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Cardiac Manganese-Enhanced Magnetic Resonance Imaging

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BM BMedSci

A Thesis Presented for the Degree of Doctor of Philosophy
The University of Edinburgh
2022
To Dadu,
though you never got to see this
you are in every page.
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i. DECLARATION

This thesis represents the research I undertook at the Centre of Cardiovascular Sciences at the University of Edinburgh, based at the Chancellor's Building and Queen's Medical Research Institute between August 2019 and February 2022.

I contributed to study design, drafted the protocol, obtained Research Ethics Committee and NHS R&D approval, undertook all recruitment, trial management, supervised all scanning, performed all image analysis and follow-up for the early clinical studies and subsequent reproducibility (Chapters 4).

I personally designed, drafted the protocol, obtained Research Ethics Committee and NHS R&D approval, undertook all recruitment, trial management, supervised all scanning, performed all image analysis and follow-up for all clinical studies (Chapters 3-5) with the exception of patients with COVID-19 (Chapter 6). A proportion of these patients were co-recruited from the Leicester Biomedical Research Centre (Prof Gerry McCann, Dr Alastair Moss, Dr Ranjit Arnold and Dr Thomas Kite) using protocols provided by us, but all analyses of these data were carried out by myself. The script used for the compartmental modelling in these studies was initially written by Dr Giorgos Papanastasiou and later amended by Dr Lucy Kershaw. I engaged
closely with this process to ensure the modelling accurately reflected previous published work. I performed all modelling and the statistical analyses for all included data independently.

Chapters 1, 4, 6 have been published in high impact peer-reviewed journals and I was first author for these papers. Chapter 3 and 5 is currently under review with a high impact peer-reviewed journal, for which I am first author. I have also compiled and contributed significantly to the clinical study which further investigates which are currently recruiting (Chapter 7).

Chapter 4 has been included in a previous doctorate thesis presented by Dr Nicholas Spath (joint first authors). Apart from this, no other content of this thesis has been previously accepted in applications for a degree and all sources of information have been acknowledged appropriately. All research was conducted in accordance with International College of Harmonisation Good Clinical Practice, the Declaration of Helsinki and the South-East Scotland Ethics Committee.

Dr Trisha Singh

26th May 2022
ii. **ABSTRACT**

**Background**
Manganese-based contrast media can provide intracellular T1 enhancement in viable tissues that have active calcium-dependent processes. Pre-clinical and early studies in man have demonstrated that manganese-enhanced magnetic resonance imaging has potential as a surrogate marker of myocardial calcium handling. The aims of this thesis are to investigate the feasibility and utility of manganese-enhanced magnetic resonance imaging of the myocardium in different aetiologies of ventricular dysfunction.

**Methods and Results**
First, I assessed the repeatability of manganese-enhanced magnetic resonance imaging and its scan-rescan reproducibility. This has not been established and is a necessary step for its future clinical application. I assessed the intraobserver and interobserver repeatability of T1 mapping and kinetic modelling, and the overall scan-rescan reproducibility, of myocardial manganese-enhanced magnetic resonance imaging. I demonstrated excellent repeatability of manganese-enhanced T1 mapping and kinetic modelling in healthy and pathological myocardium. Furthermore, scan-rescan reproducibility of manganese-enhanced magnetic resonance imaging in healthy volunteers was excellent.
I next assessed manganese-enhanced magnetic resonance imaging in non-ischaemic cardiomyopathy in patient cohorts with either dilated or hypertrophic cardiomyopathy. Using kinetic modelling, myocardial manganese uptake demonstrated stepwise reductions across healthy myocardium, hypertrophic cardiomyopathy without fibrosis, dilated cardiomyopathy and hypertrophic cardiomyopathy with fibrosis. I demonstrated that manganese-enhanced magnetic resonance can distinguish between healthy and fibrosed myocardium, providing a non-invasive measure of myocardial calcium handling.

To date, there have been no studies assessing whether manganese-enhanced magnetic resonance imaging can detect alterations in myocardial calcium handling in acute takotsubo syndrome. I used manganese-enhanced magnetic resonance imaging to assess myocardial calcium handling in patients with takotsubo syndrome during both the acute presentation and following apparent recovery. I demonstrated that patients with takotsubo syndrome have a profound perturbation of myocardial calcium handling which is most marked in the acute setting but persists for at least 3 months despite apparent restoration of normal left ventricular ejection fraction and resolution of myocardial oedema.

Finally, the COVID-19 pandemic has had a substantial effect worldwide. Myocardial injury is common in patients hospitalised with COVID-19. However, the mechanisms underlying this are not well understood. Using both
gadolinium-enhanced and manganese-enhanced magnetic resonance imaging combined with CT coronary angiography, I sought to determine the contribution and impact of pre-existing cardiovascular disease on the cardiac abnormalities of patients recovering from COVID-19 hospitalisation. Patients demonstrated right, but not left, ventricular dysfunction consistent with the dominant pulmonary pathology of this condition. Furthermore, no evidence of myocardial calcium handling was observed in patients. Previous reports of left ventricular myocardial abnormalities following COVID-19 are likely to reflect pre-existing co-morbidities rather than a direct consequence of COVID infection itself.

**Conclusion**

I have demonstrated excellent repeatability and reproducibility of manganese-enhanced T1 mapping and kinetic modelling in healthy and diseased myocardium. Furthermore, the scan-rescan reproducibility of manganese-enhanced magnetic resonance imaging in healthy volunteers was excellent, suggesting it has potential for clinical application. I described utility and feasibility of myocardial manganese-enhanced magnetic resonance imaging as a non-invasive measure of myocardial calcium handling in patients with non-ischaemic cardiomyopathy. In patients with takotsubo syndrome, I observed dysfunctional myocardial calcium handling which is most striking during the acute episode but persists despite apparent recovery of the heart. Finally, we have shown that patients with recent severe COVID-19 infection demonstrated right but not left ventricular dysfunction. This is likely secondary
to long-term pulmonary sequelae of COVID-19. Furthermore, previous reports of left ventricular myocardial abnormalities following COVID-19 may reflect pre-existing co-morbidities.

Overall, I have demonstrated that manganese-enhanced magnetic resonance imaging holds major promise for the diagnosis, risk stratification and monitoring of cardiac disease, with the potential for the assessment of novel treatment interventions.
iii. **LAY SUMMARY**

MRI (magnetic resonance imaging) is a special type of body scanner that uses strong magnetic fields to generate pictures of the body. In patients with heart disease, it can identify a range of problems within the heart, especially when the heart muscle fails to function normally. Current scans can only assess how the heart muscle moves and whether there are any scars. However, it cannot directly see whether the heart muscle cells are working properly. Detecting abnormal behaviour of the heart muscle could allow us to identify disease early, diagnose certain types of heart disease and help select treatments that may help alleviate symptoms and improve patient outcomes.

There is a relatively new approach where manganese containing compounds can be used to light up the cells of the heart muscle during magnetic resonance imaging. However, there is little information on how robust this technique is and whether it is reproducible so that it can be of use in routine clinical practice. As such, I went on to assess how repeatable and reproducible this technique was. I found that the technique had excellent repeatability and reproducibility both within and between separate scans.

I then assessed this technique in patients who suffer from weakening of the heart muscle (dilated cardiomyopathy) and those with thickening of the heart (hypertrophic cardiomyopathy). I found that magnetic resonance imaging
using manganese can detect abnormal function of heart muscle cells in a way which has not been shown before. This may enable earlier diagnosis, more targeted treatment and improved prediction of outcomes in these complex patients.

I then assessed this technique in patients suffering from broken heart syndrome (reversible weakness of the heart muscle often due to stress). This has never been done before and we showed that the heart muscles behaved very differently during the event, which did not fully recover even after 3 or more months. This may help us understand better about the mechanisms involved in this condition and its long-term consequences. This technique could therefore help diagnose this condition as well as monitor the function of the heart.

Finally, there is major concern about the potential for long-term effects of COVID-19 on the heart. We know that those with evidence of heart damage are at higher risk of serious illness. I assessed patients who had recovered following hospitalisation with COVID-19 using magnetic resonance imaging and CT imaging of the heart arteries. I found no evidence on magnetic resonance imaging or CT scanning that patients were at higher risk of heart damage. Despite recovery, these patients had evidence of right heart injury, most likely secondary to ongoing lung injury.
In summary, I have performed clinical studies of a new technique for imaging the heart which holds major potential for enabling us to better understand heart disease, to diagnose heart disease earlier and to identify the patients most likely to benefit from established and emerging treatments.
iv. ACKNOWLEDGMENTS

This research was conducted and completed under the guidance and supervision of Professors David Newby, Marc Dweck, and Scott Semple at the University of Edinburgh. Professor Newby always made time to help me through my research and overcome, what felt like, never-ending manganese obstacles. I have learnt so much from him and will miss our “lets discuss” meetings. Professor Dweck from taught me everything about cardiac magnetic resonance imaging and Professor Semple has been unwavering in his support of this work in manganese imaging. I could not have had a more supportive environment to undertake my research and it has been a pleasure to work with them all.

I thank the Medical Research Council for their support of my research through the Clinical Research Training Fellowship. Without their generosity, this thesis would not have been possible. I also wish to acknowledge, all the volunteers and patients that have given their time to be part of clinical research.

I have been fortunate enough to have met some incredible people during my PhD. My fellow researchers, Anda Bularga, Mohammed Meah, Anna Barton, Shruti Joshi and Jennifer Nash have supported me through the highs and lows of research life. My colleagues at the Edinburgh Imaging Facility, who were so
welcoming and helpful throughout recruitment and scanning. It has been a pleasure to have worked alongside them.

Finally, to my family. To Dadu, I would not be who I am and where I am today without you. You taught me to work hard, never give up or take anything for granted. You always said that one day I would do a PhD, even if I was against it at the time. I guess you were right. To Ma, you put your children before everything and we would be lost without you! I could never say thank you enough for all that you do. To Vyom, my only regret during my PhD was that it took me away from you. And to James Richardson-Singh, no one else would tolerate me! Thank you for listening to me endlessly complain about manganese, reading all my research papers (multiple times), and always making me laugh.
Abbreviations

CI confidence interval
CT computed tomography
DCM dilated cardiomyopathy
DEMRI gadolinium delayed-enhancement magnetic resonance imaging
ECV% extracellular volume fraction
ETL echo train length
FOV field of view
HCM hypertrophic cardiomyopathy
LVEF left ventricular ejection fraction
MEMRI manganese-enhanced magnetic resonance imaging
MnDPDP manganese dipyridoxyl diphosphate
MOLLI modified Look-Locker inversion recovery
MRI magnetic resonance imaging
ROI region of interest
SD standard deviation
SERCA2a sarcoplasmic reticulum Ca2+-ATPase
ShMOLLI shortened modified Look-Locker inversion recovery
TD trigger delay
TE echo time
TI inversion time
TR repetition time
1. CHAPTER 1: INTRODUCTION

Extracts of this chapter have been published in:


Singh T et al. MINOCA: a heterogenous group of conditions associated with myocardial damage. Heart. 2021 Sep;107(18):1458-1464
1.1. **OVERVIEW**

Cardiac magnetic resonance imaging has a major role in the diagnosis, evaluation and tissue characterisation of a range of cardiovascular diseases (Rickers et al. 2005, Dass et al. 2012, Japp et al. 2016). Conventional cardiac magnetic resonance with gadolinium-based contrast media can be complemented by T1 mapping techniques which allow the quantitative assessment of myocardial tissue. Furthermore, T1 mapping can risk stratify and provide prognostic value in conditions, such as dilated or hypertrophic cardiomyopathy (Dass et al. 2012, Japp et al. 2016).

Manganese, a calcium ion analogue, has paramagnetic properties and can cross intact cell membranes via calcium channels, providing intracellular contrast of viable tissues, such as the liver, pancreas, kidneys, and heart, in response to active calcium-dependent cellular processes (Lauterbur PC 1978, Mendonca-Dias et al. 1983, Kang et al. 1984). The major interest in manganese-enhanced magnetic resonance imaging of the heart lies in its biological function as a calcium channel analogue, thus behaving as an intracellular contrast agent. In 1981, Hunter et al (Hunter et al. 1981) proposed that uptake of free manganese ions in the heart can be used to measure calcium uptake because manganese is retained intracellularly, and myocytes can be labelled without reduction in cardiac function by maintaining a very low manganese concentration in the perfusate. Manganese-enhanced magnetic resonance imaging has therefore been used as a surrogate marker for cellular
calcium handling and interest in its potential clinical applications has recently re-emerged, especially in relation to assessing cellular viability and myocardial function. Calcium homeostasis is central to myocardial contraction and dysfunction of myocardial calcium handling is present in various cardiac pathologies. Recent studies (Skjold et al. 2007, Spath et al. 2021) have demonstrated that manganese-enhanced magnetic resonance imaging can detect the presence of abnormal myocardial calcium handling in patients with myocardial infarction, providing clear demarcation between the infarcted and viable myocardium. As such, manganese-enhanced magnetic resonance imaging offers exciting potential to improve cardiac diagnoses and provide a non-invasive measure of myocardial function and contractility. This could be an invaluable tool for the assessment of both ischaemic and non-ischaemic cardiomyopathies, as well as reversible cardiomyopathies providing a measure of functional myocardial recovery, an accurate prediction of disease progression and a method of monitoring treatment response.

This chapter provides a synopsis of previously published work and discusses the potential future clinical applications of manganese-enhanced magnetic resonance imaging of the heart.
1.2. **MYOCARDIAL TISSUE CHARACTERISATION**

Magnetic resonance imaging is a well-established clinical imaging modality providing excellent soft-tissue delineation and spatial resolution. Gadolinium-enhancement magnetic resonance imaging is an essential tool in routine clinical practice, enabling identification and quantification of myocardial viability and fibrosis. Gadolinium, a paramagnetic lanthanide, is a valuable contrast agent in magnetic resonance imaging on account of its profound shortening effect on the T1 of tissues where it accumulates.

1.2.1. **Assessment of myocardial viability**

Viability refers to the potential of injured myocardium to recover and to contribute to the contractile function of the left ventricle. Being able to differentiate hibernating and viable myocardium from irreversibly damaged myocardium is crucial to patient selection for higher risk interventional or surgical revascularisation strategies. Viability imaging using gadolinium-enhanced magnetic resonance imaging is based on the principle that absence of enhancement must, by inference, mean viable myocardium: dichotomising myocardium according to the presence or absence of abnormal extracellular space. Using the kinetic properties of gadolinium-based media, T1-weighted imaging can be manually set to ensure minimal signal is generated from normal myocardium (“nulling” of the myocardium) and that regions of abnormal extracellular space appear bright white. These areas of late enhancement can be assessed qualitatively, where the pattern of enhancement will usually be able to describe the aetiology: sub-endocardial or transmural fibrosis.
suggestive of partial or full-thickness infarction respectively, and mid-wall fibrosis indicating a non-ischaemic aetiology.

When late gadolinium enhancement is used in patients with coronary artery disease, dysfunctional myocardium with normal nulled signal intensity suggests myocardial stunning or hibernation and an absence of infarction. In chronic myocardial infarction, late gadolinium enhancement characteristically involves the subendocardium or progresses to involve the entire transmural extent in myocardial segments subtended by a coronary arterial territory (Kim et al. 2000). In those with left ventricular dysfunction being evaluated for benefits of coronary revascularisation, the transmural extent of the late gadolinium enhancement provides a prediction of the stepwise decreasing likelihood of improvement in segmental myocardial contractility after coronary revascularisation.

Akinetic segments with no or minimal subendocardial infarction have a >90% chance of segmental recovery of contractile function if the involved coronary artery is successfully revascularised (Wagner et al. 2003). Those segments with >50% transmural extent of infarction have a <10% chance of segmental contractile recovery despite successful coronary revascularisation. In segments that demonstrate <50% transmural extent of infarction, functional recovery is not well predicted by the criteria using late gadolinium enhancement transmural extent alone. A cut-off for viability of <50% transmural late gadolinium enhancement shows high sensitivity (95%) and
negative predictive value (90%) but low specificity (51%) (Garcia et al. 2020). Furthermore, the transmurality of scar is assessed and quantified visually and will potentially be open to bias and inaccuracies from imaging artefact.

1.2.2. T1 mapping to quantify myocardial fibrosis

Diffuse myocardial fibrosis is thought to precede the replacement fibrosis detectable by gadolinium-enhanced imaging, representing an earlier stage of the pathophysiology owing to collagen deposition. This causes expansion of the extracellular compartment and can be detectable using established T1 weighted imaging sequences. A T1 map is generated by combining multiple images acquired in diastole, but with different inversion times, to assess T1 relaxation. In principle, this approach is attractive as it mitigates against the risk of diffuse myocardial pathology appearing normal when myocardium is nulled in conventional late gadolinium sequences, providing pixel-by-pixel T1 definition.

Several T1 mapping sequences have been developed. Perhaps the most commonly used in current clinical practice is the MOdified Look-Locker Inversion recovery (MOLLI) (Messroghli et al. 2004) which uses magnetisation inversion and then samples T1 using single shot stead-state free precession readouts several times during its recovery. The resulting data points can be used to generate a best-fit T1 decay curve from which T1 is estimated. Shortened MOdified Look-Locker Inversion recovery (ShMOLLI) is an abbreviated scheme which operates on the same principles but samples fewer
data points for curve-fitting resulting in a shorter breath-hold (Piechnik et al. 2010). SATuration recovery single-Shot Acquisition (SASHA) sequences use saturation pulses (90° rather than 180°) (Chow et al. 2014) without inversion-pulse preparation, effectively removing the previous cycle’s magnetisation and samples it once during recovery enabling a more direct estimation of T1. However, this approach is prone to artefacts and gives lower signal-noise-ratio (Chow et al. 2014). Whilst all 3 sequences demonstrate excellent reproducibility, SASHA offers greater accuracy but inferior precision to MOLLI and ShMOLLI (Roujol et al. 2014)

1.3. HISTORICAL BACKGROUND: MANGANESE

Manganese was the first element used as a contrast agent in magnetic resonance imaging (Lauterbur PC 1978, Mendonca-Dias et al. 1983, Kang et al. 1984). Certain characteristics make it a desirable contrast agent. Like gadolinium, it has paramagnetic properties thereby shortening T1 relaxation of water, enabling increased contrast in tissues where it accumulates and enhancing anatomical delineation (Lauterbur PC 1978, Massaad et al. 2011). Manganese is also a naturally occurring trace element in the human body which is required for several biochemical processes through its role as a co-factor in the activation of several classes of enzymes (Hirano et al. 1996, Santamaria 2008). Humans obtain most of their manganese requirements through the diet and it is eliminated predominantly through the kidneys and liver (Gurol et al. 2022). These properties contrast with gadolinium which is toxic in its free unbound form and has no known biological function in humans.
An important imaging characteristic of manganese ions is that they behave in a similar manner to calcium ions and are actively taken up by L-type voltage-gated calcium channels and sodium-calcium exchangers (Du et al. 2001, Wendland 2004, Spath et al. 2019). This means that uptake is seen in viable tissues, particularly those with a predominance of calcium channel activity, such as the liver, pancreas, brain, kidney and heart (Figure 1.1).

Although also an analogue of calcium ions, gadolinium ions are fully chelated in gadolinium-based contrast media, because of the need to prevent gadolinium ion dissociation and any associated toxicity. As such, gadolinium-based contrast media do not cross the cell membrane and accumulate in the extravascular extracellular space before returning to the circulation for excretion by the kidneys. Due to its strong paramagnetic properties and subsequent commercial development, gadolinium-based media became the preferred contrast agent, eventually leading to a decline in interest for manganese-based contrast media.
Figure 1.1 Manganese Uptake in the Body

Manganese-enhanced images (panel A) at native T1 and post-manganese T1 (30 min) demonstrating manganese enhancement in the liver (L), pancreas (P) and kidneys (K). Conversely, little enhancement is seen in skeletal muscle (SM). T1 decay curves (panel B) demonstrate greatest reduction in T1 in liver (green), followed by pancreas (light blue), kidney (green), heart (red) and skeletal muscle (dark blue). Dotted line represents end of manganese dipyridoxyl diphosphate infusion. Manganese uptake (panel C) in liver (green), pancreas (light blue), kidney (green), heart (red) and skeletal muscle (dark blue).
1.4. **FORMULATIONS**

The formulations of manganese-based contrast media define how they behave in vivo. They determine whether there is any associated toxicity, whether they can act as an intracellular contrast agent or whether they behave as an extracellular contrast agent comparable to gadolinium-based contrast media.

1.4.1. **Non-chelated forms of manganese**

Manganese chloride was one of the earliest manganese-based contrast media to be used in magnetic resonance imaging and can be administered orally. Once absorbed, manganese chloride freely dissociates into manganese and chloride ions. The manganese ions are strongly paramagnetic and actively enter myocytes via voltage-gated calcium channels. However, due to competition with calcium, early studies demonstrated acute cardiac compromise, especially with high doses of manganese (Fernandes et al. 2011). Lower concentrations of manganese have not been associated with serious short term adverse effects (Fernandes et al. 2011).

1.4.2. **Partially chelated**

Subsequent work has sought to overcome the potential acute toxicity of high dose manganese ions. For intracellular myocardial imaging, manganese must be freely available for cardiomyocyte uptake. To date, two different methods have been employed producing distinct clinical-grade agents, both with
excellent safety profiles: (1) co-administration with calcium, and (2) partial chelation:

**Co-administration with calcium gluconate**

To counter the adverse effects of free manganese ions, intravenous manganese co-administered with calcium gluconate can result in a short plasma half-life and rapid myocardial uptake with little redistribution (Storey et al. 2003, Storey et al. 2006). This has been developed for clinical use as EVP 1001-1 (SeeMore, Eagle Vision Pharmaceuticals, Downingtown, USA), and it has reduced the toxic effects of high dose manganese ions and achieved sufficient T1 relaxation for imaging. Currently, this formulation has not been approved for clinical imaging.

**Manganese dipyridoxyl diphosphate**

Unlike stronger chelates which are designed not to dissociate, dipyridoxyl diphosphate chelation allows manganese to uncouple and circulate as a protein-bound complex (Toft et al. 1997). The dynamics of myocardial uptake of manganese dipyridoxyl diphosphate have been described in detail elsewhere (Wendland 2004). In brief, after intravenous administration in humans, manganese dipyridoxyl diphosphate undergoes dephosphorylation and transmetallation with zinc to release manganese ions into the plasma (Gallez et al. 1996, Hustvedt et al. 1997, Toft et al. 1997, Wang et al. 1997, Schmidt et al. 2002). This controlled release of manganese ions facilitates

Myocardial and bloodpool shortening of T1 relaxation occurs using the approved dose of 5 µmol per kg bodyweight of manganese dipyridoxyl diphosphate and, shortening of T1 relaxation did not increase at higher doses (Kang et al. 1984, Scholz 1994, Toft et al. 1997, Gunter et al. 2004). In humans, 70% of cytosolic calcium is from sarcoplasmic reticulum stores and approximately 30% is from extracellular uptake (Wendland 2004). This, and the higher distribution of calcium channel activity in the liver, pancreas and kidneys, could explain the relatively lower uptake of manganese in the myocardium (Figure 1.1). To date, manganese dipyridoxyl diphosphate (Teslascan; General Electric Healthcare) is the only manganese-based contrast medium to have been approved for magnetic resonance imaging in humans, with a primary indication for hepatic tumour imaging (Sutcliffe et al. 2011).

1.4.3. Fully chelated

Biocompatible macromolecular manganese-based contrast media have been generated using O-carboxymethyl chitosan (CMCS), diethylenetriamine pentaacetate (DTPA) and manganese (Mn). According to in vitro studies, CMCS-(Mn-DTPA) exhibits good cellular and blood biocompatibility at doses
necessary for magnetic resonance imaging. The relaxivity of CMCS-(Mn-DTPA) is approximately 3.5 and 5.5 times higher than that of gadolinium-DTPA and manganese dipyridoxyl diphosphate respectively (Huang et al. 2011, Wang et al. 2019). However, this is only when it is chelated to DTPA. Similar to gadolinium-DTPA, aqueous CMCS-(Mn-DTPA) is stable enough to prevent the release of any manganese ions. Thus, partial chelation of manganese enables it to act as an intracellular agent, whereas full chelation results in a stronger paramagnetic agent but one that image the extracellular rather than intracellular compartment (Figure 1.2, Table 1.1) (Huang et al. 2011).
Figure 1.2 Types of Manganese-based Contrast Media

Manganese Chloride (A), Manganese gluconate (B), Manganese dipyridoxyl diphosphate (C) and O-carboxymethyl chitosan manganese-diethylenetriamine pentaacetate are examples of non-chelated and chelated manganese-based contrast agents respectively.

MnCl\textsubscript{2}, Manganese Chloride, EVP 1001-1, Manganese gluconate, MnDPDP, Manganese dipyridoxyl diphosphate, CMCS (Mn-DTPA), O-carboxymethyl chitosan manganese-diethylenetriamine pentaacetate
<table>
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<tr>
<th>Contrast agent</th>
<th>Clinical dose (µmol/kg)</th>
<th>Approved for clinical use</th>
<th>Previous or currently- recruiting studies</th>
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<tr>
<td>Partial: MnDPDP</td>
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<td>Yes</td>
<td>Manganese-enhanced MRI (MEMRI) of the myocardium (NCT03607669)</td>
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<td>The DAPA-MEMRI Trial (NCT04591639)</td>
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<td>PANCREAS MEMRI (NCT05298735)</td>
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<tr>
<td>Full: CMCS (Mn-DTPA)</td>
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<td>None</td>
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<tr>
<td><strong>Non-chelated</strong></td>
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</tr>
<tr>
<td>EVP1001-1</td>
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<td>No</td>
<td>Clinical Trial of MEMRI to assess peri-infarct Injury (NCT02933034)</td>
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<td>Efficacy of EVP1001-1 in the Assessment of myocardial viability in patients with cardiovascular disease (NCT01989195)</td>
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*MnDPDP, Manganese dipyridoxyl diphosphate, CMCS(Mn-DTPA), O-carboxymethyl chitosan manganese-diethylenetriamine pentaacetate, MnCl2†, Manganese chloride*
1.5. **SAFETY**

Historically, there have been safety concerns regarding the early forms of manganese-based contrast media (Brurok et al. 1995, Brurok et al. 1997). Early pre-clinical studies predominantly used manganese chloride which competed too strongly with myocardial calcium uptake, causing myocardial depression, hypotension and cardiac arrest (Brurok et al. 1997, Gavin et al. 1999, Tanaka et al. 2002). Partially chelated formulations, such as manganese dipyridoxyl diphosphate, have been used in several clinical studies in patients with varying cardiac pathologies and demonstrates an excellent safety profile (Fernandes et al. 2011).

Although gadolinium-based contrast agents are safe and well tolerated, it is important to remember that early formulations were toxic. In its free state, gadolinium is highly toxic and this is primarily attributed to its ions interfering with many calcium ion channel-dependent processes (Sieber et al. 2008). For nearly a decade, there was an association between gadolinium-based contrast media and the development of nephrogenic systemic fibrosis in patients with severe renal impairment (Grobner 2006). This risk was much higher in patients given acyclic gadolinium-based contrast media. As such, these formulations (Gd-DTPA, Gd-DTPA-BMA, and Gd-DTPA-BMEA) have been suspended by the European Medicines Agency and cases of nephrogenic systemic fibrosis have subsequently fallen dramatically.
It was previously widely believed that gadolinium-based contrast agents are rapidly and completely eliminated from the human body. However, gadolinium can accumulate in tissues, such as the brain (Murata et al. 2016), bone (Rogosnitzky et al. 2016) and kidneys (Wáng et al. 2015), in patients who have received gadolinium-based contrast media despite normal renal function. Furthermore, retention of gadolinium can be higher in those who have repeated exposure (Wahsner et al. 2019). Further work is needed to clarify the propensity of macrocyclic agents to accumulate in the central nervous system, and to establish if this is associated with any clinical sequelae. These safety concerns highlight our current dependence on gadolinium and the relative lack of alternatives.

The risk of manganese accumulation with manganese-based contrast agents is minimal as manganese is naturally eliminated from the body through several processes. Toxicity from over-exposure is recognised and can result in manganese accumulation in the globus pallidus, manifesting as headaches, gait disturbance and extra-pyramidal symptoms and is referred to as ‘manganism’ (Jiang et al. 2005). However, this has only been observed following major environmental or occupational exposure over the course of years.
1.6. **MYOCARDIAL CALCIUM HANDLING**

Myocardial contraction is controlled by excitation-contraction coupling which allows for rapid changes in calcium ion concentrations in the sarcoplasmic reticulum leading to contraction (systole) and relaxation (diastole, Figure 1.3) (Bers et al. 1999, Bers 2000). Calcium homeostasis in the myocardium is essential for this and is controlled by several mechanisms. During systole, calcium ions are actively transported into the sarcoplasmic reticulum via L-type voltage-gated calcium channels. Calcium ions bind to ryanodine receptors resulting in the efflux of an even higher concentration of calcium ions from the sarcoplasmic reticulum into the cytosol (Bers et al. 1999, Bers 2000, Wuytack et al. 2002). “Calcium induced, calcium release” in turn activates calcium-sensitive contractile proteins (troponin C, troponin NC) which leads to myocardial contraction. During diastole, sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA 2a) facilitates calcium entry back into the sarcoplasmic reticulum in addition to their exit into the extracellular space via the sodium–calcium exchanger and mitochondrial uptake (Bers et al. 1999, Blaustein et al. 1999, Bers 2000). The net result is a reduced calcium concentration in the cytosol and myocardial relaxation. Phospholamban, a regulatory protein, promotes calcium efflux, resulting in reduced myocardial contraction. When phosphorylated, it causes disinhibition of SERCA 2a and subsequent increase in myocardial contraction (Bers 2000).
1.6.1. Myocardial manganese uptake

The major interest in manganese lies in its biological functionality. In 1970, Ochi et al (Ochi 1970, Ochi 1976) first demonstrated that manganese ions were taken up by L-type voltage-gated calcium channels in cardiomyocytes (Ochi 1976). Thus, abnormal myocardial calcium handling would result in reduced manganese uptake. This led to the idea that manganese could be used as a surrogate marker of calcium handling. Once taken up, it is retained for hours, and unlike calcium ions, does not redistribute between the intracellular and extracellular compartments (Figure 1.3).
Figure 1.3 Myocardial Calcium Homeostasis and Manganese Uptake

Calcium homeostasis in normal myocardium (A) and Manganese uptake via voltage gated calcium channels (B).

Ca^{2+}, Calcium ion, Na^{+}, Sodium ion, H^{+}, Hydrogen ion, Mn^{2+}, Manganese ion, NCX, Sodium-calcium exchangers, NHE-1, Sodium hydrogen exchanger, RyR, ryanodine receptors, PLB, Phospholamban, SERCA, sarcoplasmic reticulum calcium adenosine triphosphatase
1.7. **MANGANESE T1 MAPPING AND PATLAK MODELLING**

Manganese causes shortening of T1 relaxation time of water protons due to its paramagnetic properties. Manganese-enhanced magnetic resonance is therefore best visualised and quantified using T1 weighted imaging. During the initial infusion phase, there is a reduction in blood pool T1 followed by normalisation to baseline by 30 min (Figure 1.4). Myocardial T1 values also demonstrate a rapid initial descent (infusion phase) but this is followed by a plateau phase, likely attributable to ongoing intracellular myocardial uptake and accumulation until renal and biliary excretion is complete.
Figure 1.4 Manganese-Enhanced Magnetic Resonance Imaging of Healthy Myocardium

Short axis T1 mapping in healthy myocardium with manganese dipyridoxyl diphosphate (panel A). Rapid reduction in T1 is seen in the blood pool (green, B), followed by rapid normalisation by 30 min. In contrast, the T1 value of myocardium (red) shows steady and sustained reduction throughout the imaging time period (panel B).

Dotted line represents end of manganese dipyridoxyl diphosphate infusion.
Myocardial manganese uptake can be quantified by tracer kinetic modelling. The commonest modelling approach is based on a Patlak two-compartment model formulation. This assumes the influx of manganese ions from a reversible ($v_e$, extracellular and vascular space) into a largely irreversible compartment ($v_i$, cardiomyocyte during the imaging period). This apparent unidirectional influx constant ($Ki$) for the transfer of manganese from plasma to irreversible compartments $v_i$, can be measured, using Equation 1:

$$\frac{C_t(t)}{C_a(t)} = Ki \int_0^t \frac{C_a(r)dr}{C_a(t)} + v_e$$

where $C_t$ and $C_a$ are the manganese concentration in myocardial tissue and blood pool (arterial input function) respectively. This formula is equivalent to the Patlak model (Patlak et al. 1983) and describes that if a contrast medium is irreversibly trapped in the tissue within the imaging period, the instantaneous tissue concentration (myocardial T1) divided by the instantaneous arterial concentration (bloodpool T1) plotted against the integrated arterial concentration divided by the instantaneous arterial concentration, will result in linearisation of the data (Figure 1.5) (Patlak et al. 1983, Skjold et al. 2006). Skjold et al have previously modelled the change in myocardial unidirectional influx over different administration rates. Only minor differences in myocardial manganese uptake were seen between administration of manganese over 5 or 30 minutes (Skjold et al. 2006, Spath et al. 2020, Singh et al. 2021).
Figure 1.5 Patlak modelling

Patlak formulation – schematic of (A) model compartments and transfer constant $K_i$, describing passage from reversible to irreversible compartment (B) data analysis

$C_m(t)$ Myocardial manganese concentration

$C_b(t)$ Blood manganese concentration
1.8. CLINICAL STUDIES

With increasing interest in manganese-enhanced magnetic resonance imaging, this technique has been translated to assess myocardial calcium handling in early clinical studies. Skjold et al were the first to demonstrate the effectiveness of manganese in imaging the healthy human myocardium (Skjold et al. 2004). They reported a sharp reduction in T1 relaxation after manganese dipyridoxyl diphosphate administration which was followed by a consistent reduction in myocardial T1 relaxation (34-46%) (Skjold et al. 2004).

1.8.1. Myocardial Infarction

*Myocardial calcium handling in myocardial infarction*

After a period of ischaemia and hypoxia, myocardial cells within the infarct region demonstrate major metabolic abnormalities, resulting in myocardial calcium mishandling. Intracellular hydrogen ion accumulation causes a low intracellular pH, and intracellular sodium increases through sodium-hydrogen exchange (Murphy et al. 2008). Excessive intracellular sodium will promote sodium excretion and calcium intake by sodium-calcium exchange, which increases intracellular calcium levels, leading to calcium overload. This can cause a series of irreversible cell injury responses, such as cardiac contractile dysfunction and apoptosis. Furthermore, when blood flow and oxygen supply to the cardiac tissue returns to normal, intracellular calcium overload is further aggravated (Murphy et al. 2008).
The peri-infarct zone is an area of heterogenous myocardial scar containing fibrotic tissue intermingled with viable cardiomyocytes. This region is important because, in the acute phase, cells may demonstrate myocardial stunning. This refers to persistent myocardial contractile dysfunction transiently induced by acute ischaemia despite reperfusion and absence of irreversible damage. It is clear that stunned myocardium is characterised by abnormalities in excitation-contraction coupling (Marban 1997). However, despite intense research efforts, there remains some ongoing controversy as to the exact nature of these abnormalities. Contractile abnormalities can arise from either a decrease in calcium-availability, such as from sarcoplasmic reticular dysfunction, or a decreased responsiveness of the myofilaments to calcium, such as from proteolysis of troponin I (Marban 1997).

**Acute Myocardial infarction**

Various animal models have assessed quantification of myocardial infarction with manganese-enhanced magnetic resonance imaging, using coronary artery ligation or occlusion models. Early studies describe the ability of manganese to differentiate between normal and infarcted tissue. Interestingly, areas with reduced manganese uptake were greater than histopathological quantification of infarcted myocardium (Kim et al. 2005) suggesting that stunned myocardium also has reduced manganese uptake consistent with calcium mishandling.
More recently, serial manganese-enhanced magnetic resonance imaging has been used to investigate the time course of changes in patients with acute myocardial infarction (Spath et al. 2021). Regions with transmural infarction demonstrated partial recovery of T1 values similar to that of the bloodpool (Figure 1.6). The lack of manganese uptake here likely represents absence of myocardial calcium handling in the infarct area secondary to myocardial necrosis and absent myocyte viability. Unlike late gadolinium enhancement where contrast agent accumulates in areas of myocardial necrosis, manganese uptake occurs in viable tissue and absence or reduction of manganese uptake suggests absent or impaired calcium handling, thereby behaving as an inverse of gadolinium imaging (Figure 1.6). Late gadolinium enhancement is the gold standard for visualisation and quantification of myocardial infarction size. Interestingly, manganese-enhanced magnetic resonance imaging was able to quantify infarct size more accurately than late gadolinium enhancement (Spath et al. 2021). This is likely due to the latter being non-specific and overestimating infarct territory due to acute oedema (Spath et al. 2020, Spath et al. 2021).

Manganese-enhanced magnetic resonance imaging was more sensitive than late gadolinium imaging in detecting dysfunctional myocardium and tracked more closely with abnormal wall motion. A step-wise reduction was seen in manganese uptake across remote, peri-infarct and infarcted myocardium (Spath et al. 2021). After 3 months, myocardial manganese uptake in the peri-infarct region was similar to that of the remote regions. This suggests that the
lack of manganese uptake in the peri-infarct area may represent myocardial stunning.
Figure 1.6 Manganese-Enhanced Magnetic Resonance Imaging in Acute Myocardial Infarction

Short-axis views of gadolinium-enhanced, native and 30-min post-manganese T1 maps (panel A) images of a patient with an acute anteroseptal myocardial infarction. Gadolinium-enhanced images demonstrate presence of late gadolinium in the anteroseptal wall. Conversely, manganese-enhanced images demonstrate reduced manganese uptake (abnormal calcium handling, green) in the anteroseptal wall. Mean T1 decay times in bloodpool (green), infarct (red), peri-infarct (orange) and remote region (blue) in patients with acute myocardial infarction (panel B). Mean myocardial manganese uptake (Ki- ml/min/100g of tissue) defined by Patlak modelling in patients with acute myocardial infarction and healthy volunteers (Panel C).
**Chronic Myocardial Ischaemia**

Beyond infarct quantification, manganese-enhanced magnetic resonance imaging has the potential to assess myocardial viability with potential application to myocardium with chronic myocardial contractile dysfunction secondary to ischaemia: so-called ‘hibernating’ myocardium. Such ‘hibernating’ cardiomyocytes remain viable and often restoration of blood flow will lead to some degree of improvement in left ventricular ejection fraction. Thus, identifying myocardium with potential for improvement in contractility is vital to determine the appropriateness of coronary revascularisation.

\(^{18}\text{F}-\text{Fluorodeoxyglucose positron emission tomography (}\(^{18}\text{F}-\text{FDG PET)}\) is considered the reference standard for assessing myocardial viability (Windecker et al. 2014, Dilsizian et al. 2016) offering the greatest sensitivity for viable myocardium and comparable specificity to other imaging modalities. Although imaging in this way directly assesses viability through metabolic functionality of tissues (a mechanistically similar method of myocardial viability assessment to manganese-enhanced magnetic resonance imaging), its use is often limited by availability, expense and expertise. This, combined with the properties of manganese, led to the investigation of manganese-enhanced magnetic resonance imaging to assess and to quantify myocardial viability directly. Preclinical studies have validated direct quantification of myocardial viability using manganese-enhanced magnetic resonance imaging compared
with 18F-FDG PET, suggesting both cellular calcium and glucose uptake are robust and concordant markers of myocardial viability (Spath et al. 2020).

Due to radiation exposure and lack of 18F-FDG PET availability, gadolinium became invaluable in identifying scarred non-viable myocardium. However, viability in gadolinium-enhanced imaging is simply inferred and cannot be measured directly or quantitively. In a coronary artery ligation model, ischaemia reperfusion was assessed by administering manganese dipyridoxyl diphosphate acutely with vessel occlusion. This demonstrated reduced manganese-enhancement correlated with area at risk (viable myocardium) by methylene blue but interestingly not when administered beyond 6 hours after reperfusion when reduced manganese-enhancement correlated with histopathological infarction (Yang et al. 2005). This implies that manganese-enhanced magnetic resonance imaging enhances myocardium within injured but not infarcted myocardium and could be an important biomarker of viability.

Skjold et al (Skjold et al. 2007) were the first to describe manganese-enhanced magnetic resonance imaging in patients with prior established myocardial infarction. They described the ability of manganese-enhanced magnetic resonance to characterise and to quantify viable myocardium directly (Figure 1.6). This confirmed the potential to detect viable myocardium which could prove an invaluable tool in patient selection for revascularisation therapies. Moreover, it holds particular promise as a biomarker of treatment efficacy for interventional strategies targeting ischaemia-reperfusion injury.
1.9. VALIDATION OF MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING

1.9.1. Validation of gadolinium-enhanced magnetic resonance imaging.

Reproducibility of cardiac MRI is excellent, with robust intra- and inter-observer variability for volumetric assessment (intraclass correlation coefficient >0.9 for all left and right volumetric and mass measurements, n=60) (Mooij et al. 2008). Late gadolinium enhancement has been previously validated and have demonstrated strong reproducibility in various cardiac pathologies (Spiewak et al. 2010, Flett et al. 2011). Measurement of T1 using mapping at 3T offers excellent reproducibility, with intraclass coefficients of ≥0.99 for pre- and post-contrast T1 in myocardium and blood pool (Chin et al. 2014). In particular, MOLLI and ShMOLLI have demonstrated greater precision than other T1 mapping sequences in healthy volunteer studies (Shao et al. 2017).

Whilst native T1 has good reproducibility (Dekkers et al. 2019), individual post-gadolinium T1 mapping values can be highly variable between individuals due to differing contrast kinetics. As such, a correction factor is used by calculating the partition co-efficient (l), calculated by dividing the change in myocardial T1 by the change in blood pool T1 pre- and post-contrast, effectively correcting for differences in gadolinium kinetics between individuals. It is a well-established quantitative imaging biomarker of fibrosis and early indicatory of extracellular volume expansion (Flett et al. 2010, Sado et al. 2012, Miller et al. 2013, de Meester de Ravenstein et al. 2015). Extracellular volume fraction calculation also showed robust reproducibility in addition to superior scan-
rescan reproducibility compared to other T1 mapping measures (Chin et al. 2014).

1.9.2 Validation of manganese-enhanced magnetic resonance imaging.

Repeatability and reproducibility of the assessment of manganese-enhanced magnetic resonance imaging and its scan-rescan reproducibility has not been established and is a necessary step for its future clinical application. It is therefore essential to validate the intraobserver and interobserver repeatability of T1 mapping and kinetic modelling, and the overall scan-rescan reproducibility, of myocardial manganese-enhanced magnetic resonance imaging.
1.10. **MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING IN NON-ISCHAEMIC CARDIOMYOPATHY**

1.10.1. **Myocardial calcium handling in heart failure**

Due to the fundamental role of calcium in excitation-contraction coupling, dysfunctional myocardial calcium handling is central to the pathophysiology of the failing myocardium. The amount of calcium entering the cytoplasm and its rate of removal are major factors in determining rate, intensity and duration of myocardial contraction (Yue et al. 1986).

Several mechanisms contribute to disrupted calcium handling in systolic dysfunction. First, release of calcium from the sarcoplasmic reticulum is reduced which in turn impairs calcium-induced calcium release (Beuckelmann et al. 1992, Kubo et al. 2001, Piacentino et al. 2003). Second, phosphorylation of L-type voltage-gated calcium channels is increased during heart failure which results in calcium leaking from the sarcoplasmic reticulum (Lou et al. 2012). Third, there is a substantial reduction in SERCA2a expression in patients with ischaemic cardiomyopathy and to a lesser degree with dilated cardiomyopathy (Siri-Angkul et al. 2021). On the other hand, phospholamban (PLN) expression and phosphorylation were relatively unchanged (Luo et al. 2013). This not only reduces intracellular uptake of calcium via SERCA2a but also increases PLN: SERCA ratio resulting in predominately calcium efflux and inhibition of myocardial contraction (Lou et al. 2012). Finally, although ryanodine receptor expression remains unchanged in the failing myocardium,
its function is calcium dependent and therefore affected by intra-cellular calcium concentration. Together these factors result in reduced calcium release from the sarcoplasmic reticulum (Figure 1.7) (Hasenfuss et al. 1992, Gómez et al. 1997).

Myocardial calcium handling is also an important determinant of cardiac diastology and the ability of the heart to relax and fill. To achieve relaxation, cytosolic calcium must be sequestered, mainly to the sarcoplasmic reticulum by SERCA2a (He et al. 1997). Diastolic calcium is increased in human heart failure, a condition that is likely related, at least in part, to defects in cytosolic calcium removal (Gwathmey et al. 1987, Beuckelmann et al. 1992, Piacentino et al. 2003). Elevated intracellular sodium and altered sodium channel properties are present in failing myocardium of humans (Pieske et al. 2002, Maier 2009). Changes in intracellular sodium may have a large impact on calcium homeostasis (Verdonck et al. 2004). Small elevations in sodium, increases calcium influx via reverse-mode sodium-calcium exchange during systole and limits calcium extrusion via forward mode sodium-calcium exchange during diastole. The reduced rate of calcium removal reduces the rate of recovery and is associated with marked delay in myocardial relaxation. Calcium overload contributes to arrhythmias and diastolic dysfunction (Figure 1.7) (Wendt-Gallitelli et al. 1993, Pieske et al. 2003).
Several factors cause reduced calcium ion (Ca\(^{2+}\)) release from the sarcoplasmic reticulum resulting in systolic heart failure (A). Diastolic heart failure results from reduced rate of Ca\(^{2+}\) removal causing delay in myocardial relaxation (B). Ca\(^{2+}\), Calcium ion, Na\(^{+}\), Sodium ion, H\(^{+}\), Hydrogen ion, Mn\(^{2+}\), Manganese ion, NCX, Sodium-calcium exchangers, NHE-1, Sodium hydrogen exchanger, RyR, ryanodine receptors, PLB, Phospholamban, SERCA, sarcoplasmic reticulum calcium adenosine triphosphatase.
1.10.2. Dilated Cardiomyopathy

Dilated cardiomyopathy is the commonest form of cardiomyopathy and is characterised by left ventricular dilatation and dysfunction in the absence of abnormal loading conditions or ischaemia. It predominantly affects younger adults and is the most frequent indication for cardiac transplantation. Dilated cardiomyopathy represents a wide spectrum of conditions and best regarded not as a single disease entity, but rather as a nonspecific phenotype, the final common response of myocardium to several genetic and environmental insults.

The true prevalence of dilated cardiomyopathy, and of genetically mediated dilated cardiomyopathy, is not fully known. It varies with geographic and ethnic differences and is likely underestimated. In clinical practice and current guidelines, the prevalence of familial dilated cardiomyopathy is assumed to be approximately 30% to 50% (Grünig et al. 1998, Mestroni et al. 1999, Elliott et al. 2008, Sweet et al. 2015, Bozkurt et al. 2016). In patients with familial dilated cardiomyopathy, approximately 40% have an identifiable genetic cause (Ganesh et al. 2013). In sporadic dilated cardiomyopathy, pathogenic genetic variants can be identified although the frequency of genetic causes in this population is not well defined (Ganesh et al. 2013).

The clinical manifestations of dilated cardiomyopathy ranges from none to overt heart failure. With the increase in familial and genetic screening, it is now commoner to identify the minimally to mildly affected stage in younger individuals (McNally et al. 2017). There is little in the clinical evaluation that
makes it possible to distinguish one genetic subtype of dilated cardiomyopathy from another. This phenocopying is what has driven gene panel testing because with this approach, multiple genes are screened at the same time.

Magnetic resonance imaging is gold standard for the diagnosis of dilated cardiomyopathy with assessment of chamber dimensions and function, including strain measurements. Furthermore, it can differentiate between other cardiomyopathies and exclude previous myocardial infarction. Gadolinium-enhanced magnetic resonance imaging is used to identify fibrosis and therefore provide additional information on myocardial tissue quality. In dilated cardiomyopathy, the degree of fibrosis defined by delayed gadolinium enhancement is a predictor of all-cause mortality as well as the risk of future hospitalisation (Assomull et al. 2006) and ventricular arrhythmias (Gao et al. 2012, Gulati et al. 2013, Perazzolo Marra et al. 2014). Interestingly, late gadolinium enhancement may be present even when the heart appears normal. Similarly native T1 can detect diffuse subclinical fibrosis (Nakamori et al. 2018, Nakamori et al. 2018) and is an important predictor of ventricular tachycardia and ventricular fibrillation. This may provide additive risk stratification for primary prevention implantable-cardiac defibrillator in patients with dilated cardiomyopathy (Nakamori et al. 2020).

Dysfunctional calcium handling is a central feature of left ventricular dysfunction in patients with dilated cardiomyopathy, with alterations in calcium handling proteins leading to reduced myocardial contractile function (Lou et al.
As such, the ability to detect and to monitor calcium handling non-invasively has great potential to be used as a marker for diagnosis, risk stratification, disease progression and response to therapy.

1.10.3. **Hypertrophic Cardiomyopathy**

Hypertrophic cardiomyopathy is a primary myocardial disorder leading to myocyte remodelling, disorganisation of sarcomeric proteins, impaired energy metabolism and altered cardiac contractility. It is characterised by cardiac hypertrophy in the absence of abnormal loading conditions (valvular disease, hypertension, congenital heart defects and structural disease). The prevalence of hypertrophic cardiomyopathy is estimated at 0.2% to 0.5% in the general adult population (Maron et al. 1995, Maron et al. 1999, Zou et al. 2004). However, estimating the prevalence of hypertrophic cardiomyopathy based on detection of cardiac hypertrophy, although clinically valuable, has many limitations. Notable among them is the age-dependent manifestation of cardiac hypertrophy: a half of patients with the underlying causal mutations developing hypertrophy by the third decade and three quarters by the sixth decade (Niimura et al. 1998).

Hypertrophic cardiomyopathy presents as a wide clinical spectrum, ranging from patients being asymptomatic with normal life expectancy, to those with ventricular arrhythmia, sudden cardiac death, or heart failure. Cardiac imaging is essential in the diagnosis, monitoring and prognostication of this condition. Magnetic resonance imaging is particularly useful for morphological
assessments, such as asymmetrical septal hypertrophy, apical hypertrophy and localised myocardial hypertrophy, as well as functional assessments, such as left ventricular outflow tract obstruction and mitral regurgitation. However, late gadolinium enhancement is perhaps the most valuable aspect of magnetic resonance imaging in hypertrophic cardiomyopathy. It identifies myocardial fibrosis, which substantially increases the risk of ventricular tachyarrhythmia and sudden cardiac death associated with hypertrophic cardiomyopathy (De Cobelli et al. 2009, Takeda et al. 2013). T1 mapping with extracellular volume quantification have shown potential in adding to current risk stratification (Avanesov et al. 2017). Furthermore, it can also be useful for differentiating hypertrophic cardiomyopathy from hypertensive heart disease, Fabry’s disease or cardiac amyloidosis (Sado et al. 2013, Hinojar et al. 2015).

Genetic mutations in patients with hypertrophic cardiomyopathy result in increased mitochondrial activity and altered myofilament calcium sensitivity involving both calcium-dependent and independent processes (Viola et al. 2016, Viola et al. 2016), resulting in increased left ventricular wall thickness with associated diastolic and systolic dysfunction. Furthermore, dysfunctional calcium handling appears to precede alterations in metabolic activity and is associated with an increased risk of arrhythmogenesis (Coppini et al. 2018).

There are no animal models of manganese-enhanced magnetic resonance imaging in hypertrophic cardiomyopathy, but manganese-enhanced magnetic resonance imaging has been evaluated in a murine model of myocardial
hypertrophy. Pharmacological induction of hypertrophy was achieved using an infusion of isoproterenol. Following manganese dipyridoxyl diphosphate administration, there was clear reductions in myocardial T1 relaxivity and reduced manganese uptake in both the septum and the free-wall of the left ventricle, mimicking hypertensive heart disease (Andrews et al. 2015).

Imaging techniques of late gadolinium enhancement and pre and post contrast T1 mapping do not provide information on cardiomyocyte function due to the extracellular distribution of gadolinium-based contrast media. This limits the ability to detect very early myocardial dysfunction and to understand the in vivo pathophysiology of different disease states. Therefore, assessment of myocardial calcium handling with manganese-enhanced magnetic resonance imaging would potentially be invaluable in patients with hypertrophic cardiomyopathy.
1.11. MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING IN TAKOTSUBO SYNDROME

1.11.1. Takotsubo Syndrome

Takotsubo syndrome was first named by Sato et al in 1990 (Sato TH et al. 1990) although sudden and rapid death following intense psychological stress was described by Rees and Engel more than 50 years ago (Rees et al. 1967, Engel 1971). Following some initial scepticism, awareness of this potentially fatal condition has substantially increased over the last 10-15 years (Minhas et al. 2015, Pattisapu et al. 2021) and is now an established increasingly frequent and important cause of acute cardiac presentations (Pattisapu et al. 2021).

Takotsubo syndrome has several unique characteristics, which distinguish it from other acute cardiac emergencies. It is typified by non-obstructed coronary arteries associated with a characteristic antero-septal-apical dyskinetic “ballooning” of the left ventricle with hyperkinetic basal segments (Figure 1.8). These features spontaneously resolve to restore apparently normal left ventricular function (Tsuchihashi et al. 2001, Kurisu et al. 2002). Despite being described 30 years ago, its aetiology and pathophysiology remains poorly understood and the lack of treatments often presents a clinical dilemma for physicians.
Figure 1.8 Takotsubo Syndrome: Anatomical variants

Left ventriculogram demonstrating apical (A, B) ballooning of the left ventricle, similar to the shape of a Japanese octopus trap I. Basal (D, E), mid-ventricular (F, G) and focal (H,I) variations of Takotsubo syndrome.
Takotsubo syndrome is increasing in incidence, which may reflect the rising prevalence of modern life ‘stressors’ as well as the greater awareness and detection of the condition by the clinical cardiology community (Templin et al. 2015, Pattisapu et al. 2021). Takotsubo syndrome accounts for approximately 2-3% of all and 5-6% of female patients presenting with acute coronary syndrome (Akashi et al. 2008, Templin et al. 2015, Ghadri et al. 2018), although it may be under appreciated and under diagnosed especially in patients who have co-existing coronary artery disease (Napp et al. 2020). Takotsubo syndrome typically (80-90%) affects women, and although it can present at any age, it characteristically occurs in post-menopausal women. The mechanism underlying this large sex and age disparity is unknown.

Takotsubo syndrome had previously been viewed as an interesting anomaly that ran a benign course. As such, patients were often given reassurances that they were fortunate not to have suffered a heart attack, and that their heart will recover completely back to normal with an excellent prognosis. However, we now know this is not the case. Despite recovery of left ventricular ejection fraction and the absence of major coronary artery disease, patients with takotsubo syndrome have outcomes that are considerably worse than the general population (Brinjikji et al. 2012, Templin et al. 2015, Tornvall et al. 2016, Ghadri et al. 2018, Uribarri et al. 2019, Redfors et al. 2021). Takotsubo syndrome has an in-hospital mortality that is comparable to acute ST segment elevation myocardial infarction (Templin et al. 2015, Ghadri et al. 2018, Uribarri et al. 2019, Redfors et al. 2021). Beyond the acute event, patients with
takotsubo syndrome have a rate of all-cause death of 5.6% per patient-year and a rate of major adverse cardiac and cerebrovascular events of 9.9% per patient-year (Templin et al. 2015).

As many as 1 in 8 patients will experience a repeat acute takotsubo syndrome episode within 5 years of the index event (Ghadri et al. 2018, El-Battrawy et al. 2019), often precipitated by a further (and often different) stressful event although no known clinical or psychological factors can predict the likelihood of recurrence. Moreover, many patients report substantial morbidity following takotsubo syndrome. Symptoms of dyspnoea, lethargy, palpitation and fleeting chest pains can persist for 2 or more years after the index event despite ‘normalisation’ of left ventricular ejection fraction.

1.11.2. Diagnosis in Takotsubo Syndrome

The diagnosis of takotsubo syndrome can be challenging because clinical features have many similarities with acute coronary syndrome and immediate early cardiac imaging is needed because of the rapid normalisation of left ventricular ejection fraction. The most widely used diagnostic criteria are those proposed by the Mayo Clinic in 2004 (Abe et al. 2003) and subsequently revised in 2008 (Prasad et al. 2008) (Figure 1.9). Traditionally, the presence of coronary artery disease may have deterred the clinician from a diagnosis of takotsubo syndrome. Despite this uncertainty, it has become evident that takotsubo syndrome can co-exist in the presence of fixed coronary artery
disease and can even be triggered by acute coronary syndrome (Ghadri et al. 2018).

The diagnosis of takotsubo syndrome is often made once an invasive coronary angiogram has been performed and normal or non-obstructive coronary artery disease documented. Co-existing coronary artery disease is present in approximately 15% of patients with takotsubo syndrome (Uribarri et al. 2019, Redfors et al. 2021) and careful correlation between angiography and the wall motion abnormalities is required. Where doubt exists, advanced intravascular imaging techniques, such as optical coherence tomography and intravascular ultrasound, may help to exclude plaque rupture, which is not a characteristic of takotsubo syndrome (Gerbaud et al. 2020). Left ventriculography usually confirms the diagnosis due to the characteristic ballooning of the left ventricle. In the majority (50-80%) of cases, there is a typical pattern of apical and mid-ventricular dyskinesis, akinesia or hypokinesis with basal sparing (Figure 1.8) (Ghadri et al. 2018).
**Figure 1.9 Diagnostic Criteria and Pathway for Takotsubo Syndrome**

- **Clinical Presentation as Acute Coronary Syndrome**
  - Electrocardiogram ± Echocardiography
  - *Further assessment

- **Invasive Coronary Angiography + Left Ventriculography**
  - Consider OCT/IVUS

- **Obvious Disease (obstructive/non-obstructive)**
  - Yes
  - Normal Coronaries/Minor Plaque
  - Yes
  - Cardiac Magnetic Resonance

- **MINOCA**
  - Infarct Pattern rule in/out or consider dual pathology

- **Takotsubo Cardiomyopathy**
  - Non-Infarct Pattern

- **Myocarditis**

- **Cardiomyopathies**

**OCT**, optical coherence tomography, **IVUS**, intra-vascular ultrasound

**Mayo Clinic Diagnostic Criteria: Takotsubo Syndrome**

<table>
<thead>
<tr>
<th>Compulsory</th>
<th>Optional</th>
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<tr>
<td><strong>Morphology</strong></td>
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<tr>
<td>- Left ventricular wall motion abnormalities, extending beyond a single epicardial vascular distribution.</td>
<td>- Involvement of apical and mid-ventricular segments (“Apical”)</td>
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<tr>
<td>- Involvement mid-ventricular (with or without apical involvement)</td>
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<tr>
<td><strong>Time Course</strong></td>
<td></td>
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<tr>
<td>- Transient left ventricular dysfunction/ resolution of wall motion abnormalities</td>
<td>- Mild-Moderate rise in cardiac biomarkers</td>
</tr>
<tr>
<td><strong>Evidence of myocardial injury</strong></td>
<td></td>
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<tr>
<td>- New and dynamic ST-segment deviation or T-wave inversion</td>
<td>- Hypertrophic Cardiomyopathy</td>
</tr>
<tr>
<td>- Potential coronary culprit (obstructive disease, plaque rupture, dissection, thrombosis)</td>
<td>- Other pathological condition that may explain regional dysfunction</td>
</tr>
<tr>
<td>- Myocarditis</td>
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<td>- Rheomycinopathy</td>
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1.11.3. Cardiac magnetic resonance in takotsubo syndrome

Cardiac magnetic resonance with gadolinium contrast administration is an invaluable tool, distinguishing takotsubo syndrome from acute myocardial infarction and myocarditis (Agewall et al. 2017). Unlike these latter conditions, fibrosis depicted by late gadolinium enhancement is usually not a feature of takotsubo syndrome. Rarely, a characteristic pattern of takotsubo syndrome appears as a thin transmural band of fibrosis at the hinge points between the hyperkinetic base and dyskinetic apex or mid-cavity. This can be seen both acutely (at the time of presentation) and 4-5 months later at follow up (Figure 1.10). This possibly results from the opposing strong shear forces applied to the left ventricular wall. Cardiac magnetic resonance also provides a reliable assessment of right ventricular involvement, identification of left and right ventricular thrombi, and often shows the presence of a small pericardial effusion (as well as pleural effusions), particularly if the test is performed early after presentation.

Intense myocardial oedema is an important feature of takotsubo syndrome (Figure 1.10). Oedema is not only confined to regions of abnormal contractility but is present to a lesser extent within the entirety of the ventricular myocardium. Myocardial oedema resolves gradually over weeks or months following the index event, typically taking much longer to recover than myocardial contractility (Citro et al. 2020). Reflecting this, left ventricular mass is markedly elevated and native T1 and T2 mapping values are increased during the acute phase, gradually resolving over 5-6 months during
convalescence (Figure 1.10) (Scally et al. 2018). Both myocardial oedema and acute inflammation are detectable at presentation, but it remains unclear whether they are a consequence of takotsubo syndrome or if they represent a primary causal inflammatory stimulus.

Ultimately, the hallmark of takotsubo syndrome is the reversibility in systolic function which occurs within hours, days or weeks, in the absence of infarct-specific myocardial fibrosis (Kurisu et al. 2002, Akashi et al. 2008). The clinical implication of recovery time remains unknown and there is emerging evidence that full recovery may be slower and less complete than initially thought.
Figure 1.10: Cardiac Magnetic Resonance Imaging and Computed Tomography Findings in Takotsubo Syndrome
Short axis T2 maps (A), T2 polar map (B) and short-axis T1 maps (D), demonstrating elevated T2 and T1 values circumferentially in the mid and apical regions (outside of a coronary territory). Long axis two-chamber view demonstrating transmural fibrotic band pattern typical of takotsubo syndrome 4 months after the index event (C). Long axis four-chamber and short axis views of mid-ventricle demonstrating elevated left ventricular mass in acute phase (E, F) and normalisation during convalescence (G, H). Hybrid positive emission tomography with cardiac computed tomography angiography depicting a small left ventricular thrombus in a patient with takotsubo syndrome with no clinically apparent thrombus on conventional imaging. There is subtle hypoattenuation on the computed tomography angiogram (magnified inserts) and increased uptake of an activated platelet and thrombus-specific radiotracer ($^{18}$F-GP-1; yellow-red) in long axis four-chamber (I, J) and short axis (K, L) views on positron emission tomography.
1.11.4. Myocardial calcium handling in takotsubo syndrome

Endogenous adrenergic surge is the most established theory for the pathogenesis of takotsubo syndrome and is intuitive given the strong association with sudden unexpected stress and major physical illness or trauma. Iga and colleagues were the first to describe takotsubo syndrome in a patient with pheochromocytoma (Iga et al. 1989). This particular case report was important as it first established the relationship between takotsubo syndrome and elevated catecholamine concentrations. The stimulation of the β-adrenoceptors by catecholamines is widely known to result in disturbances of contractility and alteration in gene expression of calcium regulatory proteins (Boluyt et al. 1995, Stein et al. 1996). Pre-clinical studies have demonstrated that an intense β1-adrenoceptor-Gs and β2-adrenoceptor-Gs signalling might be responsible for initiating alteration in mRNA levels of regulatory proteins (phospholamban and sarcoendoplasmic reticulum calcium ATPase) in the mammalian heart (Linck et al. 1998).

The presence of myocardial calcium mishandling has in heart failure has been established (Gwathmey et al. 1987, Hasenfuss et al. 1992, Gómez et al. 1997, Hasenfuss et al. 1997, Lehnart et al. 1998, Task Force of the European Society of Cardiology 1998, Hasenfuss et al. 2002, Lehnart et al. 2009, Dybkova et al. 2018). In takotsubo syndrome, there is regional sudden and complete abolition of myocardial contractility. As such, one would expect there to be a degree of myocardial calcium mishandling during the acute setting.
Under normal condition, the action potential opens voltage-gated L-type calcium channels. The ensuing calcium entry triggers even greater release of calcium from the sarco-endoplasmic reticulum via ryanodine receptors type 2. This calcium induced calcium release generates a transient increase of the cytoplasmic calcium concentration that activates myofilament cross-bridge formation (Bers 2000, Bers 2002). To terminate contraction, calcium is removed from the cytoplasm into the sarco-endoplasmic reticulum by the sarcoendoplasmic reticulum calcium ATPase (SERCA2a) and to the extracellular space by the sodium-calcium exchangers (Bers et al. 1999, Bers 2002). Under pathological conditions with excitation–contraction uncoupling (heart failure, takotsubo syndrome), the sarcoplasmic reticulum calcium load is reduced (SERCA2a inhibition) together with the mitochondrial calcium concentration (due to the alteration of sodium-calcium exchanger) and the cytoplasmic calcium concentration increases (Hobai et al. 2001, Bers 2002).

Immunohistochemistry studies have demonstrated changes in intracellular calcium turnover and its role in the pathogenesis of takotsubo syndrome (Nef et al. 2009). Sarcolipin and phospholamban bound to the sarco-endoplasmic reticulum are critical regulators of cardiac contractility. In cardiac biopsies of patients with takotsubo syndrome, SERCA2a activity and calcium affinity was significantly down-regulated (Figure 1.11). Furthermore, an increased phospholamban/SERCA2a ratio represents a major determinant of contractile dysfunction (Nef et al. 2009). Thus, takotsubo syndrome is associated with
specific alteration of calcium handling proteins which might be crucial for contractile dysfunction. To date, there has not been an assessment of in-vivo myocardial calcium mishandling in patients with takotsubo syndrome. The application of manganese-enhanced magnetic resonance imaging will establish whether there is underlying myocardial calcium mishandling and whether this plays a role in its pathophysiology. More importantly, does this fully recover and could it explain the adverse outcomes associated with takotsubo syndrome?
Figure 1.11 Myocardial calcium dysfunction in Takotsubo syndrome
1.12. **Coronavirus-19 pandemic**

Coronaviruses have been identified as human pathogens since the 1960s. These are enveloped, positive stranded RNA viruses, which have a characteristic surface appearance. Sequencing of the COVID-19 virus determined that this is a novel coronavirus, which shares 79.5% sequence identity with the previously identified severe acute respiratory syndrome-related coronavirus (SARS-CoV) (Yan et al., 2020; Zhou et al., 2020). Furthermore, sequencing has identified similarities between the binding protein for these two strains of viruses (SARS-CoV and SARS-CoV-2). The entry receptor into the cell utilised by the novel coronavirus is Angiotensin Converting Enzyme-2 (ACE-2) as previously identified for SARS-CoV (Yan et al., 2020). ACE-2 is a transmembrane metallopeptidase, with wide distribution in lung alveolar epithelial cells and enterocytes in the small intestine, which provides insight into the entry routes of the virus (Hamming et al., 2004). Furthermore, endothelial cells in the vascular bed have large distribution of ACE-2 receptors, and as such, the endothelium may play a key role in pathogenesis of cardiac and systemic organ injury (Hamming et al., 2004).

1.12.1. **COVID-19 and clinical implications**

The novel coronavirus disease (COVID-19) has had a profound impact on all aspects of life. Healthcare services worldwide have encountered an unprecedented rapidly evolving pandemic and have adapted in real-time to meet the needs of patients. Our understanding of the clinical manifestations of
COVID-19 has grown significantly since its emergence in 2020. In severe cases, COVID-19 is associated with bilateral interstitial pneumonia, the development of acute respiratory distress syndrome, septic and cardiogenic shock (Huang et al., 2020; Zhou et al., 2020). Higher morbidity and mortality have been observed in elderly patients and those with comorbidities, with 14.8% mortality in those over the age of 80 in China (Wu et al., 2020). Patients with known cardiovascular risk factors or established cardiac problems are at higher risk of contracting SARS-CoV-2, and this confers a worse prognosis in COVID-19 infection (Fang et al., 2020; Zhou et al., 2020). A recent meta-analysis aimed to evaluate the association between cardiovascular comorbidities and the novel COVID-19 infection. The prevalence of hypertension, cardiac and cerebrovascular disease, and diabetes was 17.1%, 16.4%, and 9.7%, respectively (Li et al., 2020). Patients who had these comorbidities were more likely to have a more severe course of the disease associated with poorer outcomes. Furthermore, case studies suggest that cardiac complications are common and associated with severe illness necessitating critical care input. The aetiology of myocardial injury and its consequences are not yet understood.

1.12.2. **COVID-19, myocardial injury and infarction**

Myocardial injury is defined as an elevated cardiac troponin concentration above the 99th centile of a healthy reference population (Thygesen et al., 2018). This can be acute (where troponin elevation is dynamic; >20% change on serial testing) or chronic. There are several mechanisms for acute
myocardial injury, which may occur due to ischaemia as a result of atherothrombotic coronary artery occlusion (type 1 myocardial infarction) or in the context of prolonged myocardial oxygen supply or demand imbalance (type 2 myocardial infarction). However, it is recognised that acute myocardial injury may occur in the absence of ischaemia due to a variety of cardiac and non-cardiac causes (Chapman et al., 2017). The presence of acute myocardial injury is common in patients without acute coronary syndrome and is associated with poor clinical outcomes (Chapman et al., 2018; Kadejso et al., 2019). It is recognised that critically unwell patients are susceptible to myocardial injury, and this is predictive of mortality (Quenot et al., 2005; Frencken et al., 2018). Determining the underlying mechanism of myocardial injury may therefore help guide clinical care and treatment with the potential to improve clinical outcomes.

Myocardial injury is increasingly recognised in patients with COVID-19 and correlates with severe cases and poor outcomes (Zhou et al., 2020). In univariable analysis, the odds of in-hospital death were 80-fold higher in patients with elevated cardiac troponin above the 99th centile upper reference limit and was a predictor of mortality [OR 80.07, 95%CI 10.34-620.36, P<0.0001]. The mechanism of myocardial injury is not understood. Some suggesting indirect mechanisms of injury similar to that of other severe respiratory illnesses (Smeeth et al. 2004, Clerkin et al. 2020, Kotecha et al. 2021, Pellegrini et al. 2021). Others have proposed direct myocardial injury due to myocarditis, stress cardiomyopathy, endothelial injury, thrombo-
inflammation or the result of profound ongoing myocardial oxygen supply or demand imbalance (Lindner et al. 2020, Hu et al. 2021). Unlike parainfluenza, this coronavirus increases risk particularly in patients with vascular rather than pulmonary disease, raising the possibility that the cardiac effects of COVID-19 are mediated directly through ACE-2 receptor mediated injury to the vascular endothelium (Hamming et al. 2004, Yan et al., 2020). Determining the underlying mechanism of myocardial injury may therefore help guide clinical care and treatment with the potential to improve clinical outcomes.

1.12.3. **Cardiac Magnetic Resonance Imaging in COVID-19**

Widespread myocardial abnormalities have been described on cardiac magnetic resonance imaging have been reported in patients with COVID-19 (Huang et al. 2020, Knight et al. 2020, Puntmann et al. 2020, Kotecha et al. 2021). These range from subclinical changes such as, elevated native T1 to the presence of myocardial scar and gross cardiac dysfunction. A large proportion of these patients have significant co-morbidities and the presence of coronary artery disease is likely to have an impact. It is therefore essential to understand whether such cardiac abnormalities are the result of underlying co-morbidities or the direct impact of COVID-19.

There are increasing reports of persistent and prolonged multi-organ effects after acute COVID-19 illness (Ayoubkhani et al. 2021, Nalbandian et al. 2021). More importantly, many patients continue to have debilitating symptoms during convalescence: so-called “long COVID” (Nalbandian et al. 2021). It is
important to understand whether cardiac damage observed in the acute phase of COVID-19 will translate into subsequent cardiac dysfunction and morbidity. Traditional imaging techniques, such as gadolinium-enhanced magnetic resonance imaging and computed tomography coronary angiography (CTCA), will help assess the extent of myocardial damage and the burden of underlying coronary artery disease in this population. Furthermore, manganese-enhanced magnetic resonance imaging may demonstrate more subtle cardiac dysfunction.
1.13. **SUMMARY**

The potential clinical applications of manganese-enhanced magnetic resonance imaging are numerous and include diagnosis, risk stratification and management of a range of patients with cardiac disease. This technique has particular implications for diagnosis, especially for those with an uncertain or subclinical cardiomyopathy. The ability to diagnose an underlying cardiomyopathy prior to gross left ventricular dysfunction, will allow for early detection and prompt treatment initiation, which may have an impact on outcomes.

The detection of altered calcium handling over time and quantification of cellular myocardial function directly may transform our ability to assess myocardial function, enabling early detection and prognostication. This may be an invaluable non-invasive method of monitoring disease progression in various non-ischemic cardiomyopathies. With optimisation, this technique has potential to allow individualisation of heart failure treatment, assessment of treatment efficacy and targeting optimal therapy to those most likely to benefit.

It was long thought that there is complete recovery of cardiac function in patients with takotsubo syndrome. We now know that not only do patients demonstrate ongoing symptoms with abnormalities of cardiac energetics, but they are also at higher risk of morbidity and mortality, similar to those with acute myocardial infarction (Ghadri et al. 2016, Ghadri et al. 2018). The application of manganese-enhanced magnetic resonance imaging will
establish whether there is underlying myocardial calcium mishandling and whether this plays a role in its pathophysiology. More importantly, does this fully recover and could it explain the adverse outcomes associated with takotsubo syndrome?

Since withdrawal from the European market in 2012 by the marketing-authorisation holder, no manganese contrast medium has been clinically available. The reasons for this are multifactorial but the decision was principally driven by the lack of large-scale commercial interest, despite promising clinical pilot data. It is important to highlight that no safety concern caused its withdrawal, simply the lack of clinical demand in hepatobiliary imaging. Furthermore, early clinical studies have established that manganese is safe in various cardiac conditions (Spath et al. 2020, Singh et al. 2021, Spath et al. 2021). There are currently no available preparations of manganese-based contrast media for widespread clinical use. However, the formulation of manganese dipyridoxyl diphosphate for clinical use is clearly feasible, as evidenced by its current use in clinical studies (Table 1). The provision and availability of manganese-based contrast media is likely to change with the re-emergence of commercially available preparations anticipated in the near future.

With a large body of preclinical data and emerging clinical work in the field, the stage is now set for wider clinical translation of this exciting non-invasive imaging technique. Manganese-enhanced magnetic resonance imaging offers
the potential to improve diagnosis in a range of conditions and to provide a non-invasive measure of myocardial calcium handling. This represents an invaluable tool for the assessment of functional recovery, accurate prediction of disease progression and monitoring of treatment response.
1.14. **AIMS**

The aims of this thesis are:

1. To assess the reproducibility and repeatability of manganese-enhanced magnetic resonance imaging.

2. To assess the ability of manganese-enhanced magnetic resonance imaging to detect myocardial calcium-handling dysfunction in patients with dilated and hypertrophic cardiomyopathy.

3. To investigate calcium handling using manganese-enhanced magnetic resonance imaging as surrogate marker during acute and recovery phases of takotsubo syndrome.

4. To determine the contribution and cardiac impact of co-morbidities on the reported widespread myocardial abnormalities in patients with recent COVID-19.
1.15. **HYPOTHESES**

The hypotheses of this thesis are:

1. Manganese-enhanced magnetic resonance imaging has potential as a surrogate marker of myocardial calcium handling. We hypothesise that it is a robust repeatable and reproducible technique (Chapter 3).

2. Manganese-enhanced magnetic resonance imaging can detect disordered calcium-handling in symptomatic and asymptomatic patients with non-ischaemic cardiomyopathy (Chapter 4).

3. Manganese-enhanced magnetic resonance imaging can detect abnormal calcium handling in patients with acute and convalescent phase of takotsubo syndrome (Chapter 5).

4. Hospitalised patients with COVID-19 will have underlying co-morbidities that will impact on cardiac abnormalities seen on cardiac magnetic resonance imaging (Chapter 6).
CHAPTER 2: METHODOLOGY
2.1. **ETHICAL AND REGULATORY CONSIDERATIONS**

All clinical studies were conducted with ethical approval from the South-East Scotland Ethical Review Board (REC002 17/SS/0055).

All studies were conducted in accordance with the Declaration of Helsinki.
2.2. **AGENTS AND MATERIALS**

2.2.1. **Gadolinium-based contrast medium**

For clinical studies (Chapters 3-6), 0.1 mmol/kg of gadobutrol (Gadovist®, Bayer Pharma AG, Germany) was used, as per manufacturers’ recommended administration parameters for clinical use.

2.2.2. **Manganese dipyridoxyl diphosphate (MnDPDP)**

The formulation of manganese dipyridoxyl diphosphate (MnDPDP, mangafodipir trisodium) permits the binding of manganese ions to a chelating agent (dipyridoxyl diphosphate, DPDP, fodipir). This results in lower effective free concentrations of manganese ions in the blood, reducing risk of acute toxicity. MnDPDP was previously marketed by GE Healthcare for imaging of the liver and pancreas under the trade name Teslascan™ for imaging of hepatobiliary and pancreatic neoplasms (Sutcliffe et al. 2011), but was withdrawn from both European [EU/1/97/040/001, (Agency 2012)] and United States [FDA UNII: 129FW80TG4, (Administration 2012)] markets on account of lack of demand and it is now a generic product. Teslascan™ contains 10 μmol MnDPDP/mL. Whilst not in current commercial manufacture, clinical-grade MnDPDP (Chapters 3 to 6) was reconstituted (Exova SL Pharma, Wilmington, Delaware, USA) to identical pharmaceutical form, production standards and pharmaceutical particulars of Teslascan™ (see Appendix) with the sole exception of being 5 times more concentrated, using active pharmaceutical ingredient sourced from an independent pharmaceutical
company (Albany Molecular Research Inc. New York, USA) (see Appendix). The final product constitutes a concentration 50 μmol MnDPDP/mL and the dose administered was 0.1 mL/kg (5 μmol/kg) bodyweight given at a rate of 0.4–1.2 mL/min (20–60 μmol/min).

2.3. STUDY PARTICIPANTS AND CONDITIONS

All study participants gave written informed consent prior to enrolment. A participant information sheet was provided for all clinical studies (see Appendix) and subjects' general practitioners could be informed in writing of their participation.

2.3.1. Patients with non-ischaemic dilated cardiomyopathy

Adult patients (≥ 18 years of age) with dilated cardiomyopathy were recruited from the Edinburgh Heart Centre. Non-ischaemic dilated cardiomyopathy was defined by the presence of impaired left ventricular systolic function (ejection fraction ≤ 50% within 12 months) and left ventricular dilatation (left ventricular end-diastolic volume > 117% adjusted for age and body-surface area), in the absence of abnormal loading conditions (hypertension and valvular disease) or coronary artery disease (Elliott et al. 2008). As described in section 2.9.3, all patients were required to have stable New York Heart Association class I-III heart failure, without change in therapy in the preceding month. Exclusion criteria for all participants were any contraindication to magnetic resonance
imaging, contraindications to manganese dipyridoxyl diphosphate administration (high degree atrioventricular block, history of torsades de pointes or prolonged QTc interval, obstructive liver disease, maintenance on calcium-channel blockade or digoxin therapy), renal failure (estimated glomerular filtration rate <30 mL/min/1.73 m^2), New York Heart Association class IV heart failure, and women of child-bearing potential without a negative pregnancy test.

2.3.2. Patients with hypertrophic cardiomyopathy

Adult patients (≥ 18 years of age) with hypertrophic cardiomyopathy were recruited from the Edinburgh Heart Centre. Diagnosis of hypertrophic cardiomyopathy was based on echocardiography or MRI according to European Society of Cardiology guidelines, defined as left ventricular hypertrophy (left ventricular wall thickness ≥ 15 mm in any segment) in the absence of haemodynamic stresses or abnormal loading conditions (Elliott et al. 2008). Presence of diastolic dysfunction in hypertrophic cardiomyopathy was defined on transthoracic echocardiography as per British Society of Transthoracic Echocardiography guidelines (Nagueh et al. 2009). All patients were required to have New York Heart Association class I-III heart failure, with stable symptoms and no change in maintenance therapy in the preceding month. Exclusion criteria were as described in section 2.4.1.
2.3.3. Patients with takotsubo syndrome

Adult patients (≥18 years of age) with takotsubo syndrome were recruited from the Edinburgh Heart Centre between March 2020 and October 2021. Diagnosis of takotsubo syndrome was based on the Mayo clinic (Abe et al. 2003) and the Heart Failure Association Takotsubo Syndrome Taskforce of the European Society of Cardiology criteria (Maron et al. 2006). This comprises of new electrocardiographic (ECG) changes (ST-segment elevation, ST-segment depression, T-wave inversion, and QTc prolongation), presence of transient left ventricular dysfunction (hypokinesia, akinesia, or dyskinesia) presenting as apical ballooning or mid-ventricular, basal, or focal wall motion abnormalities, and absence of obstructive coronary artery disease or acute plaque rupture. Patients usually have an emotional or physical stressful trigger. Exclusion criteria were as described in section 2.4.1. We specifically excluded patients with pheochromocytoma, myocarditis or a primary isolated diagnosis of acute myocardial infarction.

2.3.4. Patients with COVID-19

Adult patients recovering from hospitalisation with COVID-19 were recruited prospectively from the Edinburgh Heart Centre between May 2020 and November 2020 and Glenfield Hospital, Leicester between November 2020 and February 2021. The diagnosis of COVID-19 was based on a positive polymerase chain reaction (PCR) test. Exclusion criteria were as described in section 2.4.1.
2.3.5. Comorbidity-matched volunteers

Patients with takotsubo syndrome were propensity matched in a ratio of 1:1 to control volunteers who were of a similar age, sex and cardiovascular risk factor profile, such as hypertension, known coronary artery disease, hypercholesterolemia and diabetes mellitus.

Patients with COVID-19 and co-morbid volunteers were propensity matched 1:2 to cardiovascular risk factors including hypertension, known ischaemic heart disease, hypercholesterolaemia and diabetes mellitus. Matched volunteers were recruited from general cardiology admissions, outpatient clinic or those recruited for other cardiac studies. The latter control group were scanned at Glenfield Hospital prior to January 2020 (n=16) or at the University of Edinburgh (n=10) between September 2020 and January 2021. Exclusion criteria were as described in section 2.4.1.

2.3.6. Healthy subjects

Healthy volunteer subjects (≥ 18 years of age) were recruited through local advertisement at the Edinburgh Heart Centre. Subjects had no known pre-existing medical conditions and exclusion criteria were as described in section 2.4.1.
2.4. MAGNETIC RESONANCE IMAGING

2.4.1. Principles of magnetic resonance imaging

Magnetic resonance imaging began with the discovery of nuclear magnetic resonance by Bloch (Bloch 1953), followed by the world’s first imaging using nuclear magnetic resonance by Lauterbur (Lauterbur 1989). Magnetic resonance imaging is an essential technique in modern medicine. This technique utilises a magnetic field to obtain tomographic images of the human body with high tissue resolution without the use of radiation. A magnetic resonance imaging scanner is based on the same principle as electromagnets, which produce a magnetic field by passing an electrical current through a large coil. To reduce electrical resistance, the coil is enclosed by liquid helium to bring it into a superconducting state. The strength of the magnetic field of the superconducting magnets in mainstream machines today is 1.5 or 3.0 Tesla (Tesla or T, a unit quantifying magnetic field density).

More than 80% of the human body consists of water and fat, which contain many hydrogen atoms. Magnetic resonance scanners produce images based on the nuclei of hydrogen atoms (protons) which have a magnetic field. Protons are generally oriented in a variety of directions within the body. However, when entering a powerful magnetic field, they change from having a scattered orientation to being aligned within the magnetic field. If a radio frequency (RF) pulse is applied in this state, the protons become deflected by 90°. This phenomenon is known as nuclear magnetic resonance. When the RF pulse is applied, the deflected protons then continue to store the energy of
the RF pulse. If the RF pulse is suspended, the deflected protons return to their previous orientation while emitting their stored energy. The duration of this return differs depending on the type of tissue (muscle, blood, fat, etc.) in which the protons are bonded. This energy is detected by a receiver as a signal, and an image is formed by analysing this signal and the time duration of the return. After leaving the MRI scanner, the protons become reoriented in scattered directions within the body (Ridgway 1999, Ridgway 2010, Biglands et al. 2012).

2.4.2. Magnetic resonance imaging

Magnetic resonance imaging was performed using a Siemens MAGNETOM Skyrafit 3T scanner (Siemens Healthineers, Erlangen, Germany) in the Edinburgh Imaging Facility at the Queen’s Medical Research Institute, combining elements of the spine array coil and body array coil. Images were acquired with ECG-gating and during held expiration. Localiser images were obtained to ascertain individual patient anatomy. Cine imaging was acquired with standard steady-state free precession sequences (TrueFISP) in long and short-axis orientations. A stack of short-axis slices was planned from the mitral valve annulus to left ventricular apex.

For the cohort of patients with COVID-19 scanned in Glenfield Hospital, Leicester (Chapter 6), magnetic resonance imaging was performed using a Siemens MAGNETOM Skyra 3T scanner (Siemens Healthineers, Erlangen, Germany) with an 18-channel cardiac coil.
2.4.3. T1 Mapping

T1 was quantified for each voxel using T1 mapping was performed prospectively with modified Look-Locker inversion recovery in patients with dilated and hypertrophic cardiomyopathy (Chapter 4) and shortened modified Look-Locker inversion recovery in patients with takotsubo syndrome and COVID-19 (Chapter 5-6). Quantitative estimation of native T1 was performed in a full short-axis stack from mitral valve annulus to apex and standard long-axis slices (TR = 388.8 ms; TE = 1.07 ms; matrix = 192± 75%; slice thickness = 8 mm with 1.6 mm gap). Field of view was 360± 288 mm² adjusted for patient body habitus as required. T1 relaxation times were measured before and after administration of gadolinium (Chin et al. 2014) and manganese contrast media.

2.4.4. T2 Mapping

T2 is the time taken for the transverse magnetisation to decay to 37% of baseline. T2 weighted imaging can detect oedema, as well as inflammatory processes such as sarcoidosis or myocarditis, where there is an abundance of interstitial free water in the target tissues. As with all parametric mapping, T2 mapping produces a 2-dimensional pixel-wise map where regional differences in T2 can be both visualised and quantified. Three separate T2-weighted images are acquired following a T2 preparation pulse of increasing duration, but with the same trigger delay (Kim et al. 2017). Long repetition times are used to ensure complete T1 recovery between T2 acquisitions,
which otherwise may lead to inaccurate estimation of T2 (Lota et al. 2017). T2 mapping was used to quantify T2, performed with T2 prepared balanced steady-state free precession acquisition (TR = 207.39 ms; TE = 1.32 ms; matrix = 192 ± 100; slice thickness = 8 mm with 1.6 mm gap), with T2 evolution times of 0, 0.30 and 0.55 ms.

2.4.5. Late gadolinium enhancement
Following gadolinium administration, myocardial oedema and rupture of cell membranes result in partition of gadolinium into the interstitial and extracellular spaces. This is visualised as delayed enhancement in regions of pathology. Following intravenous administration of gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany), a single breath held phase-sensitive inversion recovery short-axis stack, and long axis orientations were acquired (TR = 820 ms; TE = 1.04 ms; matrix = 192 ± 72, slice thickness = 8 mm with 2 mm gap). A standardised inversion time of 400 ms was used and adjusted only if required for optimal myocardial nulling. Late gadolinium enhancement images were acquired following intravenous gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany) using a single breath hold per slice with a short-axis stack, and long-axis orientations. T1 mapping was acquired prior to and 10 min post-contrast.

2.4.6. Manganese-enhanced magnetic resonance imaging
Manganese-enhanced magnetic resonance imaging was achieved using intravenous infusion of MnDPDP (5 μmol/kg, 1 mL/min; Exova SL Pharma, Wilmington, Delaware, USA). T1 mapping was performed pre-contrast with full
short-axis T1 stack as above. For patients, a single short-axis slice was identified by the supervising cardiologist, guided by the late gadolinium enhancement, native T1 maps and cine images to characterise pathology. For healthy and matched volunteers, a single mid-ventricular slice was chosen. A single short-axis T1 map was then acquired at this slice location every 2.5 min for 40 min after starting manganese infusion in patients with dilated and hypertrophic cardiomyopathy (Chapter 4) and 30 min after starting manganese infusion in patients with takotsubo syndrome and COVID-19 (Chapter 5-6) at which point a full short-axis T1 stack was repeated post-contrast (Figure 2.1).

Further details on Manganese-enhanced magnetic resonance imaging are described in relevant results chapters.
Figure 2.1 Manganese-enhanced magnetic resonance imaging protocol

Manganese-enhanced magnetic resonance imaging protocol in patients with dilated and hypertrophic cardiomyopathy (A) and patients with takotsubo syndrome and COVID-19 (B).

MnDPDP, manganese dipyridoxyl diphosphate, MEMRI, Manganese-enhanced magnetic resonance imaging
2.5. **IMAGE ANALYSIS**

2.5.1. Magnetic Resonance Imaging analysis

All analysis of T1 maps, late gadolinium enhancement and cine-derived volumetric and functional sequences was performed using Circle CVI (Circle Cardiovascular Imaging, CVI42 v5.3.6, Calgary, Canada). Endocardial and epicardial borders were manually defined on the short-axis cine images for volumetric and wall motion measurements and were then copied to all corresponding late gadolinium, T2 and T1 map sequences for analysis with minimal manual adjustments. Regions of interest were determined using the standard 16-segment cardiac model with global myocardial values derived from an average of all 16 segments. After contouring, an additional epicardial and endocardial offset of 20% was applied automatically to minimise tissue interface for all T1 map analyses and artefact was excluded manually for a minority of cases (Figure 2.2). In patients, additional regions of interest (ROIs) were drawn according to regional pathology, corresponding to myocardial infarction as defined by late gadolinium enhancement, or in areas of morphologically abnormal myocardium. For serial T1 imaging post-MnDPDP, manually drawn ROIs from the pre-contrast image were transferred to all subsequent post-contrast images to ensure consistency. Haematocrit from the day of scanning was used to calculate extracellular volume fraction for gadolinium-enhanced scans.
Further details on image analysis are described in relevant results chapters.
Figure 2.2 Imaging analysis

Panel A-C. Endocardial and epicardial borders for measurement of cardiac volumes in basal, mid and apical slice. Panel D. Septal region of interest in the mid-septal wall. Panel E. Six-segment model of mid-ventricular sl
2.6. **KINETIC MODELLING**

To derive quantitative estimates and to assess differential manganese uptake, kinetic model analysis was performed, as we previously described (Spath et al. 2020, Spath et al. 2021). Kinetic modelling was based on a Patlak two-compartment model formulation (Patlak et al. 1983, Skjold et al. 2006). In brief, the model consists of (i) a reversible compartment \(v_e\), comparable to intravascular and interstitial space and (ii) an irreversible compartment \(v_i\) comparable to the intracellular space, in which irreversible accumulation of the contrast agent is anticipated during the imaging period (30 min). The arterial concentration (derived from blood pool T1) represents contrast agent delivery into myocardial tissue and constitutes the arterial input function.

Skjöld et al previously derived a Patlak model formulation for cardiac manganese-enhanced magnetic resonance imaging (Skjold et al. 2006), demonstrating that an apparent unidirectional influx constant \((Ki)\) for the transfer of manganese from plasma to irreversible compartments \(v_i\), can be measured, using Equation 1:

\[
\frac{c_e(t)}{c_a(t)} = Ki \int_0^t \frac{c_u(\tau)d\tau}{c_a(t)} + v_e
\]

\[1\]
where $C_t$ and $C_a$ are the manganese concentration in myocardial tissue and blood pool (arterial input function), respectively. This formulation is equivalent to the Patlak model (Patlak et al. 1983) and describes that if a contrast medium is irreversibly trapped in the tissue within the imaging period, the instantaneous tissue concentration divided by the instantaneous arterial concentration plotted against the integrated arterial concentration divided by the instantaneous arterial concentration, will result in linearisation of the data. The gradient of this line represents the apparent unidirectional influx constant $K_i$, which equals:

$$K_i = \frac{k_1 k_2}{k_2 + k_3}$$

(2)

where $k_1$, $k_2$, and $k_3$ are the individual rate constants of the compartmental model presented. A visual representation of the influx constant $K_i$ is given in mL/100 g of tissue/min (Figure 2.3).
Figure 2.3 Patlak formulation

Patlak formulation – schematic of (A) model compartments and transfer constant $K_i$, describing passage from reversible to irreversible compartment (B) data analysis.

$k_1$ transfer into reversible compartment (transfer constant)
2.6.1. Further development of kinetic modelling

After performing manganese-enhanced magnetic resonance in patients with non-ischaemic cardiomyopathy (Chapter 4), we assessed the possibility of reducing the imaging time point. First, this would help patients tolerate the scan better. We felt that patients with takotsubo syndrome and recent COVID-19 would be more likely to suffer from dyspnoea, therefore, shorter scanning time may benefit them. Second, for this technique to be translated into wide-spread clinical practice, shorter scan times would be beneficial.

We found that myocardial manganese uptake ($K_i$, mL/100 g of tissues/min) was not affected by reducing scan times from 40 to 30 min post manganese infusion. This was seen in healthy volunteers, patients with dilated and hypertrophic cardiomyopathy (Figure 2.4)
Figure 2.4 Mean myocardial manganese uptake versus acquisition time

(A) Graph and (B) table demonstrating mean myocardial uptake over time in healthy volunteers (blue) and patients with dilated (grey) and hypertrophic cardiomyopathy (yellow).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean myocardial manganese uptake (Ki, ml/100g tissue/min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Healthy volunteer</td>
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<tr>
<td>40</td>
<td>10.0</td>
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<tr>
<td>37.5</td>
<td>10.1</td>
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<td>35</td>
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<td>32.5</td>
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<td>30</td>
<td>9.9</td>
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<tr>
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<td>9.2</td>
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</table>
2.7. **COMPUTED TOMOGRAPHY CORONARY ANGIOGRAPHY**

2.7.1. **Principles of computed tomography**

Computed tomography (CT) was developed in the 1970s and revolutionised medical imaging. CT uses ionising radiation, or x-rays, coupled with a rotating electronic detector array to create cross-sectional images of tissue. The x-ray beam rotates around the patient within the scanner such that multiple x-ray beams pass through them (Caldemeyer et al. 1999). As x-rays pass through the patient, they are attenuated and the degree of attenuation that occurs depends on the type of tissue. As a result, contrast is generated due to the differences in attenuation between adjacent tissues. The higher the attenuation of the x-ray beam, the brighter the tissue on CT images, and the lower the attenuation, the darker the tissue on CT images. For example, bone and calcification cause high attenuation of the x-ray beam whilst air causes very little attenuation. Fat and water lead to more intermediate levels of x-ray attenuation.

2.7.2. **Computed tomography coronary angiography imaging**

Cardiac CT imaging is not a novel concept, with early examples delivered by Godfrey Hounsfield in 1979 (Hounsfield 1980). However, images have only been produced with consistent quality since 2004 with the advent of 64-slice multidetector CT scanners. Electrocardiograph (ECG) gated CT has granted the ability to image the heart during specific phases of the cardiac cycle (Rubin et al. 2014). The diastolic phase can be precisely and prospectively targeted, as this is the phase when the heart is most still and coronary blood flow at its
highest. In doing so, the heart can be imaged in several slices over several beats, and then reconstructed as a single structure creating three-dimensional images of cardiac and coronary anatomy with excellent spatial resolution (Kashiwagi et al. 2009). The use of iodine-based contrast media enhances differences in attenuation between target tissues and the surrounding structures and is employed in CT angiography to delineate the vascular lumen and identify the presence of luminal and mural disease of the vasculature. CT coronary angiography is excellent accuracy for the detection of obstructive coronary artery disease with a sensitivity of 97% and specificity of 86% respectively (Leber et al. 2005, Miller et al. 2013).

In our study, computed tomography coronary angiography (CTCA) which was performed with a 128-multidetector row scanner (Siemens Biograph, Siemens Healthcare, Erlangen, Germany) according to SCCT guidelines (Abbara et al. 2016). Patients with a heart rate over 60 /min received intravenous metoprolol and all patients received sublingual glyceryl trinitrate prior to imaging. CCTA imaging was reviewed on a dedicated post processing workstation (Vitrea Advanced, v6.9.68.1, Vital Images, US). Obstructive coronary artery disease was defined as a luminal cross-sectional area stenosis of >70% in a major epicardial vessel or >50% in the left main stem. Prognostically significant coronary artery disease was defined as left main stem stenosis >50%, three-vessel disease or two-vessel disease including stenosis of the proximal left anterior descending coronary artery. Lung windows were reviewed for pulmonary COVID-19 involvement or persistent parenchymal lung abnormalities (atelectasis/scarring or ground glass opacification) (Simpson et al. 2020).
2.8. **STATISTICAL ANALYSIS**

All statistical analysis was performed with GraphPad Prism (GraphPad Software, San Diego, California, USA). Data are presented as mean ± standard deviation unless otherwise stated. Normality of continuous data was assessed using the D'Agostino-Pearson test. Comparisons were made using paired and unpaired t-tests, Mann-Whitney, Wilcoxon tests and analysis of variance/co-variance or Kruskal-Wallis tests as appropriate. Categorical baseline variables were compared using Fisher’s exact test. Statistical significance was taken as two-sided P < 0.05. Coefficient of variation (%) was defined as the average of means divided by the standard deviation of mean difference. Repeatability and reproducibility were determined using Bland-Altman analysis and bias (mean difference) is presented alongside 95% limits of agreement (Chapter 3).

Despite there being no published data or pre-clinical models on manganese-enhanced magnetic resonance imaging in hypertrophic cardiomyopathy, the pathological process is one known to cause regional fibrosis, similarly to myocardial infarction. Based on porcine models of myocardial infarction with reperfusion, manganese-enhanced magnetic resonance imaging demonstrated infarct volumes of 14 ± 4% compared to 23 ± 4% with DEMRI (Dash et al. 2015). For a conservative difference in infarct volume of 5%, 14 subjects are required at 90% power and two-sided P < 0.05. We have previously described T1 mapping reproducibility in healthy volunteers and patients with aortic stenosis with a normal pre-contrast T1 value of 1180 ± 28
ms and post-contrast T1 value of 672 ± 56 ms. We anticipate a more subtle between group difference in post-contrast T1 values between the pathological subgroups. Assuming an absolute between group difference of 60 ms, we will require at least 18 subjects per group for 90% power at two-sided P < 0.05. To account for dropouts, greater variation in infarct size and suboptimal imaging, we will recruit 20 subjects for hypertrophic cardiomyopathy cohort. Whilst no comparable measures have been made in patients with dilated cardiomyopathy and takotsubo syndrome, we aimed to recruit 20 patients for each cohort based on similar assumptions.

For the COVID-19 cohort, we have a number of exploratory end-points and our initial sample size calculation was based on our manganese-enhanced magnetic resonance imaging. To calculate the initial sample size, we used the gradient of T1 values as a measure of calcium uptake and utilisation from our previous study. We have found that the mean gradient of change in T1 over 30 minutes in healthy volunteer myocardium was -3.749±1.015, compared to -2.540±0.583 in dilated cardiomyopathy. For detecting a conservative difference in rate of change of 5%, we required at least 15 subjects at 90% power and two-sided P<0.05.
3. CHAPTER 3: REPEATABILITY AND REPRODUCIBILITY OF CARDIAC MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING.

Extracts of this chapter have been submitted for publication as:

Singh t et al. Repeatability and Reproducibility of Cardiac Manganese-Enhanced magnetic Resonance Imaging.
3.1. **SUMMARY**

Manganese-enhanced magnetic resonance imaging can be used as a surrogate marker for myocardial calcium handling. Its reproducibility is currently unknown. The aim of this post-hoc analysis was to establish the reproducibility of cardiac manganese-enhanced magnetic resonance imaging.

In this observational study, sixty-eight participants: 20 healthy volunteers, 20 with acute myocardial infarction, 18 patients with hypertrophic and 10 with non-ischemic dilated cardiomyopathy underwent manganese-enhanced magnetic resonance imaging. A subgroup of the healthy volunteers (n=10) were re-scanned 3 months following baseline imaging. Native and post manganese T1 and myocardial manganese uptake measurements were assessed for intra and inter-observer repeatability and overall scan-rescan reproducibility (only in healthy volunteers).

There was excellent intra-observer and inter-observer correlation in healthy volunteers for mean native T1 mapping (Lin’s coefficient 0.97 and 0.97 respectively) and myocardial manganese uptake (Lin’s coefficient 0.99 and 0.96 respectively) with narrow limits of agreement. There was excellent intra-observer correlation for native T1 and myocardial manganese uptake in patients with acute myocardial infarction (Lin’s coefficient 0.97 and 0.97 respectively), hypertrophic (Lin’s coefficient 0.98 and 0.97 respectively) and dilated cardiomyopathy (Lin’s coefficient 0.99 and 0.95 respectively), with narrow limits of agreement. Similarly, there was excellent inter-observer correlation for native T1 and myocardial manganese uptake in all patient cohorts. As expected, limits of agreement for inter-observer repeatability were
wider than intra-observer measurements in all cohorts. All patient cohorts demonstrated wider limits of agreement for intra- and inter-observer repeatability compared to healthy volunteers. There were no substantial scan-rescan differences for native T1 (P=0.60) and myocardial manganese uptake (P=0.89) in healthy volunteers. There was excellent scan-rescan correlation for native T1 mapping and myocardial manganese uptake (Lin’s coefficient 0.94 and 0.97 respectively).

We have demonstrated excellent repeatability of manganese-enhanced magnetic resonance imaging in healthy and pathological myocardium. Furthermore, scan-rescan reproducibility of manganese-enhanced magnetic resonance imaging in healthy volunteers is excellent.
3.2. INTRODUCTION

Cardiac magnetic resonance imaging has a major role in the diagnosis, evaluation of myocardial function and tissue characterisation of a range of cardiovascular diseases (Rickers et al. 2005, Dass et al. 2012, Japp et al. 2016). Conventional cardiac magnetic resonance with gadolinium enhancement allows for quantification of myocardial fibrosis which has utility in the assessment of viability in ischemic cardiomyopathy. Furthermore, it allows for prognostication in a variety of cardiac conditions including myocarditis, dilated cardiomyopathy, hypertrophic cardiomyopathy, amyloidosis (Migrino et al. 2009, Alba et al. 2020, Greulich et al. 2021), arrhythmogenic right ventricular dysplasia (Murphy et al. 2010) and Fabry’s disease (Tower-Rader et al. 2019). However, gadolinium-based contrast media only allow for the assessment of the extracellular space.

Magnetic resonance imaging using manganese-based contrast media has the ability to provide intracellular contrast of viable myocardium. Manganese was the first clinical magnetic resonance imaging contrast medium to be used in vivo, resulting in shortening of T1 relaxation in the heart, liver, kidneys and pancreas (Spath et al. 2019). In brief, after intravenous administration in humans, manganese dipyridoxyl diphosphate undergoes dephosphorylation and transmetallation with zinc to release manganese ions into the plasma (Kang et al. 1984, Wendland 2004). Being, a calcium analogue, is actively taken up by voltage-gated calcium channels in viable myocardium whereas abnormal myocardium has reduced or no uptake. Myocardial manganese uptake can be calculated using Patlak kinetic modelling (Patlak et al. 1983, Skjold et al. 2006, Jynge et al. 2020), thereby providing a measure for
myocardial calcium handling (Spath et al. 2020, Spath et al. 2021). Indeed, manganese-enhanced T1 mapping can detect dysfunctional myocardial calcium handling in patients with cardiomyopathies and can distinguish between normal and pathological myocardium (Spath et al. 2020, Spath et al. 2021).

Repeatability of the assessment of manganese-enhanced magnetic resonance imaging and its scan-rescan reproducibility have not been established and is a necessary step for its future clinical application. The aims of this study were to establish the intraobserver and interobserver repeatability and the overall scan-rescan reproducibility of manganese-enhanced T1 mapping and kinetic modelling of myocardial manganese uptake.
3.3. METHODS

3.3.1. Study population

Adult (≥18 years of age) healthy volunteers (n=20) were recruited as part of the MEMORY study [NCT04623788]. Patients with acute myocardial infarction (n=20), hypertrophic cardiomyopathy (n=18) or non-ischemic dilated cardiomyopathy (n=10) were recruited from the Edinburgh Heart Centre as part of the MEMRI study [NCT03607669]. Patients with acute myocardial infarction were required to have a ST-segment elevation myocardial infarction according to the universal definition of myocardial infarction (Thygesen et al. 2012) and angiographically proven coronary artery disease. Patients were required to be clinically stable with reduced left ventricular ejection fraction (≤50% by echocardiography) secondary to one or more acute ischaemic events.

The diagnosis of hypertrophic cardiomyopathy and dilated cardiomyopathy were based on echocardiography or magnetic resonance imaging according to European Society of Cardiology guidelines (Authors/Task Force et al. 2014, Bozkurt et al. 2016). Hypertrophic cardiomyopathy was defined as left ventricular hypertrophy (left ventricular wall thickness ≥15 mm in any segment) in the absence of hemodynamic stresses (Authors/Task Force et al. 2014). Non-ischemic dilated cardiomyopathy was defined by the presence of impaired left ventricular systolic function (ejection fraction ≤50% within 12 months) and left ventricular dilatation (left ventricular end-diastolic volume >105 mL/m² for men and >96 mL/m² for women, adjusted for age and body-surface area), in
the absence of abnormal loading conditions (hypertension and valvular disease) and coronary artery disease (Bozkurt et al. 2016).

3.3.2. Magnetic Resonance Imaging

All participants were scanned on a Siemens MAGNETOM Skyrafit 3T scanner (Siemens Healthineers, Erlangen, Germany) with a 30-channel body matrix coil. Electrographic gated breath-hold steady-state free procession long-axis cine images in two, three and four chamber views were acquired. Short axis cine images covering the entire left ventricle were taken at 8-mm slice thickness, 2-mm gap, field of view 300 x 400 mm, matrix 208 x 256, repetition time 2.9 ms, echo time 1.2 ms, flip angle 64-790, temporal resolution <50 ms, with 30 phases per cardiac cycle, in-plane image resolution 1.1 x 1.5 mm to 1.3 x 1.7 mm.

All participants underwent magnetic resonance imaging with gadolinium and manganese enhancement, which were performed at least 48 hours apart. Additionally, healthy volunteers underwent repeat manganese-enhanced magnetic resonance imaging, 3 months following baseline imaging (Figure 3.1)

T1 mapping

For healthy volunteers, T1 mapping was acquired with a modified Look-Locker inversion recovery sequence (Messroghli et al. 2004, Messroghli et al. 2007) and in patients, T1 mapping was acquired with shortened modified Look-Locker inversion recovery (WIP #1048 Siemens Healthineers), with a 5(3)3 sampling pattern and the following typical parameters: slice thickness 8.0 mm with 1.6-mm gap, field of view=360x280 mm, repetition time 388.8 ms; echo
time 1.07 ms, matrix 256×115. To minimise artefacts, acquisition was performed with the region of interest at isocentre, a small shim volume was applied around the myocardium, a large field of view (400 mm) was used.

**Figure 3.1 Consort Diagram**
Late Gadolinium Enhancement

Late gadolinium enhancement images were acquired following intravenous gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany) using a single breath hold per slice with a short-axis stack, and long-axis orientations. T1 mapping was acquired prior to and 10 min after contrast administration as described previously (Spath et al. 2020, Spath et al. 2021).

Manganese infusion

Manganese-enhanced magnetic resonance imaging was achieved using intravenous infusion of manganese dipyridoxyl diphosphate (5 µmol/kg (0.1 mL/kg) at 1 mL/min; Exova SL Pharma, Wilmington, Delaware, USA) and has been described previously (Spath et al. 2020, Spath et al. 2021). Following a full short-axis native T1 stack, a single mid-ventricular short-axis slice was identified and performed at this location every 2.5 min for 30 min after starting manganese infusion, at which point a full short-axis T1 stack was repeated.

A single mid-ventricular slice was chosen for healthy volunteers. For patients, the short-axis slice was identified by the supervising cardiologist, guided by late gadolinium enhancement imaging, native T1 maps and cine images to represent abnormal myocardium (Figure 3.2). For patients with acute myocardial infarction, infarct area was assessed by late gadolinium images and to reduce variability, automated reference regions of interest were generated in the infarct region.

For patients with hypertrophic cardiomyopathy, regions of maximal hypertrophy and fibrosis were selected and for dilated cardiomyopathy, a mid-ventricular
short-axis slice was selected. The chosen slice was matched visually by the same supervising cardiologist for repeat scanning.
**Figure 3.2 Regions of interest in manganese-enhanced magnetic resonance imaging.**

Late gadolinium enhanced (A) and 30 min post-manganese T1 maps (B) in healthy volunteers, patients with acute myocardial infarction, hypertrophic and dilated cardiomyopathy. Demonstrating regions of interest in healthy volunteers (septum) and patients with acute myocardial infarction (infarct, I, peri-infarct, PI, and remote, R), hypertrophic cardiomyopathy (hypertrophied, non-fibrotic, H and fibrotic, F) and dilated cardiomyopathy (septum).
3.3.3. Image analysis

All analyses of T1 maps, late gadolinium enhancement and cine-derived volumetric and functional sequences were performed using Circle CVI (Circle Cardiovascular Imaging, CVI42 v5.3.6, Calgary Canada) as described previously (Spath et al. 2020, Spath et al. 2021). Image analysis was performed by two trained observers who were blinded to participant details.

All images were assessed for artefacts caused by susceptibility effects and cardiac or respiratory motion. The presence of artefacts led to the exclusion of all affected myocardial segments. Endocardial and epicardial borders were manually defined on all conventional short-axis images for volumetric and wall motion measurements and were then copied to corresponding T1 map images for analysis with minimal manual adjustments. The left ventricular basal short axis slice was identified as the image containing at least 50% of circumferential myocardium at end diastole. Papillary muscles were included in the mass and excluded from volumetric analysis.

For healthy volunteers, T1 values were derived from segments 7-12 (mid-ventricular slice) of a standard 16-segment model, as well as septal (region of interest in mid-septal wall) and global (average of all 6 segments from mid-ventricular slice) values. After contouring, an additional epicardial and endocardial offset of 20% was applied automatically to minimise partial volume effect for all T1 map analyses.

For patients with myocardial infarction, a reference region of interest was manually generated in the remote myocardium, with minimal manual
adjustment based on the opposing wall from the late gadolinium enhancement-defined infarct and wall motion by cine sequences where necessary. Given the lack of established consensus on quantification, a threshold of 2 x SD above remote myocardium was used for area at risk (Bulluck et al. 2015). Peri-infarct tissue was defined as late gadolinium enhancement negative but with elevated T1 in the area at risk (>2 x SD) in the infarct related artery territory. For patients with hypertrophic cardiomyopathy, regions of interest were drawn in areas with hypertrophy and fibrosis (guided by late gadolinium enhancement). In patients with dilated cardiomyopathy, regions were drawn in the mid-septal wall, due to lack of late gadolinium enhancement (Figure 3.2). For serial T1 imaging post manganese, manually drawn regions of interest from the pre-contrast image were transferred to all subsequent post-contrast images to ensure consistency.

3.3.4. Kinetic modelling

To derive quantitative estimates and to assess differential manganese uptake, kinetic model analysis was performed, as described previously (Spath et al. 2020, Singh et al. 2021, Spath et al. 2021). Kinetic modelling was based on a Patlak two-compartment model formulation (Patlak et al. 1983, Skjold et al. 2006). In brief, the model consists of (i) a reversible compartment ($v_e$), comparable to intravascular and interstitial space and (ii) an irreversible compartment ($v_i$) comparable to the intracellular space, in which irreversible accumulation of the contrast agent is anticipated during the imaging period (30 min). The arterial concentration (derived from blood pool T1) represents contrast agent delivery into myocardial tissue and constitutes the arterial input function.
Skjöld et al previously derived a Patlak model formulation for cardiac manganese-enhanced magnetic resonance imaging (Skjold et al. 2006), demonstrating that an apparent unidirectional influx constant ($K_i$) for the transfer of manganese from plasma to irreversible compartments $v_i$, can be measured, using Equation 1:

$$\frac{C_t(t)}{C_a(t)} = K_i \frac{\int_0^t C_a(t)dt}{C_a(t)} + v_e$$

(1)

where $C_t$ and $C_a$ are the manganese concentration in myocardial tissue and blood pool (arterial input function) respectively. This formulation is equivalent to the Patlak model (Patlak et al. 1983) and describes that if a contrast medium is irreversibly trapped in the tissue within the imaging period, the instantaneous tissue concentration divided by the instantaneous arterial concentration plotted against the integrated arterial concentration divided by the instantaneous arterial concentration, will result in linearisation of the data. The gradient of this line represents the apparent unidirectional influx constant $K_i$, which equals:

$$K_i = \frac{k_1k_2}{k_2+k_3}$$

(2)

where $k_1$, $k_2$, and $k_3$ are the individual rate constants of the compartmental model presented. A visual representation of the influx constant $K_i$ is given in Figure 2.3.

3.3.5. Intra and inter-observer repeatability

To test for intra-observer repeatability, scans were analysed twice by the same operator in random order and 6 months apart to reduce the risk of recall bias.
To test for inter-observer repeatability, ten random datasets from each patient cohort and healthy volunteer data set were analysed by a second observer.

**3.3.6. Scan-rescan reproducibility**

Scan-rescan analysis was performed on a subset of healthy volunteers (n=10) who underwent repeat manganese-enhanced cardiac magnetic resonance imaging 3 months after baseline scanning.

**3.3.7. Statistical analysis**

Data are expressed as mean ± standard deviation or mean (95% confidence interval) for continuous variables or median [interquartile range] where not normally distributed. Categorical variables are presented as number (percentage). Data were analysed using paired or unpaired Student’s t-tests, mixed effects model, linear regression analysis and Lin’s concordance correlation coefficients. Group variance was examined with the Brown-Forsythe test. Coefficient of variation (%) was defined as the average of means divided by the standard deviation of mean difference. Repeatability and reproducibility were determined using Bland-Altman analysis and bias (mean difference) is presented alongside 95% limits of agreement. Statistical analysis was performed using GraphPad Prism (Version 8.0, GraphPad Software, San Diego, California, USA). Statistical significance was taken as a two-sided P-value <0.05.
3.4. RESULTS

3.4.1. Healthy Volunteers

Intra-observer repeatability

In twenty healthy volunteers, a total of 120 segments were analysed. Six segments, predominantly the infero-lateral wall (segment 11), were excluded due to artefact. The mean septal native T1 was $1218\pm24$ ms (Figure 3.3). There was regional difference in myocardial native T1, with septal T1 appearing to be higher than global T1 ($1218\pm24$ versus $1215\pm22$ ms, P=0.02). Furthermore, myocardial manganese demonstrated similar segmental variation across the myocardium (P= 0.04, Figure 3.3).
Figure 3.3 Native T1 and myocardial manganese uptake across myocardial segments

(A) Mean native T1 values (m/s) and (B) mean myocardial manganese uptake (Ki/ml/100g/min) per segment in healthy volunteers (n=20). Septal and global values (C).

<table>
<thead>
<tr>
<th>Healthy Volunteer</th>
<th>Septal</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Global</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Native T1 (ms)</td>
<td>1218 ±27</td>
<td>1214 ±41</td>
<td>1230 ±26</td>
<td>1226 ±23</td>
<td>1212 ±30</td>
<td>1201 ±34</td>
<td>1208 ±41</td>
<td>1215 ±25</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean Ki (ml/100g/min)</td>
<td>8.4±0.7</td>
<td>8.4±0.9</td>
<td>8.5±0.9</td>
<td>8.4±0.7</td>
<td>8.3±0.8</td>
<td>8.2±1.1</td>
<td>8.0±1.1</td>
<td>8.3±1.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

There were no differences between mean myocardial septal native and post-manganese T1 on repeat analysis by the same observer (p= 0.75 and 0.63 respectively), with excellent correlation (Lin’s concordance correlation: 0.97 and 0.95 respectively, Table 3.1). Bland-Altman plots demonstrate excellent intra-observer agreement; however, limits of agreement (LoA) were wider for post-manganese T1 compared with native T1 (880±24 ms, bias: +3.0ms, LoA: - 9.1 to 14.8 versus 1218±24ms, bias +2.2ms, LoA: -12.5 to 8.0, respectively, Table 3.1).

Mean myocardial manganese (septal) uptake in healthy volunteers was 8.4±0.7 mL/100 g of tissue/min and similarly had excellent correlation on repeated measurement (Lin’s correlation coefficient: 0.99, Table 3.1). Coefficient of variation was higher for manganese uptake compared to native
and post-manganese T1 (7.1, 1.3 and 3.3 % respectively). Despite this, Bland-Altman plots highlighting limits of agreement (Figure 3.4) demonstrate excellent intra-observer agreement.

**Inter-observer repeatability**

There were no differences in myocardial native T1 (1218±24 versus 1221±27 ms, bias: +2.9 ms, p= 0.96) and estimates of mean myocardial manganese uptake (8.4±0.7 versus 8.2±0.7 mL/100 g of tissue/min, bias: -0.04, p=0.96) in healthy volunteers, with all showing excellent correlations (Table 3.1). Bland-Altman plots highlight excellent inter-observer agreements for native T1, post-manganese T1 and myocardial manganese uptake (Figure 3.4).

**Scan-rescan Reproducibility**

Ten healthy volunteers underwent repeat manganese-enhanced imaging 88 [range: 62-124] days following baseline imaging. There were no differences between repeated scans for mean native T1 (1230±23 versus 1226±15, P=0.60) and myocardial manganese uptake (8.4±0.7 versus 8.5±0.7, P=0.89, Table 3.1). There was excellent correlation between the paired scans for mean native T1 and myocardial manganese uptake (Lin's correlation coefficient: 0.94 and 0.97 respectively, Table 3.1). Similarly, Bland-Altman plots demonstrate narrow limits of agreement between the two paired scans (Figure 3.4, Table 3.1).
TABLE 3.1. Intra-observer, inter-observer repeatability and scan-rescan reproducibility for healthy volunteers.

<table>
<thead>
<tr>
<th>Intra-observer (n=20)</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman 95% LoA (95% CI)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1 (ms)</td>
<td>1218±24 [1189 - 1270]</td>
<td>1220±22 [1187 - 1273]</td>
<td>+2.2</td>
<td>0.75</td>
<td>0.97 (0.92-0.99)</td>
<td>-12.5 (-16.8, -8.2) to 8.0 (3.7, 12.3)</td>
<td>1.3</td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>880±24 [823 - 922]</td>
<td>883±17 [851 - 918]</td>
<td>+3.0</td>
<td>0.63</td>
<td>0.95 (0.78-0.97)</td>
<td>-9.1 (9.14, -3.9) to 14.8 (9.6, 19.9)</td>
<td>3.3</td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>8.4±0.7 [7.2 – 10.2]</td>
<td>8.3±0.7 [8.0 – 8.7]</td>
<td>-0.01</td>
<td>0.91</td>
<td>0.99 (0.95-0.99)</td>
<td>-0.6 (-0.8, -0.3) to 0.5 (0.31, 0.79)</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-observer (n=20)</th>
<th>First observer</th>
<th>Second observer</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman 95% LoA (95% CI)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1 (ms)</td>
<td>1218±24 [1189 - 1270]</td>
<td>1221 ± 27 [1183 - 1279]</td>
<td>+2.9</td>
<td>0.96</td>
<td>0.98 (0.91-0.99)</td>
<td>- 10.9 9-14.6, -7.2) to 6.8 (2.6, 9.9)</td>
<td>1.8</td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>880±24 [838 - 930]</td>
<td>885± 28 [809 - 933]</td>
<td>+4.9</td>
<td>0.80</td>
<td>0.91 (0.78-0.96)</td>
<td>- 19.7 (-23.4, -9.4) to 29.3 (34.5,15.3)</td>
<td>3.9</td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>8.4±0.7 [7.2 – 10.2]</td>
<td>8.2±0.7 [7.1- 10.0]</td>
<td>-0.04</td>
<td>0.96</td>
<td>0.96 (0.91-0.99)</td>
<td>- 0.7 (-0.9, -0.3) to 0.6 (0.4, 0.90)</td>
<td>8.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scan-Rescan (n=10)</th>
<th>Baseline scan</th>
<th>Repeat scan</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman 95% LoA (95% CI)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1 (ms)</td>
<td>1230±23 [1213- 1247]</td>
<td>1226±15 [1213 - 1239]</td>
<td>-4.0</td>
<td>0.60</td>
<td>0.94 (0.76-0.98)</td>
<td>-12.6 (-25.1, -0.07) to 22.6 (10.1, 35.3)</td>
<td>1.4</td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>899±26 [871 - 927]</td>
<td>894±27 [867 - 921]</td>
<td>-4.5</td>
<td>0.71</td>
<td>0.92 (0.64-0.95)</td>
<td>-26.8 (-43.2, -12.6) to 15.7 (0.15, 31.3)</td>
<td>2.6</td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>8.4±0.7 [8.0 – 8.6]</td>
<td>8.5±0.7 [7.9 - 9.1]</td>
<td>+0.04</td>
<td>0.89</td>
<td>0.97 (0.91-0.99)</td>
<td>- 0.3 (-0.6, -0.1) to 0.3 (-1.7, 1.5)</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Mean± standard deviation [95% confidence interval], LCC, Lin’s concordance correlation, LoA, limits of agreement, CoV, coefficient of variation.
Figure 3.4 Intra, Inter- Observer Repeatability and Scan-Rescan Reproducibility for Myocardial Manganese uptake in Healthy Volunteers.

Linear regression analysis and Bland-Altman plots demonstrating intra-observer repeatability (A, B), inter-observer repeatability (C, D) and scan-rescan reproducibility (E, F) in healthy volunteers.
3.4.2. Patient cohorts

**Intra-observer repeatability**

The mean native T1 for patients with acute myocardial infarction (infarct), hypertrophic (non-fibrosis) or dilated cardiomyopathy were 1395 ± 72, 1185±35 and 1208±60 ms respectively. On repeated analysis by the same observer, there were no differences between mean myocardial T1 values (p= 0.87, 0.96 and 0.92 respectively, Table 3.2). Mean myocardial manganese uptake in patients with acute myocardial infarction (infarct) hypertrophic (non-fibrosis) and dilated cardiomyopathy was 5.3±1.4, 7.6±1.6 and 7.2±1.5 mL/100 g of tissue/min respectively. Repeated analyses demonstrated excellent correlation across all patient cohorts (Lin’s correlation coefficient: 0.98, 0.97 and 0.95 respectively, Table 3.2). Bland-Altman plots demonstrate excellent agreement (Figures 3.5- 3.7), however, patients with dilated cardiomyopathy demonstrated the widest limits of agreement for native, post-manganese T1 and myocardial manganese uptake (-15.6 to 11.4, -22.5 to 18.4 and -0.9 to 0.6 respectively, Table 3.2).

There was no difference in mean native T1 and myocardial manganese uptake in patients with acute myocardial infarction (peri-infarct and remote) and hypertrophic cardiomyopathy (fibrosis) on repeat analysis. Similarly, there was excellent correlation and Bland-Altman plots demonstrate narrow limits of agreement (Table 3.2). Peri-infarct regions in patients with acute myocardial infarction and fibrotic regions in patient with hypertrophic cardiomyopathy
demonstrated wider limits of agreement compared to infarct region and non-fibrotic regions in the same cohorts (Table 3.3).
TABLE 3.2 Intra-observer repeatability for patients with myocardial infarction (infarct), hypertrophic cardiomyopathy (non-fibrosis) or dilated cardiomyopathy.

<table>
<thead>
<tr>
<th>Myocardial infarction (n=20)</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman (95% LoA)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Native T1 (ms)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Infarct</td>
<td>1395 ± 72 [1246-1501]</td>
<td>1391 ± 74 [1241-1485]</td>
<td>-3.8</td>
<td>0.87</td>
<td>0.97 (0.91-0.99)</td>
<td>-20.0 (-27.4, -13.1) to 12.9 (5.8, 20.1)</td>
<td>4.1</td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>1137±79 [996 - 1280]</td>
<td>1133±80 [1009 - 1284]</td>
<td>-4.2</td>
<td>0.87</td>
<td>0.97 (0.91-0.99)</td>
<td>-18.4 (-24.8, -12.7) to 8.9 (2.86, 14.82)</td>
<td>6.9</td>
</tr>
<tr>
<td>Manganese uptake (Ki, mL/100 g of tissue/min)</td>
<td>5.3±1.4 [2.0 – 7.3]</td>
<td>5.4±1.4 [3.0 – 7.4]</td>
<td>+0.06</td>
<td>0.95</td>
<td>0.98 (0.94-0.99)</td>
<td>-0.4 (-0.8, -0.3) to 0.6 (0.3, 0.7)</td>
<td>8.1</td>
</tr>
<tr>
<td><strong>Hypertrophic cardiomyopathy (n=18)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1185 ± 35 [1104 -1237]</td>
<td>1188 ± 33 [1112 - 1240]</td>
<td>+ 2.9</td>
<td>0.96</td>
<td>0.98 (0.93-0.99)</td>
<td>- 15.1 (-20.7, -9.5) to 9.2 (3.6, 14.7)</td>
<td>2.9</td>
</tr>
<tr>
<td>Non fibrosis</td>
<td>922±44 [838 -994]</td>
<td>919±40 [848 - 980]</td>
<td>-3.0</td>
<td>0.80</td>
<td>0.95 (0.81-0.98)</td>
<td>- 11.2 (-17.4, -4.8) to 15.1 (8.83, 21.4)</td>
<td>4.1</td>
</tr>
<tr>
<td>Manganese uptake (Ki, mL/100 g of tissue/min)</td>
<td>7.6±1.6 [4.0 – 9.9]</td>
<td>7.7±0.7 [7.4- 9.4]</td>
<td>+0.07</td>
<td>0.96</td>
<td>0.97 (0.93-0.99)</td>
<td>- 0.6 (-0.8, -0.1) to 0.4 (0.2, 0.7)</td>
<td>7.4</td>
</tr>
<tr>
<td><strong>Dilated Cardiomyopathy (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1208 ± 6 [1164 -1252]</td>
<td>1206 ± 61 [1162 - 1250]</td>
<td>-2.7</td>
<td>0.92</td>
<td>0.99 (0.97- 1.0)</td>
<td>-15.6 (-25.6, -6.1) to 11.4 (1.78, 20.9)</td>
<td>5.9</td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>981±58 [923 -1039]</td>
<td>985±61 [924 - 1046]</td>
<td>+5.1</td>
<td>0.85</td>
<td>0.97 (0.83-0.97)</td>
<td>-22.5 (-25.5, -13.2) 18.4 (1.1, 24.5)</td>
<td>6.3</td>
</tr>
<tr>
<td>Manganese uptake (Ki, mL/100 g of tissue/min)</td>
<td>7.2 ± 1.5 [6.2 - 8.3]</td>
<td>7.4 ± 1.3 [6.4- 8.3]</td>
<td>+0.2</td>
<td>0.81</td>
<td>0.95 (0.84-0.98)</td>
<td>- 0.9 (-1.5, -0.4) to 0.6 (0.04, 1.16)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Mean± standard deviation [95% confidence interval], LCC, Lin’s concordance correlation, LoA, limits of agreement, CoV, coefficient of variation
Table 3.3 | Intra-observer repeatability for patients with myocardial infarction and hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
<th>Myocardial infarction (n=20)</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman (95% LoA)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1 (ms) Peri-infarct</td>
<td>1263±49 [1166-1361]</td>
<td>1265±50 [1169-1356]</td>
<td>+2.1</td>
<td>0.89</td>
<td>0.98</td>
<td>-20.6 (-28.7, -12.5) to 17.0 (8.9, 25.1)</td>
<td>3.8</td>
</tr>
<tr>
<td>Native T1 (ms) Remote</td>
<td>1147 ± 36 [1071-1218]</td>
<td>1144 ± 38 [1076-1207]</td>
<td>-2.8</td>
<td>0.81</td>
<td>0.95</td>
<td>-20.4 (-30.1, -10.5) to 23.4 (15.7, 31.2)</td>
<td>3.9</td>
</tr>
<tr>
<td>Post Manganese T1 (ms) Peri-infarct</td>
<td>1009±20 [973-1058]</td>
<td>1010±21 [971-1041]</td>
<td>+1.6</td>
<td>0.86</td>
<td>0.90</td>
<td>-13.1 (-27.7, -10.5) to 22.1 (13.2, 31.0)</td>
<td>3.1</td>
</tr>
<tr>
<td>Post Manganese T1 (ms) Remote</td>
<td>886 ± 37 [816 – 944]</td>
<td>889 ± 34 [812 – 947]</td>
<td>+2.2</td>
<td>0.83</td>
<td>0.94</td>
<td>-17.6 (27.8, 16.3) to 21.6 (11.1, 32.2)</td>
<td>4.4</td>
</tr>
<tr>
<td>Manganese uptake- (Ki, mL/100 g of tissue/min) Peri-infarct</td>
<td>6.7 ±1.2 [4.0-8.6]</td>
<td>6.5 ± 1.1 [4.1-8.6]</td>
<td>-0.09</td>
<td>0.79</td>
<td>0.92</td>
<td>-0.8 (-1.2, -0.4) to 1.0 (0.6, 1.4)</td>
<td>17.3</td>
</tr>
<tr>
<td>Manganese uptake- (Ki, mL/100 g of tissue/min) Remote</td>
<td>8.6±1.2 [6.0-11]</td>
<td>8.7±1.3 [6.0-11.3]</td>
<td>+0.05</td>
<td>0.90</td>
<td>0.98</td>
<td>-0.5 (-0.6, -0.2) to 0.4 (0.2, 0.5)</td>
<td>15.3</td>
</tr>
</tbody>
</table>

| Hypertrophic cardiomyopathy (n=18) |
|-----------------------------------|-------------------|--------------------|-----------------|---------|--------------|----------------------|---------|
| Native T1 (ms) Fibrosis (n=11)    | 1332 ±91 [1219 – 1477] | 1335 ± 86 [1224 – 1465] | + 3.0           | 0.93    | 0.97         | -11.8 (22.0, -1.6) to 19.2 (9.0, 29.3) | 3.4     |
| Post Manganese T1 (ms) Fibrosis (n=11) | 1083 ± 87 [1006-1261] | 1086 ±90 [1010-1251] | +3.7            | 0.95    | 0.94         | -17.7 (-36.7, -8.9) to 9.7 (0.7, 18.7) | 4.3     |
| Manganese uptake- (Ki, mL/100 g of tissue/min) Fibrosis (n=11) | 5.2±1.5 [3.2-7.3] | 5.1 ± 1.2 [3.0 – 7.4] | -1.0           | 0.88    | 0.97         | -0.3 (-0.5, -0.2) to 0.1 (-0.5, 0.2) | 14.1    |

Mean± standard deviation [95% confidence interval], LCC, Lin’s concordance correlation, LoA, limits of agreement, CoV, coefficient of variation
Figure 3.5 Intraobserver and Interobserver repeatability of Myocardial Manganese uptake in Acute Myocardial Infarction.

Intra-observer linear regression analysis (A) and Bland-Altman plots for myocardial manganese uptake (B) in patients with acute myocardial infarction. Inter-observer linear regression analysis (C) and Bland-Altman plots for myocardial manganese uptake (D) in patients with acute myocardial infarction.
Figure 3.6 Intraobserver and Interobserver repeatability of Myocardial Manganese uptake in Hypertrophic Cardiomyopathy.

Intra-observer linear regression analysis (A) and Bland-Altman plots for myocardial manganese uptake (B) in patients with hypertrophic cardiomyopathy. Inter-observer linear regression analysis (C) and Bland-Altman plots for myocardial manganese uptake (D) in patients with hypertrophic cardiomyopathy.
Figure 3.7 Intraobserver and Interobserver repeatability of Myocardial Manganese uptake in Dilated Cardiomyopathy.

Intra-observer linear regression analysis (A) and Bland-Altman plots for myocardial manganese uptake (B) in patients with dilated cardiomyopathy. Inter-observer linear regression analysis (C) and Bland-Altman plots for myocardial manganese uptake (D) in patients with dilated cardiomyopathy.
Inter-observer repeatability

Similar levels of interobserver repeatability were also seen for patients with acute myocardial infarction, hypertrophic or dilated cardiomyopathy (Table 3.4, Figures 3.5-3.7). Across all cohorts, coefficient of variation was higher for myocardial manganese uptake compared to native T1. Similarly, there was excellent correlation with narrow limits of agreement in peri-infarct and remote myocardium in patients with acute myocardial infarction and fibrotic myocardium in patients with hypertrophic cardiomyopathy (Table 3.5).
**TABLE 3.4 Interobserver repeatability for patients with myocardial infarction (infarct), hypertrophic cardiomyopathy (non-fibrosis) or dilated cardiomyopathy.**

<table>
<thead>
<tr>
<th></th>
<th>First observer</th>
<th>Second observer</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman (95% LoA)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial infarction (n=20)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1395 ± 72</td>
<td>1390 ± 74</td>
<td>-4.4</td>
<td>0.83</td>
<td>0.98</td>
<td>(-32.2, -10.5) to 28.9 (18.1, 39.8)</td>
<td>5.2</td>
</tr>
<tr>
<td>Infarct</td>
<td>[1246-1501]</td>
<td>[1222-1484]</td>
<td></td>
<td></td>
<td>(0.70- 0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>1137±79</td>
<td>1141 ± 74</td>
<td>+3.3</td>
<td>0.88</td>
<td>0.99</td>
<td>(-32.5, -14.7) to 17.6 (8.7, 26.5)</td>
<td>6.9</td>
</tr>
<tr>
<td>Infarct</td>
<td>[996 - 1280]</td>
<td>[1001-1271]</td>
<td></td>
<td></td>
<td>(0.97-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>5.3±1.4</td>
<td>5.2±1.3</td>
<td>-0.08</td>
<td>0.79</td>
<td>0.98</td>
<td>(-1.03, -0.4) to 0.9 (0.5, 1.2)</td>
<td>9.9</td>
</tr>
<tr>
<td>(Ki, mL/100 g of tissue/min)</td>
<td>[2.0 – 7.3]</td>
<td>[2.9 – 7.3]</td>
<td></td>
<td></td>
<td>(0.96-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypertrophic cardiomyopathy (n=18)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1185 ± 35</td>
<td>1189±33</td>
<td>+3.9</td>
<td>0.88</td>
<td>0.97</td>
<td>(-37.6, -16.2) to 19.1 (8.5, 27.7)</td>
<td>3.1</td>
</tr>
<tr>
<td>Non fibrosis</td>
<td>[1104 -1237]</td>
<td>[110 - 1206]</td>
<td></td>
<td></td>
<td>(0.84-0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>922± 44</td>
<td>917 ± 45</td>
<td>-4.8</td>
<td>0.82</td>
<td>0.97</td>
<td>(-19.8, -3.4) to 22.7 (914.5, 31.0)</td>
<td>4.3</td>
</tr>
<tr>
<td>Non fibrosis</td>
<td>[838 -994]</td>
<td>[844 - 975]</td>
<td></td>
<td></td>
<td>(0.94-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>7.6±1.6</td>
<td>7.4±1.6</td>
<td>-0.2</td>
<td>0.89</td>
<td>0.98</td>
<td>(-0.8, -1.0) to 0.7 (0.5, 0.9)</td>
<td>8.2</td>
</tr>
<tr>
<td>(Ki, mL/100 g of tissue/min)</td>
<td>[4.0 – 9.9]</td>
<td>[3.9 – 9.8]</td>
<td></td>
<td></td>
<td>(0.96-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dilated cardiomyopathy (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1208 ± 60</td>
<td>1206 ± 68</td>
<td>-2.2</td>
<td>0.94</td>
<td>0.98</td>
<td>(-31.2, -4.1) to 20.3 (6.7, 33.2)</td>
<td>5.8</td>
</tr>
<tr>
<td>Infarct</td>
<td>[1164 - 1252]</td>
<td>[1157 - 1256]</td>
<td></td>
<td></td>
<td>(0.94-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>981±58</td>
<td>977±57</td>
<td>-4.6</td>
<td>0.88</td>
<td>0.98</td>
<td>(-32.3, -8.9) to 12.4 (90.65, 24.1)</td>
<td>6.5</td>
</tr>
<tr>
<td>Infarct</td>
<td>[923 -1039]</td>
<td>[920 - 1034]</td>
<td></td>
<td></td>
<td>(0.98-0.699)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake</td>
<td>7.2 ± 1.5</td>
<td>7.1 ± 1.3</td>
<td>-0.1</td>
<td>0.90</td>
<td>0.95</td>
<td>(-1.1, -0.2) to 0.9 (0.2, 1.1)</td>
<td>13.2</td>
</tr>
<tr>
<td>(Ki, mL/100 g of tissue/min)</td>
<td>[6.2 - 8.3]</td>
<td>[6.2 - 8.0]</td>
<td></td>
<td></td>
<td>(0.79-0.99)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean± standard deviation [95% confidence interval], LCC, Lin's concordance correlation, LoA, limits of agreement, CoV, coefficient of variation.
**Table 3.5 Inter-observer repeatability for patients with myocardial infarction and hypertrophic cardiomyopathy.**

<table>
<thead>
<tr>
<th></th>
<th>First observer</th>
<th>Second observer</th>
<th>Mean Difference</th>
<th>P value</th>
<th>LCC (95% CI)</th>
<th>Bland-Altman (95% LoA)</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial infarction</strong> (n=20)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Native T1 (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peri-infarct</td>
<td>1263±49</td>
<td>1267±50</td>
<td>+4.1</td>
<td>0.87</td>
<td>0.97</td>
<td>-33.6 (-43.7, -5.1) to 28.2 (14.9, 41.3)</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>[1166-1361]</td>
<td>[1169-1373]</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1147 ± 36</td>
<td>1144 ± 34</td>
<td>-2.6</td>
<td>0.82</td>
<td>0.89</td>
<td>-19.4 (-29.3, 9.8) to 25.0 (15.6, 35.1)</td>
<td>3.5</td>
</tr>
<tr>
<td>Remote</td>
<td>[1071-1218]</td>
<td>[1071-1209]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>1010 ± 20</td>
<td>1015 ± 25</td>
<td>+4.6</td>
<td>0.65</td>
<td>0.88</td>
<td>-28 (-36.5, -14.3) to 19.3 (13.5, 32.6)</td>
<td>7.1</td>
</tr>
<tr>
<td>Peri-infarct</td>
<td>[973-1058]</td>
<td>[971-1058]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>886 ± 37</td>
<td>882 ± 41</td>
<td>-4.9</td>
<td>0.72</td>
<td>0.86</td>
<td>-21.4 (-26.5, -9.5) to 31.6 (22.6, 54.3)</td>
<td>6.4</td>
</tr>
<tr>
<td>Remote</td>
<td>[916 – 944]</td>
<td>[809 – 941]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake- (Ki, mL/100 g of tissue/min)- Peri infarct</td>
<td>6.7 ±1.2</td>
<td>6.5 ± 1.1</td>
<td>- 0.09</td>
<td>0.82</td>
<td>0.94</td>
<td>-0.9 (-1.37, -0.5) to 1.1 (0.6, 1.6)</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>[4.0-8.6]</td>
<td>[3.2-8.3]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake- (Ki, mL/100 g of tissue/min)- Remote</td>
<td>8.6±1.2</td>
<td>8.5 ±1.2</td>
<td>-0.04</td>
<td>0.88</td>
<td>0.97</td>
<td>-0.5 (-0.7, -3) to 0.6 (0.4, 0.8)</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>[6.0-11]</td>
<td>[6.3-10.2]</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Hypertrophic cardiomyopathy</strong> (n=18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1 (ms)</td>
<td>1332 ±91</td>
<td>1337 ± 86</td>
<td>+ 5.3</td>
<td>0.89</td>
<td>0.94</td>
<td>-24.4 (-43.7, -5.1) to 43.2 (14.9, 53.3)</td>
<td>6.5</td>
</tr>
<tr>
<td>Fibrosis (n=11)</td>
<td>[1219 – 1477]</td>
<td>[1221 – 1476]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Manganese T1 (ms)</td>
<td>1083 ± 87</td>
<td>1079 ± 90</td>
<td>-4.9</td>
<td>0.95</td>
<td>0.94</td>
<td>-18.4 (-24.3, -9.4) to 23.2 (11.5, 31.3)</td>
<td>7.3</td>
</tr>
<tr>
<td>Fibrosis (n=11)</td>
<td>[1006-1261]</td>
<td>[1001-1249]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese uptake- (Ki, mL/100 g of tissue/min)- Fibrosis (n=11)</td>
<td>5.2 ±1.5</td>
<td>5.3 ± 1.4</td>
<td>+0.2</td>
<td>0.80</td>
<td>0.97</td>
<td>-0.5 (-0.8, 0.3) to 0.2 (-0.02, 0.4)</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>[3.2-7.3]</td>
<td>[3.2 – 7.5]</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Mean± standard deviation [95% confidence interval], LCC, Lin’s concordance correlation, LoA, limits of agreement, CoV, coefficient of variation*
3.5. **DISCUSSION**

Cardiac manganese-enhanced magnetic resonance imaging holds major promise in the assessment of myocardial calcium handling. In this study of cardiac manganese-enhanced magnetic resonance imaging, we demonstrate for the first time that myocardial T1 mapping and kinetic modelling of manganese uptake is repeatable and reproducible in both healthy and diseased myocardium. We found excellent intra-observer and inter-observer repeatability as well as scan-rescan reproducibility for measures of manganese uptake in the myocardium. This suggests that this technique is sufficiently robust for application in clinical care.

As expected, septal native T1 values for healthy volunteers and patient cohorts demonstrated less variability between intra and inter observer measurements compared to post-manganese T1 values. Previous studies have described greater variation in post-contrast T1 values with gadolinium and therefore similar effects with manganese are not unexpected (Rogers et al. 2013, Kellman et al. 2014, Singh et al. 2015, Aus dem Siepen et al. 2018). Contrast-enhanced T1 mapping is a function of contrast agent dispersion and volume of distribution which may differ across individuals. This can explain why post-manganese T1 values had greater variability compared to native T1, although these differences were small.
Coefficients of variation for myocardial manganese uptake in healthy volunteers and patient cohorts for both intra- and inter-observer repeatability and scan-rescan reproducibility were higher than for native and post-manganese T1. Kinetic modelling of myocardial manganese uptake is dependent on variables such as native T1, post-manganese T1 and blood pool signal. As such, variability in any of those factors will result in greater variability in myocardial manganese uptake. Furthermore, heterogeneities in cardiac perfusion, contrast agent kinetics and dispersion will also lead to greater bias and variability. Despite this, we observed very strong correlation with no significant variation in intra and inter-observer and inter-scan measurements of myocardial manganese uptake in healthy volunteers and patient cohorts.

Similar to previous cardiac magnetic resonance reproducibility studies (Messroghli et al. 2004, Rogers et al. 2013, Dabir et al. 2014, Rauhalammi et al. 2016, Lin et al. 2018), we demonstrate that intra-observer agreements for native T1 and myocardial manganese uptake were stronger than inter-observer and scan-rescan agreements for healthy volunteers and patient cohorts. However, the main differences seem to be the 95% confidence intervals rather than the mean. Interestingly, intra-observer repeatability demonstrated broader limits of agreement in patients with dilated cardiomyopathy. This likely reflects the difficulty in defining a region of interest in the mid-septal wall in patients with dilated cardiomyopathy due to myocardial thinning. Despite this, there was very strong intra-observer and inter-observer correlation in T1 mapping and kinetic modelling in patients with dilated cardiomyopathy.
Compared to the other study populations and myocardial regions, peri-infarct regions in patients with acute myocardial infarction and fibrotic regions in patients with hypertrophic cardiomyopathy demonstrated broader limits of agreement and higher coefficients of variation for intra and interobserver repeatability. This likely reflects the subtle variations in discriminating peri-infarct and fibrotic regions as well as the manual delineation of endocardial and epicardial borders. Despite this, we continued to observe strong intra-observer and inter-observer correlations for such regions.

Manganese-enhanced magnetic resonance imaging has shown promise as a surrogate marker of myocardial calcium uptake in patients with ischemic and non-ischemic cardiomyopathies (Spath et al. 2020, Spath et al. 2021) demonstrating its potential for clinical application. As such, it is important to validate this technique before use in clinical practice. Current studies are underway assessing its utility in reversible myocardial dysfunction (NCT04623788) and patients at risk of developing heart failure (NCT04591639). Similar to traditional imaging with gadolinium, there is variation in measurements between vendors and different T1 mapping techniques, and further work is required to ensure consistency across different platforms and scanners.
3.5.1. Limitations

Our study has some limitations. First, we were not able to perform scan-rescan measurements on patients with hypertrophic and dilated cardiomyopathy. It would be important to confirm that this technique has similar reproducibility in diseased states if it is to be used for serial scanning to assess disease progression or treatment interventions. Second, healthy volunteers were scanned using MOLLI T1 and patient cohorts underwent ShMOLLI T1 mapping. Our aim was to assess the reproducibility of manganese-enhanced magnetic resonance imaging in healthy and pathological myocardium. As such, we are not comparing patients with healthy volunteers. Furthermore, we have demonstrated that manganese-enhanced magnetic resonance imaging is reproducible using either MOLLI or ShMOLLI T1 mapping reinforcing its generalisability irrespective of the method used. Third, performing a statistical test for defining cut-off for normal versus abnormal myocardial manganese uptake (Ki) was not in the scope of our study. A future study with larger numbers of patients with myocardial infarction and/ or non-ischaemic cardiomyopathy with adequate numbers of remote/healthy regions of interest (ROI’s) would support a ROC analysis for defining a normal range for the tracer kinetic parameters. Indeed, it is in our future interests to perform such work. Finally, manganese dipyridoxyl diphosphate is currently not readily or widely available for clinical use although we anticipate that this is likely to change in the future.
3.6. **CONCLUSION**

In conclusion, we have demonstrated the excellent repeatability and reproducibility of manganese-enhanced T1 mapping and kinetic modelling in healthy and diseased myocardium. Furthermore, the scan-rescan reproducibility of manganese-enhanced magnetic resonance imaging in healthy volunteers is excellent, suggesting it has potential for clinical application.
4. **CHAPTER 4: MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING IN NON-ISCHAEMIC CARDIOMYOPATHY**

Extracts of this chapter have been published in:


*Authors contributed equally*
4.1. **SUMMARY**

The aim of this study is to quantify altered myocardial calcium-handling in non-ischaemic cardiomyopathy using magnetic resonance imaging.

Patients with dilated cardiomyopathy (n=10) or hypertrophic cardiomyopathy (n=17) underwent both gadolinium and manganese contrast-enhanced magnetic resonance imaging and were compared with healthy volunteers (n=20). Differential manganese uptake (Ki) was assessed using a two-compartment model.

Compared with healthy volunteers, reduction in T1 with manganese-enhanced magnetic resonance imaging was lower in patients with dilated cardiomyopathy [mean reduction 257 ± 45 (21%) versus 288 ± 34 (26%) ms, P < 0.001], with higher T1 at 40 min (948 ± 57 versus 834 ± 28 ms, P < 0.0001). In patients with hypertrophic cardiomyopathy, reductions in T1 were less than healthy volunteers [mean reduction 251 ± 86 (18%) and 277 ± 34 (23%) versus 288 ± 34 (26%) ms, with and without fibrosis respectively, P < 0.001]. Myocardial manganese uptake was modelled, rate of uptake was reduced in both dilated and hypertrophic cardiomyopathy in comparison with healthy volunteers (mean Ki 19 ± 4, 19 ± 3, and 23 ± 4 mL/100 g/min, respectively; P = 0.007). In patients with dilated cardiomyopathy, manganese uptake rate correlated with left ventricular ejection fraction (r² = 0.61, P = 0.009). Rate of myocardial manganese uptake demonstrated stepwise reductions across healthy myocardium, hypertrophic cardiomyopathy without fibrosis and
hypertrophic cardiomyopathy with fibrosis providing absolute discrimination between the healthy myocardium and fibroed myocardium (mean Ki 23 ± 4, 19 ± 3, and 13 ± 4 mL/100 g/min, respectively; P < 0.0001). In patients with dilated cardiomyopathy, manganese uptake rate correlated with left ventricular ejection fraction ($r^2 = 0.61$, P = 0.009).

The rate of manganese uptake in both dilated and hypertrophic cardiomyopathy provides a measure of altered myocardial calcium-handling. This holds major promise for the detection and monitoring of dysfunctional myocardium, with the potential for early intervention and prognostication.
4.2. **INTRODUCTION**

Cardiomyopathy represents a broad spectrum of clinical diseases which cause myocardial dysfunction leading to symptoms and signs of congestive cardiac failure. Whilst the aetiology is well-characterised in ischaemic cardiomyopathy, non-ischaemic cardiomyopathies encompass a broad range of diseases that includes genetic, acquired and mixed aetiologies (Wu 2007). Advances in cardiac magnetic resonance imaging can help establish the aetiology of, and risk stratify patients with, dilated cardiomyopathy using techniques that include detection of fibrosis with late gadolinium enhancement and global longitudinal strain analysis (Japp et al. 2016, Halliday et al. 2017). However, there remains a relative lack of features discriminating the causes of some forms of non-ischaemic cardiomyopathy as well as the assessment of myocardial pathophysiology, disease severity and disease progression (Assomull et al. 2011, Japp et al. 2016).

Hypertrophic cardiomyopathy represents another population of patients at-risk of heart failure, but with a distinct pathophysiology. These genetic disorders of sarcomeric proteins cause abnormal myocyte architecture and increased left ventricular wall thickness resulting in diastolic and systolic dysfunction (Reza et al. 2019). Tissue characterisation with cardiac magnetic resonance imaging has improved discrimination of hypertrophic cardiomyopathy phenocopies and defines macroscopic myocardial fibrosis with late gadolinium enhancement (Dass et al. 2012, Swoboda et al. 2016, Halliday et al. 2017). T1 mapping with extracellular volume quantification has also shown potential in adding to
current risk stratification (Hinojar et al. 2015, Swoboda et al. 2016). However, the techniques of late gadolinium enhancement and pre and post contrast T1 mapping do not provide information on cardiomyocyte function due to the extracellular distribution of gadolinium-based contrast media. This limits the ability to detect very early myocardial dysfunction and understand the in vivo pathophysiology of different disease states.

Manganese-enhanced magnetic resonance imaging has been well described in animal and human models showing the potential to directly assess myocardial calcium handling (Skjold et al. 2007, Spath et al. 2018, Spath et al. 2020). Here, manganese behaves as an analogue of calcium ions and is rapidly taken up by viable myocardium (Toft et al. 1997, Wang et al. 1997). We have shown that manganese enhancement tracks with myocardial contractility and uptake is absent following acute myocardial infarction (Spath et al. 2018). Furthermore, we have demonstrated direct quantification of myocardial viability using manganese-enhanced magnetic resonance imaging against can assess viability F-FDG PET imaging (Spath et al. 2020).

We have previously reported the potential for manganese-enhanced magnetic resonance imaging to quantify myocardial calcium handling directly (Du et al. 2001, Massaad et al. 2011, Spath et al. 2018, Spath et al. 2020). However, to date, there have been no studies assessing whether manganese-enhanced magnetic resonance imaging can detect alterations in myocardial calcium handling and contractility in non-ischaemic cardiomyopathies. The objectives
of this proof-of-concept study were to evaluate the ability of manganese-enhanced magnetic resonance imaging to detect altered myocardial calcium handling in patients with dilated or hypertrophic cardiomyopathy.

4.3. METHODS

This was a single centre open-label observational cohort study (NCT03607669, EudraCT number 2016-003782-25) which was carried out in accordance with the Declaration of Helsinki, the favourable ethical opinion of the South East Scotland Research Ethics Committee 2 (17/SS/0055) and with written informed consent from all participants.

4.3.1. Patients

Adult patients (≥18 years of age) with non-ischaemic dilated cardiomyopathy and hypertrophic cardiomyopathy were recruited from the Edinburgh Heart Centre between May 2018 and July 2019. Diagnosis of dilated cardiomyopathy and hypertrophic cardiomyopathy was based on echocardiography or magnetic resonance imaging according to European Society of Cardiology guidelines (Elliott et al. 2008). Non-ischaemic dilated cardiomyopathy was defined by the presence of impaired left ventricular systolic function (ejection fraction ≤50% within 12 months) and left ventricular dilatation (left ventricular end-diastolic volume >117% adjusted for age and body-surface area), in the absence of abnormal loading conditions (hypertension and valvular disease) and coronary artery disease (Elliott et al. 2008). Hypertrophic cardiomyopathy
was defined as left ventricular hypertrophy (left ventricular wall thickness ≥15 mm in any segment) in the absence of haemodynamic stresses (Elliott et al. 2008). Presence of diastolic dysfunction in hypertrophic cardiomyopathy was based was defined on transthoracic echocardiography as per British Society of Transthoracic Echocardiography guidelines (Nagueh et al. 2016).

All patients were required to have New York Heart Association class I-III heart failure, with stable symptoms and no change in maintenance therapy in the preceding month. Healthy volunteers were recruited as a control population and had no known pre-existing medical conditions. Exclusion criteria for all participants were any contraindication to magnetic resonance imaging, contraindications to manganese dipyridoxyl diphosphate administration (high degree atrioventricular block, history of torsades de pointes or prolonged QTc interval, obstructive liver disease, maintenance on calcium-channel blockade or digoxin therapy), renal failure (estimated glomerular filtration rate <30 mL/min/1.73 m²), New York Heart Association class IV heart failure, and women of child-bearing potential without a negative pregnancy test.

4.3.2. Magnetic Resonance Imaging

Magnetic resonance imaging was performed using a Siemens MAGNETOM Skyrafit 3T scanner (Siemens Healthineers, Erlangen, Germany), with a dedicated 60-channel body array coil. All study participants underwent scanning with both late gadolinium enhancement and manganese-enhanced magnetic resonance imaging, ≥48 hours apart and in random order. Images
were acquired with ECG-gating and were breath-held in expiration. Cine imaging was acquired with retrospective ECG-gating, with standard steady-state free precession sequences (TrueFISP) in long and short-axis orientations as described previously (Spath et al. 2018). T1 mapping was performed prospectively with shortened modified Look-Locker inversion recovery (WIP #1048 Siemens Healthineers). Quantitative estimation of T1 was performed in full short-axis stack from mitral valve annulus to apex and standard long-axis slices, with additional slices positioned appropriately to characterise pathology (repetition time = 388.8 ms; echo time = 1.07 ms; matrix = 256×115; slice thickness = 8 mm with 1.6-mm gap). T1 relaxation times were estimated before and after administration of gadolinium (Papanastasiou et al. 2015) and manganese-based contrast media.

**Late Gadolinium Enhancement**

Late gadolinium enhancement images were acquired following intravenous administration of gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany) using a single breath held phase-sensitive inversion recovery short-axis stack, and long axis orientations (repetition time = 820 ms; echo time = 1.04 ms; matrix = 192×81; slice thickness = 8 mm with 2-mm gap). A standardised inversion time of 400 ms was used and adjusted as required for optimal myocardial nulling. Post-contrast T1 mapping was performed prospectively with short-axis shortened modified Look-Locker inversion recovery stack 20 min post-contrast. Serum haematocrit from the day of scanning was used to calculate extracellular volume for late gadolinium enhancement scans.
Manganese-enhanced magnetic resonance imaging

Manganese-enhanced magnetic resonance imaging was achieved using intravenous infusion of manganese dipyridoxyl diphosphate (5 µmol/kg, 1 mL/min, 0.1 mL/kg; Exova SL Pharma, Wilmington, Delaware, USA). T1 mapping was performed pre-contrast with full short-axis shortened modified Look-Locker inversion recovery stack as above (Figure 2.1). For patients, a single short-axis slice was identified by the supervising cardiologist, guided by the late gadolinium enhancement, native T1 maps and cine images to represent hypertrophied myocardium. For patients with hypertrophic cardiomyopathy, regions of maximal hypertrophy and fibrosis were selected and for dilated cardiomyopathy, a mid-ventricular short-axis slice was selected. A single short-axis T1 mapping was then performed at this slice location every 2.5 min for 40 min after starting contrast infusion, at which point a full short-axis shortened modified Look-Locker inversion recovery stack was repeated post-contrast (Figure 2.1). For healthy volunteers, a mid-ventricular slice was chosen for serial T1 mapping after manganese dipyridoxyl diphosphate infusion. Heart rate and blood pressure were measured for the duration of the scans.
4.3.3. Image Analysis

All analysis of T1 maps, late gadolinium enhancement and cine-derived volumetric and functional sequences was performed using Circle CVI (Circle Cardiovascular Imaging, CVI42 v5.3.6, Calgary Canada) as previously validated and described in animal and human models (Spath et al. 2018). The operator was blinded to patients details and manganese-enhanced images were analysed separately from late gadolinium images.

Endocardial and epicardial borders were manually defined on all the conventional short-axis images for volumetric and wall motion measurements and were then copied to all corresponding late gadolinium enhancement and T1 map sequences for analysis with minimal manual adjustments. Regions of interest (ROIs) were determined using the standard 16-segment cardiac model with global myocardial values derived from an average of all 16 segments. After contouring, an additional epicardial and endocardial offset of 20% was applied automatically to minimise tissue interface for all T1 map analyses and artefact was excluded manually for a minority of cases. In patients, additional ROIs were drawn according to regional pathology, corresponding to macroscopic myocardial fibrosis as defined by late gadolinium enhancement, or in areas of morphologically abnormal myocardium. For serial T1 imaging post-MnDPDP, manually drawn ROIs from the pre-contrast image were transferred to all subsequent post-contrast images to ensure consistency. Serum haematocrit from the day of scanning was used to calculate extracellular volume for late gadolinium enhancement scans.
4.3.4. Quantification of myocardial fibrosis with late gadolinium enhancement

Macroscopic myocardial fibrosis was assessed by late gadolinium enhancement. All images were analysed independently in a single batch by one expert operator. Myocardial fibrosis was quantified using the full-width-at-half-maximum technique (Flett et al. 2010) expressed as percentages of the left ventricle as a whole. More diffuse myocardial fibrosis was assessed by extracellular volume (ECV) using pre- and post-contrast myocardial T1 corrected for blood-pool T1 and serum haematocrit on the day of scanning (Kwiecinski et al. 2018). The ECV was calculated according to equation 1:

\[
ECV = \lambda [1 - Hct]
\]

(1)

where Hct is serum haematocrit on the day of scanning and \( \lambda \) is the partition coefficient (2):

\[
\lambda = \frac{1/T_1\text{Myocardium}}{1/T_1\text{Bloodpool}}
\]

(2)

calculated using pre- and post-gadolinium T1 values derived from T1 mapping.
4.3.5. Kinetic Modelling

Functional impairment by reduced calcium-channel activity was assessed on manganese-enhanced magnetic resonance imaging T1 maps. To quantify change in T1 over time regions of interest (ROI's) were drawn in areas of pathological myocardium copied automatically to all slices from 0 to 40 min. In patients with hypertrophic cardiomyopathy, ROIs were drawn in regions of macroscopic fibrosis by late gadolinium enhancement where present, as well as pathologically hypertrophied myocardium without clear fibrosis and non-hypertrophied non-fibrosed regions where present. For patients with dilated cardiomyopathy, ROIs were drawn in the mid-ventricular septal segments.

In brief, the model consists of (i) a reversible compartment ($v_e$), comparable to intravascular and interstitial space, and (ii) an irreversible compartment ($v_i$) where irreversible accumulation of the contrast agent is anticipated, comparable to the intracellular space. The arterial concentration (derived from blood pool T1) represents contrast agent delivery into myocardial tissue and constitutes the arterial input function (Figure 4.2).

Skjöld and colleagues have previously derived a Patlak model adaptation for cardiac manganese-enhanced magnetic resonance imaging (Skjold et al. 2006), demonstrating an apparent unidirectional influx constant ($K_i$) for the transfer of manganese from plasma to irreversible compartments ($v_i$) can be measured using equation (1) (Skjold et al. 2006):
\[
\frac{C_t(t)}{C_a(t)} = Ki \int_0^t \frac{c_a(\tau)}{c_a(t)} d\tau + v_e
\]

(1)

where \( C_t \) and \( C_a \) are the manganese concentration in myocardial tissue and blood pool (arterial input function) respectively. This expression is equivalent to the Patlak formulation (Logan 2000) and describes that if a contrast medium is irreversibly trapped in the tissue within the imaging time frame, the instantaneous tissue concentration divided by the instantaneous arterial concentration plotted against the integrated arterial concentration divided by the instantaneous arterial concentration, will result in linearisation of the data. The gradient of this line represents the apparent unidirectional influx constant \( Ki \), which in turn equals:

\[
Ki = \frac{k1 \cdot k3}{k2 + k3}
\]

(2)

where \( k1 \), \( k2 \) and \( k3 \) are the individual rate constants of the compartmental model presented (Figure 4.2). To derive the manganese concentrations \( C_t \) and \( C_a \) as a function of time to be used in equation (1), the following equation was used:

\[
R_1(t) = R_1(0) + r_1 C(t)
\]

(3)
where $R_1=1/T_1$, $R_1(0)$ is the native longitudinal relaxation rate and $R_1(t)$ is the longitudinal relaxation rate at time $t$ of manganese contrast enhancement, $r_1$ is the relaxivity and $C(t)$ is the concentration of the contrast agent at time $t$. Using equation (3), $C_t$ and $C_a$ were calculated for each successive $T_1$ map derived in the tissue and blood pool before, during and after contrast infusion for the 40 min period of the manganese-enhanced magnetic resonance imaging protocol (Figure 4.1). The Patlak model employed here has previously been shown as an effective method of estimating intracellular influx of manganese in the context of imaging with MnDPDP in the same dose and formulation used in the present study (Logan 2000, Skjold et al. 2006).

Contrast kinetics modelling was performed using in-house software (Papanastasiou et al. 2015) developed in Matlab (MathWorks Inc. Version R2016a, Natick, MA, USA) based on a two-compartment model identical to that previously applied to cardiac manganese-enhanced magnetic resonance imaging with manganese dipyridoxyl diphosphate (Skjold et al. 2006).

4.3.6. Statistical analysis

All statistical analysis was performed with GraphPad Prism (GraphPad Software v8.0.2, San Diego, California, USA). Continuous data were assessed for normality using the D’Agostino-Pearson test. Categorical baseline variables were compared using Fisher’s exact test. Values are mean±standard deviation unless otherwise stated. To compare cardiac function and change in
myocardial manganese uptake in patients and healthy volunteers, volumetric assessment and parametric mapping values were compared using paired or unpaired t-tests, Wilcoxon or Mann-Whitney tests, and analysis of variance or Kruskal-Wallis tests as appropriate. Statistical significance was taken as two-sided $p<0.05$. 
Two compartment model used for Patlak formulation consisting of (i) a reversible compartment, comparable to intravascular and interstitial space, (ii) an irreversible compartment where accumulation of the contrast agent is anticipated, comparable to the intracellular space and (iii) the plasma concentration, equal to the input function (A) where individual transfer constants are represented (k1-3.) By assuming the reversible component is in steady-state with the plasma (Logan 2000) the former behaves as an extension of the input function and the uptake into the irreversible compartment describes the effective net uptake rate or influx constant (Ki, B).
4.4. **RESULTS**

Ten patients with non-ischaemic dilated cardiomyopathy and 20 patients with hypertrophic cardiomyopathy were recruited. Three patients in the hypertrophic cardiomyopathy cohort withdrew (2 had claustrophobia and 1 developed high degree atrio-ventricular block prior to infusion), leaving 17 who completed the study protocol. A cohort of 20 healthy volunteers was used as a contemporaneous control group (*Table 4.1*). All patients completed late gadolinium-enhanced and manganese-enhanced magnetic resonance imaging scans, at least 48 hours apart.

Most patients had idiopathic non-ischaemic dilated cardiomyopathy, with 3 with history of familial dilated cardiomyopathy. Over half of patients with hypertrophic cardiomyopathy had genetic testing with 7 gene positive cases. The patient groups were older than healthy volunteers but were well matched for gender and body-mass index (*Table 4.1*). Patients had higher left ventricular mass index and extracellular volume fraction (P=0.0002 and P<0.0001 respectively, *Table 4.2*).

Administration of manganese dipyridoxyl diphosphate was well tolerated by all participants, with a mean infusion time of 8 mins. No adverse events or side-effects were recorded by 7 days of follow-up in any participant. There were no changes in ECG variables, heart rate or blood pressure during infusion of manganese dipyridoxyl diphosphate (*Figure 4.3*, all P>0.1).
Table 4.1 Baseline clinical characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Healthy Volunteers (n=20)</th>
<th>Patients with Non-Ischaemic Cardiomyopathy (n=27)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Dilated Cardiomyopathy (n=10)</td>
<td>Hypertrophic Cardiomyopathy (n=17)</td>
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<tr>
<td>Male</td>
<td>13 (65)</td>
<td>6 (60)</td>
<td>10 (59)</td>
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<tr>
<td>Age (years)</td>
<td>42±11</td>
<td>57±10</td>
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</tr>
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<td>Body-mass index (kg/m²)</td>
<td>26.0±2.9</td>
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<td>Aetiology</td>
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</tr>
<tr>
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<tr>
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<td>4 (24)</td>
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<tr>
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<td>6 (35)</td>
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<td>Echocardiographic Findings</td>
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<tr>
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<tr>
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<td>Beta-Blocker therapy</td>
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<td>ACE inhibitor/ARB therapy</td>
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<td>6 (60)</td>
<td>6 (35)</td>
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<td>MRA therapy</td>
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<tr>
<td>Statin therapy</td>
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<td>2 (12)</td>
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<tr>
<td>Anticoagulation therapy</td>
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<td>Non-smoker</td>
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<td>6 (60)</td>
<td>13 (76)</td>
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<td>Ex-smoker</td>
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<td>4 (24)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0</td>
<td>1 (10)</td>
<td>0</td>
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</tbody>
</table>

LV, left ventricular; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist.

n (%) or mean±standard deviation.
Table 4.2 Baseline magnetic resonance imaging characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Healthy Control (n=20)</th>
<th>Non-Ischaemic Cardiomyopathy (n=27)</th>
<th>ANCOVA P value</th>
<th>P value (DCM vs HCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCM (n=10)</td>
<td>HCM (n=17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDVI (mL/m²)</td>
<td>74.7±14.4</td>
<td>111.4±39.4</td>
<td>&lt;0.0001</td>
<td>0.00052</td>
</tr>
<tr>
<td>LVESVI (mL/m²)</td>
<td>26.8±7.3</td>
<td>74.0±39.2</td>
<td>&lt;0.0001</td>
<td>0.00002</td>
</tr>
<tr>
<td>Stroke Volume Index (mL/m²)</td>
<td>47.9±9.1</td>
<td>37.3±8.3</td>
<td>0.0011</td>
<td>0.002</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>64.4±5.5</td>
<td>34.7±13.6</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>57.9±13.1</td>
<td>76.8±20.7</td>
<td>0.0002</td>
<td>0.329</td>
</tr>
<tr>
<td>Extracellular Volume (%)</td>
<td>27.1±3.6</td>
<td>33.8±2.7</td>
<td>&lt;0.0001</td>
<td>0.022</td>
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<tr>
<td>Native T1- septum (ms)</td>
<td>1123±36</td>
<td>1206±57</td>
<td>&lt;0.0001</td>
<td>0.239</td>
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<tr>
<td>MEMRI T1- septum (ms)</td>
<td>835±28</td>
<td>927±43</td>
<td>&lt;0.0001</td>
<td>0.073</td>
</tr>
<tr>
<td>Native T1- fibrosis (ms)</td>
<td>-</td>
<td>-</td>
<td>1392±215</td>
<td></td>
</tr>
<tr>
<td>MEMRI T1- fibrosis (ms)</td>
<td>-</td>
<td>-</td>
<td>1141±209</td>
<td></td>
</tr>
<tr>
<td>late gadolinium enhancement (% LV)</td>
<td>-</td>
<td>-</td>
<td>13±4</td>
<td></td>
</tr>
<tr>
<td>Influx constant (Ki/mL/100 g/min)</td>
<td>Myocardial septum</td>
<td>23±4</td>
<td>&lt;0.0001</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>19±3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HCM, hypertrophic cardiomyopathy; DCM, non-ischaemic cardiomyopathy; LVEDVI, indexed left ventricular end-diastolic volume; LVESVI, indexed left ventricular end-systolic volume; MEMRI, manganese-enhanced magnetic resonance imaging; LV, left ventricular; ANOVA, analysis of variance; Mean±standard deviation or median±interquartile range.
Figure 4.3 Haemodynamic and Electrocardiography monitoring with Manganese-Enhanced Magnetic Resonance Imaging

Blood pressure and heart rate after administration of MnDPDP in healthy volunteers (A) and patients with non-ischaemic cardiomyopathy (B). ECG parameters pre- and post-Manganese-Enhanced Magnetic Resonance Imaging (C).

MnDPDP, manganese dipyridoxyl diphosphate, ECG, electrocardiography, SBP, systolic blood pressure, DBP, diastolic blood pressure, HR, heart rate.
4.4.1. Patients with Non-ischaemic Dilated Cardiomyopathy

In comparison to healthy volunteers, patients with dilated cardiomyopathy had lower ejection fraction and higher cardiac volumes (Table 4.2). Native myocardial T1 was higher in patients with non-ischaemic dilated cardiomyopathy (1206±57 versus 1123±36 ms; Figure 4.4). During manganese dipyridoxyl diphosphate infusion, myocardial T1 values demonstrated a rapid initial descent followed by a gradual and sustained reduction that was similar to the myocardium of healthy volunteers. However, mean reductions in T1 values were less marked in patients with dilated cardiomyopathy (mean reduction 257±45 (21%) versus 288 ±34 (26%) ms, ANCOVA P=0.03), resulting in higher T1 values at 40 min (927±43 versus 834±28 ms, P=0.0011; Figure 4.5).

Kinetic modelling demonstrated a lower rate of myocardial manganese uptake in patients with dilated cardiomyopathy compared to healthy volunteers (mean Ki 19±4 and 23±4 mL/100 g/min respectively, P=0.0068; Figure 4.6). The rate of myocardial manganese uptake (by influx constant) correlated with left ventricular ejection fraction in patients with dilated cardiomyopathy (r2=0.61, P=0.009, Figure 4.7).
**Figure 4.4 Late gadolinium enhanced, native T1 and manganese-enhanced magnetic resonance imaging in healthy volunteers and patients with non-ischaemic cardiomyopathy**

<table>
<thead>
<tr>
<th></th>
<th>LGE</th>
<th>NATIVE T1</th>
<th>MEMRI T1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HEALTHY SUBJECTS</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>DILATED CARDIOMYOPATHY</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>HYPERTROPHIC CARDIOMYOPATHY</strong></td>
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<td></td>
</tr>
</tbody>
</table>

Representative late gadolinium enhancement, native and manganese-enhanced magnetic resonance imaging T1 mapping in (A) healthy volunteer, (B) patient with dilated cardiomyopathy and (C) patient with hypertrophic cardiomyopathy following manganese dipyridoxyl diphosphate infusion.
Figure 4.5 Native and manganese-enhanced magnetic resonance imaging T1 profiles in patients with non-ischaemic cardiomyopathy

Representative mean T1 profiles of myocardial regions of interest over time following manganese dipyridoxyl diphosphate in (A) patient with dilated cardiomyopathy (green, n=10), compared to healthy volunteers (blue, n=20) and (B) patient with hypertrophic cardiomyopathy (red, n=17), compared to healthy volunteers (blue, n=20). Manganese dipyridoxyl diphosphate was infused from time 0 to 10 min (dashed line). Error bars are standard deviation of the mean.
Figure 4.6 Differential manganese uptake in patients with non-ischaemic cardiomyopathy

Patlak plot (A) and Kinetic modelling of manganese uptake (B) (Ki, influx constant) in patients with non-ischaemic cardiomyopathy. Each line represents a single representative individual.

Healthy volunteers (blue), patients with dilated cardiomyopathy (green), patients with hypertrophic cardiomyopathy without fibrosis (red), and patients with hypertrophic cardiomyopathy with fibrosis (black).
Figure 4.7 Manganese uptake versus left ventricular ejection fraction in patients with dilated cardiomyopathy.

Myocardial manganese uptake (by influx constant) in patients with dilated cardiomyopathy.
4.4.2. Patients with Hypertrophic Cardiomyopathy

In comparison to healthy volunteers, patients with hypertrophic cardiomyopathy had higher ejection fraction, lower cardiac volumes and diastolic dysfunction to varying degrees (Table 4.1, Table 4.2). Native T1 values of the myocardium were higher in patients with hypertrophic cardiomyopathy compared to healthy volunteers (1183±42 versus 1123±36 ms; Figure 4.4). In patients, there were discrete foci of bright late gadolinium enhancement indicating dense fibrosis which showed markedly different T1 profiles following manganese dipyridoxyl diphosphonate infusion, with T1 recovering towards baseline values similar to the blood pool (Figure 4.8). In areas of hypertrophied myocardium without discrete late gadolinium enhancement, T1 values following manganese dipyridoxyl diphosphonate infusion demonstrated a gradual but continued reduction in T1 values over the 40-min period. Manganese-enhanced T1 values were higher in regions with and without discrete late gadolinium enhancement (1141±209 and 899±34 ms versus 835±28 ms in myocardium of healthy volunteers, both ANCOVA P<0.0001). Mean reductions in T1 were lower in patients with and without fibrosis compared to healthy volunteers (mean reduction 252±86 (18%) and 277±34 (23%) ms versus 288±34 (26%) ms respectively, ANCOVA P<0.001).

Kinetic modelling of myocardial manganese influx demonstrated stepwise reductions across healthy myocardium, hypertrophic cardiomyopathy without fibrosis and hypertrophic cardiomyopathy with fibrosis, providing clear
demarcation between the healthy myocardium and fibroed myocardium (mean Ki 23±4, 19±3 and 13±4 mL/100 g/min; ANCOVA P<0.0001; Figure 4.6).
Figure 4.8 Manganese-enhanced magnetic resonance imaging in patients with hypertrophic cardiomyopathy

Representative manganese-enhanced magnetic resonance imaging T1 mapping image and T1 profile in a patient with hypertrophic cardiomyopathy in (A) hypertrophied myocardium without fibrosis (red), compared to bloodpool (light blue) and (B) in hypertrophied myocardium with fibrosis (red), compared to bloodpool (light blue).

Manganese dipyridoxyl diphosphate was infused from time 0 to 10 min (dashed line). Error bars are standard deviation of the mean.
4.5. DISCUSSION

This is the first description of manganese-enhanced magnetic resonance imaging T1 mapping to detect abnormal myocardial cellular physiology in patients with non-ischaemic dilated cardiomyopathy or hypertrophic cardiomyopathy. Our findings suggest that alterations in myocardial calcium handling in cardiomyopathies can be detected and are associated with left ventricular systolic dysfunction as well as regions of fibrosis and scar formation. This novel approach has major potential for the detection, investigation and management of patients with a range of cardiomyopathies.

4.5.1. Manganese-enhanced MRI in non-ischaemic dilated cardiomyopathy

Myocardial contraction is dependent on excitation-contraction coupling for which calcium homeostasis is essential. Dysfunctional calcium handling is a central feature of left ventricular dysfunction in patients with dilated cardiomyopathy, with alterations in calcium handling proteins (Meyer et al. 1995) leading to reduced myocardial contractile function (Ren et al. 1997, Hasenfuss et al. 2002). We have shown that manganese-enhanced magnetic resonance imaging detects clear abnormalities in myocardial calcium handling of patients with non-ischaemic dilated cardiomyopathy. Moreover, kinetic modelling demonstrated a direct correlation between myocardial manganese uptake and left ventricular ejection fraction. As such, this study confirms the presence of dysfunctional calcium handling in the myocardium of patients with
non-ischaemic dilated cardiomyopathy. Furthermore, it provides a non-invasive imaging technique that has the potential to be used as a marker for risk stratification, disease progression and response to therapy (Reza et al. 2019).

Detection of myocardial fibrosis with late gadolinium enhancement and global measures of cardiac performance, such as left ventricular ejection fraction, can help in monitoring disease and prognostication (Kwong et al. 2019). However, subtle myocyte dysfunction is difficult to detect and could be beneficial in highlighting patients who are at risk of deteriorating left ventricular function: a feature which is associated with poor cardiac outcomes (Nabeta et al. 2019). In the present study, manganese-enhanced magnetic resonance imaging detected altered manganese uptake reflecting abnormal calcium handling in patients with severe phenotypes of non-ischaemic cardiomyopathy. Manganese-enhanced magnetic resonance imaging therefore has potential to go beyond myocardial fibrosis imaging and identify subclinical changes in calcium handling, a feature that could also be utilised in the early detection of patients who are at risk of heart failure prior to developing symptoms or signs of overt left ventricular dysfunction. This needs to be confirmed in future studies across a range of clinical phenotypes of cardiac disease.

4.5.2. Manganese-enhanced MRI in hypertrophic cardiomyopathy

In patients with hypertrophic cardiomyopathy, the left ventricular ejection fraction was preserved or supranormal with diastolic dysfunction seen in all
cases. Despite this, myocardial manganese-enhanced magnetic resonance imaging T1 and native T1 were higher in regions of hypertrophy without discrete late gadolinium enhancement. The rate of manganese influx into pathologically hypertrophied myocardium was reduced. This indicates the presence of abnormal myocardial calcium handling in hypertrophied myocardium with predominantly diastolic impairment. Diastolic dysfunction in hypertrophic cardiomyopathy is multifactorial, and includes prolonged and disordered ventricular relaxation, decreased chamber compliance and abnormal calcium cycling (Geske et al. 2018). Increases in calcium entry through L-type calcium channels combined with reduced calcium extrusion and lower sarcoplasmic reticulum calcium adenosine triphosphotase expression result in elevated intracellular calcium concentration. Furthermore, dysfunctional calcium handling appears to precede alterations in metabolic activity (Kenny et al. 2019) and is associated with an increased risk of arrhythmogenesis (Coppini et al. 2018). As such, manganese-enhanced magnetic resonance could be used to detect the earlier signs of altered calcium handling, a surrogate marker of disease progression and prognostication (Viola et al. 2019).

In patients with hypertrophic cardiomyopathy, the recovery of myocardial T1 values within dense regions of severe fibrosis was similar to that observed in the blood pool. We have previously described a similar profile of recovery in regions of transmural acute myocardial infarction (Spath et al. 2018). This is consistent with the marked reduction in manganese uptake due to the
presence of non-viable myocardium and non-functional fibrotic tissue scar. This ability to differentiate and track viable myocardium could play a role in the assessment of reversible causes of cardiomyopathies which show transient myocardial dysfunction.

After administration, biotransformation of manganese dipyridoxyl diphosphate occurs by dephosphorylation and transmetallation with zinc, enabling intracellular manganese uptake as demonstrated in vitro where tissue uptake and renal clearance occur rapidly (Toft et al. 1997, Spath et al. 2019). Manganese-based contrast media have previously been reported to cause acute myocardial suppressant effects. In preclinical studies, unchelated manganese chloride produced very high free manganese ion concentrations causing negative inotropy and cardiovascular instability (Jiang et al. 2005). We used a custom manufactured dipyridoxyl diphosphate chelation of manganese which markedly reduces free unbound manganese ions. This formulation of manganese was previously approved for clinical use (Teslascan®) (Sutcliffe et al. 2011) but was withdrawn from the US market in 2003 (Administration 2012) and the EU market in 2010 (Agency 2012) because it was not commercially viable. In phase 3 clinical trials (Federle et al. 2000), mild to moderate side effects were reported in 1-7% of participants and included headache and flushing. In the present study, we observed no adverse events or side effects despite including patients with marked left ventricular dysfunction and ejection fractions of <25%. Furthermore, there were no instances of haemodynamic compromise or cardiac dysrhythmia from administration of the agent to 30
minutes following scanning, and no adverse symptoms or side effects report at 7 day follow up. This suggests that this formulation of manganese is safe and relatively straightforward to manufacture.

4.5.3. Clinical Implications

What are the clinical implications of our findings? In this study, we have undertaken manganese-enhanced T1 mapping in two populations of non-ischaemic cardiomyopathies and have demonstrated its ability to detect and to quantify altered myocardial calcium handling over and above existing methods of identifying myocardial fibrosis. This is not specific to non-ischaemic cardiomyopathies and could potentially be used to assess a wider range of cardiomyopathy phenotypes, although this has yet to be established. As expected, manganese-enhanced magnetic resonance imaging correlated with a range of left ventricular ejection fraction suggesting it has potential to track and to quantify more subtle gradations of myocardial dysfunction. Late gadolinium enhancement and extracellular volume fraction imaging relies on abnormal tissue structure to identify regions of pathological myocardium. The ability of manganese-enhanced magnetic resonance imaging to detect altered calcium handling over time and quantify cellular myocardial function directly may transform our ability to assess myocardial function directly, enabling early detection, prognostication and assessment of treatment efficacy. With optimisation, this technique has potential to allow individualisation of heart failure treatment, targeting optimal therapy to those most likely to benefit. Furthermore, early detection of altered calcium-handling in established or at-
risk cardiomyopathy may enable aggressive initiation of therapy earlier than previously possible, which has potential to improve long-term clinical outcomes. We therefore suggest that further investigation of reversible cardiomyopathies, such as stress cardiomyopathy and myocarditis, is a crucial next step to further our understanding of calcium dysfunction seen in the acute and chronic setting.

4.5.4. Limitations
Our study has several limitations that should be considered. First, there have been prior concerns regarding toxicity as well as current lack of a marketed formulation of manganese. The chelated form used here retains the necessary properties for intracellular myocardial imaging whilst cardiac function remains uncompromised as demonstrated by the absence of haemodynamic or arrhythmic effects in published safety data (Jynge et al. 1997, Marti-Bonmati et al. 2003). Furthermore, we anticipate that the availability of such agents will increase following continued demonstration of its clinical and research utility. Second, calcium channel antagonists and digoxin reduce uptake of manganese dipyridoxyl diphosphate and as a result these medications were excluded. However, patients were maintained on other cardiac medications including beta-blocker, angiotensin converting enzyme inhibitor, mineralocorticoid receptor antagonist, statin and anti-platelet therapies. We cannot exclude an effect of these medications on our findings but in our previous work in patients with acute myocardial infarction, concomitant use of these medications did not influence manganese uptake in the myocardium.
remote from the site of infarction (Spath et al. 2021). Finally, we cannot rule out that calcium handling deteriorates with age and the fact that control subjects were younger than those with cardiomyopathy could explain some of the observed reductions in manganese uptake. However, we found no correlation between age and manganese uptake in the healthy volunteers or our individual patient cohorts (data not shown).
4.6. **CONCLUSION**

In conclusion, we have described the first proof-of-concept T1 mapping study of manganese-enhanced magnetic resonance imaging in patients with non-ischaemic cardiomyopathy. Using kinetic modelling, we have shown that manganese-enhanced magnetic resonance imaging can detect dysfunctional myocardial calcium handling and can directly distinguish normal from pathological myocardium. We believe that manganese-enhanced magnetic resonance imaging holds major promise for the detection and monitoring of disease progression in non-ischaemic cardiomyopathy, with the potential for early intervention, tracking of response to therapy and overall prognostication. Finally, its application to other areas of disease, such as reversible cardiomyopathies or detection of sub-clinical cardiomyopathies, warrants further investigation.
5. CHAPTER 5: MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING IN TAKOTSUBO SYNDROME

Extracts of this chapter have been submitted for publication as:

5.1. **SUMMARY**

Takotsubo syndrome is an acute cardiac emergency characterised by transient left ventricular systolic dysfunction typically following a stressful event. Despite its rapidly rising incidence, its pathophysiology remains poorly understood. Furthermore, it may pass unrecognised especially if timely diagnostic imaging is not performed. Defective myocardial calcium homeostasis is a central cause of contractile dysfunction and has not been explored in takotsubo syndrome. We aimed to investigate myocardial calcium handling using manganese-enhanced magnetic resonance imaging during the acute and recovery phases of takotsubo syndrome.

Twenty patients with takotsubo syndrome (64 ± 12 years, 90% female) and 20 age, sex and cardiovascular risk factor matched volunteers (59 ± 11 years, 70% female) were recruited from the Edinburgh Heart Centre between March 2020 and October 2021. Patients underwent gadolinium and manganese-enhanced magnetic resonance imaging during index hospitalisation with repeat manganese-enhanced magnetic resonance imaging performed after at least 3 months.

Compared to matched control volunteers, patients had a reduced left ventricular ejection fraction (51±11 versus 67±8 %, P<0.001), increased left ventricular mass (89±11 versus 57±14 g/m², P<0.01) and, in affected myocardial segments, elevated native T1 (1358±49 versus 1211±28 ms,
P<0.001) and T2 (60±7 versus 38±3 ms, P<0.001) values at their index presentation. During manganese-enhanced imaging, kinetic modelling demonstrated a substantial reduction in myocardial manganese uptake (5.1±0.5 versus 8.2±1.1 mL/100 g of tissue/min respectively, P<0.0001) consistent with markedly abnormal myocardial calcium handling. Following recovery, left ventricular ejection fraction, left ventricular mass and T2 values were comparable to matched control volunteers. Despite this, native and post-manganese T1 and myocardial manganese uptake remained abnormal compared to matched control volunteers (6.6±0.5 versus 8.2±1.1 mL/100 g of tissue/min, P<0.0001).

In patients with takotsubo syndrome, there is a profound perturbation of myocardial calcium handling which is most marked in the acute phase but persists for at least 3 months despite apparent restoration of normal left ventricular ejection fraction and resolution of myocardial oedema. Abnormal myocardial calcium handling appears to be implicated in the pathophysiology of takotsubo syndrome and manganese-enhanced magnetic resonance imaging has major potential to assist in the diagnosis, characterisation and risk stratification of patients with takotsubo syndrome.
5.2. **INTRODUCTION**

Takotsubo syndrome is an acute cardiac emergency that is often triggered by a stressful event and is characterised by transient and profound left ventricular systolic dysfunction, typically due to marked ‘ballooning’ of the left ventricular apex (Sato TH et al. 1990, Akashi et al. 2008, Ghadri et al. 2018). The pathology of this condition is poorly understood and we lack targeted treatments. Moreover, it can be challenging to recognise due to its phenotypical similarities with acute myocardial infarction and is often considered when invasive coronary angiography fails to identify major obstructive coronary artery disease (Kurisu et al. 2002). However, the characteristic left ventricular abnormalities can be brief, and opportunities to document them with diagnostic imaging are often missed (Citro et al. 2020).

Cardiac magnetic resonance imaging is an essential diagnostic tool in the assessment of takotsubo syndrome. In addition to visualising the hallmark regional wall motion abnormalities, it can identify complications, such as left ventricular outflow tract obstruction, mitral regurgitation, pericardial effusion and left ventricular thrombus. It is particularly useful in establishing the diagnosis of acute cardiac conditions of uncertain origin, such as myocardial infarction with non-obstructive coronary arteries and myocarditis. Previous cardiac magnetic resonance imaging studies have demonstrated the presence
of acute myocardial oedema in patients with takotsubo syndrome as well as some reports of persistent subtle cardiac abnormalities following recovery of normal left ventricular ejection fraction (Scally et al. 2019). However, there is a need to develop more sensitive and discriminatory imaging techniques that are less time sensitive and more specific to takotsubo syndrome.

Manganese-enhanced magnetic resonance imaging is a novel technique that assesses manganese uptake into viable cardiomyocytes and has shown promise as a surrogate marker of myocardial calcium handling. We have previously demonstrated differences in manganese uptake in patients with dilated, hypertrophic and ischemic cardiomyopathy, suggesting abnormal myocardial calcium handling (Spath et al. 2020, Spath et al. 2021). However, there have been no studies assessing whether manganese-enhanced magnetic resonance imaging can detect alterations in myocardial calcium handling in takotsubo syndrome and whether this recovers during convalescence. The objectives of this proof-of-concept study were to evaluate the ability of manganese-enhanced magnetic resonance imaging to detect altered myocardial calcium handling in patients with takotsubo syndrome during both the acute presentation and following apparent recovery.
5.3. METHODS

This was a single centre case-control observational longitudinal cohort study (NCT04623788) which was conducted in accordance with the Declaration of Helsinki, the favourable ethical opinion of the Southeast Scotland Research Ethics Committee 2 (20/SS/0001) and with the written informed consent from all participants.

5.3.1. Study Populations

Adult patients (≥18 years of age) with takotsubo syndrome were recruited from the Edinburgh Heart Centre between March 2020 and October 2021. Diagnosis of takotsubo syndrome was based on the Mayo clinic (Abe et al. 2003) and the Heart Failure Association Takotsubo Syndrome Taskforce of the European Society of Cardiology criteria (Maron et al. 2006). This comprises of new electrocardiographic (ECG) changes (ST-segment elevation, ST-segment depression, T-wave inversion, and QTc prolongation), the presence of transient left ventricular dysfunction presenting as apical ballooning or focal mid-ventricular/basal wall motion abnormalities, and the absence of obstructive coronary artery disease or acute plaque rupture. Patients usually have an emotional or physical stressful trigger. We specifically excluded patients with pheochromocytoma, myocarditis or a primary isolated diagnosis of acute myocardial infarction. Control volunteers were matched for
age, sex and cardiovascular risk factor profile, such as hypertension, known coronary artery disease, hypercholesterolemia and diabetes mellitus. All participants were scanned at the University of Edinburgh between September 2020 and August 2021.

Exclusion criteria for all participants were any contraindication to magnetic resonance imaging, contraindications to manganese dipyridoxyl diphosphate administration (high degree atrioventricular block, history of torsades de pointes or prolonged QTc interval, obstructive liver disease, maintenance on calcium-channel blockade or digoxin therapy), renal failure (estimated glomerular filtration rate <30 mL/min/1.73 m²), New York Heart Association class IV heart failure, and women of child-bearing potential without a negative pregnancy test.

5.3.2. Magnetic Resonance Imaging
Magnetic resonance imaging was performed using a 3T scanner (MAGNETOM Skyrafit, Siemens Healthineers, Erlangen, Germany) using a 30-channel anterior body matrix coil and elements of a posterior spine matrix coil. Images were acquired during expiratory breath hold with ECG gating. Cine imaging was acquired with standard steady-state free precession sequences in long and short-axis orientations as described previously (Spath et al. 2020, Singh et al. 2021, Spath et al. 2021). All study participants underwent scanning with both late gadolinium enhancement and manganese-enhanced magnetic resonance imaging, at least 48 hours apart. Where
possible, gadolinium imaging was performed first, with at least 48 hours between the two scans (Figure 5.1).

**Figure 5.1: Consort Diagram**
T1 mapping was performed prospectively with modified Look-Locker inversion (MyoMaps) recovery (repetition time 388.8 ms; echo time 1.07 ms; matrix 256×115; slice thickness 8 mm with 1.6-mm gap, FOV=360×288 mm, sampling pattern 5(3)3). Quantitative estimation of T1 was performed in full short-axis stack from mitral valve annulus to apex and standard long-axis slices, with additional slices positioned appropriately to characterise pathology.

T2 mapping was performed with T2 Myomaps (TR 207.39 ms; TE 1.32 ms; matrix 192×100; slice thickness 8 mm with 1.6 mm gap, with T2 evolution times of 0, 0.30 and 0.55 ms. Field of view (FOV) was 360×288 mm adjusted for patient body habitus as required (MyoMaps, Siemens Healthineers, Erlangen, Germany). This was acquired in long and short-axis orientation covering the entire left ventricle.

Late gadolinium Enhancement

Late gadolinium enhancement images were acquired following intravenous administration of gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany) using a single breath held phase-sensitive inversion recovery short-axis stack, and long axis orientations (TR 820 ms; TE 1.04 ms; matrix 192×81; slice thickness 8 mm with 1.8-mm gap, FOV 380 mm). A standardised inversion time of 400 ms was used and adjusted as required for optimal myocardial nulling. Post-
contrast T1 mapping was performed prospectively with short-axis modified Look-Locker inversion recovery stack 10 min after contrast administration.

**Manganese-enhanced magnetic resonance imaging**

Manganese-enhanced magnetic resonance imaging was achieved using intravenous infusion of manganese dipyridoxyl diphosphate (5 μmol/kg (0.1 mL/kg, up to a maximum of 10 mL) and 1 mL/min; Exova SL Pharma, Wilmington, Delaware, USA). T1 mapping was performed pre-contrast with full short-axis modified Look-Locker inversion recovery stack as above. For patients, a single short-axis slice of the diseased myocardium was identified by the supervising cardiologist and guided by native T1, T2 maps and cine images (mid-ventricular or basal in all patients). Single short-axis T1 mapping was then performed at this slice location every 2.5 min for 30 min after commencing contrast infusion. At 30 min, a full short-axis T1 stack was repeated (Figure 5.2). For controls, a mid-ventricular slice was chosen for serial T1 mapping after manganese dipyridoxyl diphosphate infusion.
Figure 5.2 Manganese-enhanced magnetic resonance imaging protocol

MEMRI, Manganese-enhanced magnetic resonance imaging, MnDPDP, manganese dipyridoxyl diphosphate
5.3.3. Image Analysis

All analyses of T1, T2 maps, late gadolinium enhancement and cine-derived volumetric and functional sequences were performed using Circle CVI (Circle Cardiovascular Imaging, CVI42 v5.3.6, Calgary Canada) as previously validated and described in animal and human models (Spath et al. 2020, Spath et al. 2020, Spath et al. 2021). Image analysis was conducted blind to patient’s details (analysed in groups after the end of scanning period) and manganese-enhanced images were analysed separately from late gadolinium images. Endocardial and epicardial borders were manually defined on all conventional short-axis images for volumetric and wall motion measurements and were then copied to corresponding T1 map images for analysis with minimal manual adjustments. The left ventricular basal short axis slice was identified as the image containing at least 50% of circumferential myocardium at end diastole. Papillary muscles were included in the mass and excluded from volumetric analysis.

After contouring, an additional epicardial and endocardial offset of 20% was applied automatically to minimise partial volume effect for all T1 map analyses. In patients, T1 and T2 measurements were taken from septal segments in both pathological (area with regional wall motion abnormality) and remote regions (no regional wall motion abnormality). In control volunteers, T1 and T2 measurements were made in the septal wall of the midventricular slice (Figure 5.3). We also measured global T1 (mean of 6 segments from a mid-ventricular segment) in patients and volunteers. For this, patients with focal takotsubo and
dual pathology were excluded (n=4). Left ventricular wall thickness was measured in the pathological and remote myocardium (septal walls for both) for acute and follow-up scans. For matched volunteers, wall thickness was measured in the mid-ventricular septal wall (Figure 5.4).

**Figure 5.3 T1 Mapping of manganese-enhanced magnetic resonance imaging**

*T1 maps 30 min after manganese-based contrast media administration demonstrating regions of interest in pathological myocardium (blue semicircle) in a matched volunteer (A) and a patient with apical (B), basal (C) and focal (D) takotsubo syndrome.

*BP, bloodpool*
**Figure 5.4 Left ventricular oedema in patients with takotsubo syndrome**

Left ventricular wall thickness measurement in remote and pathological myocardium in a patient with takotsubo syndrome during acute (panel A) and follow-up (panel B).

*LV, left ventricle*
5.3.4. Manganese Kinetic Modelling

Myocardial calcium handling was assessed using T1 maps during manganese-enhanced magnetic resonance imaging. To quantify the change in T1 over time, regions of interest were drawn as described above. For serial T1 imaging post manganese, manually drawn regions of interest from the pre-contrast image were transferred to all subsequent post-contrast images to ensure consistency. The rate of myocardial manganese uptake was determined by Patlak modelling as described previously (Singh et al. 2021); (Patlak et al. 1983, Skjold et al. 2006).

5.3.5. Statistical analysis

All statistical analysis was performed with GraphPad Prism (GraphPad Software v8.0.2, San Diego, California, USA). Categorical baseline variables were presented as number (%) and compared using Chi-squared test. Continuous data were assessed for normality using the D'Agostino-Pearson test and presented as mean±standard deviation or median [interquartile interval]. Cardiac function, myocardial manganese uptake, volumetric assessment and parametric mapping values were compared using paired or unpaired Student’s t-tests and Wilcoxon or Mann-Whitney tests. Correlations were assessed using linear regression analysis. Statistical significance was taken as two-sided p<0.05.
5.4. **RESULTS**

5.4.1. **Study Populations**

Twenty-five patients with takotsubo syndrome were recruited into the study although five patients were withdrawn: 3 were unable to complete the cardiac magnetic resonance scan due to claustrophobia and 2 had an infarct pattern of late gadolinium enhancement on magnetic resonance imaging and an isolated primary diagnosis of acute myocardial infarction (Figure 5.4). The patient cohort was predominantly middle-aged women (Table 5.1). All patients had symptoms (chest pain in 70% and dyspnea in 30%) requiring emergency hospitalisation with the majority having an identifiable precipitating stressor (Table 5.2). Twenty control volunteers were well matched for age, sex and co-morbidities (Table 5.1) although there was a higher prevalence of pre-existing psychiatric or neurological disorders and antidepressant therapy in those with takotsubo syndrome (Table 5.3).
Figure 5.4: Anatomical Types of Takotsubo Syndrome

Short-axis and long-axis manganese-enhanced T1 maps and long-axis late gadolinium images (10 min post contrast) in a matched control volunteer (panel A), and patients with apical (panel B), basal (panel C) and focal (panel D) takotsubo syndrome. Blue represents normal manganese uptake and green represents reduced manganese uptake and abnormal calcium handling.
### Table 5.1. Baseline characteristics of the study populations.

<table>
<thead>
<tr>
<th></th>
<th>Patients with Takotsubo syndrome (n=20)</th>
<th>Matched Control Volunteers (n=20)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>63 [42-80]</td>
<td>59 [29-70]</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Female sex</strong></td>
<td>18 (90)</td>
<td>14 (70)</td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index (kg/m²)</strong></td>
<td>25 [22-33]</td>
<td>27 [21-35]</td>
<td>0.67</td>
</tr>
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<td><strong>Past Medical History</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>8 (40)</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>Known coronary artery disease</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>5 (25)</td>
<td>5 (25)</td>
<td></td>
</tr>
<tr>
<td>Acute neurological/psychiatric disorder</td>
<td>1 (5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Past of chronic neurological/psychiatric disorder</td>
<td>14 (70)</td>
<td>4 (20)</td>
<td></td>
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<tr>
<td>Diabetes Mellitus</td>
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<td>Thyroid disorder</td>
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<tr>
<td><strong>Medications</strong></td>
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<td>Antiplatelet therapy</td>
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<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Beta-Blocker therapy</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor or ARB therapy</td>
<td>2 (10)</td>
<td>4 (35)</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blockers</td>
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<td>1 (10)</td>
<td></td>
</tr>
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<td>Statin therapy</td>
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<td>5 (25)</td>
<td></td>
</tr>
<tr>
<td>Anti-glycaemic therapy</td>
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<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Previous/Current Antidepressant therapy</td>
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<td>2 (10)</td>
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<tr>
<td><strong>Acute takotsubo episode</strong></td>
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<td></td>
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<tr>
<td>Presenting symptom</td>
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<td>Dyspnoea</td>
<td>6 (30)</td>
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<td></td>
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<tr>
<td>Chest pain</td>
<td>14 (70)</td>
<td>-</td>
<td></td>
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<tr>
<td>Stressor</td>
<td></td>
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</tr>
<tr>
<td>Emotional</td>
<td>13 (65)</td>
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<tr>
<td>Physical</td>
<td>4 (20)</td>
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<tr>
<td>None</td>
<td>3 (15)</td>
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<tr>
<td>Peak plasma cardiac high sensitive troponin I concentration (ng/L)</td>
<td>6,981</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>ECG</strong></td>
<td></td>
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<tr>
<td>ST-segment elevation</td>
<td>8 (40)</td>
<td>-</td>
<td></td>
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<tr>
<td>ST-segment depression</td>
<td>2 (10)</td>
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<td></td>
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<tr>
<td>T-wave changes</td>
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<tr>
<td>QTc (ms)</td>
<td>552 ± 42</td>
<td>387 ± 13</td>
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<tr>
<td>Index left ventricular ejection fraction</td>
<td></td>
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<td>-----------------------------------------</td>
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<tr>
<td>Borderline (50-54%)</td>
<td>4 (20)</td>
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<tr>
<td>Impaired (36-49%)</td>
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<tr>
<td>Normal coronaries</td>
<td>10 (50)</td>
<td>-</td>
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<tr>
<td>Non-obstructive arteries</td>
<td>8 (40)</td>
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<td>Obstructive disease</td>
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<th>Left ventriculography completed</th>
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<td></td>
<td>13 (65)</td>
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<tr>
<th>Takotsubo syndrome sub-type</th>
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<td>Apical</td>
<td>17 (85)</td>
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<tr>
<td>Basal</td>
<td>1 (5)</td>
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<tr>
<td>Focal</td>
<td>2 (10)</td>
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<td>Antiplatelet therapy</td>
<td>10 (50)</td>
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<td>Beta-Blocker therapy</td>
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<td>ACE inhibitor or ARB therapy</td>
<td>16 (80)</td>
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<td>Diuretic therapy</td>
<td>6 (30)</td>
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<td>Statin therapy</td>
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<td>Days since acute episode</td>
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<td>Symptoms at follow-up</td>
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<tr>
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<td>Dyspnoea</td>
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<td>Palpitations</td>
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<td>Fatigue</td>
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<tr>
<td>Death</td>
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</table>

*Number (%), median [interquartile range], mean ± standard deviation*
Table 5.2 Precipitating stressors in patients with takotsubo syndrome

<table>
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<tr>
<th>Type of stressor</th>
<th>Example</th>
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<tr>
<td>Emotional (n=13)</td>
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<tr>
<td></td>
<td>Bereavement of family member/friend (x4)</td>
</tr>
<tr>
<td></td>
<td>Bereavement of family pet (came home to find dog had attacked cat)</td>
</tr>
<tr>
<td></td>
<td>Argument with husband</td>
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<td>Argument with daughter-in-law</td>
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<td></td>
<td>Argument with friend (x2)</td>
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<td></td>
<td>Receiving hostile email from landlord</td>
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<td>Driving the wrong way on the motorway</td>
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<td>Workplace stress (x2)</td>
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<td>Physical (n=4)</td>
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<td>Endoscopy procedure</td>
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<td>Acute cholecystitis</td>
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<td>Loch swimming</td>
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<td></td>
<td>Direct current cardioversion procedure</td>
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</table>

Table 5.3 Myocardial manganese uptake (Ki) during acute and follow up takotsubo syndrome by pre-existing psychiatric, neurological disorder or anti-depressant use.

<table>
<thead>
<tr>
<th>Myocardial Manganese uptake (Ki) mL/100 g of tissue/min</th>
<th>Patients with pre-existing psychiatric, neurological disorders or antidepressant use (n=9)</th>
<th>Patients without pre-existing psychiatric, neurological disorders or antidepressant use (n=11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute event</td>
<td>5.0± 1.1</td>
<td>5.1±0.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Follow-up</td>
<td>6.7±0.8</td>
<td>6.6±1.0</td>
<td>0.53</td>
</tr>
</tbody>
</table>
5.4.2. Characterisation of Takotsubo Syndrome

Electrocardiography demonstrated either ST-segment deviation or T-wave changes in all patients, with evidence of QT interval prolongation in some patients. All patients had a degree of left ventricular impairment on baseline echocardiographic imaging and invasive coronary angiography demonstrated normal coronary arteries in half of the population, with the remaining having non-obstructive or obstructive coronary artery disease (Table 5.1, Table 5.4). Of those with obstructive disease (n=2), one had spontaneous coronary artery dissection in the first obtuse marginal and the second had plaque rupture in the distal left anterior descending artery. In both cases, there were extensive regional wall motion abnormalities involving all mid-ventricular and apical segments which were not in keeping with myocardial infarction alone. Most patients had an ‘apical’ pattern of takotsubo syndrome (Figure 5.4). Two patients demonstrated a ‘focal’ pattern of takotsubo: one of whom had normal coronary arteries whilst the other had mild plaque in the left anterior descending artery and underwent intra-coronary imaging (demonstrated stable plaque) and a pressure wire study (fractional flow reserve during maximal hyperaemia, 0.92). Both patients demonstrated rapidly resolving left ventricular function and had no evidence of late gadolinium enhancement. All patients were commenced on heart failure treatment, with a smaller proportion being started on diuretic therapy. Patients who had evidence of coronary artery disease were commenced on anti-platelet therapy, and two patients were started on dual anti-platelet therapy (Table 5.1). During follow up, one patient
suffered a stroke and another from recurrence of takotsubo syndrome within a year of their index event.
Table 5.4 Coronary angiography and left ventriculography in patients with takotsubo syndrome

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<tr>
<th>ID</th>
<th>Sex</th>
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<th>Diagnosis</th>
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<td>Systole</td>
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<td>1</td>
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<td>Basal takotsubo</td>
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![Images of coronary angiography and left ventriculography in basal takotsubo syndrome](attachment:image.png)
<p>| | | | | | |</p>
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<td>62</td>
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<td>F</td>
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<td>F</td>
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</table>
5.4.3. Magnetic Resonance Imaging

Acute Index Presentation

Most patients underwent cardiac magnetic resonance imaging within a median of 4 days (range 1-18 days) of symptom onset. Compared to matched control volunteers, patients with takotsubo syndrome had reduced left ventricular ejection fraction (Table 5.4), increased left ventricular mass (Figure 5.5) and comparable right ventricular systolic function. Interestingly left ventricular wall thickness was elevated in pathological and remote regions (Table 5.4). Two patients had late gadolinium enhancement consistent with acute myocardial infarction (dual pathology, Figure 5.6). Two patients demonstrated hazy ‘incomplete nulling’ of the late gadolinium enhancement imaging in mid-ventricular and apical segments consistent with the marked oedema characteristic of takotsubo syndrome (Figure 5.6).

Compared to matched control volunteers, patients with takotsubo syndrome had elevated native T1 and T2 values in both the pathological and remote myocardial segments (Table 5.4). Following manganese infusion, T1 shortening was less pronounced in patients with takotsubo syndrome (Table 5.4) with kinetic modelling demonstrating marked reductions in myocardial manganese uptake (5.1±0.5 versus 8.1±1.1 mL/100 g of tissue/min, P<0.0001). One patient underwent cardiac magnetic resonance imaging 18 days after the onset of presentation symptoms, and there was complete resolution of regional wall motion abnormalities and normal left ventricular systolic function. Despite this, manganese-enhanced T1 mapping
demonstrated a pattern of reduced myocardial manganese uptake consistent with apical takotsubo syndrome (Figure 5.7). Global values are given in Table 5.5.
Figure 5.5: Myocardial Oedema and Left Ventricular Mass in Takotsubo Syndrome

Changes in left ventricular mass (panel A), native T2 (panel B) and native T1 (panel C), demonstrating reduction in all three parameters between acute and follow up scans in patients with takotsubo syndrome. Correlations are seen between left ventricular mass and native T2 (D), left ventricular wall thickness and native T2 (E), myocardial manganese uptake and left ventricular mass (F) and myocardial manganese uptake and native T2 (G) during acute (red) and follow up (blue) scans.
### Table 5.4  Cardiac magnetic resonance measures.

<table>
<thead>
<tr>
<th></th>
<th>Matched Control Volunteers (n=20)</th>
<th>Patients with takotsubo syndrome: index event (n=20)</th>
<th>Patients with takotsubo syndrome: follow-up (n=18)</th>
<th>P value*</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time between symptom onset and scan (median, days)</td>
<td>-</td>
<td>4 [1-18]</td>
<td>99 [85-368]</td>
<td>0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume index (mL/m²)</td>
<td>79±15</td>
<td>71±20</td>
<td>72±11</td>
<td>0.03</td>
<td>0.89</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume index (mL/m²)</td>
<td>27±7</td>
<td>36±11</td>
<td>23±9</td>
<td>0.002</td>
<td>0.83</td>
</tr>
<tr>
<td>Stroke volume index (mL/m²)</td>
<td>51±11</td>
<td>39±12</td>
<td>46±7</td>
<td>&lt;0.001</td>
<td>0.61</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>67±8</td>
<td>51±11</td>
<td>69±4</td>
<td>&lt;0.001</td>
<td>0.71</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m²)</td>
<td>57±14</td>
<td>86±11</td>
<td>55±13</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>Left ventricular wall thickness- pathological (mm)</td>
<td>7.0±0.9</td>
<td>13.6±1.5</td>
<td>7.8±1.3</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Left ventricular wall thickness- remote (mm)</td>
<td>6.9±0.9</td>
<td>10.4±1.8</td>
<td>7.4±1.1</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Measure</td>
<td>Controls</td>
<td>Patients</td>
<td>Patients</td>
<td>p-value</td>
<td>p-value</td>
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<tr>
<td>Right ventricular ejection fraction (%)</td>
<td>63±6</td>
<td>63±10</td>
<td>66±4</td>
<td>0.47</td>
<td>0.09</td>
</tr>
<tr>
<td>Global longitudinal strain (%)</td>
<td>-18±1</td>
<td>-12±6</td>
<td>-16±3</td>
<td>0.003</td>
<td>0.09</td>
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<td>Late gadolinium enhancement pattern</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>-</td>
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<tr>
<td>Ischemic</td>
<td>-</td>
<td>1 (5)</td>
<td>-</td>
<td></td>
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<tr>
<td>Native T1 in pathological segment (ms)</td>
<td>1211±28</td>
<td>1358±49</td>
<td>1238±35</td>
<td>&lt;0.0001</td>
<td>0.02</td>
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<tr>
<td>Native T1 in remote segment (ms)</td>
<td>1211±28</td>
<td>1255±56</td>
<td>1209±27</td>
<td>0.02</td>
<td>0.90</td>
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<tr>
<td>Native T2 in pathological segment (ms)</td>
<td>38±3</td>
<td>60±7</td>
<td>39±2</td>
<td>&lt;0.0001</td>
<td>0.33</td>
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<tr>
<td>Native T2 in remote segment (ms)</td>
<td>38±3</td>
<td>43±5</td>
<td>36±3</td>
<td>0.007</td>
<td>0.55</td>
</tr>
<tr>
<td>Global extracellular volume (%)</td>
<td>26±3</td>
<td>34±5</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Myocardial T1 30 min after manganese pathological segment (ms)</td>
<td>884±26</td>
<td>1030±48</td>
<td>919±31</td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial T1 30 min after manganese remote segment (ms)</td>
<td>884±26</td>
<td>920±16</td>
<td>900±9</td>
<td>&lt;0.001</td>
<td>0.005</td>
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<tr>
<td>Manganese influx (Ki; mL/100 g of tissue min)</td>
<td>8.1±1.1</td>
<td>5.1±0.5</td>
<td>6.6±0.5</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Number (%), median [interquartile range], mean ± standard deviation
*Controls v patients with takotsubo syndrome at index presentation.
** Controls v patients with takotsubo syndrome at follow-up
Short-axis views of inferior late gadolinium enhancement in a patient with spontaneous coronary artery dissection of the obtuse marginal branch of the left circumflex artery (A) and apical takotsubo (dual pathology). During acute imaging reduced myocardial manganese uptake (green) extends beyond the infarct region (B). Follow-up imaging demonstrates recovery of manganese uptake (blue) in regions affected by takotsubo syndrome with persistent abnormal manganese uptake (green) in the infarct region (C). Long-axis, four chamber view demonstrating characteristic hazy “incomplete nulling” in late gadolinium enhancement imaging in a patient with apical takotsubo (D). Corresponding native T1 map during acute event demonstrates elevated native T1 in mid-ventricle and apical segments (E), with resolution on follow-up scans (F).
Resolution of left ventricular systolic dysfunction (A) and apical ballooning (B and C) in a patient with takotsubo syndrome scanned 18 days after symptom onset. Long-axis (D) and short-axis (E) manganese-enhanced T1 map demonstrating typical apical pattern of takotsubo syndrome with abnormal myocardial manganese uptake (green) in mid-ventricular and apical segments with normal uptake (blue) in basal segments despite apparent restoration of normal cardiac function.
Table 5.5 Global native T1, post-manganese T1 and myocardial manganese uptake (Ki) values

<table>
<thead>
<tr>
<th></th>
<th>Matched Control Volunteers (n=20)</th>
<th>Patients with takotsubo syndrome: index event (n=16) †</th>
<th>Patients with takotsubo syndrome: follow-up (n=14) †</th>
<th>P value*</th>
<th>P value**</th>
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<tr>
<td>Native T1 (ms)-</td>
<td>1211±28</td>
<td>1358±49</td>
<td>1237±30</td>
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<td>Septal</td>
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<tr>
<td>Native T1 (ms)-</td>
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<td>1360±59</td>
<td>1231±23</td>
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<tr>
<td>Myocardial T1</td>
<td>884±26</td>
<td>1030±48</td>
<td>914±22</td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
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<td>30 min after</td>
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<td>manganese (ms)-</td>
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<tr>
<td>Septal</td>
<td>892±22</td>
<td>1029±31</td>
<td>908±21</td>
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<td>Manganese influx</td>
<td>8.0±1.0</td>
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<td>6.8±0.6</td>
<td>&lt;0.0001</td>
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<td>(Ki; mL/100 g/</td>
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<td>tissue min)-</td>
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<td>8.2±1.1</td>
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*Controls v patients with takotsubo syndrome at index presentation.
** Controls v patients with takotsubo syndrome at follow-up.
† Patients with focal takotsubo and dual pathology excluded (n=4)
Follow Up at 3 Months

Twenty patients returned for follow-up, with 18 undergoing manganese-enhanced magnetic resonance imaging 99 days [median, range: 85-368] after symptom onset (Table 5.4) with 11 (65%) patients describing ongoing symptoms (Table 5.1). There was restoration of normal left ventricular ejection fraction and resolution of regional wall motion abnormalities in almost all patients (Table 5.4). Of the patients who were diagnosed with dual pathology (n=2), one demonstrated hypokinesis in the inferior wall despite normalisation of left ventricular ejection fraction (acute myocardial infarction and takotsubo syndrome) and the other had resolution of regional wall motion abnormality.

In patients with takotsubo syndrome, native and post-manganese T1 values were reduced compared to those obtained after the acute index event but remained higher than the matched control volunteers (P≤0.001 and P<0.02 respectively). T2 values in the previously pathological myocardium normalised and were comparable to matched control volunteers (P=0.33). Left ventricular mass and left ventricular wall thickness (pathological and remote) were also reduced and correlated with the reduction in myocardial native T2 values (r²= 0.6 and 0.7 respectively, Figure 5.5). Myocardial manganese uptake demonstrated some improvement but remained persistently abnormal at follow-up (Table 5.4, Figure 5.8). Furthermore, manganese uptake correlated with improvement in left ventricular mass and T2 values (Figure 5.5).
Figure 6: Myocardial Calcium Handling in Takotsubo Syndrome

Short-axis and long-axis manganese-enhanced T1 map in a (panel A) matched control volunteer and (panel B) patient with acute takotsubo syndrome demonstrating reduced manganese uptake (green). Short-axis and long-axis manganese-enhanced T1 maps at follow-up (panel C) demonstrating improvement (patchy) myocardial manganese uptake (as calculated by Patlak modelling) but persistent abnormalities compared to matched control volunteers (D).
5.5. **DISCUSSION**

We provide the first description of manganese-enhanced magnetic resonance imaging in detecting abnormal myocardial cellular physiology in patients with takotsubo syndrome. Our findings demonstrate that there is a profound disturbance in myocardial calcium handling, and this is most marked in the acute setting but remains abnormal following apparent recovery of left ventricular function. This abnormal myocardial calcium handling may contribute to the underlying the pathophysiology of this condition, and perhaps explains persistent symptoms reported by some patients and the high rate of recurrence of takotsubo syndrome (El-Battrawy et al. 2018, Ghadri et al. 2018, El-Battrawy et al. 2019, Singh et al. 2022)

For the first time, we have demonstrated a profound *in vivo* abnormality of myocardial calcium handling in patients with takotsubo syndrome. Our findings support previously described abnormalities of intracellular calcium handling in endomyocardial biopsies obtained from patients with takotsubo syndrome and acute left ventricular dysfunction (Nef et al. 2009). Here, calcium-regulating proteins, such as phospholamban, sarcoendoplasmic reticulum calcium-adenosine triphosphatase (SERCA) and sarcolipin, were markedly altered suggesting that this may be responsible for the associated ventricular dysfunction. Using myocardial manganese uptake as a measure of the flux of intracellular calcium ions, we confirm that marked alterations in myocardial calcium handling appear to play a major pathophysiological role in takotsubo
syndrome, especially in the acute high-risk period. Indeed, such a mechanism could explain why levosimendan can improve cardiogenic shock during acute severe cases of takotsubo syndrome since it augments myocardial calcium binding to troponin C. Although this is an important fundamental mechanistic observation, we cannot determine whether these alterations are a consequence or a cause of the takotsubo syndrome and this will require further study.

Takotsubo syndrome is partly defined by the apparent dramatic improvement of left ventricular function following the acute dramatic emergent presentation. The rapid transition from severe left ventricular systolic dysfunction and low ejection fraction to apparently normal left ventricular function and ejection fraction within days is a characteristic feature of this condition. However, this can create challenges for the diagnosis of this condition since unless the index of suspicion is high and the diagnosis is considered early, these typical features will resolve and the opportunity to undertake diagnostic imaging may have passed. In this regard, manganese-enhanced magnetic resonance imaging may provide an opportunity to identify the typical distribution of myocardial abnormalities despite normalisation of regional wall motion abnormalities. This provides a unique opportunity to diagnose this condition at later time points and help resolve potential diagnostic uncertainties especially where the clinical suspicion of the diagnosis was initially low. There can also be major diagnostic uncertainty in cases of dual pathology where acute myocardial infarction triggers a takotsubo syndrome as demonstrated by two
examples in our case series (Daghem 2021 [In Press]). Manganese-enhanced magnetic resonance imaging could therefore prove invaluable in identifying and discriminating cases of takotsubo syndrome, especially when the precipitating events is caused by another primary cardiac condition.

There are major implications for the protracted abnormalities in myocardial calcium handling. First, these findings could also account for the persistence of symptoms and reduced exercise capacity reported by patients who have apparently recovered from takotsubo syndrome (Scally et al. 2018). The majority of patients continue to complain of fatigue, tiredness and reduced exercise tolerance despite the presence of a normal left ventricular ejection fraction. Continued long-term impairment in cardiac energetic status and reduced exercise maximal oxygen capacity has previously been described and are likely to be linked to the abnormalities of myocardial calcium handling (Scally et al. 2018). Indeed, 60% of our cohort described ongoing symptoms at follow up, compatible with a heart failure-like syndrome with a substantial impact on quality of life.

In contrast to the prior belief that the heart recovers spontaneously and completely without clinical sequelae, patients with takotsubo syndrome are now recognised to have substantial long-term morbidity and mortality which is comparable to that of acute myocardial infarction (Templin et al. 2015, Ghadri et al. 2018, Di Vece et al. 2019, Uribarri et al. 2019, Redfors et al. 2021, Singh et al. 2022). Indeed, long-term rates of cerebrovascular and cardiac events
and recurrent takotsubo syndrome approach 10% and 2% per year (Templin et al. 2015, Ghadri et al. 2018). We have recently reported abnormal myocardial manganese uptake in patients with dilated and hypertrophic cardiomyopathy (Spath et al. 2020). Interestingly, levels of myocardial manganese uptake were more impaired in patients who had recovered from takotsubo syndrome than those with dilated cardiomyopathy despite the latter having marked left ventricular systolic dysfunction (Spath et al. 2020). Whilst it would have been ideal to assess patients with takotsubo syndrome prior to their incident event, the longer-term persistence of abnormal myocardial calcium handling does suggest an underlying cardiomyopathy which is only brought to light following an acute stressful event. As such, we may never see normalisation of myocardial calcium handling, and is again in keeping with previous studies describing a heart failure-like phenotype in this patient cohort (Scally et al. 2018).

**5.5.1. Clinical Implications**

There are potential opportunities for manganese-enhanced magnetic resonance imaging to play an important role in prognostication and the assessment of novel treatment interventions. Persistent perturbation of myocardial calcium-handling may identify those patients who are at risk of incident or recurrent cases of takotsubo syndrome. Whilst this would seem intuitive, large prospective patient cohort studies are required to establish whether this is indeed the case. In addition, there are currently no proven treatments to improve the symptoms and clinical outcomes of patients with
takotsubo syndrome. The assessment of myocardial calcium handling using manganese-enhanced magnetic resonance imaging could provide a very useful surrogate biomarker of treatment efficacy. This is particularly important since standard measures of cardiac function, such as left ventricular ejection fraction, appears to be normal in patients who have recovered from takotsubo syndrome, and this has limited the field in terms of assessing the efficacy of potential preventative therapeutic interventions. It is noteworthy that persistent abnormalities of myocardial calcium handling were present despite the initiation of angiotensin converting enzyme inhibitor or beta-blocker therapies in many of our patients.

5.5.2. Limitations

Our study has several limitations that should be acknowledged. First, there have been prior concerns regarding toxicity of unchelated forms of manganese. However, the chelated form of manganese used here retains the necessary properties for intracellular myocardial manganese uptake without any demonstrable adverse hemodynamic or arrhythmic effects (Jynge et al. 1997, Marti-Bonmati et al. 2003). Second, our study population size was modest, although we detected substantial and large abnormalities in myocardial calcium handling. Future multicentre studies of larger patient populations are needed to demonstrate the robustness and generalisability of our findings. Third, the presence of anti-depressant therapy use was greater in the patients compared to matched control volunteers. However, there was no demonstrable differences in myocardial manganese uptake according to
the use of anti-depressant therapies (Table 5.3). Finally, there are no currently available preparations of manganese-based contrast medium for widespread clinical use. However, this is likely to change with commercially available preparations anticipated in the near future.
5.6. **CONCLUSION**

In conclusion, we have conducted the first proof-of-concept study of manganese-enhanced magnetic resonance imaging in patients with takotsubo syndrome. Using kinetic modelling, we have observed dysfunctional myocardial calcium handling in patients with takotsubo syndrome which is most striking during the acute episode but persists despite resolution of oedema and recovery of myocardial function. We believe that manganese-enhanced magnetic resonance imaging holds major promise for the diagnosis, risk stratification and monitoring of disease, with the potential for the assessment of novel treatment interventions.
6. **CHAPTER 6: MAGNETIC RESONANCE IMAGING AND COMPUTED TOMOGRAPHIC CORONARY ANGIOGRAPHY IN SURVIVORS OF COVID-19.**

Extracts of this chapter have been published in:

6.1. **SUMMARY**

The aim of this study was to determine the contribution of co-morbidities on the reported widespread myocardial abnormalities in patients with recent COVID-19. In a prospective two-centre observational study, patients hospitalised with confirmed COVID-19 underwent gadolinium and manganese-enhanced magnetic resonance imaging and computed tomography coronary angiography (CTCA). They were compared to healthy and co-morbidity-matched volunteers after blinded analysis.

In 52 patients (median age:54 [IQR 51-57] years, 39 male) who recovered from COVID-19, one-third (n=15, 29%) were admitted to intensive care and a fifth (n=11, 21%) were ventilated. Twenty-three patients underwent CTCA, with one third having underlying coronary artery disease (n=8, 35%). Compared with younger healthy volunteers (n=10), patients demonstrated reduced left (EF: 57.4±1 (95% CI: 68.1-78.1) versus 66.3±5 (95% CI: 62.4-69.8) %; P=0.02) and right (EF: 51.7±9.1 (95% CI: 53.9-60.1) versus 61±5 (95% CI: 42.1-52.9) %; P≤0.0001) ventricular systolic function with elevated native T1 values (1225±46 (95% CI: 1205-1240) versus 1197±30 (95% CI: 1178-1216) ms; P=0.04) and extracellular volume fraction (ECV) (31±4 (95% CI: 29.6-32.1) versus 24±3 (95% CI: 22.4-26.4) %; P<0.0001) but reduced myocardial manganese uptake (6.9±0.9 (95% CI: 6.5-7.3) versus 7.9±1.2, (95% CI: 7.4-8.5) mL/100g/min; P=0.01). Compared to co-morbidity-matched volunteers (n=26), patients had
preserved left ventricular function but reduced right ventricular systolic function (EF: 51.7±9.1 (95% CI: 53.9-60.1) versus 59±3 (95% CI: 51-66.5)%; P=0.0005) with comparable native T1 values (1225±46 (95% CI: 1205-1240) versus 1227±51 (95% CI: 1208-1246)ms; P=0.99), ECV (31±4, (95% CI: 29.6-32.1) versus 29±5 (95% CI: 27.0-31.2)%; P=0.35), presence of late gadolinium enhancement and manganese uptake. These findings remained irrespective of COVID-19 disease severity, presence of myocardial injury or ongoing symptoms.

Patients with COVID-19 demonstrated right but not left ventricular dysfunction. Furthermore, no evidence of myocardial calcium handling was observed. Previous reports of left ventricular myocardial abnormalities following COVID-19 may reflect pre-existing co-morbidities.
6.2. INTRODUCTION

In patients with COVID-19, there remains major concern surrounding the extent of cardiac involvement and its consequences. Myocardial injury is common in patients hospitalised with COVID-19 and correlates with disease severity and worse clinical outcomes (Clerkin et al. 2020, Zhou et al. 2020). The mechanisms underlying this are not well understood, with some suggesting indirect mechanisms of injury similar to that of other severe respiratory illnesses (Smeeth et al. 2004, Lindner et al. 2020, Hu et al. 2021, Kotecha et al. 2021, McCracken et al. 2021, Pellegrini et al. 2021). Others have proposed direct myocardial injury due to myocarditis, stress cardiomyopathy, endothelial injury, thrombo-inflammatory or the result of profound ongoing myocardial oxygen supply or demand imbalance (Buzon et al. 2012, Huang et al. 2020, Knight et al. 2020, Puntmann et al. 2020, Starekova et al. 2021).

There are increasing reports of persistent and prolonged multi-organ effects after acute COVID-19 illness (Ayoubkhani et al. 2021, Nalbandian et al. 2021). More importantly, many patients continue to have debilitating symptoms during recovery (Nalbandian et al. 2021) and it is important to understand whether cardiac damage observed in the acute phase of COVID-19 will translate into subsequent cardiac dysfunction and morbidity. Widespread myocardial abnormalities seen on cardiac magnetic resonance imaging have been reported in patients with COVID-19 (Knight et al. 2020, Puntmann et al. 2020,
Kotecha et al. 2021). However, a large proportion of these patients have co-morbidities and the presence of coronary artery disease had not been excluded. It is therefore essential to understand whether such cardiac abnormalities are the result of underlying co-morbidities or the direct impact of COVID-19.

Manganese-enhanced magnetic resonance imaging has shown promise in assessing myocardial calcium handling (Spath et al. 2020) and may measure more subtle disturbances in myocardial function. Using both gadolinium and manganese-enhanced MRI combined with CT coronary angiography, we sought to determine the contribution and impact of pre-existing cardiovascular disease on the cardiac abnormalities of patients recovering from COVID-19 hospitalisation.
6.3. METHODS

6.3.1. Participants

Adult patients recovering from hospitalisation with COVID-19 were recruited prospectively from the Edinburgh Heart Centre between May 2020 and November 2020 and Glenfield Hospital, Leicester between November 2020 and February 2021. The diagnosis of COVID-19 was based on a positive polymerase chain reaction (PCR) test. Comparisons were made with healthy volunteers (10 participants) and volunteers matched for age, sex and co-morbidities (26 participants). Patients and co-morbid volunteers were propensity matched 1:2 to cardiovascular risk factors including hypertension, known ischaemic heart disease, hypercholestrolaemia and diabetes mellitus. Matched volunteers were recruited from general cardiology admissions, outpatient clinic or those recruited for other cardiac studies. The latter control group were scanned at Glenfield Hospital prior to January 2020 (n=16) or at the University of Edinburgh (n=10) between September 2020 and January 2021. Matched (n=6) and healthy volunteers (n=10) scanned during the pandemic were eligible once previous COVID-19 infection or current symptoms of COVID-19 were excluded. The majority of this cohort (matched n=6, healthy n= 8) underwent regular PCR testing during hospital admission or due to occupational requirements. Exclusion criteria for all participants included contraindications to magnetic resonance imaging or manganese dipyridoxyl diphosphate administration, High degree atroventricular block, torsades de pointes, prolonged QTc interval, liver failure, calcium-channel
blockers or digoxin, renal failure, New York Heart Association class IV heart failure, and pregnancy.

Myocardial injury was defined as plasma cardiac troponin I concentration above the 99th centile (female: 16 ng/L, male: 34 ng/L) but was not necessary for inclusion. Quick COVID-19 severity index (qCSI) was used to assess severity with severe cases defined as having an index of >4 (>30% risk of critical illness) (Haimovich et al. 2020). The Quick COVID-19 Severity Index (qCSI) has been validated to risk-stratify patients with COVID-19 during the first 24 hours of hospital admission and assess their risk of critical illness. The formula is based on 3 variables: respiratory rate (breaths/min), pulse oximetry (lowest value recorded, %) and O2 flow rate (L/min) (Table 6.1).

Table 6.1 qCSI score and its associated risk of critical illness is shown below:

<table>
<thead>
<tr>
<th>qCSI</th>
<th>Risk Level</th>
<th>Risk of Critical Illness at 24 hours (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td>4-6</td>
<td>Low-intermediate</td>
<td>30</td>
</tr>
<tr>
<td>7-9</td>
<td>High-intermediate</td>
<td>44</td>
</tr>
<tr>
<td>10-12</td>
<td>High</td>
<td>57</td>
</tr>
</tbody>
</table>
6.3.2. Magnetic Resonance Imaging

Magnetic resonance imaging in Edinburgh was performed using a Siemens MAGNETOM Skyrafit 3T scanner (Siemens Healthineers, Erlangen, Germany) with a dedicated 30-channel body matrix coil. Magnetic resonance imaging in Leicester was performed using a Siemens MAGNETOM Skyra 3T scanner (Siemens Healthineers, Erlangen, Germany) with an 18-channel cardiac coil. All study participants underwent imaging with late gadolinium enhancement. Patients from Edinburgh and all healthy volunteers underwent additional manganese-enhanced magnetic resonance imaging, at least 48 hours apart. Patients were scanned during convalescence. Images were acquired during expiratory breath hold with ECG gating. Cine imaging was acquired with standard steady-state free precession sequences in long and short-axis orientations as described previously (Spath et al. 2020, Spath et al. 2021). T2 mapping (MyoMaps) was acquired in the short-axis orientation covering the entire left ventricle. Native T1 mapping was acquired with a Modified Look-Locker Inversion recovery short-axis stack. COVID-19 Patients scanned in Leicester underwent native T1 mapping with a shortened modified Look-Locker inversion recovery short-axis stack.

Late Gadolinium Enhancement

Late gadolinium enhancement images were acquired following intravenous gadobutrol (0.1 mmol/kg; Gadovist, Bayer, Germany) (Papanastasiou et al. 2015) using a single breath hold per slice with a short-axis stack, and long-axis orientations. T1 mapping was acquired prior to and 10 min post-contrast.
Haematocrit was measured on the day of scanning and was used to calculate global extracellular volume fraction.

**Manganese-enhanced magnetic resonance imaging**

Manganese-enhanced magnetic resonance imaging was carried out using intravenous infusion of manganese dipyridoxyl diphosphate (5 µmol/kg, 1 mL/min, 0.1 mL/kg; Exova SL Pharma, Wilmington, Delaware, USA). T1 mapping was performed pre-contrast with a full short-axis Modified Look-Locker Inversion recovery stack, which have been described previously (Spath et al. 2020, Spath et al. 2021). For patients with COVID-19, a single short-axis slice was then identified to represent pathological myocardium, guided by the late gadolinium enhancement, native T1 maps and cine images. For all participants with no obvious abnormality, a single mid-ventricular short-axis slice was selected. A single short-axis T1 mapping was then performed at this slice location every 2.5 min for 30 min after starting manganese contrast infusion, at which point a full short-axis shortened modified Look-Locker inversion recovery stack was repeated post-contrast.

**6.3.3. Image Analysis**

Cardiovascular magnetic resonance studies were analysed offline using Circle CVI (Circle Cardiovascular Imaging, CVI42 v5.3.6, Calgary Canada). T1, T2 maps, late gadolinium enhancement and cine-derived volumetric and functional sequences was analysed by experienced observers (MRD, TS, TK). Endocardial and epicardial borders were manually defined on all the
conventional short-axis images for volumetric and wall motion measurements and were then copied to all corresponding late gadolinium enhancement and T1 map sequences for analysis with minimal manual adjustments. After contouring, an additional epicardial and endocardial offset of 20% was applied automatically to minimise tissue interface for all T1 map analyses and artefact was excluded manually for a minority of cases.

Regions of interest (ROIs) were determined using the standard 16-segment cardiac model with septal native T1 values. For patients with previous myocardial infarction or a new diagnosis of infarction, region of interest (ROIs) was defined in the remote myocardium. For those with a non-ischaemic pattern of late-enhancement native T1 was defined in the septal wall. Native T1 and T2 measurements were taken from septal segments, although we also report results for global T1. T1 mapping acquired using shortened modified Look-Locker inversion recovery were excluded from native T1 analysis but not from extracellular volume analysis. Areas of late gadolinium enhancement were included for calculating global extracellular volume. Late gadolinium enhancement was adjudicated by consensus of expert observers (GPM, JRA) who were blinded to all participant details (including whether scans were from patients or volunteers) and classified as ischaemic or non-ischaemic (mid-wall or epicardial) pattern. Right ventricular insertion point enhancement in isolation was not considered pathological.
6.3.4. Manganese Kinetic Modelling
Myocardial calcium handling was assessed using T1 maps during manganese-enhanced magnetic resonance imaging (Spath et al. 2018) Regions of interest were drawn in areas of myocardial abnormalities or the mid-ventricular septum in those without myocardial abnormalities (mean size: 0.7±0.13 cm²). The rate of myocardial manganese uptake was determined by Patlak modelling as described previously (Spath et al. 2020, Spath et al. 2021).

6.3.5. Computed Tomography Coronary Angiography
Patients scanned in Edinburgh underwent computed tomography coronary angiography (CTCA) which was performed with a 128-multidetector row scanner (Siemens Biograph, Siemens Healthcare, Erlangen, Germany) according to SCCT guidelines and have been described previously (Abbara et al. 2016). Patients with a heart rate over 60 /min received intravenous metoprolol and all patients received sublingual glyceryl trinitrate prior to imaging. CCTA imaging was reviewed on a dedicated post processing workstation (Vitrea Advanced, v6.9.68.1, Vital Images, US) by experienced observers (MCW, EJRB). Obstructive coronary artery disease was defined as a luminal cross-sectional area stenosis of >70% in a major epicardial vessel or >50% in the left main stem. Prognostically significant coronary artery disease was defined as left main stem stenosis >50%, three-vessel disease or two-vessel disease including stenosis of the proximal left anterior descending coronary artery. Lung windows were reviewed for pulmonary COVID-19
involvement or persistent parenchymal lung abnormalities (atelectasis/scarring or ground glass opacification) (Simpson et al. 2020).

6.3.6. Statistical analysis

All statistical analysis was performed with GraphPad Prism (GraphPad Software v8.0.2, San Diego, California, USA). Categorical baseline variables were presented as number (%) and compared using Chi-squared test. Continuous data were assessed for normality using the D'Agostino-Pearson test and presented as mean±standard deviation or median [interquartile range]. Cardiac function, myocardial manganese uptake, volumetric assessment and parametric mapping values were compared using paired or unpaired Student's t-tests, Wilcoxon or Mann-Whitney tests and ANOVA ± Dunnett's as appropriate. Statistical significance was taken as two-sided p<0.05.
6.4. **RESULTS**

6.4.1. **Study populations**

Fifty-four patients recovering from COVID-19 were recruited into the study with two patients withdrawing due to problems with vascular access or claustrophobia (Figure 6.1). Ten healthy volunteers and 26 volunteer patients matched for age, sex and co-morbidities were recruited as comparator groups (Table 6.2).

6.4.2. **Characteristics of patients with COVID-19**

All patients with COVID-19 were symptomatic and required hospitalisation, with the commonest symptom being dyspnoea (87%) and a smaller proportion presenting with chest pain (13%). Twenty-seven (52%) patients had severe disease with a Quick COVID-19 severity score of greater than 4, 15 (29%) requiring admission to the intensive care unit and 11 (21%) undergoing non-invasive or invasive ventilation. Overall, 29 (56%) patients had pre-existing cardiovascular disease or risk factors including hypertension, diabetes mellitus, and hypercholesterolaemia. Seventeen (33%) had an elevation in plasma high-sensitivity cardiac troponin I concentration above the normal upper reference limit (Table 6.3). Of those who underwent clinically indicated echocardiography during their hospital admission, 10 (19%) had an abnormal echocardiogram with 6 demonstrating right ventricular dilatation and 3 left ventricular dilatation (Table 6.4). Eight of the 10 patients with an abnormal baseline echocardiogram received either non-invasive or invasive ventilation.
Of those who had computed tomography pulmonary angiography (CTPA) as part of clinical care (n=19), 2 had evidence of pulmonary emboli.
CTCA, Computed tomography coronary angiography, PCR, Polymerase chain reaction, PIL, Patient information leaflet.
**Table 6.2: Baseline characteristics of study populations**

<table>
<thead>
<tr>
<th></th>
<th>Patients with COVID-19 (n=52)</th>
<th>Co-Morbidity-Matched Volunteers (n=26)</th>
<th>Healthy Volunteers (n=10)</th>
<th>P value a</th>
<th>P value b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median [IQR] (years)</strong></td>
<td>54 [51-57]</td>
<td>53 [47-57]</td>
<td>35 [29-40]</td>
<td>0.58</td>
<td>&lt;0.0001</td>
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<tr>
<td>Male</td>
<td>39 (75)</td>
<td>19 (73)</td>
<td>9 (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index, median [IQR]</strong> (kg/m²)</td>
<td>28 [18- 42]</td>
<td>27 [22-41]</td>
<td>21 [22-27]</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Time of scan post symptoms onset median± IQR (days)</strong></td>
<td>90 ± (7- 290)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent symptoms</td>
<td>20 (38)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive care admission</td>
<td>15 (29)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-tracheal intubation</td>
<td>5 (10)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-invasive ventilation</td>
<td>6 (12)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quick COVID-19 severity index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>21 (40)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-intermediate</td>
<td>13 (25)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-intermediate</td>
<td>14 (27)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>4 (8)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Past Medical History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (35)</td>
<td>8 (31)</td>
<td>-</td>
<td></td>
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<tr>
<td>Ischaemic Heart Disease</td>
<td>8 (15)</td>
<td>5 (19)</td>
<td>-</td>
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<tr>
<td>Hypercholestrolaemia</td>
<td>16 (31)</td>
<td>8 (31)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients with COVID-19</td>
<td>Cohort b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------</td>
<td>----------</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial Fibrillation/Flutter</td>
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<td>1 (4)</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>Previous Cerebro-vascular event</td>
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<td>0</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>Diabetes mellitus</td>
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<td>11 (38)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-cardiac</td>
<td>17 (33)</td>
<td>2 (8)</td>
<td>-</td>
<td></td>
<td></td>
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**Medications**

<table>
<thead>
<tr>
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<th>Patients with COVID-19</th>
<th>Cohort b</th>
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<td>Antiplatelet therapy</td>
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<td>6 (23)</td>
<td></td>
</tr>
<tr>
<td>Beta-Blocker therapy</td>
<td>8 (15)</td>
<td>4 (15)</td>
<td></td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy</td>
<td>14 (27)</td>
<td>9 (35)</td>
<td></td>
</tr>
<tr>
<td>Diuretic therapy</td>
<td>2 (4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Statin therapy</td>
<td>16 (31)</td>
<td>10 (38)</td>
<td></td>
</tr>
<tr>
<td>Anti-glycaemic therapy</td>
<td>18 (35)</td>
<td>11 (42)</td>
<td></td>
</tr>
</tbody>
</table>

**Smoking status**

<table>
<thead>
<tr>
<th></th>
<th>Patients with COVID-19</th>
<th>Cohort b</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Smoker</td>
<td>43 (83)</td>
<td>14 (54)</td>
<td>0</td>
</tr>
<tr>
<td>Ex-Smoker</td>
<td>8 (15)</td>
<td>10 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0</td>
<td>1 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Patients with COVID-19 versus co-morbidity-matched volunteers

\(^b\) Patients with COVID-19 versus healthy volunteers

SD, standard deviation, IQR, interquartile range.
### Table 6.3 Laboratory biomarkers

<table>
<thead>
<tr>
<th>Laboratory Findings</th>
<th>Recovered COVID-19 (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak white-cell count (x10³/L)</td>
<td>11.6 (4 - 11)</td>
</tr>
<tr>
<td>Peak neutrophil count (x10⁹/L)</td>
<td>7.9 (2 - 7.5)</td>
</tr>
<tr>
<td>Lowest lymphocyte count (x10⁹/L)</td>
<td>0.94 (1.5 - 4.5)</td>
</tr>
<tr>
<td>Peak C-reactive protein (mg/L)</td>
<td>150 (0 – 5)</td>
</tr>
<tr>
<td>Peak D-dimer (ng/L)</td>
<td>1563 (0 - 230)</td>
</tr>
<tr>
<td>Peak ferritin (ug/L)</td>
<td>1058 (20- 300)</td>
</tr>
<tr>
<td>Peak procalcitonin (ug/L)</td>
<td>0.22 (&gt;0.15)</td>
</tr>
<tr>
<td>Elevated troponin (&gt;99th centile)</td>
<td>17</td>
</tr>
<tr>
<td>Peak high-sensitivity troponin I (ng/L)</td>
<td>1068 (female&lt;16, male:&lt;34)</td>
</tr>
</tbody>
</table>

n (reference range)

### Table 6.4 Electrocardiographic and Echocardiographic Findings

<table>
<thead>
<tr>
<th></th>
<th>Recovered COVID-19 (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECG changes</strong></td>
<td></td>
</tr>
<tr>
<td>Rhythm disturbance</td>
<td>4 (25)</td>
</tr>
<tr>
<td>ST segment deviation</td>
<td>4 (25)</td>
</tr>
<tr>
<td>T wave deviation</td>
<td>10(50)</td>
</tr>
<tr>
<td><strong>Echocardiogram</strong></td>
<td></td>
</tr>
<tr>
<td>Right ventricle dilatation</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Right ventricle dysfunction</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Left ventricle dilatation</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Left ventricle dysfunction</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Regional wall motion abnormalities</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

n (%)
6.4.3. Computed tomography coronary angiography

Twenty-three patients underwent CT coronary angiography and were scanned 90 days [IQR: 7-290 days] following symptom onset. Sixty-five percent of patients (15 of 23) had normal coronary arteries and 35% (8 of 23) had evidence of coronary artery disease with one patient having obstructive disease (Table 6.5). Within this sub-group, eight patients (35%) described persistent dyspnoea at the time of imaging, of whom seven demonstrated persistent parenchymal lung abnormalities at the time of imaging (Figure 6.2).

6.4.4. Cardiac magnetic resonance imaging

Patients with COVID-19 compared with healthy volunteers

In comparison to younger healthy volunteers, patients with COVID-19 had reduced biventricular systolic function (Table 6.6). Septal native myocardial T1 values (1225±46 (95% confidence interval (CI):1205-1240) versus 1197±30 (95% CI: 1178-1216) ms; P=0.04; Figure 6.3), global native T1 values (Table 6.6) and extracellular volume fraction (31±4 (95% CI:29.6-32.1) versus 24±3 (95% CI:33.4-25.4) %; P=0.0003; Figure 6.3) were higher in patients with COVID-19. None of the healthy volunteers had evidence of late gadolinium enhancement. Manganese-enhanced magnetic resonance imaging demonstrated reduced uptake of myocardial manganese uptake in patients recovering from COVID-19 (mean Ki 6.9±0.9 (95% CI:6.5-7.3) versus 7.9±1.2 (95%CI:7.4-8.5) mL/100g/min; P=0.01, Figure 6.3).
Table 6.5 Coronary computed tomography angiography findings

<table>
<thead>
<tr>
<th>Patients with COVID-19 (n=23)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15 (65)</td>
</tr>
<tr>
<td>Non-Obstructive Disease</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Mild (&lt;50%)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Moderate (50-70%)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Obstructive Disease</td>
<td></td>
</tr>
<tr>
<td>One vessel</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Two vessels</td>
<td></td>
</tr>
<tr>
<td>Three vessels</td>
<td></td>
</tr>
<tr>
<td>Other Cardiac Findings</td>
<td></td>
</tr>
<tr>
<td>LV thrombus</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Anomalous coronary anatomy</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Non-Cardiac Findings</td>
<td></td>
</tr>
<tr>
<td>Parenchymal Scarring/Atelectasis</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Peripheral ground glass opacification</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Pulmonary Mass or Nodule</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Emphysema</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Hiatus Hernia</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Liver Pathology</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Pulmonary Embolism</td>
<td>0</td>
</tr>
</tbody>
</table>

n (%)
Figure 6.2 Chest Computed Tomography in Severe COVID-19.

Typical COVID-19 appearance with ground glass opacification (long arrow) and peripheral basal consolidation (short arrow) on during hospital admission (A) and 4 months later (B) with residual atelectasis (short arrow) and subtle ground glass opacification (long arrow) in a patient with severe COVID-19 with ongoing symptoms compared to a patient with COVID-19 without symptoms (C).
<table>
<thead>
<tr>
<th></th>
<th>Patients with COVID-19 (n=52)</th>
<th>Matched Co-Morbidity-Matched Volunteers (n=26)</th>
<th>Healthy Volunteers (n=10)</th>
<th>P value (^a)</th>
<th>P value (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDVI , mean ± SD (95% CI), (mL/m²)</td>
<td>73.1±18.1 (68.1-78.1)</td>
<td>78.5±20 (67-81.0)</td>
<td>79.2±18.3 (72.6-43.7)</td>
<td>0.99</td>
<td>0.17</td>
</tr>
<tr>
<td>LVESVI, mean ± SD (95% CI), (mL/m²)</td>
<td>32.1±16.1 (27.6-36.4)</td>
<td>31.7±16.2 (26.0-37.9)</td>
<td>25.8±7.9 (22.5-30.1)</td>
<td>0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>Stroke Volume Index, mean ± SD (95% CI), (mL/m²)</td>
<td>41.3±10.8 (38.3-44.1)</td>
<td>44.9±8.7 (40.0-50.4)</td>
<td>52.1±10.6 (46.5-56.3)</td>
<td>0.12</td>
<td>0.001</td>
</tr>
<tr>
<td>LV Ejection Fraction, mean ± SD (95% CI), (%)</td>
<td>57.4±11.1 (54.0-60.1)</td>
<td>61.6±9.9 (56.1-65.2)</td>
<td>66.3±5.3 (62.4-69.8)</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>LV Mass Index, mean ± SD (95% CI), (g/m²)</td>
<td>53.5±11.0 (50.4-56.6)</td>
<td>56.6±12.2 (51.8-60.2)</td>
<td>55.7±15.2 (50.6-61.4)</td>
<td>0.21</td>
<td>0.81</td>
</tr>
<tr>
<td>RVEDVI, mean ± SD (95% CI), (mL/m²)</td>
<td>79.3±16.2 (74.8-83.8)</td>
<td>75.4±12.3 (70.4-80.4)</td>
<td>74.5±9.8 (68.6-80.4)</td>
<td>0.27</td>
<td>0.06</td>
</tr>
<tr>
<td>RVESVI, mean ± SD (95% CI), (mL/m²)</td>
<td>39.9±15.3 (34.7-43.3)</td>
<td>30.1±7.8 (28.2-33.5)</td>
<td>29.3±5.4 (26.1-32.6)</td>
<td>0.006</td>
<td>0.02</td>
</tr>
<tr>
<td>RV Stroke Volume Index, mean ± SD (95% CI), (mL/m²)</td>
<td>39.8±9.7 (37.2-42.6)</td>
<td>45.1±9.8 (45.2-55.7)</td>
<td>47.5±9.3 (42.1-52.9)</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>RV Ejection Fraction, mean ± SD (95% CI), (%)</td>
<td>51.7± 9.1 (53.9-60.1)</td>
<td>59.3± 4.9 (51.0-66.5)</td>
<td>60.5 ± 4.9 (57.1-63.2)</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Main Pulmonary Artery, mean ±SD (mm)</td>
<td>20.7±3.1</td>
<td>22.8 ± 6.6</td>
<td>18.5±4.0</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>Late Gadolinium Enhancement pattern, n (%)</td>
<td>18 (35)</td>
<td>9 (35)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic</td>
<td>9 (17)</td>
<td>5 (19)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-ischaemic</td>
<td>9 (17)</td>
<td>4 (15)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1- Septum, mean ± SD (95% CI), (ms)</td>
<td>1225±46* (1205-1240)</td>
<td>1227±51** (1208-1246)</td>
<td>1197±30 (1178-1216)</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Global T1- midventricular, mean ± SD (95% CI), (ms)</td>
<td>1210±38* (1193-1226)</td>
<td>1208±33** (1191-1228)</td>
<td>1184±24 (1168-1200)</td>
<td>0.88</td>
<td>0.04</td>
</tr>
<tr>
<td>Extracellular Volume, mean ± SD (95% CI), (%)</td>
<td>31±4 (29.6-32.1)</td>
<td>29 ±5 (27.0-31.2)</td>
<td>24±3 (22.4-26.4)</td>
<td>0.35</td>
<td>0.0003</td>
</tr>
<tr>
<td>T2 Septum, mean ± SD (95% CI), (ms)</td>
<td>37.3±4.6</td>
<td>38.5±5.9 (36.1-40.1)</td>
<td>38.7±3 (37.4-40.1)</td>
<td>0.35</td>
<td>0.18</td>
</tr>
<tr>
<td>Manganese Influx constant, mean ± SD (95% CI), (Ki/mL/100 g/min)</td>
<td>(35.9-38.6)</td>
<td>6.9±0.9* (6.5-7.3)</td>
<td>7.3±1.3** (6.7-7.9)</td>
<td>7.9±1.2 (7.4-8.5)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Abbreviation: LVEDVI, indexed left ventricular end-diastolic volume; LVESVI, indexed left ventricular end-systolic volume; RVEDVI, indexed right ventricular end-diastolic volume; RVESVI, indexed right ventricular end-systolic volume; LV, left ventricular; RV, right ventricular, SD, standard deviation, CI, confidence interval.

a Patients with COVID-19 versus co-morbidity-matched volunteers
b Patients with COVID-19 versus healthy volunteers
*n=23
** n=20
Figure 6.3 Cardiac Magnetic Resonance Imaging in Patients with COVID-19 Compared with Matched Volunteers and Healthy Volunteers

Left ventricular (LV) ejection fraction (A), right ventricular (RV) ejection fraction (B), native T1 values (C) and extracellular volume (D) in healthy control volunteers (n=10, green), matched control volunteers (n=26, blue) and patients with COVID-19 (n=52, red).
Patients with COVID-19 compared with co-morbidity-matched volunteers

There were no major differences in left ventricular volumes and systolic function between patients and co-morbidity-matched volunteers. However, patients recovering from COVID-19 did have reduced right ventricular systolic function (51.7±9.1 (95% CI:53.9-60.1) versus 59±3 (95% CI:51-66.5)%; P=0.0005, Table 6.6). When compared to co-morbidity matched volunteers, septal native myocardial T1 values (1225±46 (95% CI:1205-1240) versus 1227±51 (95% CI:1208-1246)ms; P=0.99, Figure 6.3), global native T1 values (Table 6.6) and extracellular volume fraction (31±4 (95%CI:29.6-32.1) versus 29±5 (95% CI:27.0-31.2) %; P=0.35, Figure 6.3) were similar. Myocardial manganese uptake (mean Ki 6.9±0.9 (95% CI:6.5-7.3) versus 7.3±1.3 (95% CI:6.7-7.9)mL/100 g/min;P=0.45, Figure 6.3) was also comparable. Late gadolinium enhancement was seen in 18 (35%) patients with COVID-19, with 9 demonstrating a non-ischaemic pattern and 9 with an ischaemic pattern (Figure 6.6). None of the patients with a non-ischaemic pattern of late gadolinium enhancement had a history of prior cardiac disease and all had normal T2 values at the site of late enhancement (mean T2,40.3±3.9 ms). Only one patient with an ischaemic pattern of enhancement had elevated T2 values in the corresponding region (45ms). The prevalence of late gadolinium enhancement was similar in the co-morbidity-matched volunteers including right ventricular insertion point enhancement (Table 6.6, Table 6.7).
Figure 6.4 Cardiac Magnetic Resonance features in hospitalised COVID-19 survivors.

Magnetic resonance imaging findings in patients recovering from COVID-19 infection compared to age, sex and co-morbidity matched volunteers

*Statistically significant
Table 6.7 RV insertion point late gadolinium enhancement.

<table>
<thead>
<tr>
<th>Presence of right ventricular insertion point late gadolinium enhancement</th>
<th>Patients with COVID-19 (n=52)</th>
<th>Matched Co-Morbidity-Matched Volunteers (n=26)</th>
<th>Healthy Volunteers (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 (35)</td>
<td>10 (38)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

LGE, Late gadolinium enhancement n (%)
Influence of disease severity, myocardial injury and ongoing symptoms

In patients with severe COVID-19 (27 of 52, 52%), left ventricular function was preserved compared to matched volunteers. In contrast, right ventricular systolic function was reduced (ejection fraction 52.2±10.2 (95% CI:48.1-56.2) versus 59.3±4.9 (95% CI:51.0-66.5)%; P=0.0012). Native myocardial T1 values, extracellular volume fractions, myocardial manganese uptake and prevalence of late gadolinium enhancement were similar to co-morbidity-matched volunteers (Figure 6.5, Table 6.8).

A similar pattern was seen in patients with COVID-19 with evidence of myocardial injury (Table 6.8). Native T1, extracellular volume fractions and myocardial manganese uptake were similar to co-morbidity-matched volunteers (Figure 6.5, Table 6.8). There was a higher prevalence of late gadolinium enhancement in this cohort compared to matched volunteers (9 of 17, 53% versus 9 of 26, 35%), with the majority demonstrating an ischaemic pattern of injury (6 of 9, 66%).

Twenty of 52 patients (38%) had ongoing symptoms at the time of scanning. While these patients had comparable left ventricular systolic function to matched volunteers, they had reduced right ventricular systolic function (ejection fraction 49.0±6.5 (95% CI:45.9-52.2) versus 59.3±4.9 (95% CI: 51.0-66.5) %; P<0.0001; Figure 6.5, Table 6.8). Native T1 values, extracellular volume fractions, myocardial manganese uptake and prevalence of late
gadolinium enhancement were similar to co-morbidity-matched volunteers (Table 6.8).
Figure 6.5 Cardiac Magnetic Resonance Imaging in Subgroups of Patients with COVID-19 Compared with Matched Volunteers.

Left ventricular (LV) ejection fraction (A), right ventricular (RV) ejection fraction (B), native T1 values (C) and extracellular volume (D) in matched control volunteers (n=26, green) and patients with COVID-19 and severe COVID-19 disease (n=27, red), myocardial injury (n=17, orange) or ongoing symptoms (n=20, blue).
Table 6.8 Subgroup analysis of magnetic resonance imaging findings

<table>
<thead>
<tr>
<th></th>
<th>Matched Volunteer (n=26)</th>
<th>Recovered COVID-19 (n=52)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe COVID-19 (n=27)</td>
<td>P value</td>
<td>Myocardial Injury (n=17)</td>
<td>P value</td>
<td>Ongoing Symptoms (n=20)</td>
</tr>
<tr>
<td>LV Ejection Fraction , mean ± SD (95% CI) (%)</td>
<td>61.6 ±9.9 (56.1-65.2)</td>
<td>0.35</td>
<td>57.5± 13.1 (52.5-64.6)</td>
<td>0.08</td>
<td>57.9±9.2 (53.5-62.3)</td>
</tr>
<tr>
<td>RV Ejection Fraction , mean ± SD (95% CI) (%)</td>
<td>59.3± 4.9 (51.0-66.5)</td>
<td><strong>0.0012</strong></td>
<td>51.4 ±2.1 (47.4-55.4)</td>
<td><strong>0.0017</strong></td>
<td>49.0±6.5 (45.9-52.2)</td>
</tr>
<tr>
<td>Late Gadolinium Enhancement pattern</td>
<td>9 (35)</td>
<td>9 (33)</td>
<td>9 (53)</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>Ischaemic</td>
<td>5 (56)</td>
<td>5 (56)</td>
<td>6 (67)</td>
<td>5 (71)</td>
<td></td>
</tr>
<tr>
<td>Non-Ischaemic</td>
<td>4 (44)</td>
<td>4 (44)</td>
<td>3 (33)</td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>Native T1-Septum , mean ± SD (95% CI) (ms)</td>
<td>1227±51** (1208-1246)</td>
<td>0.99</td>
<td>1221±24* (1190-1251)</td>
<td>0.88</td>
<td>1230±23* (1202-1276)</td>
</tr>
<tr>
<td>Global T1-midventricular , mean ± SD (95% CI) (ms)</td>
<td>1208±33** (1191-1228)</td>
<td>0.81</td>
<td>1208±42* (1190-1225)</td>
<td>0.91</td>
<td>1228±28* (1200-1274)</td>
</tr>
<tr>
<td>Extracellular Volume , mean ± SD (95% CI) (%)</td>
<td>31±4 (29.6-32.1)</td>
<td>0.99</td>
<td>32±2 (27.0-34.3)</td>
<td>0.66</td>
<td>30±5 (27.7-33.1)</td>
</tr>
<tr>
<td>T2, mean ± SD (95% CI) (ms)</td>
<td>37.3±4.6 (35.9-38.6)</td>
<td>0.94</td>
<td>38.9±3.1 (37.1-40.1)</td>
<td>0.51</td>
<td>38.7±2.0 (37.8-39.7)</td>
</tr>
<tr>
<td>Manganese Influx constant, mean ± SD (95% CI) (KIL/ml/100g/min)</td>
<td>6.9±0.9* (6.5-7.3)</td>
<td>0.46</td>
<td>6.2±0.5* (5.9-6.5)</td>
<td>0.22</td>
<td>6.3±0.8* (5.6-6.8)</td>
</tr>
</tbody>
</table>

*n= 23
**n= 20
6.5. DISCUSSION

In this prospective multimodality two-centre observational study, we have shown that patients recovering from severe COVID-19 do not have evidence of left ventricular dysfunction or a major excess in persistent myocardial injury compared to co-morbidity-matched volunteers. These patients have a high prevalence of cardiovascular comorbidity which may account for much of the reported myocardial abnormalities on cardiac magnetic resonance. However, some patients recovering from COVID-19 did have evidence of mild persistent right ventricular dysfunction which likely reflects recovery from a severe life-threatening respiratory viral illness. It is possible that the cardiac consequences of COVID-19 may relate to the severity of the pulmonary effects of COVID-19 rather than direct cardiac effects of coronavirus infection.

Several studies have reported cardiac abnormalities on cardiac magnetic resonance imaging in patients who have recovered from COVID-19. These range from elevated native T1 and T2 values, to presence of cardiac dysfunction and late gadolinium enhancement (Knight et al. 2020, Puntnmann et al. 2020, Kotecha et al. 2021, Ojha et al. 2021, Raman et al. 2021, Starekova et al. 2021). Raman and colleagues describe myocardial injury in a third of patients with moderate to severe COVID-19, but they lacked a well-matched control population (Raman et al. 2021). Huang and colleagues described abnormalities in 58% patients who had recently recovered from COVID-19 with ongoing cardiac symptoms although only a third of cases had evidence of late gadolinium enhancement (Huang et al. 2020). Similarly, Puntnmann and colleagues suggested that 70% of patients with COVID-19 have ongoing
cardiac damage, with 32% of patients having late gadolinium enhancement (Puntmann et al. 2020), and the majority having elevations in native T1 values or extracellular volume fraction. However, native T1 values vary between individuals and are affected by co-morbidities, such as hypertension and diabetes mellitus (Rodrigues et al. 2016, Shang et al. 2017). It is therefore not surprising that whilst we observed increased native T1 values in patients with COVID-19, these differences disappeared once comparisons were made with co-morbidity-matched volunteers. Similar observations were made for extracellular volume fraction, T2 mapping and manganese assessments of myocyte function indicating that differences in these measures may relate to underlying co-morbidities rather than persistent damage or injury from COVID-19. Similar to previous studies (Puntmann et al. 2020, Raman et al. 2021), a third of our cohort demonstrate late gadolinium enhancement, which was comparable to our co-morbidity matched volunteer group which included those with coronary artery disease. This begs the question whether individuals with cardiovascular risk factors, who are at higher risk of developing severe COVID-19, demonstrate late gadolinium enhancement as a result of their pre-existing conditions rather than COVID-19 itself.

Kotecha and colleagues described similar native T1 and T2 values in COVID-19 survivors and co-morbidity-matched volunteers (Kotecha et al. 2021) However, they demonstrated a higher rate of late gadolinium enhancement (49%) in patients with COVID-19 compared to our study and their matched control group. However, all their patients had elevated cardiac troponin concentrations and had been referred for cardiac magnetic resonance imaging
for clinical indications. Both factors will have introduced a case-selection bias that will likely have increased their rate of late gadolinium enhancement. It has been suggested that non-ischaemic late gadolinium enhancement could represent COVID-19-related myocarditis, although without evidence on corresponding oedema imaging during the acute illness or other forms of validation, this cannot be established. An inflammatory cardiomyopathy has previously been linked with several viruses including influenza (Mavrogeni et al. 2011) but its association with COVID-19 remains unclear. Moreover, similar patterns of non-ischaemic late gadolinium enhancement are commonly observed in patients with hypertension and diabetes mellitus (Shang et al. 2017) in the absence of myocarditis. Regardless of the mechanism, the prognostic value of late gadolinium enhancement across multiple disease states probably warrants long-term follow up.

Similar to previous studies (Puntmann et al. 2020, Kotecha et al. 2021), we observed that patients had evidence of increased right ventricular dysfunction, particularly in those with severe COVID-19, myocardial injury and ongoing symptoms. Development of pulmonary fibrosis in severe acute respiratory syndrome coronavirus-1 (SARS-CoV) and adult respiratory distress syndrome have been reported in patients during recovery. Given SARS-CoV-2 affinity for lung and heart tissues, it is possible severe lung injury in the setting of SARS-CoV-2 infection may lead to pulmonary fibrosis and elevated pulmonary pressure. These are all risk factors for the development of right ventricular dysfunction (Venkataraman et al. 2017). Furthermore, the persistence of symptoms in our patient cohort also related to persistent lung abnormalities
observed on CT. However, due to the study size, this cannot be assumed, and further research should focus on patients with persistent symptoms.

We should consider the strengths and weaknesses of our study. We have undertaken a thorough assessment of cardiac structure and function with the use of multiple imaging modalities. Whilst echocardiography is more practical and most widely used clinically, we chose to use contrast-enhanced cardiac magnetic resonance imaging to identify and to characterise any myocardial abnormalities since it is the most sensitive non-invasive measure to achieve this. Importantly, images were analysed by readers blinded to participant details and COVID-19 status, a rigorous approach that was not always applied in previous studies. Manganese-enhanced magnetic resonance imaging is a novel and sensitive measure of myocyte function (Scally et al. 2019, Spath et al. 2020, Spath et al. 2021), which allowed us to assess subtle changes alongside more traditional assessments of cardiac function. The use of computed tomography coronary angiography also allowed assessment of both coronary artery disease and the presence of persistent lung damage. Despite this, we observed no left ventricular dysfunction when compared to appropriate comparator groups.

6.5.1. Limitations
This study is limited by its modest sample size and the heterogeneity of patients surviving COVID-19. However, we have focused upon the patient cohort of most interest, patients recovering following hospitalisation with severe COVID-19. Other limitations of our study include the use of evolving
therapeutic interventions and, perhaps more importantly, survival bias. As the pandemic evolved, new therapeutic interventions, such as dexamethasone, were introduced to reduce mortality and this could also have influenced our findings. Over half of the patients who were eligible for the study died, and it is certainly possible that more extensive abnormalities could have been observed had earlier imaging been possible in these patients. As a consequence, our findings are limited to the population of patients who recover from severe COVID-19. It is difficult to ascertain whether the abnormalities seen in patients with COVID-19 were present prior to hospitalisation, however, it is reassuring that there was no excess in left ventricular abnormalities when compared to matched volunteers. Lastly, in an ideal world, a comparator group should include patients with non-COVID-19 viral or bacterial pneumonitis patients with a similar incidence of intensive care admissions and this is an area for future studies to focus on.
6.6. CONCLUSION

We found no evidence that patients who had recovered from severe COVID-19 had substantially higher left ventricular dysfunction or abnormal myocardial calcium handling. However, there was evidence of persistent right ventricular dysfunction that presumably reflects the recent severe viral pneumonia and consequent pulmonary hypertension. In conclusion, concomitant comorbidities and risk factors play a major role in prior reports of left ventricular abnormalities associated COVID-19.
7. CHAPTER 7: CONCLUSIONS
7.1. **SUMMARY OF FINDINGS**

Contrast-enhanced magnetic resonance imaging is almost exclusively reliant on gadolinium-based contrast agents. Whilst gadolinium-enhanced magnetic resonance imaging is the gold-standard in the diagnosis, monitoring and prognostication in various cardiac pathologies, it acts as a passive marker of the extra-cellular space. The uniqueness of manganese lies in its biological function as a calcium ion analogue, thus behaving as an intracellular contrast agent and acting as a surrogate marker for myocardial calcium handling.

In this thesis, I have assessed the robustness of manganese-enhanced magnetic resonance imaging in healthy myocardium and patients with ischaemic and non-ischaemic cardiomyopathy. I demonstrated excellent repeatability of manganese-enhanced magnetic resonance imaging in healthy and pathological myocardium. Furthermore, scan-rescan reproducibility of manganese-enhanced magnetic resonance imaging in healthy volunteers.

I undertook the first clinical study of manganese-enhanced magnetic resonance imaging in patients with dilated and hypertrophic cardiomyopathy, demonstrating that cardiac manganese-enhanced magnetic resonance imaging quantifies myocardial calcium-handling dysfunction and tracks with established imaging markers of left ventricular dysfunction. I then proceeded to assess, for the first time, manganese-enhanced magnetic resonance imaging in patients during acute takotsubo syndrome and convalescence. This demonstrated a profound abnormality in myocardial calcium handling during...
the acute phase, which improves but does not fully recover during convalescence.

Finally, I assessed the contribution and cardiac impact of co-morbidities on myocardial abnormalities seen in patients with recent COVID-19 infection, using gadolinium and manganese-enhanced magnetic resonance imaging and computed tomography coronary angiography (CTCA). I determined that patients were not at higher risk of left ventricular dysfunction or abnormal myocardial calcium handling. Previous reports of left ventricular myocardial abnormalities following COVID-19 may reflect pre-existing co-morbidities.
Cardiac magnetic resonance imaging is an established imaging modality which is key in clinical cardiology. It has a major role in diagnosis, evaluation of myocardial function and tissue characterisation. Furthermore, it has been shown to be a robust imaging tool. The potential of manganese-enhanced magnetic resonance imaging as an intracellular contrast agent and surrogate marker of myocardial calcium handling has been established. Recent clinical studies have now assessed its use in various cardiac pathologies and have demonstrated its ability to not only detect myocardial calcium mishandling but quantify it. Repeatability and reproducibility of manganese-enhanced magnetic resonance imaging has not been established and is a necessary step for its future clinical application.

For the first time, I have demonstrated that myocardial T1 mapping and kinetic modelling of manganese uptake is repeatable and reproducible in both healthy and diseased myocardium. We found excellent intra-observer and inter-observer repeatability as well as scan-rescan reproducibility for measures of manganese uptake in the myocardium. This suggests that this technique is sufficiently robust for application in clinical care.
7.1.2. Manganese-Enhanced magnetic Resonance imaging in dilated cardiomyopathy and hypertrophic cardiomyopathy

Dilated and hypertrophic cardiomyopathy represent significant ongoing clinical challenges in terms of diagnosis, prognosis and optimisation of therapies. Early diagnosis is often key to primary prevention of adverse cardiovascular endpoints, as well as family screening and genetic testing. Current magnetic resonance imaging techniques using gadolinium assist in risk stratification and can provide valuable prognostic information. However, early markers of disease are lacking. With calcium-handling central to myocyte dysfunction in cardiomyopathy, characterisation and detection of this with manganese-enhanced magnetic resonance imaging has potential to significantly add to the diagnostic armoury of the cardiologist.

For the first time, I have conducted proof-of-concept clinical study into the role manganese-enhanced magnetic resonance imaging in patients with non-ischaemic cardiomyopathy. I demonstrated clear differences in manganese uptake in dilated and hypertrophic cardiomyopathy compared to healthy volunteers. Importantly, this also correlated with established markers of cardiac function in dilated cardiomyopathy and could distinguish between hypertrophic and fibrotic areas in hypertrophic cardiomyopathy. This has relevance to early diagnosis and risk stratification as well as future investigation into subtle and sub-clinical cardiomyopathy phenotypes.
7.1.3. Manganese-Enhanced imaging in takotsubo syndrome

Takotsubo syndrome is a cardiac emergency that is often triggered by a stressful event and is characterised by transient and profound left ventricular systolic dysfunction, typically due to marked ‘ballooning’ of the left ventricular apex. It can be challenging to diagnose due to its phenotypical similarities with an acute myocardial infarction and brief left ventricular abnormalities, making it difficult to document these characteristic changes. Traditional cardiac magnetic resonance imaging can aid in excluding differential diagnoses and highlight acute complications. However, there is a need to develop more sensitive and discriminatory imaging techniques.

In this proof-of-concept study, I observed dysfunctional myocardial calcium handling in patients with takotsubo syndrome which is most striking during the acute episode but persists despite resolution of oedema and recovery of myocardial function. Myocardial mishandling may be involved in the pathogenesis of takotsubo syndrome. This holds major promise in improving diagnosis and disease monitoring, with the potential for the assessment of novel treatment interventions.

7.1.4. Magnetic resonance imaging and computed tomography in survivors of COVID-19

Myocardial injury is increasingly common in patients with COPVID-19 infection. As expected, this is associated with disease severity and poorer outcomes. The mechanism behind myocardial injury is poorly understood, with some suggesting indirect mechanisms, whilst others have proposed direct
myocardial injury due to myocarditis, stress cardiomyopathy, endothelial injury and thrombo-inflammation. Several studies reported abnormalities on magnetic resonance imaging, ranging from abnormal T1/T2 values to extensive late gadolinium enhancement and ventricular dysfunction. It has been well established that those with underlying co-morbidities are more likely to have severe disease. As such, it is important to establish the role of pre-existing cardiovascular disease on the cardiac abnormalities of patients recovering from COVID-19 hospitalisation.

I assessed patients with recent COVID-19 hospitalisation with gadolinium and manganese-enhanced magnetic resonance imaging and computed tomography coronary angiography (CTCTA). I observed a significant proportion of the cohort with underlying coronary artery disease. Patients were not at higher risk of developing left ventricular dysfunction or abnormal myocardial calcium handling. Concomitant comorbidities and risk factors may play a role in prior reports of COVID-19 associated left ventricular abnormalities. Moreover, there was evidence of persistent right ventricular dysfunction that presumably reflects recent severe viral pneumonia and consequent pulmonary hypertension.
7.2. **CLINICAL PERSPECTIVES**

In the modern era, cardiac magnetic resonance imaging has become an essential tool for the clinical cardiologist. In recent decades a significant body of work has developed extracellular contrast imaging of the myocardium with great clinical progress in recognition of aetiology of myocardial disease, viability assessment in ischaemic heart disease and prognostication. However, there remain ongoing and significant shortcomings in our abilities as doctors to target the right therapies to the right patients. With many innovative techniques emerging into clinical practice, such as high-sensitivity troponin, myocardial strain and other novel imaging markers of myocardial decompensation, biomarkers of early or subclinical disease are increasingly called for. Intracellular tissue characterisation with manganese-enhanced magnetic resonance imaging offers important potential clinical applications in diagnosis, risk stratification and management of a range of patients with cardiac disease.

7.2.1. **Screening**

The ability to detect altered calcium handling over time and quantify cellular myocardial function directly may transform our ability to assess myocardial function, enabling early detection and prognostication. This may be an invaluable non-invasive method of monitoring disease progression in various non-ischemic cardiomyopathies. With optimisation, this technique has potential to allow individualisation of heart failure treatment, assessment of treatment efficacy and targeting optimal therapy to those most likely to benefit. A randomised controlled trial has confirmed that 40% of patients that have
recovered from dilated cardiomyopathy (resolution of left ventricular ejection fraction) will ‘relapse’ following discontinuation of heart failure medication (Halliday et al. 2019). The ability to detect subclinical myocardial calcium mishandling in this population could potentially identify which individuals should continue with their heart failure treatment. Such applications are conceptual and speculative but manganese-enhanced magnetic resonance imaging does now provide the opportunity to explore such approaches in future studies and potentially provide a more targeted or personalised approach to the treatment and management of our patients with cardiomyopathy.

7.2.2. Diagnostics

This technique has particular implications for diagnosis, especially for those with an uncertain or subclinical cardiomyopathy. This could include a range of cardiomyopathies including genetic causes of cardiomyopathy with variable penetrance or athlete’s heart. Athlete’s heart is a term referring to a constellation of electrical, functional and structural remodelling that accompanies regular athletic training. This is an important physiological adaption which helps the physical performance of athletes. What was initially thought to be a benign adaptation to endurance training, we now know that 20% of elite athletes demonstrate residual cardiac chamber enlargement despite detraining (Pelliccia et al. 2002). This raises the question of whether athlete’s heart is truly benign and how best to identify those at risk. Thus, the ability to diagnose an underlying cardiomyopathy prior to gross left ventricular dysfunction, will allow for early detection and prompt treatment initiation, which
may have an impact on outcomes. However, this has yet to be established for manganese-enhanced magnetic resonance imaging.

7.2.3. Regenerative Therapy

There remains considerable interest in the development of therapies to improve the recovery of the cardiac function following myocardial infarction and coronary revascularisation. Manganese-enhanced magnetic resonance imaging can identify viable myocardium within the peri-infarct region (Dash et al. 2011) and therefore presents a biomarker measure of novel treatment interventions to reduce infarct size. In the field of myocardial stem cell therapy, manganese uptake can be used as a measure of successful delivery and function of implanted stem cells as demonstrated following human amniotic mesenchymal stem cell delivery in a porcine model of acute myocardial infarction (Dash et al. 2015).

7.2.4. Prognostication and surveillance

In the past, we have been unable to quantify cellular myocardial function. With manganese-enhanced magnetic resonance imaging, we are now able to assess intracellular myocardial calcium handling. The presence of late gadolinium enhancement has been proven to carry a poor prognosis in different cardiac conditions such as ischemic cardiomyopathy, dilated cardiomyopathy and hypertrophic cardiomyopathy (Alba et al. 2020, Greulich et al. 2021). However, to date, there has been no assessment of the prognosis of patients with dysfunctional myocardial calcium handling and it will be important to establish whether abnormal myocardial manganese uptake is
associated with adverse outcomes and can provide useful prognostic information.
7.3. **FUTURE DIRECTIONS**

In-depth study of the application of manganese-enhanced magnetic resonance imaging patients at risk of developing heart failure and reversible ischaemia is required, as well as application to other organs. In addition, in-depth study into the sensitivity of manganese-enhanced magnetic resonance imaging to detect myocardial recovery in patients treated for heart failure is required. To that end, we have proposed, designed and secured funding for three further clinical studies; MEMORY (Manganese-Enhanced Magnetic resonance imaging in reversible Cardiomyopathy), DAPA-MEMRI (Effect of DAPAgliflozin on myocardial calcium-handling in patients with heart failure by Manganese-Enhanced Magnetic Resonance Imaging) and Pancreas MEMRI (Pancreas Manganese-Enhanced Magnetic Resonance Imaging).

7.3.1. **Optimisation of imaging**

In the clinical translational work presented in this thesis, imaging has been performed in such a way as to enable description of manganese uptake kinetics. However, this approach leads to prolonged imaging protocols and optimisation is required for greater clinical applicability. The next step in the development of manganese-enhanced magnetic resonance imaging of the heart would be to use this technique in larger clinical programmes and multi-centre trials. This will allow for further assessment of its use across a wider population, different T1 mapping sequences and magnetic resonance scanners, as well as providing best evidence of effectiveness. This is essential in progressing manganese-enhanced magnetic resonance imaging and is
needed to establish the role, value and clinical impact of this very promising approach.

### 7.3.2. MEMORY study- Myocarditis

We have shown that manganese-enhanced magnetic resonance imaging can detect myocardial calcium mishandling in reversible heart failure. Furthermore, demonstrating ongoing subclinical dysfunction despite resolution of left ventricular ejection fraction and regional wall motion abnormalities. It would be of interest to see whether manganese-enhanced magnetic resonance imaging can detect myocardial calcium handling in myocarditis, a reversible, focal pathology. More importantly, whether this as well as conventional measures of cardiac function recover during convalescence. One in twenty patients with acute myocarditis will go on to develop heart failure (Tschöpe et al. 2021) and earlier detection of abnormal myocardial calcium handling may help guide earlier treatment to prevent gross left ventricular systolic dysfunction and thereby improve outcomes.

We will therefore explore the role of this manganese-enhanced magnetic resonance imaging in patients with acute myocarditis and during convalescence (NCT04623788). These responses will be compared with age and sex matched control subjects. We will recruit 20 patients with acute myocarditis and 20 age and sex matched control subjects. All patients will undergo 2 magnetic resonance scans (gadolinium and manganese-enhanced) at least 48 hours apart at baseline. Patients will undergo repeat manganese-enhanced magnetic resonance imaging at 3-6 months. Control subjects will
undergo a single manganese-enhanced magnetic resonance imaging scan at baseline.

We hypothesise that there will be marked alterations in myocardial manganese uptake during the acute myocarditis. We also anticipate that manganese uptake will recover following clinical recovery. To this end the following study will be carried out (Figure 7.1):

*Figure 7.1 MEMORY- Myocarditis flow chart*
7.3.3. DAPA-MEMRI

Diabetes mellitus is among the top 10 causes of death worldwide and its incidence is increasing rapidly (WHO 2014). Patients with diabetes are at risk of developing heart failure, even in the absence of ischaemic heart disease (Nichols et al. 2004), a condition called diabetic cardiomyopathy. Diabetic cardiomyopathy is characterised by both structural and function changes in the myocardium manifesting as myocardial fibrosis and diastolic dysfunction respectively, even if gross systolic function is not always impaired (Levelt et al. 2018). The use of manganese-enhanced magnetic resonance is being assessed in the detection of pre-clinical cardiac dysfunction in patients with diabetes mellitus with normal left and right ventricular function. Early detection of altered calcium-handling in at-risk cardiomyopathy may enable initiation of preventative or disease-modifying therapy earlier than previously possible, which has potential to improve long-term clinical outcomes. Myocardial calcium handling is central to the effective myocardial contraction. The failing myocardium is characterised by loss of contractility due to reduced calcium supply to the myofilaments (Harding 2005), reduced sarcoendoplasmic reticulum calcium release and increased ryanodine receptor opening probability (Gwathmey et al. 1987, Hasenfuss 1998). Importantly, this complex calcium-cycling becomes disordered and is an early feature of disease observed in both ischemic and non-ischemic cardiomyopathies, with impairment of the myocyte's ability to increase and to decrease intracellular calcium concentration impacting on systole and diastole accordingly (Harding 2005).
The mechanism in which SGLT-2 inhibitors exert beneficial outcomes is not fully understood and may involve improvements in cardiac energetics (Verma et al. 2018). There is evidence from preclinical models that sodium-glucose co-transporter-2 (SGLT2) inhibitor therapy can reverse some of this dysfunction through interactions between the sodium-glucose transporter and SERCA2a (Hammoudi et al. 2017). Recently, SGLT2 inhibitor therapy in patients with diabetes mellitus was observed to improve clinical and cardiovascular outcomes, especially reductions in rehospitalisation for heart failure (Packer et al. 2020, Nassif et al. 2021, Spertus et al. 2022). These benefits are independent of diabetes mellitus and this has been shown in a recent landmark trial, the DAPA-HF trial of patients with heart failure with or without type 2 diabetes mellitus (McMurray et al. 2021).

We have designed an observational cross-sectional study and a double-blind placebo controlled randomised controlled trial to assess the effect of dapagliflozin therapy in the treatment of heart failure patients with and without diabetes mellitus with the use of manganese-enhanced magnetic resonance imaging (NCT04591639). To this end we will conduct the following study (Figure 7.2).

We aim to recruit patients with symptomatic heart failure with reduced ejection fraction for at least 2 months (50% with type 2 diabetes mellitus on stable therapy) with elevated N-terminal pro-B-type natriuretic peptide (NT-ProBNP; >125 pg/mL) will be recruited. Age and sex matched populations of patients with diabetes but no heart failure and healthy volunteers will also be recruited.
Key exclusion criteria are 1) unstable or grade IV heart failure, 2) 2\textsuperscript{nd} or 3\textsuperscript{rd} degree atrioventricular block, 3) type I diabetes mellitus, 4) restrictive cardiomyopathy, active myocarditis, constrictive pericarditis, hypertrophic cardiomyopathy or primary valvular heart disease and 5) ischaemic heart disease in diabetic patients without heart failure.

With optimisation, this technique has potential to track treatment response non-invasively. The benefits could include individualisation of heart failure treatment and targeting optimal therapy to those most likely to benefit. Furthermore, this can potentially be used to assess a wider range of cardiomyopathy phenotypes. First, this offers potential to expand current understanding of cellular abnormalities in diabetic cardiomyopathy. Second, it may give further insights into subclinical myocyte dysfunction in this important patient group. Finally, and perhaps most importantly, this could allow for individualisation of heart failure treatment and targeting optimal therapy to those most likely to benefit. Furthermore, this can potentially be used to assess a wider range of cardiomyopathy phenotypes.
Figure 7.2 DAPA-MEMRI flow chart

Healthy volunteers (n=20)

- Manganese-enhanced magnetic resonance imaging

Patients with heart failure without diabetes mellitus (n=60)
Patients with diabetes mellitus and heart failure (n=60)

- **Baseline visit:** Screening, bloods, ECG, ECHO, gadolinium and manganese-enhanced MRI (48 hours apart)
- Randomisation 1:1 to dapagliftozin 10mg daily or matched placebo
- **1 Month:** ECG, ECHO, bloods, manganese-enhanced MRI
- **6 month:** Bloods, ECG, ECHO, gadolinium and manganese-enhanced MRI (48 hours apart)

*ECG, electrocardiogram, ECHO, echocardiogram, MRI, magnetic resonance imaging*
7.3.4. PANCREAS MEMRI

Beyond the myocardium, manganese-based contrast media have the potential to provide functional assessment in other organs, such as the kidneys and the pancreas. Both pancreatic and renal tissue have substantial manganese uptake, and this could provide a non-invasive measure of cellular calcium handling prior to the onset of pancreatic insufficiency or renal failure.

Approximately 400,000 people are living with Type 1 Diabetes Mellitus (T1DM) in the United Kingdom: one of the highest rates in the world. It is characterised by autoimmune loss of pancreatic beta cell mass leading to metabolic dysregulation, requiring lifelong insulin therapy (Rodriguez-Calvo et al. 2018). The destruction of beta cells in T1DM precedes the clinical manifestation of the disease and impaired insulin secretion can be detected several years prior to the onset of hyperglycaemia (Kloppel et al. 1985). The degree of beta cell dysfunction at this time often exceeds the percentage beta cell loss, suggesting additional functional impairment in insulin secretion in these patients. Both beta cell mass and function further decline over time, but not always reflected in C-peptide response (Madsbad et al. 1981). The beta cell deficit in T1DM provides a rationale for novel therapeutic strategies aimed at restoring or at least preventing further loss of beta cell mass. Furthermore, enhancement of endogenous insulin secretion may provide several physiological advantages over the administration of exogenous insulin. The opportunity to preserve or to enhance the function of beta cells in T1DM early may maximise endogenous beta cell activity and minimise its destruction.
In a pre-clinical setting, manganese-based contrast agents have been used to study murine diabetes models, showing decreased manganese-based signal enhancement in the pancreas of diabetic mice that correlated with loss of beta cell mass (Antkowiak et al. 2009, Antkowiak et al. 2013). Proof of concept studies have demonstrated that manganese-enhanced magnetic resonance imaging can distinguish between patients with type 2 diabetes mellitus and normoglycemic control subjects (Botsikas et al. 2012). We have shown that manganese-enhanced magnetic resonance imaging can quantify manganese uptake in healthy and diseased myocardium. Furthermore, we have simultaneously shown that there is marked manganese uptake in the normal pancreas which greatly exceeds that of the myocardium (Figure 1.1). Therefore, its sensitivity to detect potential abnormalities in pancreatic islet cell function will be extremely high. We will therefore use of manganese-enhanced magnetic resonance imaging as a measure of islet beta cell function and active insulin secretion (Antkowiak et al. 2009). This study will be a translational project in patients with type 1 diabetes and could be instrumental in shaping the future of type 1 diabetes assessment and management.

We will recruit 30 patients with type 1 diabetes mellitus and compare them to age and sex-matched healthy volunteers (n=30). Patients with type 1 diabetes and healthy volunteers will undergo blood tests including haematology, clinical chemistry, HbA1c, C-peptide, glucose. Patients will undergo manganese-enhanced magnetic resonance imaging of the pancreas. To this end we will conduct the following study (Figure 7.3).
Figure 7.3 Pancreas MEMRI flow chart

Patients with T1DM and 1) detectable C-peptide (n=22), 2) no detectable C-peptide (n=8)
Healthy Volunteers (n=30)

Clinical haematology, clinical biochemistry, C-peptide, HbA1c, glucose

Baseline manganese enhanced MRI (MEMRI) of pancreas

MRI, magnetic resonance imaging, HbA1c, glycated haemoglobin, T1 DM, type 1 diabetes mellitus.
7.3.5. Concluding remarks

Since withdrawal from the European market in 2012 by the marketing-authorisation holder, no manganese contrast medium has been clinically available. The reasons for this are multifactorial but the decision was principally driven by the lack of large-scale commercial interest, despite promising clinical pilot data. It is important to highlight that no safety concern caused its withdrawal, simply the lack of clinical demand in hepatobiliary imaging. Furthermore, early clinical studies have established that manganese is safe in various cardiac conditions (Spath et al. 2020, Singh et al. 2021, Spath et al. 2021). There are currently no available preparations of manganese-based contrast media for widespread clinical use. However, the formulation of manganese dipyridoxyl diphosphate for clinical use is clearly feasible, as evidenced by its current use in clinical studies (Table 7.1). The provision and availability of manganese-based contrast media is likely to change with the re-emergence of commercially available preparations anticipated in the near future.

The next step in the development of manganese-enhanced magnetic resonance imaging of the heart would be to use this technique in larger clinical programmes and multi-centre trials. This will allow for further assessment of its use across a wider population, different T1 mapping sequences and magnetic resonance scanners, as well as providing best evidence of effectiveness. This is essential in progressing manganese-enhanced magnetic resonance imaging and is needed to establish the role, value and clinical impact of this very promising approach.
With a large body of preclinical data and emerging clinical work in the field, the stage is now set for wider clinical translation of this exciting non-invasive imaging technique. Manganese-enhanced magnetic resonance imaging offers the potential to improve diagnosis in a range of conditions and to provide a non-invasive measure of myocardial calcium handling. This represents an invaluable tool for the assessment of functional recovery, accurate prediction of disease progression and monitoring of treatment response.
Table 7.1: Manganese based contrast agents

<table>
<thead>
<tr>
<th>Contrast agent</th>
<th>Clinical dose (µmol/kg)</th>
<th>Approved for clinical use</th>
<th>Previous or currently- recruiting studies</th>
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<tbody>
<tr>
<td><strong>Chelated</strong></td>
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<td></td>
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<tr>
<td>Partial: MnDPDP</td>
<td>5</td>
<td>Yes</td>
<td>Manganese-enhanced MRI (MEMRI) of the myocardium (NCT03607669)</td>
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<td></td>
<td>The DAPA-MEMRI Trial (NCT04591639)</td>
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<td></td>
<td></td>
<td></td>
<td>PANCREAS MEMRI (NCT05298735)</td>
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<tr>
<td>Full: CMCS (Mn-DTPA)</td>
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<td>None</td>
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<tr>
<td><strong>Non-chelated</strong></td>
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<tr>
<td>MnCl2†</td>
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<tr>
<td>EVP1001-1</td>
<td>1-10</td>
<td>No</td>
<td>Clinical Trial of MEMRI to assess peri-infarct Injury (NCT02933034)</td>
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<td>Efficacy of EVP1001-1 in the Assessment of myocardial viability in patients with cardiovascular disease (NCT01989195)</td>
</tr>
</tbody>
</table>

*MnDPDP, Manganese dipyridoxyl diphosphate, CMCS(Mn-DTPA), O-carboxymethyl chitosan manganese-diethylenetriamine pentaacetate, MnCl2†, Manganese chloride*
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