiffness. However, the difficulty arises in the mechanism of measurement as well as the timing and nature of diastole. Flow-based measurements rely on a change in volume to occur, and so they are unable to quantify isovolumic relaxation as they assess only the last stage of diastole. There is also no universal measurement of diastole (equivalent to EF in systole) and torsion and dyssynchrony are difficult to quantify.

E-wave deceleration time is the rate at which the atrial and ventricular pressures equilibrate after onset of the E-wave and is shorter in compliant ventricles (160–240ms in adults). The IVRT is the period between closure of the aortic valve and opening of the mitral valve. This is normally 70–90ms long in adults and is prolonged in the case of decreased LV compliance. It is also affected by heart rate and ventricular function. It is best recorded from the apical five-chamber view with the cursor placed to record LV outflow tract velocities and LV inflow simultaneously.

Tissue Doppler imaging directly measures myocardial wall velocities by focusing on the high-amplitude, low-frequency signals reflected by the myocardium rather than the blood pool. The areas sampled include the lateral aspect of the mitral annulus in the apical four-chamber view, the basal septal region in the same view, and the lateral tricuspid valve annulus. This serves to minimise translational artefact and to align the probe with the direction of
movement. Three waves are usually seen—the systolic (s’) wave, the early diastolic (e’) wave, and the late diastolic wave caused by atrial contraction (a’). Normal values and Z-scores are available for each age group in paediatrics⁴⁹.

Nagueh et al. were the first to show that E/e’ ratio (ratio of transmitral E velocity and TDI mitral annular e’ velocity) corresponded to pulmonary capillary wedge pressure (PCWP)⁵⁰. In 125 patients classified by systolic and diastolic function and symptoms, PCWP correlated strongly with E/e’ ratio \( r = 0.87 \). PCWP correlated only weakly with E velocity but not e’ velocity. Patients with abnormal relaxation and pseudonormalisation of the mitral inflow E/A ratio had a decreased e’ velocity \( (P < 0.001) \). In patients with diastolic dysfunction, a saline bolus affects the E/A wave as measured by transmitral Doppler measurement but did not affect the e’ or e’/a’ ratios⁵¹. These studies show that e’ acts as a preload-independent marker of LV relaxation. E wave velocity on mitral inflow Doppler, corrected for e’, correlates strongly with PCWP, and can be used to estimate left atrial pressure non-invasively”.

1.2.1 Heart Failure with Preserved Ejection Fraction

The following is quoted from the same published paper⁴⁸ “One theory of the mechanism of heart failure with preserved EF (HFpEF) is that it is caused by diastolic dysfunction. Increased LV filling pressures cause back pressure on the pulmonary circulation, leading to symptoms of HF, including breathlessness. This is assumed to be the case as EF remains in the normal range, which is thought to denote normal systolic function⁵². However, there have been studies which show that symptoms of HF in these patients correlate
with left ventricular end-diastolic volume (LVEDV) and that the stroke volume (SV) is only maintained due to LV dilatation. The mechanism postulated is that of excessive LV diastolic dilatation by fibre slippage and creep\textsuperscript{53}.

The process of LV remodelling to compensate for decreased systolic function in these patients occurs due to feedback from the periphery, causing the heart to adapt with an increase in volume to maintain SV\textsuperscript{54}. Therefore, an EF of 20% in a dilated ventricle may produce the same SV of a normally sized ventricle with a normal EF\textsuperscript{55}. Patients with LVH manage to avoid this excessive distension and may be more prone to HF with reduced EF\textsuperscript{56}. Symptoms of HF, such as breathlessness on exertion, are not related to PCWP\textsuperscript{57} or systolic function\textsuperscript{58}. Instead, the determinants appear to be musculoskeletal status, body composition, motivation, and tolerance of discomfort\textsuperscript{57}. Therefore, using symptoms alone to determine whether a patient has HF may not be valid.

A number of problems with the definition of HFpEF have been highlighted above; HF may not be reliably diagnosed using symptoms alone, and a preserved EF does not always correlate with normal systolic function. The notion of this type of disease being the definitive model for diastolic dysfunction is flawed".

\subsection{1.3 Circulating Biomarkers}

The only commonly used blood biomarker in paediatric practice is brain natriuretic peptide (BNP). Detailed below is the current state of knowledge of BNP and a panel of novel biomarkers, chosen to describe the pathophysiology
of paediatric CMP and for their promising role in the diagnosis and prognosis in adult CMP. Some of these have been investigated previously by our group in a more diverse patient population including children with congenital heart disease\textsuperscript{59}. The small numbers and diverse diagnoses may have masked the utility of these markers in systolic CMP.

### 1.3.1 Brain Natriuretic Peptide

BNP is a 32-amino acid peptide synthesised in ventricular myocytes in response to cardiac stretch and shear stress. It is stored in and released by atrial and ventricular myocytes when chronically stimulated\textsuperscript{60}. BNP is widely used as a diagnostic and prognostic marker in HF. Its use in paediatric patients is age-dependent and it has been evaluated in congenital heart disease\textsuperscript{61} and HF\textsuperscript{62}. Levels are known to rise in the first few days of life before declining in patients without cardiac disease\textsuperscript{61}. BNP also correlates with clinical parameters and echocardiographic FS in children with DCM and serial measurement predicts a change in these parameters\textsuperscript{63}.

In adults, BNP is used for the diagnosis of HF in patients with dyspnoea\textsuperscript{64} and prognosis of patients with acute\textsuperscript{65} and chronic HF\textsuperscript{66}. However, there is some evidence that the majority of circulating BNP is the inactive NT-proBNP rather than the active cleavage product, BNP\textsuperscript{67}. There are many potential causes of raised BNP apart from systolic heart failure, including diastolic dysfunction\textsuperscript{68}, acute\textsuperscript{69} or chronic ischaemic heart disease\textsuperscript{70}, LVH\textsuperscript{71}, inflammatory cardiac diseases, arterial hypertension with LVH, pulmonary hypertension\textsuperscript{72}, acute or
chronic renal failure\textsuperscript{73}, sepsis\textsuperscript{74}, ascitic liver cirrhosis\textsuperscript{75}, endocrine disorders and cardiac toxicity\textsuperscript{76}.

There are also some conditions in which HF is present where BNP levels are not raised including well-compensated HF, obesity\textsuperscript{77}, acute mitral regurgitation, pulmonary oedema of less than 1 hour duration, constrictive pericarditis and upstream causes including mitral stenosis. BNP varies with age\textsuperscript{78}, gender\textsuperscript{79} (women have twice the level at any compared to men) and ethnic origin (although the latter has a smaller effect than cardiac function when corrected for this)\textsuperscript{80}.

1.3.2 Diagnosis

In the ‘Breathing Not Properly’ study, BNP proved diagnostic of patients with HF of those attending the emergency department with dyspnoea\textsuperscript{64}. In the PRIDE study, which included 600 patients, it seemed there were age-specific cut-offs for the diagnosis of HF, but a BNP of <300pg/ml ruled out HF at any age with a negative predictive value of 99\%\textsuperscript{81}. BNP does not appear to be useful for screening of an asymptomatic population for heart failure, according to the Framingham study, but this study was relatively small\textsuperscript{82}.

In the paediatric population, BNP has been used to diagnose patients with congenital heart disease (CHD). The levels were found to rise in neonates in the first days of life, with a subsequent decline in those without heart disease and a plateau in those with CHD. Optimal diagnostic cut-off values vary with age\textsuperscript{61}. 


1.3.3 Prognosis

In the hospital setting, BNP can help to predict long-term outcome in chronic HF. Patients whose BNP was lower than 350pg/ml by the time of discharge had a much lower risk of death or rehospitalisation over 6 months than those whose levels remained above 700pg/ml\textsuperscript{83}. The ValHeft study showed that the magnitude of the change in BNP levels may be a more important factor than simply the direction, with larger increases (>360%) or decreases (>55%) associated with a higher mortality\textsuperscript{84}.

1.3.4 Guiding Treatment

The TIME-CHF study randomised 500 patients to conventional treatment vs. BNP-guided therapy and showed a non-significant reduction in all-cause mortality of 24% in those on BNP-guided therapy\textsuperscript{85}. An individual patient data meta-analysis of 9 trials on this topic showed this approach is more beneficial to those aged <75 years. This may be due to comorbidities in older patients confounding the effect of treatment, or to aggressive treatment causing toxicity in frail older patients\textsuperscript{86}.

1.4 Novel Biomarkers

There are many novel biomarkers which have been investigated in adult practise. These have been discovered as markers associated with processes involved in the development of CMP and HF in adult patients including inflammation, ischaemia, apoptosis and neurohormonal activation. The main
The aim of this project was to understand the pathophysiology of paediatric CMP and to compare this with the data in existing (mainly adult) studies.

The model of paediatric heart failure which was used for this project is detailed in Figure 1. The initial step in the development of CMP is injury to the myocardium, leading to cell death. This releases troponin and other intracellular proteins and ions. This leads to activation of inflammatory cascades in response to the cell death/necrosis. Events can then be either protective and anti-inflammatory or involve further activation and cell damage.

**Figure 1**: Simplified model of paediatric heart failure\(^2\).

Common causes for CMP in adults differ from paediatrics, with diabetes, ischaemic heart disease and hypertension commonly to blame\(^87\). However,

whether the response to injury to the myocardium is similar in both age groups is not known. Measuring levels of circulating biomarkers is one way of investigating similarities and differences between these age groups. This is one step in allowing us better understand pathophysiology and compensatory mechanisms with a view to developing/using better therapies for paediatric CMP.

In normal cardiac physiology, SV (the amount of blood pumped per heart beat) is affected by three main factors: preload, afterload and contractility. The main cause of cardiac failure in children is loss of functional myocardium due to cell death. This can be due to infection, genetic causes, toxins and rarely, ischaemia. This leads to a decrease in organ perfusion and activates the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS).

The RAAS is activated in order to maintain cardiac output (SV x heart rate) by increasing total peripheral resistance and increasing water retention. The SNS mediates catecholamine release in order to increase heart rate, contractility and peripheral vasoconstriction. The result of these mechanisms is to temporarily improve organ perfusion and decrease the damaging effects of HF. However, eventually, the compensatory mechanisms themselves lead to further problems.

Somewhat counterintuitively, LV mass in DCM is increased in paediatric patients with worse function and the persistence of symptoms. It may be that hypertrophy is a response to increased wall stress but leads to adverse
remodelling and increased systolic and diastolic dysfunction. Hypertrophy itself may lead to decreased compliance and increased diastolic dysfunction.

The next section will look at each pathophysiological process in turn and summarise the novel biomarkers measured in this study involved in each process, although there remains much to learn.

1.4.1 Myocardial Injury

1.4.1.1 Troponin

In order to be a marker for cardiac cell death, the protein must be tissue-specific, something which has been proven in the case of troponin in heart disease. Troponin is a complex of three globular contractile regulatory proteins (T, I and C). They occur at regular intervals along the thin filament of striated muscle and inhibit contraction by blocking the interaction of actin and myosin. The forms of troponin T (TnT) and I (TnI) differ from skeletal muscle, allowing for their use as cardiac-specific biomarkers. In the 1990s, troponin started to be used for diagnosis of myocardial infarction (MI). More recently, interest in high sensitivity assays has grown, with the idea that subclinical myocardial damage may predict adverse outcome in subjects with and without known cardiovascular risk factors and disease.

The mechanism of release of cardiac troponin in CMP is unclear. One postulated mechanism of release involves subclinical ischaemia, but results of stress tests have had differing results. Other possibilities include cell death (apoptosis), microvascular dysfunction or other structural abnormalities.
Patients with pre-existing HF or coronary artery disease (CAD) with detectable levels of troponin on high-sensitivity assays, have an increased rate of adverse cardiovascular events. In a representative sample of the population, 3557 asymptomatic subjects (age 30-65 years), only 1.1% had an elevation of high sensitivity TnT (hsTnT). Heart failure, diabetes, LVH and chronic renal disease were all independently associated with raised hsTnT levels.

In a review of the use of high sensitivity hsTnT to risk stratify in chronic stable HF, elevated baseline hsTnT was associated with higher risk of mortality with a hazard ratio (HR) of 2.85 and combined adverse cardiovascular outcome with a HR of 2.38. Interestingly, this review showed no difference between high- and low-sensitivity assays. In a study of 285 patients with chronic HF, hsTnI and BNP were independent significant predictors of poor outcome, but hsTnT was not. The HR of high BNP and hsTnI was 5.74. Serial levels have been shown to be of importance with a rise in hsTnI levels in a cohort of patients with stable chronic HF having a HR of 3.59 in patients with increased levels which then increased further vs those who showed a subsequent decline.

In a small (n=83) group of neonates, including 54 with CHD and 29 healthy controls, TnT was significantly higher in patients than control subjects. There was no correlation between TnT and Ross classification or echocardiographic markers of LV dysfunction.
1.4.2 Inflammation

The role of the inflammatory markers in HF is well recognised\textsuperscript{102}. Due to the high frequency of myocarditis as a cause of paediatric DCM, inflammation was an important process to investigate. It is known that a proportion of those with myocarditis will progress to chronic DCM, with others recovering completely\textsuperscript{103}. It is not clear how many patients with DCM have had myocarditis in the past, estimates range from 0.5-67\%\textsuperscript{104}. Cardiac injury occurs as a result of direct damage by pathogenic infiltration of cells, the inflammatory response and autoimmune cell damage. Cytokines have also been known to influence fibrogensis (as is the case with GDF-15). The markers we used in this study which pertain to inflammation included hsCRP, sST2 and GDF-15.

1.4.2.1 Growth Differentiation Factor-15

Growth differentiation factor-15 (GDF-15) is a stress-responsive cytokine produced in situations of tissue injury or inflammation. Interestingly, despite being found in rat myocardium, it is not found in human myocardium in physiological conditions but is produced by a variety of cell types in response to stress and ischaemic injury (Figure 2)\textsuperscript{105}. There is some evidence (although contradictory reports exist) that GDF-15 is protective in the case of ischaemia/reperfusion injury\textsuperscript{106}. The exact mechanism of action of GDF-15 is unknown.
Figure 2: Cellular sources of GDF-15 in the cardiovascular system.

Circulating levels of GDF-15 are elevated in patients with heart failure. Kempf et al. showed 75% of patients presented with levels >1200ng/L. GDF-15 levels were correlated with NYHA class, NT-proBNP levels and risk of death during follow-up increased with increasing quartiles of GDF-15\textsuperscript{107}. Increased circulating levels of GDF-15 are associated with a higher risk of developing heart failure in otherwise healthy individuals\textsuperscript{108}. The first paper on GDF-15 found higher levels at baseline in women who experienced a cardiovascular event compared to those who did not over a 4 year period\textsuperscript{109}.

1.4.3 Soluble Suppression of Tumourgenicity 2

SST2 is a decoy receptor for interleukin-33 (IL-33), which functions as an alarmin, signalling the presence of tissue damage to local immune cells. Although ST2 ligand is activated by IL-33 and effects intracellular signalling pathways, sST2 works to effectively remove IL-33 from the circulation, decreasing its ability to induce the immune response (Figure 3\textsuperscript{110}). “Damage

\textsuperscript{3} Data obtained from rat cardiomyocytes, otherwise in human cell types. LDL: low-density lipoproteins, TG: triglycerides, VSMCs: vascular smooth muscle cells.
to stromal cells can induce necrosis and release full length IL-33 (active IL-33) which can activate the heterodimeric ST-2/IL-1RAcP complex on a variety of immune cells or can be neutralised by sST2, which acts as a decoy receptor for IL-33. Upon activation of the ST2L/IL-1RAcP complex signalling through the Toll-IL-1 receptor (TIR) domain is induced. By activation of diverse intracellular kinases and factor this leads to an inflammatory gene transcription and ultimately to the production of inflammatory cytokines/chemokines and an immune response\textsuperscript{110}."

SST2 is increased in a number of different pathologies such as pulmonary disease\textsuperscript{111} and autoimmune disease\textsuperscript{112}. IL-33 and ST2 and sST2 are upregulated in fibroblasts and cardiomyocytes after MI in mice\textsuperscript{113}. ST2 is most probably produced in endothelial cells with evidence against cardiac production in humans (no transmyocardial gradient)\textsuperscript{114}. sST2 is increased in response to LVED pressure and may be produced by the systemic vasculature in response to diastolic load on the ventricle, thus helping to counteract the maladaptive neurohumoral activation seen in HF\textsuperscript{115}. It appears to act to regulate the inflammatory response to tissue damage as a result of various disease processes.

ST2 is not a useful \textit{diagnostic} biomarker for heart failure due to the lack of disease specificity\textsuperscript{116}, however, it is a strong predictor of all-cause mortality in patients with acute dyspnoea\textsuperscript{117}. This includes mortality due to cardiac and pulmonary disease\textsuperscript{118}. The normal adult reference ranges for sST2 are 4-31ng/ml in males and 2-21ng/ml in females\textsuperscript{119}.
sST2 is strongly correlated with measures of cardiac damage in MI such as creatinine kinase (CK)\textsuperscript{113} and troponin I (Tnl)\textsuperscript{120}. It has also been shown to have prognostic utility in patients with MI, predicting risk of death or HF; it increases across quartiles of sST2\textsuperscript{120}.

Increase in levels of sST2 over 2 weeks have been shown to be predictive of death or transplantation in patients with severe chronic HF\textsuperscript{121}. A large, multicentre study on ambulatory chronic heart failure patients showed that those with increased sST2 had a markedly increased risk of death or transplantation at 2.8-year median follow up\textsuperscript{122}.

In patients with acute dyspnoea, sST2 was the most powerful predictor of mortality at one year from a panel of 11 biomarkers including NT-proBNP and C-reactive protein\textsuperscript{123}. In a study of 346 patients with acutely decompensated
HF, sST2 was correlated with worse NYHA score, BNP and CRP. sST2 was an independent predictor of mortality\textsuperscript{124}.

1.4.4 High-Sensitivity C-reactive Protein

The role of the inflammatory markers in HF is well recognised\textsuperscript{102}. Pro-inflammatory biomarkers have been shown to be elevated in cardiac disease no matter what the aetiology. Part of this immune response is C-reactive protein (CRP), which is a ring-shaped pentameric protein released by the liver in response to IL-6 secretion by macrophages and T-cells. Its role is to bind lysophosphatidylcholine on the surface of dead and dying cells and the surface of some bacteria in order to activate the complement system via the C1Q complex\textsuperscript{125}. High sensitivity assays are being tested in CMP as biomarkers of disease severity.

In a study of 545 patients with stable congestive HF, mortality was increased in patients with CRP >3mg/L. When adjustment was made for known risk factors and biomarkers, the association remained only for those with ischaemic CMP\textsuperscript{126}. In patients with DCM, high sensitivity CRP (hsCRP) was found to be significantly higher in patients than healthy controls. Furthermore, it increased significantly with increasing NYHA and decreasing EF\textsuperscript{127}.

In a study in the hospital setting, CRP levels on discharge increased in relation to NYHA class p<0.05. Those with a CRP level >0.9mg/dL were identified as candidates for earlier readmission\textsuperscript{128}. In a Korean study, of 1608 patients with acute HF, elevated CRP and NT-proBNP predicted a worse prognosis over 1
year follow up. Patients with increased CRP and NT-proBNP had a HR of 2.4 compared to patients who did not have raised markers\textsuperscript{129}.

1.5 Neurohormonal Activation

As detailed above, neurohormonal activation of the RAAS and SNS pathways is part of the response to decreased cardiac output. The initial phase of activation of these pathways leads to the maintenance of end-organ perfusion, however, in the case of chronic CMP, these systems can be over-stimulated and cease to be useful. The markers which reflect the activation of these systems in our study show secondary effects of neurohormonal activation.

ANP is released in response to atrial stretch which occurs due to increased fluid retention and LV end-diastolic pressure (leading to increased left atrial pressure). ADM appears to work against these changes, having natriuretic, hypotensive and vasodilatory effects. Conversely, ET-1 acts with the neurohormonal response, causing vasoconstriction and positive ionotropy. Each of these markers and the evidence base for each is discussed below.

1.5.1 Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is so-named because it is produced in atrial tissue in response to atrial stretch and produces a profound natriuresis when injected into experimental animals\textsuperscript{130}. The peptide itself has a short half-life and the more stable pro-hormone, MR-proANP is often measured instead\textsuperscript{131}.
In 68 hypertensive patients with systolic dysfunction, ANP levels were significantly higher in patients with DCM than in controls and decreased with angiotensin receptor blocker (ARB) treatment\(^\text{132}\). In 48 paediatric patients with LV overload, ANP levels were measured before and 3 months after ACEi therapy. Levels were higher in patients with DCM as compared to controls and varied with NYHA class. ANP decreased significantly with treatment\(^\text{133}\).

ANP predicts poor outcome in chronic HF. In 525 patients, elevated MR-proANP levels predicted poor survival even when adjusted for BNP, age, LVEF, NYHA class, creatinine and body mass index (BMI)\(^\text{134}\).

### 1.5.2 Adrenomedullin

Adrenomedullin (ADM) is a peptide hormone with natriuretic, vasodilatory and hypotensive effects mediated by cyclic adenosine monophosphate (cAMP), nitric oxide and renal prostaglandin systems. It is expressed in many tissues including cardiovascular, renal, pulmonary, cerebrovascular, gastrointestinal and endocrine tissues. It was first isolated from human phaeochromocytoma tissue in 1993\(^\text{135}\). It acts as a circulating hormone and local autocrine and paracrine hormone. It is increased in chronic renal disease, hypertension and HF. MR-proADM correlates with levels as ADM itself is unstable\(^\text{136}\).

Immunostaining of atrial and ventricular tissues showed presence of ADM in both structures in patients with heart failure and control subjects. However, expression of ADM appeared increased in the ventricles of patients with HF\(^\text{137}\).
ADM decreases arterial tone when administered to sheep with pacing-induced CMP\textsuperscript{138}. Infusion into humans results in reduction of LV end-systolic volume (LVESV) and decreased arterial blood pressure (BP)\textsuperscript{139}. Cardiac output is maintained by a compensatory increase in heart rate. Infusion at higher doses results in increased cardiac index and reduced pulmonary capillary wedge pressure while augmenting urine volume and sodium excretion and inhibiting plasma aldosterone levels\textsuperscript{140}.

Levels of ADM are higher in humans with congestive HF than normal controls\textsuperscript{137}. Levels have been shown to be consistently higher in patients with HF, with a study of 44 patients showing levels of from 14.4 ± 2.7pg/ml in control subjects and 39.8 ± 3.6pg/ml in patients with HF (P<0.001)\textsuperscript{141}. This showed an increase with worsening HF. There was also evidence of cardiac and particularly ventricular release of the peptide on direct catheter measurement.

Of 1641 patients presenting with dyspnoea, 34.6% were found to have HF. Those who subsequently died had a significantly higher median MR-proADM 1.57nmol/L (1.02-3.21) vs. 0.84nmol/L (0.55-1.35) P<0.0001. Higher quartiles were associated with higher mortality. MR-proADM showed excellent short-term mortality prediction (outperforming BNP over 14 days)\textsuperscript{142}.

In adult ischaemic heart disease, raised ADM levels are associated with an increased risk of mortality and admission to hospital with HF over 18 months (P<0.001)\textsuperscript{143}.
1.5.3 Endothelin-1

ET-1 was characterised in 1988 and is a peptide converted from big-endothelin by the endothelin-converting enzyme family\textsuperscript{144}. There are 3 isoforms and ET-1 is generated by the heart (myocytes), kidney, central nervous system and human aortic smooth muscle cells and acts mainly via two receptor subtypes ET-A and ET-B\textsuperscript{145}. ET-1 has vasoconstrictive and ionotropic actions\textsuperscript{146}; maintaining blood pressure in normal individuals\textsuperscript{147}. It appears to have an autocrine/paracrine role rather than an endocrine action due to the concentrations found circulating.

Plasma ET-1 levels are increased in HF of all causes\textsuperscript{148,149}. Levels of ET-1 increase with increased level of clinical impairment and decreased exercise capacity\textsuperscript{150,151}. Plasma levels of ET-1 correlate inversely with LVEF, cardiac index, LV end-diastolic volume (LVEDV) and pulmonary hypertension\textsuperscript{149}. Increase in ET-1 is due to elevation of big ET-1, which occurs disproportionately in patients with heart failure\textsuperscript{152}. Higher levels of big-ET-1 are predictive of worse clinical condition, transplantation or death. Levels of big-ET-1 predict 1 year mortality better than ANP, norepinephrine, New York Heart Association (NYHA) class, age and echo parameters\textsuperscript{153}.

Because of its apparent role in the pathogenesis of HF, endothelin-receptor antagonists were trialled as a treatment for congestive HF. A randomised, placebo-controlled, double blind trial of Bosentan in chronic HF found an increased number of adverse events in the treatment arm in the first 3
months\textsuperscript{154}. Although, outcomes seemed to improve in the treated patients by 6 months, Bosentan is not currently in use as a treatment for HF in children.

### 1.6 Fibrosis – Circulating Biomarkers

Myocardial fibrosis is defined as an increase in the collagen volume fraction of the interstitial space and is a common feature in CMP of any aetiology, including DCM\textsuperscript{3, 155}. Myocardial fibrosis can be focal (replacement) or diffuse (interstitial). In paediatric patients, the prevalence of focal fibrosis is lower (as measured by late gadolinium enhancement) at 16\%\textsuperscript{156} than in adults at around 48\%\textsuperscript{157}. It is a marker of remodelling, which is often maladaptive, and is associated with increased ventricular stiffness, resulting in first diastolic and then systolic dysfunction\textsuperscript{158}. There is some evidence of reversibility with therapy\textsuperscript{159, 160}.

The major imaging biomarkers for diffuse fibrosis in this study were T1 mapping and ECV and the main circulating marker was GDF-15. GDF-15 is increased in patients with end-stage HF prior to ventricular assist device (VAD) insertion and decreases after VAD insertion\textsuperscript{161}. GDF-15 is moderately correlated with the amount of fibrosis in biopsy specimens from these patients and is considered a marker of remodelling. GDF-15 seems to enhance myocyte growth and collagen deposition by cardiac fibroblasts\textsuperscript{162}.

Increased levels of ET-1 are associated with increased fibrosis in mouse hearts\textsuperscript{173}. The physiological and pathological functions of ET-1 in adult HF/CMP are as yet unknown. BNP is higher in patients with HCM and LGE on
MRI scanning\textsuperscript{174}. It is raised in patients with severe HF and may be increased in those with fibrosis as both are markers of disease severity.

1.6.1 Multiple Biomarkers

The utility of multiple biomarkers may be to add incremental prognostic power to BNP alone. Another important role for these panels would be to provide information about the pathophysiological processes not covered by BNP, e.g. inflammatory, extracellular volume remodelling, myocyte injury and angiogenesis. The Appsala longitudinal study of adult men followed a community-based cohort of 1135 elderly men. Of a range of biomarkers tested, those with the best performance in predicting CV death were NT-proBNP, hsTnT, tissue inhibitor of metalloproteinase -1 (TIMP-1), GDF15 and \textit{IBP}-4. Their predictive power was superior to BNP alone and increased significantly and progressively with the number of biomarkers\textsuperscript{163}.

1.7 T1 mapping and Extracellular Volume

1.7.1 Fibrosis- MRI

Fibrosis is a major independent predictor of clinical outcome\textsuperscript{164}. Currently, fibrosis is measured using endomyocardial biopsy and LGE on MRI imaging. Endomyocardial biopsy is invasive and not routinely performed in children in our centre. The patchy distribution and the fact that the right ventricle is more commonly sampled also make this a problematic technique for quantifying fibrosis. However, fibrosis has been shown to be a common feature in the biopsies of patients with DCM and to correlate with LV systolic dysfunction\textsuperscript{165}.  

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Techniques have evolved more recently to non-invasively quantify fibrosis using MRI and gadolinium contrast agents. LGE exploits the extracellular nature and prolonged washout of the agent from the extracellular space\textsuperscript{166}. Gadolinium shortens T1 time and is seen as a bright area once the normal myocardium is ‘nulled’ on conventional inversion-recovery echo sequences. This technique allows visualisation of focal (replacement) fibrosis as well as any cause of increased extracellular volume (ECV). However, the nature of fibrosis in adult DCM appears more diffuse and novel, non-invasive techniques to quantify diffuse fibrosis have been developed, including T1 mapping and calculation of ECV.

\subsection*{1.7.1.1 Diagnosis}

In the adult population, CAD can lead to a similar degree of systolic dysfunction as DCM. These entities can be difficult to differentiate. In a study of 93 adults with HF, investigators were able to differentiate between CAD and DCM due to the pattern of LGE. Indeed, some patients with a putative diagnosis of DCM were reassigned to the CAD group due to the pattern LGE indicative of an ischaemic event, despite patent coronary arteries. This is thought to be due to a process of recanalisation after the event\textsuperscript{167}.

\subsection*{1.7.1.2 Prognosis}

The pattern and frequency of fibrosis differs in adults and children with DCM, with up to 35\% of adults reported to have mid-wall fibrosis. This is associated with a higher rate of the combined end-points of all cause death and hospitalisation for a cardiovascular event with HR 3.4 (P=0.01). Mid-wall
fibrosis remains the sole predictor of death or hospitalisation in multivariate analysis in this study of 101 patients. In another study of 184 patients with DCM, LGE was found in 39% and was associated with a higher LV mass, lower LVEF and higher LVEDV (all significant). Those with LGE were more likely to suffer an adverse outcome.

1.7.2 T1 Mapping

The T1 time is the longitudinal relaxation time constant that is unique for each tissue type and increases with increased interstitial space. The measurement of the T1 time requires measuring the longitudinal relaxation time after it has been disturbed from its equilibrium state by a radiofrequency pulse (other methods can also be used, such as saturation pulses). The equilibrium magnetisation is inverted using radiofrequency pulses at certain intervals and T1 is allowed to return to normal in between these experiments. In general, several images are acquired with different T1 weightings and the signal intensities are then fit to the equation for T1 relaxation.

The sequence used in this study was the Modified Look-Locker Inversion Recovery (MOLLI) introduced by Messroghli et al. in 2004. Images were acquired in diastole for 3 or 5 R-R intervals after the inversion pulse. Multiple inversions with slightly different T1s were used to more thoroughly sample the T1 relaxation curve. The specific timing of the sequence is denoted as 3(3)3(3)5, meaning 3 images were acquired after the first inversion, 3 after the second and 5 after the final inversion. This resulted in a map of the myocardium where the value of each pixel represented the T1 of each voxel.
There have since been many modifications to this sequence including techniques to introduce motion correction\textsuperscript{171}, and shorten breath-hold times\textsuperscript{172}.

The timing of radiofrequency pulses is important, especially in paediatric patients with higher heart rates, as T1 times can be inaccurate if complete relaxation is not allowed before the next inversion pulse\textsuperscript{173}. Therefore, different sequence timings have been tried including 5(3)3 for higher heart rates, which would more fully allow relaxation between sampling. Unfortunately, we did not have access to these protocols during the study. T1 mapping is also limited by changes in gadolinium-clearance time, time of sampling, injected gadolinium dose, body composition, haematocrit and other disease processes\textsuperscript{174}.

As the extracellular space of the myocardium increases, so the $T_{1\text{native}}$ and calculated ECV increase. Gadolinium contrast enhances this distinction by remaining in the extracellular space and further shortening T1. It should be understood that T1 times increase with any cause of increased ECV including fibrosis, oedema and inflammation. However, in the case of DCM, where fibrosis is an integral part of the remodelling process, this is the most likely cause of increased ECV. Importantly, ECV has been shown to correlate with fibrosis in studies using endomyocardial biopsy (the current gold-standard for ECV quantification)\textsuperscript{175}.

Both $T_{1\text{native}}$ and calculated ECV have been measured in various disease states. In DCM and HCM, $T_{1\text{native}}$ times are higher in diseased patients than controls, suggesting an increase in fibrosis in these populations\textsuperscript{176}. However,
there remains overlap with normal values, decreasing the utility of this as a diagnostic test.

ECV is derived from the $T1_{\text{native}}$ and post-contrast blood and myocardial $T1$ times and haematocrit using the following formula:

$$ECV = (1 - \text{Hct}) \times \frac{1}{(\text{postcontrast } T1_{\text{myocardium}} - T1_{\text{native myocardium}})} \times \frac{1}{(\text{postcontrast } T1_{\text{blood}} - T1_{\text{native blood}})}$$

The gadolinium must have reached equilibrium in the blood pool and myocardium for the ECV to be accurately measured and the post-contrast $T1$ is therefore measured 15 minutes after injection, a technique which has been shown to have good histological correlation with ECV$^{177}$. ECV is a ratio and may be less prone to sources of systematic bias such as renal function, percentage body fat, gadolinium characteristics (i.e. dose, concentration and water exchange rate)$^{178}$ as these tend to cancel each other out in the calculation.

We decided to measure $T1$ in one mid-ventricular short-axis slice, mainly due to the time taken for acquisition and the difficulties our patients had with multiple breath-holds. Motion artefacts can also pose a problem especially in the infero-postero-lateral region$^{179}$.

**1.7.3 ECV and Heart Failure**

793 patients referred for MRI with various pathologies (not HCM or amyloidosis), had ECV measured and compared to volunteers. ECV in
patients was significantly higher than in controls (controls: 21.7-26.2% vs. patients: 21-45.8%). There were 39 deaths over 0.8 years, and 43 patients reached the composite end-point of death/cardiac transplant/ventricular assist device (VAD) insertion. On Cox-regression, ECV was related to all-cause mortality and the composite end-point with HR 1.55 and 1.27 for every 3% increase in ECV adjusted for age, LVEF and MI size\textsuperscript{180}.

ECV was higher in patients with diabetes compared to controls in another large, population-based study (n=1176). ECV was 30.2% in diabetes patients and 28.1% in controls P<0.001. This association remained when adjusted for demographics, comorbidities and medications (P<0.001). Over 1.3 years, ECV independently predicted hospital admission with HF and death with HR 1.52 per 3% increase\textsuperscript{181}.

In DCM, a large study of 637 adults showed a significant correlation between T1\textsubscript{native}, ECV and presence and extent of LGE were predictive of all-cause mortality and a combined HF end-point (cardiovascular mortality and hospitalisation)\textsuperscript{182}. 


2 Methods

2.1 Overview

The patients included in this study were recruited as part of the MD-Paedigree project from Great Ormond Street Hospital (GOSH), London, UK from February 2015-December 2017. All were diagnosed with CMP and those included in the study had a dilated phenotype.

Control subjects were adults recruited as healthy controls for a project on coarctation (n=7), who had MRI with T1 mapping, see Figure 4. 7 paediatric neuro-oncology patients had MRI and biomarker levels measured, however, they were excluded from the analysis of the T1 mapping results below.
There were varying amounts of data available for each group. Some patients with DCM had either claustrophobia or needle phobia which excluded the possibility of MRI scanning or blood biomarker analysis and contrast administration respectively. Children with claustrophobia were able to give partial consent for blood biomarkers only.

Children having MRI had blood biomarkers done only if they needed routine blood tests in clinic (to monitor renal function, for example). Those who did not require a blood test were not administered gadolinium and did not have blood

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4 Biomarker (BM) numbers are based on the maximum number of patients with biomarker levels measured as these varied by marker. 14 control subjects include 7 adults (included in analysis) and 7 children (excluded from analysis).
biomarkers tested. Similar considerations resulted in varying completion of datasets in the SOCRATES study.

Some patients (who attended clinic on the same day) had insufficient blood taken for the study and samples were instead used for clinical blood tests. NT-proBNP is used clinically in our centre, so there were higher levels of this marker tested during the study.

Neuro-oncology patients all had MRI scans and blood biomarkers measured. Healthy subjects for the coarctation study had not been consented specifically for biomarker measurement and had only T1 mapping and ECV calculation done. The MD-Paedigree study incorporated only paediatric patients and funding was otherwise unavailable.
2.1.1 Study design

This was a cross-sectional, observational study. We recruited two groups of people, one with the disease (CMP) and a group of control patients without cardiac disease. The control group included both children and young adults.

Control subjects included neuro-oncology paediatric patients and healthy adults for the T1 mapping study, obese and non-obese patients from the SOCRATES study.

2.1.2 Primary Endpoint

The combined primary end-point for the study was listing for transplant, insertion of VAD device, admission to hospital for ionotropic support, transplantation or death. Secondary end-points included worsening systolic function, clinical condition or cardiac surgery of any kind (including mitral valve repair).

2.1.3 Study Population

Patients were recruited from the heart function clinic at Great Ormond Street Hospital. Children who required an MRI scan with gadolinium contrast for non-cardiac imaging were recruited from the neurology and neurosurgery outpatient clinics. A group of adult control subjects had only T1 mapping performed as part of a study into coarctation. Some patients were recruited as healthy controls and had biomarkers measured alongside an MRI or alone (see Figure 4).
### 2.1.4 Inclusion Criteria for CMP Patients

The study cohort included children and adolescents (age 0-18 years old) of both genders with an established diagnosis of acute or chronic CMP with a dilated phenotype and the following:

- Presence of biventricular anatomy
- LV EF <50% and/or FS <25%, diagnosed by echocardiogram at some point and/or
- Increased LVEDD >2 standard deviations from the expected normal limit either currently or previously
- Written, informed consent provided

### 2.1.5 Inclusion Criteria for Control Subjects

- Normal hearts\(^5\)
- Children booked for non-cardiac MRI (neurological) with gadolinium contrast or healthy adult volunteers
- Able to cooperate with MRI scan instructions
- Written, informed consent provided

### 2.1.6 Exclusion Criteria

- Systemic hypertension (>95th percentile for age and height)
- Persistent high rate supraventricular arrhythmias

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\(^5\) No previous cardiac disease in past medical history, normal medical examination and normal structure and function on MRI.
• Pericardial disease (including restrictive and constrictive pericarditis),
• Univentricular heart
• Cor pulmonale
• Heart transplantation (at onset of study)
• Contraindication to MRI scanning
• Severe renal dysfunction
• Congenital heart disease

2.1.7 Study Protocol

Patients were recruited from the heart function clinic. The date of the study MRI and blood test was on the same day as the routine clinic visit for patient convenience. Patients were identified from the clinic list and recruited if they were over the minimum age limit and willing to participate in the study. Parents were called a week prior to the study and then again, the day before to be given information about the study. They were consented in person during the day. Consent materials were emailed to families if requested or posted.

Patients had clinical evaluation, measurement of blood biomarkers and genetic analysis on their first visit. They also had echocardiographic assessment and MRI scanning performed. Laboratory testing of blood took place at Great Ormond Street Hospital, with blood samples sent on to Ospedale Pediatrico Bambino Gesù, Rome for Genetic testing and to the Medical University of Vienna, Austria, for biomarker analysis.
2.1.8 Ethics

The study was approved by the West London & GTAC Ethics Committee (see Appendix-1 section 6). All patients had normal renal function, and the use of gadolinium contrast agent was considered to be of low risk. Those with oncological diagnoses did have higher creatinine levels than control subjects, but there was a clinical necessity for the contrast and the risk was not above that accepted in our department. Written, informed consent was obtained from parents of all individual participants included in the study and from patients themselves in the case of adults.

2.1.9 Biomarkers

The following methods are quoted from a published paper59: “Blood was collected using standard collection techniques on the day of assessment. Plasma and serum samples were spun and frozen on the day of collection and stored at −80°C for batch-analysis. Soluble ST2 (sST2; Presage® ST2 Assay, Critical Diagnostics, San Diego, CA, USA) and Growth Differentiating Factor 15 (GDF-15; Human GDF-15 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA) were measured in patient sera using a specific enzyme-linked immunosorbent assays (ELISA). The measurement range was 3.125 to 200 ng/mL for the Presage® ST2 Assay (based on a 50-fold dilution of patient samples) and 23.40 to 1,500 pg/mL for the Human GDF-15 Quantikine ELISA Kit (based on a 4-fold dilution).

An automated immunofluorescent assay (KRYPTOR® System, BRAHMS AG, Hennigsdorf/Berlin, Germany) was used to determine levels of mid-regional...
pro-Adrenomedullin (MR-proADM) and C-terminal pro-Endothelin-1 (CT-proET-1), both processed from EDTA-plasma, as well as mid-regional pro-Atrial Natriuretic Peptide (MR-proANP), processed from serum. Measurement ranges were: 2.1 to 10,000.0 pmol/L for MR-proANP; 0.05 to 100 nmol/L for MR-proADM and 3 to 5,000 pmol/L for CT-proET-1 (based on automated dilution).

NT-proBNP was assessed using an Elecsys® immunoassay on a Cobas 8000 system (Roche Diagnostics, Mannheim, Germany)." HsTnI was measured on an Abbott Architect analyser using plasma (EDTA) by immunoassay. The hsCRP was measured on an Ortho Clinical Diagnostics Vitros 5600 analyser using an immunoturbidimetric method on patient serum. Laboratory measurements were performed by investigators who were blinded to patient history.

Patients included in the biomarker part of the study were those with CMP and healthy paediatric control subjects. All adult controls were excluded for the whole analysis. For the majority of the analysis, those with DMD were excluded. Patients and control subjects who were obese were also excluded. There was a separate sub-analysis in this chapter on those with LVEF on MRI of less than or equal to 40% (see flowchart, Figure 5).
2.1.10 Echocardiography

Transthoracic echocardiography was performed using a Philips IE33 echocardiography system (Philips Healthcare, Best, Netherlands). Conventional systolic functional parameters were measured. These included: EF and FS measured in the parasternal long axis view.

Trans-mitral inflow velocities were acquired using pulsed-wave Doppler with the sample volume placed at the tip of the mitral valve leaflets in the apical 4-

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6 Biomarker (BM) numbers are based on the maximum number of patients with biomarker levels measured as these varied by marker.
chamber view. The peak E-wave velocity (cm/s), peak A-wave velocity (cm/s), and ratio of E-wave to A-wave (E/A) velocities were recorded.

Tissue Doppler Imaging myocardial velocities were measured using a standard pulsed-wave Doppler technique. Images were acquired over two consecutive cardiac cycles using low-velocity, high-intensity myocardial signals at a high frame rate (>120 MHz). The sample volume was placed at the junction of the LV wall with the mitral annulus at the septal and lateral myocardial segments in the apical 4-chamber view. Peak E’ velocities (cm/s) were measured on-line and corresponding E/e’ ratios were calculated. Diastolic dysfunction was measured using E/e’ ratio and defined as E/e’ ratio >15.

2.1.11 MRI Image Acquisition

All subjects in the study were awake and cooperative during the MRI. No sedation or anaesthetic was administered. MRI imaging was performed on a 1.5-T MR Scanner (Avanto, Siemens, Erlangen, Germany). A 12-element phased-array coil was used for signal reception and a vectorcardiogram system was used for cardiac gating.

Ventricular volumes were assessed using a real-time radial k-t SENSE sequence (field of view [FOV]: 380 mm, matrix: 128x128, voxel size: 3.0x3.0x10 mm, TE/TR: 1.14/2.3 msec, flip angle: 38, pixel bandwidth [BW]: 1500 Hz/pixel, radial spokes: 128, k-t SENSE acceleration factor: 8, scan time: 1.5 seconds per slice, temporal resolution: 35.5 msec). 11 to 13 contiguous
slices were acquired in the short axis to ensure coverage of the ventricle. This sequence was used to allow image acquisition during free-breathing.

2.1.12 Aortic and Pulmonary Flow

Through-plane pulmonary artery and aortic valve flow data were acquired using a spiral triggered flow sequence during breath-hold\textsuperscript{183}.

2.1.13 T1 Mapping

Myocardial T1 mapping was performed using a modified Look-Locker Inversion Recovery (MOLLI) sequence\textsuperscript{170}. Three successive experiments were performed with 3, 3 and 5 readouts respectively; pauses of 3 R-R intervals were allowed between experiments to allow for T1 recovery (standard notation 3(3)3(3)5). Images were captured in the standard mid-cavity short axis views in diastole, both pre-contrast (T1\textsubscript{native}) and 15 minutes post-contrast injection. Scan parameters for the MOLLI protocol were: FOV, 8mm slice thickness, flip angle 30, T1 100ms. This sequence was repeated exactly 15 minutes after gadolinium administration to create a post-contrast T1 map.

Gadopentetate dimeglumine (gadolinium or Gd-DTPA; Magnevist; Schering, Berlin, Germany) was administered at a dose of 0.15 mmol per 1 kg body weight at an injection rate of 2ml/s followed by a 10ml saline flush. Late gadolinium-enhanced images were acquired 5 minutes after intravenous injection of contrast. T1 was calculated to null the myocardium.
Haematocrit (Hct.) was measured at the time of MRI, by taking a blood sample at the time of peripheral intravenous access. Where measured Hct. was unavailable, synthetic Hct. was calculated using the following formula as per the method described by Triebel et al.\textsuperscript{184}:

$$\text{Hct.} = 0.88 - \left( \frac{T_{1\text{blood}}}{3240} \right)^{2.1}$$

2.1.14 Cardiac Magnetic Resonance Image Analysis

All images were processed using in-house plug-ins for the open-source DICOM software OsiriX (OsiriX Foundation, Geneva, Switzerland)\textsuperscript{185}. Off-line analysis was performed on departmental desktop computers or laptops. For quality control, MRI data were reviewed by a consultant in cardiovascular MRI (AT) who co-reported all clinical scans.

2.1.15 Cardiac Volumes and Function

Cardiac MRI scans were analysed and reported in the same way as any clinical scan. These data were scanned into the clinical record of the patients involved in the study. MRI data were stored anonymised in the system to allow blinded clinical analysis.

The biventricular EDV and end-systolic volume ESV were measured by manual segmentation of the short axis cine images. The endocardial borders were traced at end-diastole and end-systole, excluding trabeculations and papillary muscles from the blood pool (see Figure 6).
EDV and ESV were calculated for each ventricle using Simpson’s rule. Ventricular SV was calculated as the difference between the EDV and ESV, and ventricular EF (%) as (SV/EDV) ×100. All volume measurements were indexed for the body surface area and expressed in mL/m². Internal validation of the ventricular SV data was attained by quantifying aortic and pulmonary valve forward flow volume, using a semi-automatic vessel edge-detection algorithm with operator correction. The late-enhancement images were independently reviewed by 2 cardiologists (DP, AT) in a blinded fashion.

**Figure 6:** Region of interest created by tracing endocardial and epicardial LV border, excluding trabeculations

2.1.16 Aortic Flow

Aortic flow was measured by using phase contrast and cine images of aortic flow. Images were viewed side-by-side on the image viewer. The inner edge of the aorta was traced using the ‘closed polygon’ tool on OsiriX to create a region of interest (ROI) encompassing the lumen of the aorta (see Figure 7). This was propagated throughout the images including the entire cardiac cycle.
Manual correction of the automatic propagation was performed for accuracy. The OsiriX plug-in was then used to measure aortic flow using the phase contrast images.

**Figure 7:** 2D view of aortic cine and phase contrast images, with aortic lumen outlined.

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**2.2 T1 mapping and Extracellular Volume**

Pre-and post- contrast T1 maps were generated using a mono-exponential three-parameter fit. All maps were analysed with OsiriX (V 6.5.2 64 bit Pixmeo SARL, OsiriX Foundation, Geneva, Switzerland). Myocardial T1 values were determined by drawing regions of interest in every segment of the mid-ventricular slice according to the AHA 17 segment model (see Figure 8). The global T1 was calculated as an average of these, minus any areas including late gadolinium enhancement. T1 values for blood were obtained by drawing a region of interest in the blood pool. ECV values were calculated using the standard formula.
2.2.1 Study Population - T1 mapping

Of the study population as a whole, those included in the T1 mapping study included 55 patients with CMP, excluding those with obesity and DMD. Of these, 29 patients had T1 mapping and 26 had ECV calculated. Control subjects excluded children with oncology disease and therefore included only 7 healthy controls who were adults. The reason for excluding the paediatric controls was that their ECVs appeared much higher than expected for healthy individuals and their disease process may have affected these results.

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7 LV mid-ventricular short axis slice – segments 7-12 starting at 12 o’clock and moving anti-clockwise.
2.2.2 Statistical Analysis

Data are presented as mean ± standard deviation (SD) or median (interquartile range) as appropriate. Categorical variables are reported as number and percentage. Baseline demographic, MRI and echo parameters were compared between DCM patients and controls. A 2-sided t-test was used for normally distributed variables. Kolmogorov-Smirnov or Mann-Whitney-U tests were used for skewed data. Fisher’s exact test is used for categorical variables. A P value of less than 0.05 was considered significant, except in the case of multiple comparisons, where a Bonferroni correction was applied, in this case the P value (0.05) was divided by the number of comparisons being made.

Receiver operating characteristic (ROC) curves were constructed for binary outcome variables. Results were reported as area under the curve (AUC) along with P values, cut offs with sensitivity and specificity where appropriate. Linear regressions were performed in a forward stepwise fashion and compared by adjusted $R^2$ to previous models. Kaplan Meier and Cox-proportional Hazard models were created.

All statistical analysis is performed using IBM© SPSS© Statistics Version 24.

2.2.3 Sample Size Calculation

Sample sizes were calculated for the MRI section of the study but this proved impossible for the biomarker study as no previous data existed.
Based on a study performed at our institution on T1 measurements on patients following the Senning procedure\textsuperscript{187}, the sample size was calculated as follows:

Group A (patients): ECV 25\% ± 3.6, Group B (controls): ECV 23\% ± 3.2

Mean standard deviation: 3.4, Sampling ratio: 1

Power: 0.8, Type 1 error: 5\

Sample size: 46

Sample sizes were not calculated for biomarkers as no paediatric data were available on which to base assumptions.
2.3 Phantom Experiments

In order to assess the accuracy of the T1 mapping protocol at different heart rates, scans were done on the same MRI scanner as the study at 1.5T using the T1MES (T1 Mapping and ECV Standardisation) phantom manufactured by Resonance Health, Australia. Myocardial T1 mapping was performed using a modified Look-Locker Inversion Recovery (MOLLI) sequence with the same scan parameters as those used for patients and control subjects. Changes in heart rate only affected the value of T1 (ms) above 85bpm. Mean heart rate was 85.2 ± 17bpm for our cohort and 28.6% of patients had a mean heart rate above 85bpm. This should be borne in mind when interpreting data from our study and paediatric data (as children have higher heart rates than adults) in general.

2.3.1 Genetics

As part of the MD-Paedigree project, some patients were tested for a panel of genes\(^8\). I collected the blood samples for this analysis and helped to place the results in clinical context. All genetics testing was performed in Bambino Gesù Hospital, Rome. Coding exons and untranslated regions of 56 genes associated with inherited CMP were analysed using Next Generation Sequencing (NGS).

\(^8\) PLN, DES, LMNA, MYBPC3, MYH7, TNNT2, TNNT3, TPM1, TNNC1, MYH6, VCL, TAZ, LDB3/ZASP, SCN5A, PSEN1, PSEN2, SGCD, ACTC1, ABCC9, DMD, ANKRD1, NEXN, CSRP3, TCAP, ACTN2, PKRAG2, MYOZ2, MYL2, MYL3, CACNA2D1, LAMP2, CASQ2, PKP2, DSP, DSG2, DSC2, JUP, TGFB3, CTNNA3, TMEM43, TTN, RYR2, KCNQ1, KCNH2, CAV3, ANKB/ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, GPD1L, CACNB2, SCN1B, KCNE3, SCN3B, KCND3.
The genetics results in this study are very difficult to interpret. For ethical reasons, we were unable to test anyone other than probands recruited to the study. Therefore, due in part to the complicated nature of genetic abnormalities in DCM and the unknown pathogenicity of genes in this disease, very few conclusions can be drawn from the study. Many of the patients had multiple variations in the same gene, increasing the likelihood of benign polymorphisms, TTN being the most common gene for this.

There were some interesting findings including TNNT2 gene mutation in patient 5, one of the few to have a transplant during the study and TMEM43 in siblings with a family history of sudden death. As with many long QT syndromes, the QT interval was normal in the patients with associated genetic defects associated with this problem. However, this does not rule out a risk of long QT syndrome and further investigation and monitoring is required. It is safe to say the genetic analysis created more questions than answers.
2.3.2 Ethics and Consent

The ethics protocol and funding application was initially done by myself with support from Professor Burch and the project was authorised in 2013. The original project involved T1 mapping and measuring novel blood biomarkers in paediatric patients with DCM. During this time, I applied for a research fellowship which allowed for the project to continue with EU funding as part of the MD-Paedigree project. I designed the protocol and consent forms which were used in the final project. While I was on maternity leave, the protocol was changed and ethics were re-applied for to include follow up and a wider range of patients (see Appendix 1, section 6).

The main ethical issues faced by the project were administration of contrast in children without heart disease, taking blood samples in control patients and those with cardiovascular disease and making the consent process child-friendly. Gadolinium is routinely used in clinical practice and we have not had any adverse events recorded in study subjects or patients with cardiovascular disease to date. Therefore, it was the decision of the ethics committee that administration of contrast to patients with no contraindications was allowed for the study. The use of contrast was necessary for the calculation of ECV.

The calculation of ECV in control patients posed another problem. We needed to identify patients without cardiovascular disease but with a need for gadolinium contrast. The children also needed to be old enough to follow instructions during a scan and to have a slightly longer scan than normal. On discussion with the radiographers, it transpired that many children having post-
resection surveillance for brain tumours required contrast. I contacted the
neurology and neurosurgical teams who were very happy for their patients to
take part. Hence, some of these patients were recruited as controls for ECV
measurement. Some healthy adult volunteers who were being scanned as part
of a study into coarctation of the aorta were also used as normal controls for
our study. The use of adults was due to the ability to administer gadolinium
contrast to healthy individuals, which was considered unethical in children.
Using contrast is the only way to measure ECV (as opposed to $T_1^{\text{native}}$, which
is a pre-contrast measure).

Blood samples were taken during insertion of the peripheral cannula for
gadolinium administration. This minimised discomfort for the patients; routine
blood tests for clinic were also taken at this time. Most MRI scans were done
in the morning of the clinic appointment to avoid disruption of the children’s
routines and/or inconvenience to the parents.

Parents were contacted prior to the clinic, usually one week prior, to allow
them to discuss the process with their child. They were contacted once more
to confirm their participation and consent was taken formally on the day of the
MRI. Children with claustrophobia were excluded from the study.

The decision to make MRI results available to clinical staff was due to ethical
considerations. As T1 mapping is not routinely measured, this information was
not disclosed during the study and did not confound the results.
Written consent was taken by myself DP or JH (during my absence). Consent was obtained from parents of children too young to consent themselves. Those with competence were invited to consent for themselves. There was also the opportunity for young children to sign an assent form to allow them to take part in the consent process and make their views known. All studies had ethical approval granted prior to the commencement of recruitment (see Appendices).
3 Results - Biomarkers

3.1 Demographics

Patient demographics for the 51 patients with CMP and 57 control subjects recruited for the study are presented in Table 1.

Controls were significantly older with a greater BSA than CMP patients (see Table 1). Differences in gender, systolic blood pressure (SBP), haematocrit and creatinine were not significantly different between groups.

Most patients with CMP had stable disease, with the majority in NYHA class I (76%) and Ross class I (94%). Patients with DMD were excluded from this analysis (n=21).

Most patients, (43%), had idiopathic DCM. Patients with familial CMP had a family history of CMP in a parent or sibling. The ‘other’ group consisted of the following aetiologies: Holt Oram Syndrome, thyroid hormone resistance, vitamin D deficiency, ischaemic CMP secondary to Takayasu vasculitis, ischaemic CMP of unknown aetiology, unknown neuromuscular condition, phaeochromocytoma (post-operative), mixed type hypertrophic and dilated CMP of unknown aetiology. The majority of patients were on an ACE-inhibitor (77%).
3.1.1 Biomarker Levels Between Groups

Of the patients detailed in the whole study (CMP=76, control=88), there were significant differences in biomarker levels between patients with CMP (of all aetiologies) and control subject, see Table 1). NT-proBNP, MR-proANP, GDF-15, sST2 and hsCRP were significantly higher in patients with CMP than control subjects. Patients with DMD and obesity were included in this analysis.

Table 1: Differences in levels of biomarkers between CMP patients and control subjects. \(P<0.05\).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CMP (n)</th>
<th>Median (IQR)</th>
<th>Control (n)</th>
<th>Median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (pg/ml)</td>
<td>47</td>
<td>135.0 (61-537)</td>
<td>7</td>
<td>40.0 (36-46)</td>
<td>0.007</td>
</tr>
<tr>
<td>MR-proANP (nmol/l)</td>
<td>38</td>
<td>48.8 (27-93)</td>
<td>25</td>
<td>37.3 (26-47)</td>
<td>0.05</td>
</tr>
<tr>
<td>GDF15 (ng/ml)</td>
<td>38</td>
<td>290.7 (221-487)</td>
<td>25</td>
<td>221.4 (193-315)</td>
<td>0.006</td>
</tr>
<tr>
<td>sST2 (pg/ml)</td>
<td>38</td>
<td>19.4 (15-30)</td>
<td>25</td>
<td>16.4 (11-23)</td>
<td>0.04</td>
</tr>
<tr>
<td>MR-proADM (pmol/l)</td>
<td>38</td>
<td>0.34 (0.29-0.42)</td>
<td>25</td>
<td>0.36 (0.33-0.40)</td>
<td>0.41</td>
</tr>
<tr>
<td>CT-proET1 (pmol/l)</td>
<td>36</td>
<td>41.3 (30-50)</td>
<td>25</td>
<td>38.0 (34-44)</td>
<td>0.07</td>
</tr>
<tr>
<td>HsCRP (mg/l)</td>
<td>24</td>
<td>0.28 (0.02-1.1)</td>
<td>56</td>
<td>0.7 (0.2-1.8)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Of those described in

Table 2, i.e. with patients with DMD and those with obesity excluded (CMP=51, Control=54), only GDF-15 differed significantly between groups (CMP 281.4 (220-558ng/ml) vs. control 211.0 (187-315ng/ml), \(P=0.01\).
3.2 MRI

CMP patients had significantly lower LVEF and a higher LVEDVi compared to controls (see Table 2). LV function and diagnosis were linked in that those patients with familial DCM (61 ± 9%) had a significantly higher LVEF than those of other diagnoses. Mean (+SD) of each aetiology is as follows; iDCM 42 ± 12%, familial 61 ± 9%, myocarditis 43 ± 13%, Anthracycline 48 ± 9%, Other 48 ± 15%, controls 63 ± 5%.

For this reason, patients with LVEF =/=<40% on MRI (n=14) were included in a separate sub-analysis. On multiple linear regression, only control and familial patients remained significantly associated with MRI LVEF on stepwise analysis using changes in adjusted $R^2$ to assess significance.

Table 2: Demographics of study participants\(^9\).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CMP patients (n=51)</th>
<th>Controls (n=54)</th>
<th>Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.5 ± 4</td>
<td>16.1 ± 2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>17 (33%)</td>
<td>24 (44%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Overweight</td>
<td>3 (6%)</td>
<td>12 (22%)</td>
<td>0.02</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.1 ± 0.4</td>
<td>1.7 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>104.0 ± 13</td>
<td>109 ± 10</td>
<td>0.48</td>
</tr>
</tbody>
</table>

\(^9\) Figures are given as mean ± standard deviation. Or median and IQR for non-normally distributed variables. P-Values are calculated using T-test for normal data and Mann-Whitney U test for non-normal data. Patients with obesity and those with DMD have been excluded. Ethnic origin was not recorded in all control subjects. P<0.05.
<table>
<thead>
<tr>
<th></th>
<th>(I:0-5/II:6-10/III:16-20)</th>
<th>(54/0/0)</th>
<th>0.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA class (I/II/III/IV)</td>
<td>(39/8/3/1)</td>
<td>(54/0/0/0)</td>
<td>0.84</td>
</tr>
<tr>
<td>Duration of illness (months)</td>
<td>61.4 ± 68</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.37 ± 0.04</td>
<td>0.39 ± 0.02</td>
<td>0.28</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>57.4 ± 84</td>
<td>53.0 ± 12</td>
<td>0.93</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>131.0 (56-569)</td>
<td>43.0 (35-74)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Aetiology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>22 (43%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Familial</td>
<td>13 (26%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Post-myocarditis</td>
<td>4 (8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anthracycline</td>
<td>4 (8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>8 (16%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>54 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>12 (24%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II converting enzyme inhibitor</td>
<td>39 (77%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mineralocorticoid receptor antagonist</td>
<td>17 (33%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>26 (51%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>13 (26%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>11 (22%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnic Origin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>28 (55%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>9 (18%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>5 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>3 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6 (12%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>48.9 ± 14</td>
<td>63.3 ± 6</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>LVEDVi (ml/m²)</td>
<td>91.5 (79-100)</td>
<td>71.5 (67-73)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>LVESVi (ml/m²)</td>
<td>38.5 (32-60)</td>
<td>25.5 (22-31)</td>
<td><strong>0.006</strong></td>
</tr>
</tbody>
</table>

### 3.3 Biomarker levels:

On ROC analysis, markers that distinguished between children with normal vs. low LVEF (=/≤40%), were GDF-15 (cut off 184.5ng/ml, sensitivity 87.5%, specificity 96.4%, P=0.03), MR-proANP (cut off 18.9nmol/l, sensitivity 87.5%, specificity 96.4%, P=0.004), sST2 (cut off 13.3pg/ml, sensitivity 87.5%, specificity 78.9%, P=0.01), see Figure 9.

**Figure 9**: ROC curve of biomarkers distinguishing between normal and low LVEF (=/≤40%)
3.4 Aetiology

76 patients included are included in the following section, with aetiologies as follows; 23 iDCM, 14 familial DCM, 4 myocarditis, 5 anthracycline, 21 DMD, 9 other and 54 controls (all obese controls were removed). Obese patients and those with DMD were included in this section.

Most biomarker levels were significantly different between patients of different aetiologies of CMP (Kruskall-Wallis test NT-proBNP P=0.012; GDF-15 P=0.002; MR-proANP P=0.006; CT-proET1, P=0.014; hsTnI, P=0.007).

Patients with iDCM had the highest levels of NT-proBNP, GDF-15, ET-1 and sST2. Patients with anthracycline toxicity had especially high levels of MR-proADM, CT-proET1 and hsCRP.

NT-proBNP had the highest Z-scores in general and those for iDCM were highest (147.2, S.D. 2.1-56). The difference was statistically significant between aetiologies by the Kruskall Wallis test (P=0.004). Other biomarkers with significant differences between aetiologies included sST2 (P=0.009), for which Z scores were highest in iDCM (0.6, S.D -0.01-1.1), MR-proANP (P=0.009), also highest in iDCM (2.3, S.D. 0.6-6) and GDF-15 (P=0.02), highest in iDCM (1, S.D 0.02-2.65).

HsTnI was markedly elevated in patients with DMD. Z-scores for TnI were calculated using published values in adults as no controls had TnI measured in our study (see Figure 10).
Figure 10: Levels of biomarkers by aetiologies\textsuperscript{10}.

3.4.1 MRI Parameters and Biomarker Levels:

The patients included in the following analysis numbered 51 altogether, 23 with iDCM, 14 familial, 4 myocarditis, 5 anthracycline and 9 other. Patients with DMD were excluded. Numbers of patients who had both MRI and BM levels were different for each marker and are detailed below. NT-proBNP, hsTnI, hsCRP and MR-proANP all correlated negatively and significantly with

\textsuperscript{10} Plotted levels are Z scores calculated from control samples from this study and published data in the case of TnI\textsuperscript{244}. NT-proBNP is excluded from this graph for clarity of results.
MRI LVEF (see Figures 11-14). NT-proBNP remained significantly correlated with MRI LVEF when corrected for multiple comparisons.

**Figure 11:** NT-proBNP correlated with MRI LVEF. $\rho = -0.79$, $P < 0.0001$. (n=39)

![Log NT-proBNP vs MRI LVEF](image1)

**Figure 12:** MR-proANP correlated with MRI LVEF. $\rho = -0.57$, $P = 0.001$. (n = 32)

![Log MR-proANP vs MRI LVEF](image2)
Figure 13: HsCRP correlated with MRI LVEF. $\rho=-0.67$, $P=0.005$. (n=16)

Figure 14: HsTnI correlated with MRI LVEF. $\rho=-0.63$, $P=0.006$. (n=17)

3.4.2 Creatinine Levels

The level of creatinine was measured in a subgroup of patients and controls. The highest levels were found in patients with Anthracycline toxicity.
(anthracycline- n=5 median 68 (47-341 $\mu$mol/L) vs. all other aetiologies 42 (36-49 $\mu$mol/L) P=0.006); this difference was mainly caused by one extreme outlier (subject 24).

### 3.5 Low Left Ventricular Ejection Fraction

Of all patients, those included in the following analysis were those without DMD and with LVEF on MRI of less than or equal to 40%. This left 15 patients, of whom 5-11 had biomarkers measured on one visit (see Table 3). Control subjects initially numbered 81, however, after obese control subjects were removed from the analysis, 57 remained. Between 7 and 37 had biomarkers measured in this group. 8 patients (53%) had iDCM, 1 had familial CMP, 1 had myocarditis and 1 had anthracycline toxicity.

#### 3.5.1 Difference in Biomarker Levels Between Groups

Table 3: Difference between biomarker levels in CMP patients with low LVEF and control subjects. $P<0.05$

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CMP (n)</th>
<th>Median (IQR)</th>
<th>Control (n)</th>
<th>Median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (pg/ml)</td>
<td>11</td>
<td>1268 (426-2659)</td>
<td>7</td>
<td>40.0 (36-46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MR-proANP (nmol/l)</td>
<td>8</td>
<td>97.2 (70-150)</td>
<td>20</td>
<td>37.0 (25-51)</td>
<td>0.001</td>
</tr>
<tr>
<td>GDF15 (ng/ml)</td>
<td>8</td>
<td>436.0 (335-644)</td>
<td>20</td>
<td>219.4 (191-319)</td>
<td>0.007</td>
</tr>
<tr>
<td>sST2 (pg/ml)</td>
<td>8</td>
<td>30.9 (19-37)</td>
<td>20</td>
<td>14.3 (11-22)</td>
<td>0.01</td>
</tr>
<tr>
<td>MR-proADM (pmol/l)</td>
<td>8</td>
<td>0.37 (0.3-0.5)</td>
<td>20</td>
<td>0.36 (0.3-0.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>CT-proET1 (pmol/l)</td>
<td>8</td>
<td>39.9 (29-57)</td>
<td>20</td>
<td>36.2 (31-44)</td>
<td>0.56</td>
</tr>
<tr>
<td>HsCRP (mg/l)</td>
<td>5</td>
<td>1.5 (0.3-19.3)</td>
<td>37</td>
<td>0.39 (0.2-1.0)</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Within the group of patients with poor systolic function, those biomarkers which were significantly higher in patients with CMP versus controls were NT-proBNP, MR-proANP, GDF-15 and sST2 (see Table 3).

### 3.5.2 Correlation with Imaging Variables

Of 15 patients with low LVEF and 57 control subjects, those that correlated significantly negatively with MRI LVEF included NT-proBNP ($\rho=-0.823$, $P<0.0001$), MR-proANP ($\rho=-0.615$, $P=0.02$) and sST2 ($\rho=-0.533$, $P=0.04$).

### 3.5.3 Clinical Parameters

Of those with low LVEF, ($n=15$), most patients were in NYHA class I ($n=10/15$, 67%) and Ross class 1 ($n=13/15$, 87%).

On multiple regression analysis, the best predictors of modified Ross score (adjusted $R^2=0.857$, $P<0.0001$) was NT proBNP when corrected for age, BSA, mean heart rate and duration of illness.

### 3.5.4 Age

The relationship between age, LVEF and biomarker levels is interesting. There appears to be no effect of age on NT-proBNP when MRI LVEF is taken into account (see Figure 15). This was true for all biomarkers (data not shown).
Figure 15: Relationship between NT-proBNP and age for patients with normal (>40%) and low (<40%) LVEF

3.6 Survival

5 patients experienced one of the combined endpoints (1 was listed for transplant, 2 had VAD bridge to transplant and 2 had direct transplantation without bridging).

On Cox regression analysis, NT-proBNP remained the only independent predictor of outcome (HR=15.4, P=0.002), although caution needs to be exercised due to the low number of events. None of the novel biomarkers or any other clinical/imaging markers were predictive of outcome.
4 Results – T1 Mapping

4.1 All Aetiologies

4.1.1 Patient Characteristics

Altogether, 51 patients with CMP were included in this part of the analysis and 7 adult controls. The ages of the two groups were significantly different (P<0.0001, Table 4) and there was a corresponding and statistically significant difference in the BSA (m²) between the two groups. There was no statistically significant difference in the modified Ross score or NYHA class between groups, signifying the stability of the patients included in the study. The majority of patients were white in both groups and there was no statistically significant difference between groups by ethnicity.

The second part of this chapter describes differences between patients with idiopathic DCM and adult controls. Patients with DMD were excluded from all of the analyses and where they are included, this is made clear in the section. Children with oncological disease were also recruited as control subjects, but their ECV and T1\textsuperscript{native} measurements were found to be higher than expected for healthy subjects. Due to this, they were excluded from further analysis.

Table 4: Patient Demographics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CMP patients (n=51)</th>
<th>Controls (n=7)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.6 ± 4</td>
<td>25.7 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>19 (37%)</td>
<td>4 (57%)</td>
<td>0.12</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.1 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Modified Ross score (I:0-5/II:6-10/III:16-20) | (47/4/0) | (7/0/0) | 0.30  
---|---|---|---
NYHA class (I/II/III/IV) | (38/9/3/1) | (7/0/0/0) | 0.12  
Duration of illness (months) | 62 ± 69 | 0 |  

**Aetiology**

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Count (Percentage)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>23 (45%)</td>
<td>0</td>
</tr>
<tr>
<td>Familial</td>
<td>14 (27%)</td>
<td>0</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>4 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Anthracycline</td>
<td>5 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>9 (18%)</td>
<td>0</td>
</tr>
</tbody>
</table>
| Control           | 0                  | 7 (100%) | 0  

**Medications**

<table>
<thead>
<tr>
<th>Medications</th>
<th>Count (Percentage)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuretics</td>
<td>13 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>Angiotensin II converting enzyme inhibitor</td>
<td>43 (84%)</td>
<td>0</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>18 (35%)</td>
<td>0</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>29 (57%)</td>
<td>0</td>
</tr>
<tr>
<td>Aspirin</td>
<td>14 (27%)</td>
<td>0</td>
</tr>
<tr>
<td>Digoxin</td>
<td>12 (24%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Ethnic Origin**

<table>
<thead>
<tr>
<th>Ethnic Origin</th>
<th>Count (Percentage)</th>
<th>Count (Percentage)</th>
</tr>
</thead>
</table>
| White         | 32 (63%)           | 7 (100%)           | 0.26  
| Asian         | 9 (18%)            | 0                  |  
| Black         | 6 (12%)            | 0                  |  
| Mixed         | 2 (4%)             | 0                  |  
| Other         | 6 (12%)            | 0                  |  

**MRI**

| MRI                          | Value | Value | P-value  
|------------------------------|-------|-------|----------  
| MRI LVEF (%)                 | 48.7 ± 14 | 63.3 ± 6 | 0.005  
| MRI LVEDVi (ml/m²)           | 90.0 (80-99) | 65.8 (63-77) | <0.0001  


<table>
<thead>
<tr>
<th>MRI LVESVi (ml/m$^3$)</th>
<th>39.0 (33-58)</th>
<th>27.2 (22-28)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGE (n)</td>
<td>11 (20%)</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Average T1$_{native}$ (ms)</td>
<td>1032.0 ± 41</td>
<td>993.0 ± 41</td>
<td>0.16</td>
</tr>
<tr>
<td>Average T1$_{native}$ LGE negative (ms)</td>
<td>1029.4 ± 47</td>
<td>993.0 ± 41</td>
<td>0.22</td>
</tr>
<tr>
<td>T1$_{native}$ septum (ms)</td>
<td>1095.4 ± 68</td>
<td>984.3 ± 46</td>
<td>0.001</td>
</tr>
<tr>
<td>T1$_{native}$ septum LGE negative (ms)</td>
<td>1085.5 ± 66</td>
<td>984.3 ± 46</td>
<td>0.003</td>
</tr>
<tr>
<td>ECV average (%)</td>
<td>35.4 ± 4</td>
<td>30.3 ± 4</td>
<td>0.05</td>
</tr>
<tr>
<td>ECV average LGE negative (%)</td>
<td>34.7 ± 4</td>
<td>30.3 ± 4</td>
<td>0.05</td>
</tr>
<tr>
<td>ECV septum (%)</td>
<td>38.5 ± 5</td>
<td>30.1 ± 4</td>
<td>0.002</td>
</tr>
<tr>
<td>ECV septum LGE negative (%)</td>
<td>37.8 ± 4</td>
<td>30.1 ± 4</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### 4.1.2 Cardiac Magnetic Resonance Imaging

The MRI data of the controls and CMP patients are provided in Table 4. Control patients had a significantly higher LVEF than patients with CMP (P=0.005). Control patients also had a significantly lower LVEDVi and LVESVi than patients with CMP (P<0.0001 for both).

### 4.1.3 Comparison of CMP Patients and Control Subjects

Patients with CMP had higher average ECV, septal T1$_{native}$ and septal ECV values than controls (see Table 4). This difference remained once segments with LGE were excluded. Averaged T1$_{native}$ of the whole myocardium was not significantly different between groups.
On ROC analysis, septal T1\textsubscript{native} and ECV were predictive of the diagnosis of CMP with an area under the curve (AUC) of 0.762 (0.573-0.950, P=0.01) and 0.845 (0.693-0.997, P=0.001, see Figure 16).

**Figure 16:** ROC curve of CMP predicted by septal T1\textsubscript{native} and ECV (LGE negative)

4.2 LGE Analysis

11 patients out of 55 (20%) with CMP had LGE in at least 1 segment. There were a maximum of 6 segments affected in 1 patient with a mean of 1 segment per patient. There was a significant difference between patients with LGE positive segments and those without in levels of septal T1\textsubscript{native} (LGE positive 1136.0 ± 39ms, LGE negative 1049.1 ± 71ms, P=0.05), average ECV (LGE positive 36.1 ± 5%, LGE negative 33.8 ± 4%, P=0.04) and septal ECV (LGE positive 42.0 ± 5%, LGE negative 34.4 ± 4%, P=0.02). All segments had LGE
segments removed before analysis. Patients were considered ‘LGE positive’ if they had one or more segments of LGE on analysis.

The pattern of LGE varied. Some patients, e.g. those with DMD, had very specific patterns of LGE in the inferolateral segment (segments 10-11) of the ventricle at the mid-ventricular level on short axis imaging. This was frequently associated with thinning of the ventricle and hypokinesia (qualitative relative to the remaining myocardium). Other patients had a range of LGE locations. Figure 17 shows the higher levels of LGE in patients with DMD and ‘other’ aetiology (including ischaemic) in comparison to the rest of the group.

**Figure 17:** Average number of segments with LGE per diagnosis

![Bar chart showing the mean number of LGE positive segments per diagnosis](chart.jpg)
4.3 Correlation with Other Imaging Variables

Septal $T1_{native}$ was significantly negatively correlated with MRI LVEF ($\rho=-0.333$, $P=0.05$). However, this association disappeared when LGE segments were removed from the analysis (see Figure 18).

**Figure 18:** Relationship between MRI LVEF and septal $T1_{native}$ (LGE included)

4.3.1 Correlation with Clinical Variables

Septal $T1_{native}$ (LGE negative) was significantly correlated with modified Ross score ($\rho=0.615$, $P<0.0001$). This result was significant even when corrected for multiple comparisons.

4.3.2 Correlation with Circulating Biomarkers

Average $T1_{native}$ (LGE negative) correlated with GDF-15 ($\rho=0.573$, $P=0.001$, $n=30$). Septal $T1_{native}$ (LGE negative) correlated significantly with NT-proBNP
(ρ=0.507, P=0.006, n=28) and CT-proET1 (ρ=0.679, P<0.0001, n=26; see Figure 19). Septal ECV (LGE negative) correlated with CT-proET1 (ρ=0.683, P<0.0001, n=23). All correlations remained significant when corrected for multiple comparisons.

**Figure 19:** Correlation of septal T1\textsubscript{native} with log\textsuperscript{10} CT-proET-1.

### 4.3.3 Prognosis and Survival

5 patients experienced the primary outcome of urgent listing for transplant, there were insufficient events to evaluate whether T1\textsubscript{native}, ECV or LGE predicted outcome.

### 4.3.4 Gender Differences

Splitting the groups by gender did not significantly alter the differences between CMP and controls seen above, except in septal ECV (LGE positive)
which became non-significant in males. There were no significant differences in $T_{1\text{native}}$ or ECV between groups by gender.

4.4 Z-scores of ECV and $T_{1\text{native}}$

Patients with DMD were included but patients with myocarditis did not have sufficient numbers to be included in this analysis. Z-scores of septal and average $T_{1\text{native}}$ and ECV based on the adult control values were calculated for all aetiologies (see Figure 20). Patients with anthracycline toxicity had the highest levels of septal and average ECV and $T_{1\text{native}}$. Patients with iDCM and familial DCM also had positive Z-scores for all parameters. Patients with DMD had lower levels of mean $T_{1\text{native}}$ than control subjects. Patients with other causes of DCM had lower levels of mean ECV with and without LGE segments than control values.
**Figure 20:** Z-scores for septal and average T1\(_{\text{native}}\) and ECV for all aetiologies\(^{11}\).

4.5 Septal T1\(_{\text{native}}\) and ECV

T1\(_{\text{native}}\) was significantly different between septal and other regions on paired T-test (septal 1059.1 ± 74ms, other 1020.2 ± 45ms, P=0.001, see Figure 21). This difference was further enhanced when only patients with CMP were included in the analysis (septal 1085.5 ± 66ms, other 1022.0 ± 50ms, P<0.0001); patients with DMD were not included in this analysis.

\(^{11}\) Z-scores based on healthy adult controls from this study.
There was no significant difference between non-septal segments (mean value) in CMP and controls. There was no significant difference between septal ECV and other segments. All segments had LGE removed prior to analysis.

**Figure 21:** Differences in $T_{1\text{native}}$ values (ms) between septal and other segments and CMP and control patients.

4.6 Results with iDCM Patients Only

In this section, results from patients with iDCM ($n=23$) as an aetiology are considered separately, with healthy adult controls ($n=7$). The control subjects are those used in the study above.
4.6.1 Cardiac Magnetic Resonance Imaging

The MRI data of the healthy population and patient population are provided in (Table 5). Control patients had a significantly higher LVEF than patients with CMP. Control patients also had a significantly lower LVEDVi and LVESVi.

Table 5: MRI results by patient group for iDCM only\textsuperscript{12}.

<table>
<thead>
<tr>
<th></th>
<th>DCM (n=23)</th>
<th>Control (n=7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.0 ± 5</td>
<td>25.7 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>99.9 ± 11</td>
<td>125.4 ± 24</td>
<td>0.001</td>
</tr>
<tr>
<td>BSA (m\textsuperscript{2})</td>
<td>1.0 ± 0.5</td>
<td>1.8 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>10 (44%)</td>
<td>4 (57%)</td>
<td>0.42</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>42.3 ± 12</td>
<td>63.3 ± 6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVEDVi (ml/m\textsuperscript{2})</td>
<td>93.6 (81-144)</td>
<td>65.9 (63-77)</td>
<td>0.003</td>
</tr>
<tr>
<td>LVESVi (ml/m\textsuperscript{2})</td>
<td>53.7 (36-93)</td>
<td>27.2 (22-28)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LGE</td>
<td>6/23 (26%)</td>
<td>0/7</td>
<td>0.17</td>
</tr>
<tr>
<td>Average T\textsubscript{1} native (ms)</td>
<td>1041.1 ± 20</td>
<td>993.0 ± 41</td>
<td>0.003</td>
</tr>
<tr>
<td>Average T\textsubscript{1} native LGE neg (ms)</td>
<td>1035.4 ± 38</td>
<td>993.0 ± 41</td>
<td>0.03</td>
</tr>
<tr>
<td>T\textsubscript{1} native septum (ms)</td>
<td>1115.8 ± 66</td>
<td>984.3 ± 46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T\textsubscript{1} native septum LGE neg (ms)</td>
<td>1118.9 ± 65</td>
<td>984.3 ± 46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECV average (%)</td>
<td>36.6 ± 4</td>
<td>30.3 ± 4</td>
<td>0.003</td>
</tr>
<tr>
<td>ECV LGE negative (%)</td>
<td>35.4 ± 4</td>
<td>30.3 ± 4</td>
<td>0.02</td>
</tr>
<tr>
<td>ECV septum (%)</td>
<td>39.9 ± 6</td>
<td>30.1 ± 4</td>
<td>0.002</td>
</tr>
<tr>
<td>ECV sep LGE neg</td>
<td>39.7 ± 5</td>
<td>30.1 ± 4</td>
<td>0.002</td>
</tr>
</tbody>
</table>

\textsuperscript{12} Idiopathic dilated cardiomyopathy (iDCM), left ventricular ejection fraction (LVEF), left ventricular end diastolic volume indexed for body surface area (LVEDVi), left systolic volume indexed for body surface area (LVESVi), late Gadolinium enhancement (LGE), T1 time pre-contrast (T\textsubscript{1} native), extracellular volume (ECV). \textit{P<0.05}. 
4.6.2 T1 Mapping Data

With only iDCM patients included and compared to adult controls all $T_{1\text{native}}$ and ECV parameters were significantly different between groups. The difference between septal $T_{1\text{native}}$ (LGE negative) was especially significant (see Table 5 and Figure 22).

**Figure 22:** Difference in septal $T_{1\text{native}}$ between iDCM patients and control subjects.

As was shown in the group as a whole, septal $T_{1\text{native}}$ was higher than that of other segments (septal $T_{1\text{native}}$ 1060.0 ± 89ms, other segments 1011.1 ± 38ms, $P<0.0001$) and higher in patients with iDCM when compared to a control group (see Table 5 and Figure 23).
4.6.3  Correlation with Other Imaging Variables

No $T_1^{\text{native}}$ or ECV parameters correlated with MRI LVEF, LVEDVi or LVESVi.

4.6.4  Correlation with Clinical Variables

There was no correlation between any $T_1^{\text{native}}$ or ECV parameters and age, BSA, SBP or modified Ross score. Mean heart rate was correlated with septal $T_1^{\text{native}}$, but this did not remain significant when corrected for multiple comparisons.

4.6.5  Correlation with Circulating Biomarkers

There was no significant correlation between circulating biomarkers and $T_1^{\text{native}}$ or ECV, septal or all segments.
4.6.6 Prognosis and Survival

3 patients in this group reached the combined end point. 2 were bridged to transplant with a VAD and 1 was listed for transplant. There were insufficient events to comment on the prognostic value of $T1_{\text{native}}$ or ECV.

4.6.7 Gender Differences

In males, septal $T1_{\text{native}}$ (LGE negative) was significantly different between patients with iDCM and controls (iDCM $1083.3 \pm 48$ms, controls $956.7 \pm 64$ms, $P=0.03$). In females, LGE negative septal $T1_{\text{native}}$ (iDCM $1147.4 \pm 68$ms, control $1005.0 \pm 15$ms, $P=0.005$) and LGE negative septal ECV (iDCM $42.2 \pm 5$%, control $30.5 \pm 3$, $P=0.02$) were significantly different between those with iDCM and controls.
5 Discussion

The aim of this Thesis is to better understand the pathophysiology of heart muscle disease in children by using blood and MRI biomarkers. The hypotheses are that the biomarkers measured are different in paediatric heart muscle disease compared to controls and that they correlate with disease severity and differ with aetiology. We also hypothesise that there may be some correlation between the blood and MRI biomarkers themselves, linking, for example, inflammation in blood and fibrosis on MRI. This work has not been undertaken in paediatric heart muscle disease before and the findings of this thesis are, therefore, largely novel.

5.1 Biomarkers

5.1.1 Hypothesis

Novel circulating biomarkers are significantly higher in children patients with CMP than control subjects, they correlate with LV function (ejection fraction), vary with aetiology and reflect remodelling processes.

Of the whole study population, comprising 81 control subjects (all except 7 adults) and 76 patients with all aetiologies of CMP including those with DMD and obesity, NT-proBNP, MR-proANP, GDF-15, sST2 and hsCRP were significantly different between groups. However, when patients with DMD and anyone with obesity were removed from the analysis, only GDF-15 remained
significantly different between groups. A subanalysis of those with low systolic function (MRI LVEF $\leq 40\%$), showed that NT-proBNP, MR-proANP, GDF-15 and sST2 were higher in patients with CMP than control subjects.

5.1.2 Patient demographics

The diversity and case mix of heart failure clinics in the UK have been taken into account in the study, allowing different aetiologies to be studied. We have considered this heterogeneity and different sections of results correspond to different groups of patients. Those with CMP were further spilt into aetiologies for some sections, obese and patients with DMD were removed for the majority of the analysis (CMP=51). Control subjects with obesity and adults were removed in the whole section (control=54).

The patients included in the study were very stable with nearly normal systolic function and minimal symptoms. Of interest, even of those patients with low LVEF, there were minimal symptoms and most patients were in NYHA class I. This reflects the population of patients we commonly see in the heart function clinic and also reflects the bias inherent in the study design, with patients who were older and clinical well consenting more for the study than those who were unwell or younger.

Control subjects tended to be older than patients with CMP. This was due to the fact that younger children were reluctant to have the tests associated with the study including blood tests and MRI scanning. The control population had similar systolic blood pressure, haematocrit, creatinine and gender to the CMP group. This suggests these were not confounding factors in this study.
Patients with DMD were assumed to have a different pathophysiology to other patients and were excluded from the bulk of the study. As could be seen when levels of biomarkers were compared between aetiologies, those with DMD had a unique biomarker profile.

5.1.3 Growth Differentiation Factor-15

GDF-15 is thought to be protective and anti-fibrotic in animal models involving injury to the heart\textsuperscript{189}. GDF-15 has also been shown to be associated with mortality in heart failure\textsuperscript{107}. GDF-15 is higher in patients with HF in adult studies\textsuperscript{107}. GDF-15 may be released in conjunction with other biomarkers in a protective cascade in acute HF. The trigger for release is unclear, but there may be some subclinical ischaemia/inflammation in patients with chronic CMP causing it’s continued release.

GDF-15 was the only biomarker which was significantly higher in patients with DCM than controls, once those with DMD and obesity were excluded. This may be due to the aetiologies of CMP we included in the study. Cell death and subclinical ischaemia are triggers for the release of GDF-15\textsuperscript{105}. GDF-15 can also be released by other cell types (see Figure 2) and this is often due to oxidative stress and the release of inflammatory cytokines\textsuperscript{105}.

There may be a combination of necrosis, apoptosis, fibrotic remodelling and inflammation in our patient group accounting for the differences seen. The patients in which GDF-15 was highest were those with low LVEF (less than 40%). This suggests that those with chronic, poor function are experiencing continued cell death and/or inflammation, causing a cytokine response in the
myocardium. This suggests a disease model in which chronic CMP is an active process of remodelling and cell damage and not a burnt-out disease.

5.1.4 MR-proANP

As previously discussed, ANP causes a profound natriuresis when injected into experimental animals\textsuperscript{130}. In adult studies on DCM, ANP has been shown to be higher in patients than control subjects\textsuperscript{132, 133}. It has also been shown to decrease with treatment in adults with DCM\textsuperscript{133}. The role of ANP was explored in an elegant experiment into knockout (DCM\textsuperscript{ANP-/-}) and partial knockout (DCM\textsuperscript{ANP-/+}) mice\textsuperscript{190}. Mice with DCM and normal ANP levels survived longer than those who had a partial or total deficiency of ANP. Mice with normal ANP levels had decreased pulmonary congestion and effusions, increased EF and decreased LV volumes. They also showed decreased remodelling with less interstitial and perivascular fibrosis in the myocardium. This fits with the described role of ANP (and BNP) as opposing the renin-angiotensin-aldosterone system.

5.1.5 sST2

Soluble suppression of tumourgenicity 2 (sST2) is a decoy receptor for interleukin-33 (IL-33), which functions as an alarmin, signalling the presence of tissue damage to local immune cells. The function of this protein is to act as an anti-inflammatory. It is not useful in the diagnosis of HF in adults as there are many causes for the signs and symptoms of this condition. ST2 is increased in many disease processes including pulmonary and cardiac disease, making it non-specific as a diagnostic marker\textsuperscript{116}. 
ST2 does, however, indicate a worse prognosis in chronic HF\textsuperscript{122}; our population did not have sufficient events to test its utility in this regard. The increased levels of inflammatory proteins including GDF-15 and sST2 do indicate an ongoing inflammatory process in chronic paediatric CMP. This may be a response to subclinical and chronic cell death, or chronic/repeated myocardial infection. Given the trajectory of decline in function in our patients is linear and slowly progressive rather than in sudden, large steps, the idea of repeated further insults does not seem to fit the clinical picture. More likely, chronic inflammation occurs as a response to slow, constant cell death and remodelling with no new external stimuli. Interestingly, all of the markers described so far have the ability to be cardioprotective.

5.1.6 NT-proBNP

BNP is well established in both paediatric and adult HF as a marker or disease severity. Analysis of early data at our centre showed a cut off of >290pg/ml predicted worse outcome in acute heart failure over a three year follow up period\textsuperscript{63}. In patients with chronic LV systolic dysfunction, Price et al. showed a similar adverse outcome in patients with BNP levels >300pg/ml\textsuperscript{191}. The latter study also showed raised levels in patients with LV dysfunction compared to controls.

BNP is released from the ventricles in response to myocardial stretch. It is increased with increasing severity and symptoms in paediatric HF\textsuperscript{63}. The low levels in our patient population go some way to explaining the low event rate. Patients had a very low median NT-proBNP: 135 (61-537pg/ml). This suggests
a stability of the patient group which explains the lack of deterioration within the follow-up period of the study.

5.2 Correlation of Biomarkers with LVEF

Biomarkers which differentiated between normal and low MRI LVEF (\(\leq 40\%\)) in patients with CMP were GDF-15, MR-proANP and sST2. NT-proBNP, hsTnI, hsCRP and MR-proANP negatively correlated with MRI LVEF in the whole group. Of those with low LVEF, NT-proBNP, MR-proANP and sST2 correlated significantly and negatively with MRI LVEF.

5.2.1 NT-proBNP

The correlation of NT-proBNP with LVEF is unsurprising and has been shown on many previous occasions in both paediatric and adult HF. BNP is released from ventricular myocytes when stimulated by ventricular stretch. Serial changes in NT-proBNP measurements have been shown to be predictive of change in FS (echo measurement analogous to EF) in children\(^63\). Studies in adults with HF with reduced and preserved EF show that BNP is increased in both but more in those with reduced EF. There is a negative correlation between BNP and LVEF\(^{192}\).

5.2.2 MR-proANP

The studies on ANP’s role in cardiovascular physiology occurred around its discovery in 1981. ANP was found to be negatively correlated with LVEF calculated by radionuclide ventriculography in patients with congestive HF\(^{193}\).
Further studies showed ANP to be associated with BNP and diastolic function\textsuperscript{194}. ANP is released from the atria as a result of atrial stretch and therefore its release in situations of increased end-diastolic volume is unsurprising. The inability of the ventricle to fully eject during systole increases LVEDV and subsequently LA volume. The correlation of ANP with LVEF seems to have a clear explanation.

5.2.3 sST2

sST2 is effectively a decoy receptor for IL-33 and removes it from the circulation, acting to decrease the inflammatory response. HF is increasingly thought to involve inflammation as a key process in its progression. The finding that sST2 correlated with LVEF in our population is not in keeping with previous adult studies in this area and may reflect a difference in paediatric responses. In 247 adults with ischaemic heart disease, sST2 did not predict lower LVEF or infarct size\textsuperscript{195}, however, it is known to be prognostic in MI\textsuperscript{120}. It has been shown to correlate with LV mass index in patients with metabolic syndrome\textsuperscript{196}.

There is some evidence that sST2 is increased in situations of inflammation including cardiac damage and infarction. It has been shown to correlate with troponin I\textsuperscript{120}. It is of interest that hsTnI was also raised in our study and correlated with LVEF in all patients. There may be some cell death and associated inflammation involved in CMP in children, which appears to be specific to those with DMD and/or obesity. However, sST2 is not disease-specific and our cohort was heterogenous in terms of aetiology. It is also the
case that paediatric CMP is not an isolated disease in terms of body systems, and there is often involvement of other organs/systems including the neurohormonal system and respiratory system. This may confound these findings due to the lack of specificity of sST2.

5.2.4 Biomarkers by Aetiology

5.2.4.1 Troponin in DMD

The marker that was strikingly different in one aetiology was hsTnI, which was significantly elevated in patients with DMD. This has not been noted in DMD patients before (results differ in previous papers-197-199). None of the patients had acute HF, myocarditis or severe HF (EF was not less than 35% in all). It is likely that this reflects the mechanism of heart failure/apoptosis in patients with DMD.

A case report of two patients with DMD showed an acute troponin rise associated with ECG abnormalities suggesting acute myocardial damage200. These episodes were associated with cardiac chest pain, which had not been reported by any patients under cardiac follow up at our centre; most cardiac dysfunction was noted on routine screening. ECG changes and pain would be consistent with ischaemic cell death and there have been reports of abnormal coronary vasculature in myotonic dystrophy201 and DMD (although not Becker muscular dystrophy)202. This may be due to dystrophin-complex mediated production of nitric oxide, leading to a lack of opposition to coronary vasoconstriction200.
In a cross-sectional study of 129 patients who were carriers of DMD or Becker muscular dystrophy, 5.4% had DCM and 18% LV dilatation. No carriers had detectable TnI and 2 had detectable TnT (TnT detection limit 0.002 mcg/l)\textsuperscript{197}. Another study looked at 100 patients with DMD, 25 with Becker muscular dystrophy, and 40 carriers of either disease. A high TnI was observed in patients able to row their wheelchairs in the second decade. BNP was strongly correlated with LVEF but only weekly with TnI. LVEF and TnI were not correlated\textsuperscript{199}. The reasons for these findings are not clear.

High troponin levels have also been noted in patients with myotonic dystrophy\textsuperscript{203}, although these are not always associated with cardiac dysfunction\textsuperscript{204}.

### 5.2.5 Neurohormonal Activation

As was noted in the introduction, the renin-angiotensin-aldosterone system (RAAS) is of great importance in the initially adaptive and later maladaptive response of the body to HF. Activation of the sympathetic nervous system results in both water retention and an increased risk of arrhythmias and/or death\textsuperscript{205}. The extent of neurohormonal and sympathetic nervous system activation is difficult to quantify in our cohort, especially as most patients were on neurohormonal-axis modifying medications and the hormones themselves are very difficult to measure in vivo. The current model of chronic HF assumes activation of the RAAS and sympathetic nervous systems. The use of specific medications did not appear to affect survival in this study; however, this was difficult to assess due to the small sample size.
There is evidence of neurohormonal activation in children with CMP. BNP is a well-known marker of neurohormonal activation and it has been shown to be of use in the diagnosis and prognosis of children with heart failure (see section 30). In a small sample of 19 children, Ratnasamy et al. found elevated levels of NT-proBNP associated with increased severity of HF\textsuperscript{206}. It was indeed increased and prognostic in our study (although with a low event rate).

5.2.6 Obesity

Obesity was common in both patients and controls subjects in this study due to the methods of recruitment. Patients with DMD were also more likely to be obese due to their myopathy and steroid use. Obesity is a risk factor for cardiovascular disease and increased levels of biomarkers for oxidative stress and inflammation have been found in obese subjects without cardiovascular disease\textsuperscript{207}. For this reason, subjects with obesity in both groups were excluded from the analysis. Subjects with DMD were also excluded from the majority of analyses due to the differences in pathophysiology in this condition compared to CMP of other aetiologies.

5.2.7 Diverse Aetiologies

In terms of aetiology, the cases included in our cohort were representative of all patients being followed up in a heart muscle disease clinic. This meant that the case mix was broader than in registries including only patients with DCM. In comparison with a UK-based registry, our case mix varied\textsuperscript{208}. The prevalence of idiopathic CMP was similar in our group and in the published data (43% vs. 48%); however, we had lower rates of post-myocarditis CMP
(8% vs 22%). Many patients with new-onset myocarditis were not eligible for our study due to disease severity, so this aetiology was underrepresented.

We had no patients included in the study with metabolic disease, which is in part due to the complexity of the disease process and the inability to tolerate contrast and/or MRI scanning. We were also wary about measuring biomarkers in patients with a metabolic disease process, although this was not a strict exclusion criterion.

A large American and Canadian cohort had the following mix of aetiologies; idiopathic disease: 66%, myocarditis: 46%, neuromuscular disease: 26%\(^1\). This study included a prospective and retrospective cohort, so was more similar to our study in the mix of patients. Again, the differences are unsurprising given the selection bias inherent in our study; only patients who were old enough and well enough to tolerate a non-sedated MRI were selected. The percentage of children with neuromuscular disease in our study was 29%.

DMD was excluded from the majority of the analysis due to the differences in this disease from CMP of other causes. The mechanism of CMP in DMD is thought to be due to fibrofatty infiltration as with skeletal muscle. This is different to the ‘toxic-insult’ model seen in other forms of this condition, such as after myocarditis and anthracycline administration. There is some evidence that cardiovascular deterioration is caused by inflammation in patients with DMD as shown by MRI and biopsy\(^2\). There have been studies showing an increase in myocardial fibrosis in patients with DMD on MRI scanning\(^3\).
These findings, although interesting, would have biased the results of the study with both circulating and imaging biomarkers likely to be altered by the underlying pathophysiology of the disease.

### 5.2.8 Data Collection

The prognostic power of the study was limited by the small number of events that occurred in our population, the reasons for which are discussed in this chapter. The other main issue with the project was non-uniformity of data collection. Unfortunately, due to the chronic nature of the condition and the risk of traumatizing patients, some aspects of the study were omitted for certain patients. For example, some patients experienced claustrophobia and were unable to tolerate the MRI scan. Others had a needle-phobia and were unable to have the biomarker levels or any contrast during the scan. Patients were able to consent to all or part of the study as per our ethics approvals and this led to many incomplete datasets which confounded the analysis of data.

Variable data collection was a difficulty experienced many times in the study. The use of IV contrast certainly complicated matters and it is interesting to note that $T_1^{\text{native}}$ was significantly different in patients with CMP, thus negating the use of contrast in future studies into $T_1$ mapping. However, the problem of blood tests remains. Those with chronic CMP do not require very regular blood tests and these are often done in secondary centres rather than at ours. Further studies into biomarkers in CMP may require a larger infrastructure, allowing for regular, local blood testing and serial measurements in ambulatory patients.
5.2.9 Stable Patient Population

As previously mentioned, the patients recruited to this study had a relatively low NT-proBNP level; 135 (61-537pg/ml) and low-normal LVEF 48.9 ± 14%. The patients were ambulatory outpatients with stable CMP and few if any symptoms (NYHA class was I in 71%). This population was selected due to their ability to tolerate an MRI scan and IV contrast administration during the study. However, due to the clinical and age restrictions (breath holding is difficult for children below 8 years), there was an inherent bias in the patient selection.

Patients tended to be well, stable and older. The lack of difference in some biomarker levels may be a reflection of this stability rather than a true lack of difference between CMP and control subjects. Diffuse fibrosis (by T1 mapping) may also not be sufficiently severe in our population to show a true difference. Patients were followed up clinically, but uptake of MRI at follow up was insufficient to draw any conclusions and was not included in this Thesis. Unfortunately, ethical considerations precluded the use of gadolinium contrast at follow up and therefore many patients elected not to have further biomarkers tested at this time.

There were also very few patients who reached the combined end point of listing for transplant, heart transplantation, VAD insertion or death. In fact, there were no deaths in this cohort during the study period. This led to difficulties assigning prognostic utility to any marker both circulating or imaging. Again, this outcome appears to be due to the inherent bias in the
selection of participants to the study. Although patients were chosen at random, the age constraints and reluctance of those who were unwell to lie flat and have extra tests confounded the sample in this study.

5.2.10 Age

The control subjects were significantly older than the patients with CMP in this study, due to the process of recruitment of normal controls. There was also a selection bias inherent to the study, meaning older children who were able to cooperate with the MRI scan, which included breath-holding, were selected, thus excluding younger and potentially sicker patients.

The majority of patients presenting with DCM do so before the age of 1, so those in the study had time to recover and/or be treated prior to the study’s commencement. The most severely affected patients may already have been fitted with a ventricular assist device, have been transplanted or died prior to enrolment in the study. Of course, there were also many who had recovered and took part in the study. We were unable to include patients with decompensated heart failure who were inpatients at the time of the study, in line with the MRI protocol. One acutely unwell patient did have bloods taken, but all biomarker levels were extreme outliers in statistical analysis and it was decided to halt recruitment of severely ill patients.

5.2.11 Low Event Rate

Patients with different aetiologies have different patterns of survival. A large north American registry found neuromuscular disease and familial DCM had
the worst survival, whereas patients with myocarditis, inborn errors of metabolism and malformation syndromes had the best freedom from death or transplant\(^1\).

In our cohort, myocarditis had a worse prognosis than idiopathic DCM, followed by 'other' causes. The difference between aetiologies in our study was not significant on a Kaplan Meier plot (LR 0.566). These differences appear to represent the selection bias of our study and the inclusion criteria of the study and registry. The grouping of several different pathologies into one overarching group of ‘CMP’ may have affected the analysis and conclusions of this study.

The reason for choosing these patients were that they represented a cross-section of patients commonly seen at a heart failure clinic and similar treatments are used for these patients. However, the study group may make drawing conclusions difficult as each group seems to have a different pattern of biomarkers. Importantly, most patients do not have an identified cause for their CMP and more work needs to be done to improve diagnosis of the aetiology of CMP.

### 5.3 T1 Mapping

The results of this section were presented in two parts. Adult controls were used due to difficulties finding truly healthy paediatric controls who were able to have Gadolinium injected due to ethical considerations. The paediatric controls having gadolinium did have other pathologies. Once paediatric control
data were analysed, it was noticed they had higher levels of creatinine, suggesting renal damage and higher ECV, suggesting cardiac damage/fibrosis. Importantly, paediatric control patients had significantly higher ECV in inferior and lateral segments (10 and 11). These differences were likely to confound our results and these controls were excluded from the study. The entire patient population (excluding those with DMD) were studied and then those with iDCM were studied separately.

5.3.1 Demographics

As with the entire study, those in this analysis were very stable with low levels of NT-proBNP, normal NYHA and modified Ross scores on the whole. This population was representative of the majority of patients seen in heart function clinic. However, those with worse function and clinical deterioration were not able to be included due to the difficulties in administering contrast and reluctance in allowing them to have an MRI scan. Depending on the severity of the deterioration, they were often also too unwell for contrast injection at this time. The patients needed for the MD-Paedigree project (stable DCM/CMP with a dilated ventricle) affected the patients chosen for this study, as those with acute deterioration were not able to have MRI scanning and were therefore not funded to participate in this study.

5.3.2 Hypothesis

*Patients with CMP of all aetiologies have significantly higher levels of $T1_{\text{native}}$ and ECV than control subjects when LGE segments are removed from analysis. MRI assessment of fibrosis will correlate with both severity of disease*
(ejection fraction), and functional class and will vary with aetiology. Those blood biomarkers that reflect fibrosis and remodelling will correlate with the MRI assessments of fibrosis/remodelling.

5.3.3 T1_{native} and ECV

With adult controls, there was a significant difference in septal T1_{native} and ECV between control subjects and patients with CMP in this study. This finding is in keeping with adult studies of DCM^{176, 182}. T1_{native} and ECV are indirect measures of fibrosis and this assumption relies on the assumption that the extracellular space is expanded due to increased interstitial fibrosis rather than oedema or other infiltrates. It may be that the differences seen were due to oedema/inflammation instead, however, adult studies involving biopsy in patients with DCM show a positive correlation between histological fibrosis and T1/ECV^{211}. Although the prevalence of fibrosis appears lower in paediatric patients^{156} with CMP than adults^{157} and this has been considered a fundamental difference in the disease in children however our results suggest fibrosis is common even in mild heart muscle disease.

The myocardium in DCM is thinner than in normal hearts and MRI scans in children are more prone to movement artefacts. The difference may also lie in the inherent difficulties in performing MRI scans on un-sedated young people; with difficulties breath-holding well and staying still. However, a recent study on children following heart transplantation showed a higher septal ECV for transplant recipients than control subjects as well as a correlation between total and septal ECV with collagen volume fraction on biopsy^{212}. 
The finding that ECV was different between groups may be true or may be due to any number of reasons including Gadolinium wash-out kinetics, renal function of patients and timing of T1 measurement after Gadolinium dosing (although the latter was homogenous in our study). There may have been differences in these parameters despite a similar protocol being used. \( T1_{\text{native}} \) and ECV have been shown to be predictive of an adverse outcome\(^{213} \), which we have been unable to show in this study due to the stability of the patients included.

### 5.3.4 Late Gadolinium Enhancement

20% of patients with CMP had LGE in at least one segment. Septal \( T1_{\text{native}} \), septal and average ECV were significantly associated with remote areas of LGE. Segments with LGE were excluded from the measurements of ECV and \( T1_{\text{native}} \) (unless expressly stated) as they would have skewed the \( T1_{\text{native}} \) and ECV measurements to higher levels. LGE analysis is widely practised and the knowledge that LGE segments have higher ECV and \( T1_{\text{native}} \) values is expected. By definition, areas of high LGE show focal fibrosis, which would have higher \( T1_{\text{native}} \) and ECV.

### 5.3.5 Duchenne Muscular Dystrophy and Late Gadolinium Enhancement

It is unsurprising that patients with DMD and ischaemic heart disease had higher numbers of LGE positive segments than those with other aetiologies. The association between ischaemia and replacement fibrosis is well known. DMD has been shown to be associated with the presence of LGE in a
characteristic pattern involving the inferior and lateral myocardial segments\textsuperscript{214}. Our patients also had a similar, specific infero-lateral location of LGE. LGE has also been associated with clinical presentation in DMD\textsuperscript{215}.

The actual mechanism of fibrosis in DMD is thought to be due to fibro-fatty replacement of myocytes due to the dystrophin gene mutation. This is similar to the process taking place in skeletal muscle and has been confirmed in dystrophin-deficient mice\textsuperscript{216}.

MRI may provide a useful method of screening/monitoring patients for worsening cardiac involvement in future, especially as mobility and body habitus make echocardiography difficult in these patients.

5.3.6 Correlation of Septal T1\textsubscript{native} with Left Ventricular Ejection Fraction

Due to few events in this group, disease severity was a surrogate ‘end-point’ and septal T1\textsubscript{native} was increased with severity (worse LVEF) in our cohort. This suggests we are seeing an increased level of fibrosis with increased disease severity. However, the fact that LGE negative parameters were not correlated with LVEF suggests this correlation is explained by LGE rather than any more diffuse fibrosis. It may be that changes in LVEF occur at a later stage in disease progression where LGE is already noted. The lack of correlation of septal T1\textsubscript{native} with ECV is puzzling.

If LVEF were to truly correlate with fibrosis this supports the theory that increased remodelling is associated with increased fibrosis and longer T1\textsubscript{native}. 
Increased septal $T_{1_{\text{native}}}$ fits with the pattern of mid-wall fibrosis seen in adults with DCM, which may have as its precursor a greater concentration of diffuse fibrosis before sufficient contrast with surrounding myocardium develops.

This correlation has been noted before in patients with congenital heart disease\textsuperscript{217}. Tissue characterisation including, but not confined to T1 mapping may become more widespread as MRI scanning becomes a more routine part of monitoring cardiac disease. Patients in our centre are now having annual MRI scans for functional assessment with rapid results reported to clinic on the same day. It may be that the within-patient comparison of the amount of fibrosis will be available and will allow for characterisation of the degree and extent of remodelling.

5.3.7 Correlation with Clinical Variables

Septal $T_{1_{\text{native}}}$ was significantly correlated with modified Ross score. This has not been shown in children but in adults $T_{1_{\text{native}}}$ and ECV are correlated with all-cause mortality and hospitalisation\textsuperscript{218}, it is unsurprising that they are also correlated with modified Ross score. It may well be that the true prognostic association between T1 indices and outcome was missed in this study due to the relatively low numbers of ‘events’ occurring during follow up. This is an issue inherent in the design of this study. Improvements in the motion-correction and rapidity of scanning techniques should help to negate difficulties in patient scanning and recruitment in the future. The similarities between ECV and $T_{1_{\text{native}}}$ in our study, may lead to further studies in children using $T_{1_{\text{native}}}$ only (avoiding contrast administration).
Not all patients in this study were able to have Ross score/NYHA class recorded. Those with DMD were removed from this evaluation. The inability to gauge cardiac-related exercise intolerance versus that caused by muscle weakness is an important issue in patients with DMD. It may be that MRI becomes more important in the detection and monitoring of cardiac involvement in these patients as techniques become easier, cheaper and faster. There is evidence that presence of LGE correlates with clinical parameters in these patients and may be an early warning of cardiac involvement\textsuperscript{219}. It should also be noted that echocardiography is technically difficult in these patients due to habitus and mobility.

The relationship between modified Ross score and T1 indices is independent of LVEF. The theory that as systolic function declines, signs and symptoms of heart failure become apparent, is not borne out by our results. This is supported by the fact that those with poor systolic function continued to have a low NYHA/modified Ross score on average. The reality of the association is more complex with various psychological and conditioning factors involved.

\textbf{5.3.8 Correlation with Biomarkers}

As both blood and imaging biomarkers are related to remodelling and fibrosis, the correlation between the two was sought. Average $T1_{\text{native}}$ was correlated with GDF-15, septal $T1_{\text{native}}$ with CT-proET1 and BNP, and septal ECV with CT-proET1. As has been explained in prior sections, all of these markers correlate with fibrosis or increased remodelling.
The correlation of biomarkers and T1 indices is interesting. Both measures increase with severe HF in adult studies. The correlation here may well be linking the diffuse fibrosis, adverse remodelling and increased LV diameters in this study. The biomarkers which were correlated with T1 indices included those concerned with fibrosis including ET-1 and GDF-15. NT-proBNP was also increased, but this is already known to increase with severity in HF in children.

Cardiac biomarkers and diffuse fibrosis are both indirect ways of indicating cellular processes which may be occurring in paediatric HF. One aim of this study was to characterise HF in terms of various processes including fibrosis. Adverse remodelling appears to have a role to play in the future of these patients. It is still unclear; however, which patients will go on to get worse. Earlier prognosis remains the goal and this still eludes us.

The biomarkers which showed a correlation with T1 and ECV are detailed below:

5.3.9 Endothelin-1

ET-1 correlated with average T1 native, and septal ECV. It has been implicated in the increased expression of fibronectins and collagens in ageing mice hearts. Inhibition of ET-1 suppressed pro-fibrotic signals and synthetic ET-1 upregulated fibrosis in young mice\textsuperscript{220}. Diabetes increased ET-1 in wild-type mice; further, it promoted fibrosis and HF through accumulation of fibroblasts via endothelial to mesenchymal transition\textsuperscript{221}. The septal region was especially important in this correlation. The reason for this is unclear, but may
reflect the importance of septal fibrosis in CMP as a marker of disease progression/severity.

5.3.10 Growth Differentiation Factor-15

GDF-15 correlated with septal T1native in this study. It is linked with remodelling/ reverse remodelling in adults. LVADs cause reverse remodelling by offloading the failing heart. Levels of GDF-15 taken prior to device implantation were higher in patients with HF than in controls, and these levels decreased after LVAD implantation and remained stable over 6 months161. Interestingly, GDF-15 levels correlated with fibrosis. GDF-15 also promoted collagen secretion by fibroblasts222.

5.3.11 Brain Natriuretic Peptide

BNP was correlated with septal T1native in this study. There has been a previous study showing an independent correlation between BNP and extracellular volume and therefore fibrosis in patients without MI, amyloidosis or HCM223. Fibrosis mass, calculated as the sum of segments with LGE, correlated significantly with BNP in a study in patients with HCM224,225.

The mechanism of induction of fibrosis by BNP is unclear, however BNP is released due to myocardial stretch in the context of volume or pressure overload. BNP may not be responsible for the production of fibrosis but may instead be increased in the context of a failing heart, with remodelling and fibrosis occurring at the same time. In opposition to its role as a pro-fibrotic marker, Kapoun et al. found that BNP decreased TGF-β-induced effects on
primary human fibroblasts (including collagen production)\textsuperscript{226} and increasing BNP may be a compensatory response to remodelling.

5.3.12 T\textsubscript{1}\textsubscript{native} and ECV in Different Aetiologies

Patients with anthracycline toxicity had higher levels on average of T\textsubscript{1}\textsubscript{native} and ECV. Anthracycline toxicity remained an independent predictor of average ECV, which is a surrogate marker for diffuse fibrosis. A plausible reason for this could be remodelling secondary to cardiac damage caused by chemotherapy. Increased interstitial fibrosis could be due to the attempts at healing the damaged myocardium, which would eventually lead to diastolic dysfunction secondary to increased stiffness.

Anthracycline chemotherapeutic agents cause cell death by intercalation of nucleic acids; interfering with cell replication and leading to anti-tumour effects\textsuperscript{227}. There is some evidence that up to 50\% of people have diastolic dysfunction on echocardiogram following anthracycline-based chemotherapy\textsuperscript{228}. Diastolic dysfunction seems to precede systolic dysfunction and is useful in the earlier detection of cardiotoxicity\textsuperscript{229}. In a rat model of anthracycline-induced cardiotoxicity, the addition of an agent which reduced fibrosis (SIRT1 activator) improved cardiac dysfunction and survival following doxorubicin-induced chemotherapy\textsuperscript{230}.

Patients with iDCM also showed a similar and unique pattern of raised T\textsubscript{1}\textsubscript{native} and ECV values on average above control values. Interestingly, mean T\textsubscript{1}\textsubscript{native} was lower in all patients than septal T\textsubscript{1}\textsubscript{native}, suggesting again, that septal T\textsubscript{1}\textsubscript{native} is of particular significance in this disease process. Patients with DMD
showed an even more marked difference between septal and other regions, with levels of average $T1_{\text{native}}$ below those of control subjects. Patients with ischaemic CMP showed a similar pattern with septal ECV rather than $T1_{\text{native}}$.

The majority of patients with iDCM (non-ischaemic) in adult studies have LGE positive segments (up to 70% in some patients$^{231}$). In this particular dataset, patients were more likely to have septal than lateral mid-wall fibrosis. Septal areas of fibrosis are a predictor of sudden cardiac death and implantable cardiac defibrillator implantation$^{232}$.

5.3.13 Septal region

The septal myocardium proved the easiest to measure $T1$ in and this also yielded the most significant results. The differences seen may, therefore, be due to greater accuracy in the septal region, rather than due to true regional differences in the amount of fibrosis present. This is probably explained by a greater wall thickness in this region and less artefact due to movement in the centre of the image. This is supported by the fact that adult subjects (with presumably better ability to breath-hold and remain still) showed a greater difference from patients than paediatric controls (however, the oncological disease process in paediatric controls may be partially to blame). The findings may all be due to the movement artefact which would perturb the model-fitting process of $T1$ mapping, which assumes there is no movement between images acquisitions.

Alternatively, there is a known association with mid-wall fibrosis in DCM in adults and this is linked to an increased risk of sudden cardiac death brought
on by arrhythmias\textsuperscript{233}. Two studies detailing the extent and pattern of fibrosis in iDCM showed septal fibrosis is more prevalent than lateral mid-wall fibrosis\textsuperscript{231,232}. We may be seeing the precursor to this discrete mid-wall fibrosis as an increase in diffuse fibrosis in this region. It is of interest that adult subjects had a lower $T1_{\text{native}}$ than children with CMP, which does not support the theory that fibrosis is an inevitable age-related change (or at least that CMP causes more fibrosis than ageing). A follow up study of patients with high $T1_{\text{native}}$ in the septal region would be informative in this regard. Previous studies on adults have found higher septal $T1_{\text{native}}$ in healthy myocardium\textsuperscript{234}.

5.4 T1 Mapping in Idiopathic DCM

5.4.1 $T1_{\text{native}}$ and ECV

With iDCM patients alone included in the analysis, the significance of differences in average and septal $T1_{\text{native}}$ and ECV increased as both of these indices were higher in patients with iDCM than in those of different aetiologies (although not significantly, except for septal $T1_{\text{native}}$). The reason for including this analysis was to further investigate the differences between patients with DCM versus controls. Our patient group was initially heterogenous and our theory was that this would dilute the differences seen.

Removing all but those with one specific disease process did indeed reveal greater differences, suggesting we are unmasking a true, significant difference in the amount of diffuse fibrosis in patients with iDCM vs controls, despite the resulting decrease in numbers. This suggests a common pattern of fibrosis in
patients with iDCM including increased septal fibrosis. Whether this progresses to replacement fibrosis, giving the pattern of discrete mid-wall fibrosis seen in adults, requires further follow up.

5.5 Potential Confounders

5.5.1 Control subjects

The results have been split into two separate sections; one comprising all subjects and the other only those with iDCM. Children requiring Gadolinium enhanced MRI scans who were healthy proved difficult to find. Ethically, we were unable to recruit true ‘healthy’ controls, as giving Gadolinium was considered too risky. The compromise involved recruiting patients with non-cardiac disease and no signs of cardiovascular disease. These patients were mainly recovering from intracranial tumour excision and/or chemotherapy. We excluded those having cardiotoxic chemotherapy from the study. Unfortunately, these patients had still undergone extensive and challenging treatment for serious disease and can in no way be considered ‘healthy’ controls. Due to this, they were excluded as controls in this analysis and only adult controls were used. As an ongoing part of the project, we will measure $T1_{\text{native}}$ (without contrast) in healthy, paediatric controls in the future.

We therefore recruited another group of healthy controls, who were adults. They were truly healthy and had no previous past medical history of note. However, as noted below, age may be a significant factor influencing the degree of fibrosis in a subject/population. Of course, as mentioned, measuring
ECV in children is extremely difficult and ethically challenging, so the studies on age-related T1 values are all in adults. The findings of these studies may be able to be extrapolated backwards, or this may be a false assumption. There is some dispute as to the actual influence of age on T1 values.

The mean levels of T1\textsubscript{native} and ECV measured in our study are analogous to normal reference values in adult studies at 1.5T. Average T1\textsubscript{native} in the mid-ventricular short axis slice was 991.1 ± 34ms in an adult study and 993.0 ± 41ms in our study (P=0.11)\textsuperscript{235}. Normal reference values of ECV in one adult study (albeit using a FLASH IR sequence at 1.5T) was 28.0 ± 0.004%, whereas our normal control subjects had a value of 30.3 ± 4% (P=0.13). The only segment in which values were significantly higher were in T1\textsubscript{native} values in the septal segment 984.3 ± 46ms versus 943 ± 45ms (P=0.02)\textsuperscript{236}. The reasons for this are unclear. This may be to do with the low average age of our control subjects, although the same paper quoted a similar septal T1\textsubscript{native} for those <30 years of age of 944 ± 50ms. The reason for performing our own control scans was to negate the effects of different machines, protocols etc, which may bias these results.

\textbf{5.5.2 Age}

There is a widely held belief that ageing causes an increase in diffuse fibrosis in the myocardium\textsuperscript{237}. Studies in adults have shown a variable influence of age on T1; with some studies showing no influence of age\textsuperscript{238}, and various gender-dependent influences\textsuperscript{234, 239}. Studies vary on the proposed changes in T1\textsubscript{native} with age with increases\textsuperscript{239}, decreases\textsuperscript{240} and no change with age\textsuperscript{241} reported.
This is the reason results were reported with adults included and excluded in this Thesis. It is difficult to know what the true effect of age is on normal T1 values.

There are few studies on comparisons of $T1_{native}$ or ECV between adults and children\textsuperscript{212}. We found lower values of $T1_{native}$ in adults than children in our study. There was no significant difference between any T1 or ECV indices in control subjects when adults and children were compared, although average $T1_{native}$ did approach significance ($P=0.054$) with an independent T-test. Adult $T1_{native}$ values were lower ($1000 \pm 41$ms in adults vs. $1048.9 \pm 41$ms in children), although this was not statistically significant. These findings do not fit with the accepted wisdom that fibrosis increases with ageing. It may be due to the control children in our study had other pathologies. This may have affected the results of this study and reflects the difficulty of finding healthy paediatric controls in research. A further study on $T1_{native}$ (not requiring contrast) is being planned, using truly healthy control children.

**5.5.3 Gender**

In 75 healthy subjects aged 20-90, $T1_{native}$ and ECV were higher in age-matched females than males\textsuperscript{239}. In 231 normal controls aged 11-81 (using the ShMOLLI protocol), $T1_{native}$ was also found to be higher in females than males\textsuperscript{240}. The reason for these differences is not known.

There appeared to be some influence of gender in our study; female children appeared to have a significant difference between T1 and ECV parameters.
whereas males did not show such a great difference. The link between female gender and T1 values is interesting and is an area for further research.

5.5.4 Accuracy and Precision

T1 mapping results can be affected by many parameters including Gadolinium clearance time, time of sampling, dose of Gadolinium, body composition and haematocrit\textsuperscript{174}. We optimised our study design to minimise the effects of the majority of these factors, including a strict study protocol with the same Gadolinium dose (by weight) for each patient and the same time of sampling. The same T1 mapping sequence was used in all experiments\textsuperscript{170}. ECV is arguably more accurate and was developed to negate most of the intra-subject effects, as it is a ratio and should remain equally perturbed from the ‘true’ value of T1 both before and after Gadolinium administration.

However, the values of T1 in our patients were impossible to link directly to the amount of fibrosis present in the myocardium as we did not perform biopsies due to ethical considerations. Even if biopsies were performed, these are normally taken from the RV and do not always give an accurate indication of fibrosis in the LV. Therefore, it is difficult to truly prove the accuracy and precision of ECV and T1\textsubscript{native} in our cohort. Further studies with larger numbers of normal subjects could allow for the discovery of normal values by age and sex. This would help to design further studies using T1 to measure diffuse fibrosis in paediatric disease.
5.5.5 LV Volumes

The analysis of MRI data including LV volumes and function were done at the time of the study for clinical reasons. These data were not reanalysed prior to the results analysis in this project. The studies were all supervised by AT and performed by either JH or DP depending on the time of the study. This may have led to bias in the measurements. T1 and ECV measurements were all done by DP blinded to disease and timepoint.

5.5.6 Inflammation

The T$_1$\text{native} and ECV changes seen in patients with CMP may have been due to inflammation and not fibrosis. The presence of active inflammation in chronic heart failure is something that is not well understood. It may be of importance in DMD and herald disease progression$^{242}$, but the importance of this process has not been investigated in other aetiologies. It may well be that an increase in interstitial space due to inflammation (and therefore, oedema) is occurring in patients with CMP. HsCRP and CRP were measured in a subset of patients and did not correlate with T$_1$\text{native} or ECV, which suggest inflammation was not active in these patients. There were correlations between imaging markers and blood markers of remodelling and fibrosis, suggesting this may be the cause of the T1 and ECV changes seen. T2 mapping could help to answer this question, however, further sequences were difficult to add into the scan protocol due to the age of patients and the length of the scan required. This could be the basis of future research.
5.6 Prognosis

The lack of prognostic prediction by either biomarkers or T1 indices may be due to the relatively few events occurring during the study. A longer follow up, larger patient population and more acute cohort may be necessary for future studies. Interestingly, a patient who had biomarkers measured during an acute deterioration for HF did have very high levels of all biomarkers, several orders of magnitude higher than other patients. This patient’s data were not included in the analysis due to the skewing values. Also, patients who eventually reached the end-point had many other comorbidities and all investigations were not performed on all patients. Patient safety and comfort remained paramount during the study. The main problem with recruiting acute HF patients is safety. They are often too young and unwell to tolerate an MRI scan in the acute period.

5.7 Potential Applications and Future Directions

The potential for circulating biomarkers is great. The use of NT-proBNP as a remote-monitoring tool in acutely decompensated HF patients in paediatrics is already well established. Levels are taken in the acute setting and allow for a decision on future care/admission with no cardiac imaging required (in the short term). The other biomarkers may be able to augment these data by forming a panel for ‘screening’ for cardiac disease. This would of course be limited to the cardiac-specific biomarkers in this group.
There may be an application for a panel of biomarkers to be part of an initial acute prognosticating tool in the first stages of presentation with CMP/acute HF. The difficulties with this approach include the variable time of presentation of patients to a cardiac team after first symptoms.

The most promising biomarker for immediate practical application appears to be hsTnI in DMD. This marker is used frequently in many peripheral hospitals in adult ischaemic heart disease and is thus readily available in peripheral centres. If levels were found to be raised in patients with DMD prior to LV dysfunction, this could be used in routine outpatient screening to allow early detection and treatment in this vulnerable group. The current system of routine echocardiograms can prove time-inefficient and technically challenging due to patients’ lack of mobility and habitus. A fast blood test would be preferable to many families and would cut down clinic waiting times and allow for patient selection for further screening. Of course, further research is required to test whether the rise in troponin occurs earlier than LV dysfunction as our cohort had already had cardiac deterioration.

For those without DMD, GDF-15 elevation suggests an antifibrotic response is important in children and pursuing this in treatment strategies may be more important than neurohormonal treatments that have been unsuccessful in paediatric CMP. In those with the lowest EF, sST2 was also raised which is again consistent with a future therapeutic regime targeting fibrosis.

As MRI scanning becomes faster, cheaper and more widely available for younger patients, tissue characterisation may become a more realistic
measure in a variety of disease processes. There is already some use of T1 and T2 mapping in patients with acute inflammation and oedema. The next step would be measuring normal values in different genders and ages. This would allow for comparison to a normal range and negate the use of normal control subjects after this. However, the use of gadolinium or even IV cannulation in healthy children is not considered ethically reasonable and leads to difficulties in measuring ECV in normal children. T1\textsubscript{native}, however, should be possible and recent studies have suggested comparable ability of ECV and T1\textsubscript{native} to detect and quantify fibrosis\textsuperscript{243}.

The abnormalities of T1 and ECV in this study are consistent with the blood biomarker findings implicating fibrosis and may be useful as targets of disease progression or even therapeutic interventions in the future.

5.8 Conclusion

This study is the first to measure the levels of biomarkers in children with CMP and normal controls and the first to measure T1 indices in paediatric patients with CMP. As such, it has allowed some insight into the pathophysiology of CMP in children. The data support a process of fibrosis/remodelling following an initial insult. Patients with DMD seem to undergo constant, low-level myocyte cell death and replacement fibrosis as the mechanism of heart failure. Troponin levels may allow for biochemical screening for this population.

Circulating biomarkers and T1 indices correlate with LVEF in paediatric CMP. The chronicity of disease in this cohort makes comments on prognosis difficult,
but there is evidence that there are higher levels of biomarkers representing processes including fibrosis and inflammation in paediatric HF. Septal fibrosis appears to be especially high in paediatric CMP and may precede the septal mid-wall fibrosis seen in adults.

Whether early therapeutic intervention, targeting fibrosis in paediatric heart muscle disease, will alter the prognosis is uncertain but this thesis raises the possibility of that strategy and the thesis offers blood and MRI biomarkers that could be used to track such treatment.
6 Appendices 1- MD-Paedigree Ethics Approval

6.1 MD Paedigree Ethics Approval

02 October 2014

Professor Andrew Taylor
Professor of Cardiovascular Imaging
Centre for Cardiovascular Imaging
UCL Institute of Cardiovascular Science &
Great Ormond Street Hospital NHS Foundation Trust
30 Guilford Street
London
WC1N 1EH

Dear Professor Taylor

Study title: EVALUATION OF PREDICTORS OF CARDIAC FAILURE IN CHILDREN AND ADOLESCENTS WITH CARDIOMYOPATHY

REC reference: 14/LO/1562
IRAS project ID: 151301

Thank you for your e-mail of 01 October 2014. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 30 September 2014.

Documents received

The documents received were as follows:

- Participant information sheet (PIS) [8-12 years Cardiomyopathy Group] version 4, dated 01 October 2014
- Participant information sheet (PIS) [8-12 years Control Group] version 4, dated 01 October 2014

Approved documents

The final list of approved documentation for the study is therefore as follows:

- Covering letter on headed paper [Response to Rec] dated 24 September 2014
- GP consultant information sheets or letters [Letter to GP] version 2, dated 30 July 2014
- IRAS Checklist XML [Checklist_29052014] dated 29 August 2014
- Participant consent form [Revised Consent Parents] version 3, dated 16 September 2014
- Participant consent form [Revised Assent form] version 2, dated 16 September 2014
- Participant information sheet (PIS) [Revised PIS Parent Control Group] version 6, dated 23 September 2014
You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor’s responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

14/LO/1582

Please quote this number on all correspondence

Yours sincerely

Miss Regina Caden
REC Assistant

E-mail: rerescommittee.london-hampstead@nhs.net

Copy to: Ms Emma Pendleton, Great Ormond Street Hospital

Dr Thomas Lewis, Research Manager and Governance Officer, Great Ormond Street Hospital for Children NHS Foundation Trust

A Research Ethics Committee established by the Health Research Authority
6.2 SOCRATES Ethics Approval

05 December 2013

Dr Alexander Jones
Principal Clinical Research Associate
University College London
Institute of Cardiovascular Science
3G Guilford Street
WC1N 1EH

Dear Dr Jones

Study title: Model-Driven European Paediatric Digital Repository (MD-PAEDI) WP 4: STUDY OF CARDIOVASCULAR RISK OF ADIPOSY IN TEENAGERS (SOCRATES)

REC reference: 13/LO/1750
IRAS project ID: 136703

The Research Ethics Committee reviewed the above application at the meeting held on 21 November 2013. Thank you for attending to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Ms Louise Braley, louise.braley@nhs.net.

Ethical opinion

As you know, the following was discussed at the meeting:

- You kindly confirmed that the blood pressure cuff was the standard cuff used routinely in clinical care and that some participants will experience some unpleasantness, but that the level of discomfort will vary. You also stated that the cuff when deflated can cause a slightly different sensation, but that this was harmless.

- You confirmed the initial cohort will be in the study for 12 – 18 months and that if participants reach the age 20 whilst still in the study they will be still be included.

- You explained the recruitment process further. He confirmed that the Clinical Research Datalink was a new organisation sponsored by the NHS. The organisation provides access through the GP, to non-identifiable patient notes with the GP holding the link to identify which patients will be suitable for the study.

- You confirmed that if there was a finding of clinical significance the participant’s GP will be informed in order for the GP to follow up. You also confirmed that participants will be asked to consent for this to happen and that at Great Ormond Street Hospital it is a requirement for young
people to be told of any incidental findings.

- You stated that the genetic risk score is not a validated risk score, but is an exploratory procedure. You confirmed that the intermediate outcome was to try and find patterns of association in the gene type and phenotype.

- The Committee questioned the procedure for dealing with distress. You confirmed that he has a skilled research team who are experienced of dealing with distress as they have to deal with this on a daily basis.

- You confirmed that samples would be sent abroad and would include blood samples that will be anonymised. You also stated that the samples will be sent to the main international centre/laboratory in the Netherlands where all measures are taken to ensure no identifiable data is leaked. You informed the Committee that plasma will be spun down.

- The Committee discussed distress that may occur with individuals. You confirmed that the Questionnaires contain profiling and stress measures, that there are two questions relating to depression. You also confirmed that a trained clinical psychologist will administer the Questionnaire and that if appropriate; any incidental findings will be referred on.

After discussion, the members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Ethical review of research sites**

**NHS Sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&I office prior to the start of the study (see “Conditions of the favourable opinion” below).

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

i. The Committee asks the Chief Investigator to confirm that any results from the MRI that holds any clinical significance will immediately referred to a specialist as well as the participant’s GP.

ii. The Committee asks the Chief Investigator to review the Information Sheet as the formatting is inadequate in some areas of the document, for example the paragraphs and justification.

iii. Similarly, the Committee asks the Chief Investigator to review the Invitation Letter as it has some poor punctuation, for example, “child’s” instead of child’s.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

**Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.**

Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application...
System or at http://www.reforum.nhs.uk.

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (“participant identification centre”), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

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6.3 Appendix 3- Coarctation Study Ethics Approval

14 October 2015

Dr Vivek Muthurangu  
Senior Lecturer, Institute of Cardiovascular Imaging, UCL  
University College London - Institute of Cardiovascular Science  
30 Guilford Street  
London  
WC1N 1EH

Dear Dr Muthurangu

Study title: MR assessment of haemodynamics in patients after coarctation repair during submaximal isometric exercise compared with healthy volunteers.

REC reference: 15/LO/1603  
IRAS project ID: 181439

The Research Ethics Committee reviewed the above application at the meeting held on 05 October 2015. Thank you and Dr Abbas Khushwood for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Mrs Helen Wilson, nrescommittee.london-camdenandkingscross@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

A Research Ethics Committee established by the Health Research Authority
Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

1. The Participant Information Sheets to include:
   - a sentence, "You will be given £15 to thank you for your participation in the study".
   - the correct REC name reviewed the study, i.e. Camden and Kings Cross REC.
   - the consistent use of either 'MRI' or 'MR' in all participant documentation.
   - the words "...not much chance that anything will go wrong..." should be replaced with "very low risk".
   - any incidental findings would be reported to the participant as well as the GP.
   - the title of the Healthy Volunteer Information Sheet to be renamed, 'Coarction (a major narrowing of the aorta).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rctforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 8 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

A Research Ethics Committee established by the Health Research Authority
If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Summary of discussion at the meeting

You and Dr Abbas Khushwood joined the meeting for discussion.

Social or scientific value; scientific design and conduct of the study

Members queried how the Great Ormond Street Hospital for Children would deal with adults.

You stated that the scanner would be for the research study only. You stated that all adult scans of the congenital form are undertaken at the Hospital. You stated that if any problems arose, the adult participant would be moved to St Bart’s Hospital.

Members enquired whether all procedures would be undertaken during normal working hours.

You confirmed that this was correct.

Members queried the rationale for not including any Patient and Public Involvement in the study.

You stated that this had been an error and you had different patient groups available.

Recruitment arrangements and access to health information, and fair participant selection

Members stated that the IRAS form and PIS documentation mentioned travel expenses however a poster for healthy volunteers also mentioned a voucher of £10 or £15.

You confirmed that travel expenses would always be paid and vouchers.

Members confirmed that the same amount must be paid to all participants, ie £10 or £15.

You acknowledged this point.

Informed consent process and the adequacy and completeness of participant information

Members enquired whether there was a difference between the use of MRI and MR.
Bibliography


70. Richards M, Nicholls MG, Espiner EA, Lainchbury JG, Troughton RW, Elliott J, Frampton CM, Crozier IG, Yandle TG, Doughty R, MacMahon S and
Sharpe N. Comparison of B-Type Natriuretic Peptides for Assessment of Cardiac Function and Prognosis in Stable Ischemic Heart Disease. *Journal of the American College of Cardiology*. 2006;47:52-60.


79. Chang AY, Abdullah SM, Jain T, Stanek HG, Das SR, McGuire DK, Auchus RJ and de Lemos JA. Associations Among Androgens, Estrogens,


109. Brown DA, Breit SN, Buring J, Fairlie WD, Bauskin AR, Liu T and Ridker PM. Concentration in plasma of macrophage inhibitory cytokine-1 and risk of


118. Martinez-Rumayor A, Camargo CA, Green SM, Baggish AL, O'Donoghue M and Januzzi JL. Soluble ST2 plasma concentrations predict 1-


146. Pieske BMD, Beyermann BMD, Breu VP, Loffler BMMD, Schlotthauer KMD, Maier LSMD, Schmidt-Schweda SMD, Just HMD and Hasenfuss GMD. Functional Effects of Endothelin and Regulation of Endothelin Receptors in


and adolescents with dilated cardiomyopathy. *Journal of Cardiovascular Magnetic Resonance.* 17:34.


192. Ganesh M and Silambanan S. *Correlation between brain-type natriuretic peptide (BNP) levels & left ventricular ejection fraction (LVEF) in heart failure*; 2016.


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