Cardiac Magnetic Resonance Imaging in ST-Elevation Myocardial Infarction Patients Undergoing Primary Angioplasty

By

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Submitted to

University College London

For the degree of

MD(Res)
Declaration

I, Manish Ramlall, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature…………………………………………………………
Abstract

Numerous pre-clinical studies investigating remote preconditioning as a cardioprotective strategy using surrogate biochemical and imaging markers have yielded positive results. A large randomised trial is required to show whether or not RIC has a beneficial impact on clinical outcomes in ST-elevation myocardial infarction (STEMI) patients treated with primary angioplasty. In addition, cardiac magnetic resonance imaging derived surrogate markers are being increasingly used as prognostic tools in phase II studies. Two commonly utilised surrogate markers are infarct size and intramyocardial haemorrhage. Various imaging sequences used to assess these parameters have been utilised for clinical and research purposes. Novel motion corrected sequences, not requiring breath-holding, have been introduced to aid patient compliance and reduce motion artefacts. The performance of these sequences has not been fully investigated. The aims of this thesis are as detailed below. The first aim is to investigate whether or not remote preconditioning improves clinical outcomes in reperfused STEMI patients. I describe the set-up and findings of a large multicentre randomised trial which shows that remote preconditioning does not provide added clinical benefits after 1-year follow-up. The second aim is to compare two T2* mapping sequences used to assess intramyocardial haemorrhage. I demonstrate that the novel sequence produces fewer artefacts but has a lesser diagnostic ability than the conventional sequence. The third aim is to compare two novel motion corrected post-contrast sequences with two commonly used ones for quantifying infarct size, including the reference standard fast low-angle shot sequence. I demonstrate that there is good correlation in infarct size measured using the four sequences. In conclusion, this thesis shows that remote preconditioning does not provide additional benefit to primary angioplasty in STEMI patients and surrogate markers of adverse outcomes such as infarct size and intramyocardial haemorrhage can be measured with novel motion corrected sequences.
Impact Statement

Patients suffering from an acute ST-elevation myocardial infarction have high mortality rates, around 5% in the UK in 2017 which rises to around 40% in patients with cardiogenic shock. Primary angioplasty is an effective treatment, but it is labour intensive and costly. The National Health Service and various other western healthcare providers have been under intense pressure to improve clinical outcomes without any significant increase in resources. Research needs to focus on cost-effective means of treating disease conditions, rather than emphasising only on outcome improvement. Remote preconditioning is a cheap and non-invasive strategy which has shown promise in pre-clinical studies. Large trials in cardiac surgical patients have not shown any benefit. The CONDI-2/ERIC-PPCI trial described in this thesis was also neutral. This however should not discourage researchers worldwide to continue investigating this strategy in various other clinical scenarios. Another aspect investigated in this thesis is the performance of novel diagnostic cardiac magnetic resonance imaging sequences. Conventional image acquisition techniques have not been well tolerated in some of the sickest patients, mainly due to inability to breath-hold. There is a need to make imaging acquisition less uncomfortable. My findings will give clinicians more confidence that the new free-breathing techniques can be used effectively.
Author Contribution

I assisted in the development of the research protocol of the ERIC-PPCI arm of the study. I submitted the Integrated Research Application System (IRAS) ethics application, along with the subsequent amendments. I was in charge of harmonising governance and data collection between the ERIC-PPCI and the CONDI-2 arms of the study.

I attended regular trial management meetings, held every two weeks in set-up phase and every four weeks during recruitment, which oversaw trial set-up and its day-to-day running. I also attended the Trial Steering Committee and Data and Safety Monitoring Committee meetings (both held every 6 months). I oversaw all site initiation visits (33 sites in the UK) which included set-up and training involving the CellAegis RIC therapy and sham devices. My role was to provide advice and trouble-shooting tips to research nurses at the UK sites in terms of set-up, management and running of the trial. I produced a training video uploaded to Vimeo [https://vimeo.com/147567143]. I presented at Principal Investigators and research nurse meetings. Chapter 3 of this thesis represents aspects of my work on the CONDI-2/ERIC-PPCI trial.

I lead recruitment at the Barts Heart Centre. I set-up the CMR sub-study, conducted across 5 sites in the UK and 2 sites in Denmark. I wrote the protocol and liaised with various partners to help recruitment. I scanned patients at the Barts Heart Centre and at the Royal Free Hospital. Chapters 4 and 5 of this thesis, which includes elements of the CMR sub-study, were designed and conducted by me in their entirety.
I would like to thank Professor Derek Hausenloy and Professor Derek Yellon whose counsel has been vital to enable completion of this thesis.

I would like to thank Professor HE Botker, Aarhus, Denmark and his team for managing recruitment of the CONDI-2 arm of the study.

I would like to thank the Clinical Trials Unit, London School of Hygiene and Tropical Medicine, London, UK for helping manage patient recruitment and conducting blinded analysis of the data.

I would like to thank the Professor J Moon and the cardiac MRI department at St Bartholomew’s Hospital, London, UK for all the help and support in patient scanning. I would also like to thank the Dr M Fontana and her department at the Royal Free Hospital, London, UK for helping in patient recruitment for the CMR studies.

I would like to thank my fellow research associates Dr H Bulluck and Dr R Francis for all their advice and support while writing this thesis.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>1</td>
</tr>
<tr>
<td>Declaration</td>
<td>2</td>
</tr>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Impact Statement</td>
<td>4</td>
</tr>
<tr>
<td>Author Contribution</td>
<td>5</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>6</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>7</td>
</tr>
<tr>
<td>List of Figures</td>
<td>13</td>
</tr>
<tr>
<td>List of Tables</td>
<td>15</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>16</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>19</td>
</tr>
<tr>
<td>1.1 Background</td>
<td>20</td>
</tr>
<tr>
<td>1.2 ST-segment Elevation Myocardial Infarction (STEMI)</td>
<td>21</td>
</tr>
<tr>
<td>1.2.1 Definition of STEMI</td>
<td>21</td>
</tr>
<tr>
<td>1.2.2 Management of STEMI</td>
<td>21</td>
</tr>
<tr>
<td>1.3 Cardioprotective Strategies</td>
<td>22</td>
</tr>
<tr>
<td>1.3.1 RIC Proof-of-concept Studies</td>
<td>22</td>
</tr>
<tr>
<td>1.3.2 Section Summary</td>
<td>29</td>
</tr>
<tr>
<td>1.4 Use of CMR in STEMI patients</td>
<td>32</td>
</tr>
<tr>
<td>1.4.1 CMR in Acute and Chronic Myocardial Infarction</td>
<td>32</td>
</tr>
<tr>
<td>1.4.1.1 Acute Myocardial Infarction Imaging</td>
<td>32</td>
</tr>
<tr>
<td>1.4.1.2 LGE in Chronic Myocardial Infarction</td>
<td>34</td>
</tr>
<tr>
<td>1.4.1.3 Post-contrast Acquisition Sequences</td>
<td>39</td>
</tr>
</tbody>
</table>
Chapter 2: Research Objectives

2.1 Effect of Remote Ischaemic Conditioning on Clinical Outcomes in ST-segment Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention Study (CONDI-2/ERIC-PPCI)

2.1.1 Rationale

2.1.2 Hypothesis

2.1.3 Aim

2.2 Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence

2.2.1 Rationale

2.2.2 Hypothesis

2.2.3 Aim

2.3 Comparing Infarct Size Quantification Using Four Different Post-contrast CMR Sequences

2.3.1 Rationale

2.3.2 Hypothesis

2.3.3 Aim

Chapter 3: The CONDI-2/ERIC-PPCI Trial

3.1 Background

3.2 Methods

3.2.1 Declaration of Helsinki, Good Clinical Practice and Ethical Approval

3.2.2 Study Design and Research Collaboration
3.2.3 Inclusion Criteria
3.2.4 Exclusion Criteria
3.2.5 Recruitment Sites
3.2.6 Randomisation and Blinding
3.2.7 Trial Treatment
  3.2.7.1 Automated CellAegis autoRIC™ Devices
  3.2.7.2 Remote Ischaemic Conditioning (RIC)
  3.2.7.3 Sham RIC
  3.2.7.4 Duration of Treatment
3.2.8 Primary Endpoint
  3.2.8.1 Definition of Cardiac Death
  3.2.8.2 Definition for Hospitalisation for Heart Failure
3.2.9 Secondary Endpoints
  3.2.9.1 Definition of Stroke
  3.2.9.2 Definition of reinfarction
3.2.10 Planned Sub-group Analysis
3.2.11 Endpoint Validation
3.2.12 Power Calculations
3.2.13 Statistical Analysis
3.2.14 Consent and Ethical Considerations
  3.2.14.1 The ERIC-PPCI Arm
  3.2.14.2 The CONDI-2 arm
  3.2.14.3 Withdrawal
3.2.15 Data Collection
  3.2.15.1 Basic Information
  3.2.15.2 Medical and Other History
  3.2.15.3 Admission and Procedural Data
3.2.15.4 Medication at Admission and Discharge 66

3.2.16 Follow-up 66

3.2.17 Compliance and Loss to Follow-up 67

3.2.18 Safety Reporting 67

3.2.18.1 Definition 67

3.2.18.2 Data and Safety Monitoring Committee (DSMC) 67

3.2.18.3 Expected Adverse Events (recognised potential adverse effects of providing RIC stimulus) 67

3.2.18.4 Expected Serious Adverse Events Related to Usual Clinical Care 68

3.2.18.5 Unexpected Serious Adverse Events 68

3.2.18.6 Unexpected Non-Serious Adverse Events 69

3.2.18.7 Reporting Unexpected Adverse Events 69

3.2.18.8 Assessment of Intensity 69

3.2.18.9 Assessment of Causality 70

3.3 Results 70

3.3.1 CONDI-2 and ERIC-PPCI Data 70

3.3.2 Study Population 70

3.3.3 Primary Outcomes 71

3.3.4 Secondary Outcomes 78

3.3.5 Adverse Events 78

3.4 Discussion 78

3.5 Limitations 82

3.6 Future Directions 83

Chapter 4: Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence 85

4.1 Introduction 86
4.2 Methods

4.2.1 Study Population

4.2.2 CMR Acquisition

4.2.2.1 BH-T2* Mapping
4.2.2.2 FB-T2* Mapping
4.2.2.3 T2 Mapping
4.2.2.4 Native T1 Mapping
4.2.2.5 Gadolinium Enhanced Imaging

4.2.3 Quality Analysis

4.2.4 Intramyocardial Haemorrhage

4.2.5 Statistical Analysis

4.3 Results

4.3.1 T2* Mapping Quality Score
4.3.2 Inter and Intra Observer Variability
4.3.3 Acquisition Time
4.3.4 Pick-up Rate of IMH using FB-T2* and BH-T2* Mapping
4.3.5 Post-contrast Imaging and MVO
4.3.6 T1 and T2 Mapping

4.4 Discussion

4.5 Limitations and Future Directions

Chapter 5: Comparing Infarct Size Quantification Using Four Different Post-contrast CMR Sequences

5.1 Introduction

5.2 Methods

5.2.1 Study Population
5.2.2 CMR Acquisition
5.2.2.1 Balanced Single Shot Steady State Free Precession (bSSFP) Imaging
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.2.2 Fast Low-angle Shot (FLASH) Imaging</td>
<td>112</td>
</tr>
<tr>
<td>5.2.2.3 Bright-blood Motion Corrected (BB-MOCO) Imaging</td>
<td>112</td>
</tr>
<tr>
<td>5.2.2.4 Dark-blood Motion Corrected (DB-MOCO) Imaging</td>
<td>113</td>
</tr>
<tr>
<td>5.2.3 Quantitative Image Analysis</td>
<td>113</td>
</tr>
<tr>
<td>5.2.4 Statistical Analysis</td>
<td>114</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>114</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>120</td>
</tr>
<tr>
<td>5.5 Limitations</td>
<td>122</td>
</tr>
<tr>
<td>5.6 Conclusion and Future Directions</td>
<td>122</td>
</tr>
<tr>
<td>Chapter 6: Discussion and Conclusion</td>
<td>123</td>
</tr>
<tr>
<td>6.1 Summary of Results</td>
<td>124</td>
</tr>
<tr>
<td>6.1.1 The CONDI-2/ERIC-PPCI Trial</td>
<td>124</td>
</tr>
<tr>
<td>6.1.2 Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence</td>
<td>124</td>
</tr>
<tr>
<td>6.1.3 Comparing Infarct Size Quantification Using Four Different Post-contrast CMR Sequences</td>
<td>124</td>
</tr>
<tr>
<td>6.2 Discussion and Conclusion</td>
<td>125</td>
</tr>
<tr>
<td>References</td>
<td>127</td>
</tr>
</tbody>
</table>
List of Figures

**Figure 1.1** Figure 1.1: Mechanism of RIC

**Figure 1.2** Late-gadolinium imaging showing hyper-enhancement (arrows) due to delayed contrast wash-out following LAD territory acute myocardial infarction

**Figure 1.3** Oedema-based AAR by T1 and T2 mapping and infarction size by late-gadolinium imaging

**Figure 1.4** Temporal course of gadolinium contrast distribution

**Figure 1.5** Late post-gadolinium imaging. Short-axis acquisitions showing normal tissue (Panel A), scarring from chronic inferior infarct (Panel B) and scarring with presence of microvascular injury (Panel C)

**Figure 1.6** Schematic representation of development of intramyocardial haemorrhage

**Figure 1.7** LGE and T2* mapping in post-infarct patient with MVO and IMH

**Figure 1.8** Breath-held and free-breathing T2* mapping sequences in healthy volunteers

**Figure 3.1** Automated CellAegis autoRIC™ device

**Figure 3.2** Consort diagram of the ERIC-PPCI/CONDI-2 study

**Figure 3.3** Cumulative Incidence of Cardiac Death or HHF in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis)

**Figure 3.4** Cumulative Incidence of Cardiac Death in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis)

**Figure 3.5** Cumulative Incidence of HHF in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis)

**Figure 3.6** Forest plot for prespecified subgroup analyses of the primary endpoint in the intention-to-treat population

**Figure 4.1** Quality score for BH-T2* and FB-T2* mapped segments (n=684)

**Figure 4.2** Mean quality scores for each segment for BH-T2* (Panel A) and FB-T2* (Panel B) sequences

**Figure 4.3** Mid-level BH-T2* (A) and FB-T2* (B) images for patient with IMH in segment 10
Figure 4.4  Scatter plots of mean quality scores (QS) for each left ventricular segment for BH-T2* (A) and FB-T2* (B) sequences by two independent observers

Figure 4.5  Patient with IMH on BH sequence only

Figure 4.6  Bland-Altman plot of mean BH and FB T2* measurements against difference (BH minus FB)

Figure 4.7  Scatter plot of BH-T2* versus FB-T2* measurements of areas with intramyocardial haemorrhage (BH-T2* measurement of <20ms)

Figure 4.8  Segmental analysis of diagnostic performance of BH (A1-D1) and FB (A2-D2) T2* sequences

Figure 5.1  Mean percentage infract size as measured using the four different sequences with manual contouring

Figure 5.2  Late-gadolinium enhancement and method of manual contouring used for the bSSFP (A1-2), FLASH (B1-2), BB-MOCO (C1-2) and DB-MOCO (D1-2) sequences

Figure 5.3  Infarct size for the 12 patients as measured using the four different sequences

Figure 5.4  Relationship between infarct size measured using various modalities using the FLASH sequence as standard
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Table 1.1: Characteristics of RIC studies</td>
<td>30</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Table 1.2: Summary of studies showing effect of infarct size on clinical end-points</td>
<td>37</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Guidance on assessment of severity of adverse event</td>
<td>69</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Guidance on assessment of causality of adverse event</td>
<td>70</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Baseline characteristics of the patients in the ERIC-PPCI/CONDI-2 trial (ITT Analysis)</td>
<td>73</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Procedural details in the ERIC-PPCI/CONDI-2 trial (ITT Analysis)</td>
<td>74</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Primary and secondary outcomes in intention-to-treat population</td>
<td>75</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Baseline patient characteristics</td>
<td>92</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Procedural details</td>
<td>94</td>
</tr>
</tbody>
</table>
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-SD</td>
<td>5-Standard Deviation</td>
</tr>
<tr>
<td>AAR</td>
<td>Area-At-Risk</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>AUC</td>
<td>Area-Under-Curve</td>
</tr>
<tr>
<td>BB</td>
<td>Bright Blood</td>
</tr>
<tr>
<td>BB-MOCO</td>
<td>Bright Blood Motion Corrected</td>
</tr>
<tr>
<td>BH</td>
<td>Breath-Held</td>
</tr>
<tr>
<td>BHF</td>
<td>British Heart Foundation</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>bSSFP</td>
<td>Balanced Steady-State Free Precession</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary Artery Bypass Grafting</td>
</tr>
<tr>
<td>CCS</td>
<td>Canadian Cardiovascular Society</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive Heart Failure</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CK</td>
<td>Creatinine Kinase</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Creatinine Kinase-MB</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiac Magnetic Resonance</td>
</tr>
<tr>
<td>CONDI</td>
<td>Remote Ischemic Preconditioning in Primary PCI</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>DB</td>
<td>Dark Blood</td>
</tr>
<tr>
<td>DB-MOCO</td>
<td>Dark Blood Motion Corrected</td>
</tr>
<tr>
<td>DCCV</td>
<td>Direct-current cardioversion</td>
</tr>
<tr>
<td>DSMC</td>
<td>Data and Safety Monitoring Committee</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECV</td>
<td>Extra Cellular Volume</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection Fraction</td>
</tr>
<tr>
<td>EGE</td>
<td>Early Gadolinium Enhancement</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td>ERIC-PPCI</td>
<td>Effect of Remote Ischaemic Conditioning on Clinical Outcomes in STEMI Patients Undergoing PPCI</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>EVC</td>
<td>Event Validation Committee</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>FB</td>
<td>Free-Breathing</td>
</tr>
<tr>
<td>FLASH</td>
<td>Fast Low-Angle Shot</td>
</tr>
<tr>
<td>FMC</td>
<td>First Medical Contact</td>
</tr>
<tr>
<td>FU</td>
<td>Follow-Up</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-Width Half Max</td>
</tr>
<tr>
<td>GRE</td>
<td>Gradient-Recalled Echo</td>
</tr>
<tr>
<td>HHF</td>
<td>Hospitalisation for Heart Failure</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>hsTropT</td>
<td>High-sensitivity Troponin T</td>
</tr>
<tr>
<td>ICD</td>
<td>Implantable Cardioverter Defibrillator</td>
</tr>
<tr>
<td>ICPost</td>
<td>Intracoronary Postconditioning</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic Heart Disease</td>
</tr>
<tr>
<td>IMH</td>
<td>Intramyocardial Haemorrhage</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>IR</td>
<td>Inversion Recovery</td>
</tr>
<tr>
<td>IRI</td>
<td>Ischaemia Reperfusion Injury</td>
</tr>
<tr>
<td>IS</td>
<td>Infarct Size</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention-to-Treat</td>
</tr>
<tr>
<td>LAD</td>
<td>Left Anterior Descending</td>
</tr>
<tr>
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</tr>
<tr>
<td>LCx</td>
<td>Left Circumflex</td>
</tr>
<tr>
<td>LGE</td>
<td>Late Gadolinium Enhancement</td>
</tr>
<tr>
<td>LLA</td>
<td>Lower Limit of Agreement</td>
</tr>
<tr>
<td>LV</td>
<td>Left Ventricle</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left Ventricular Ejection Fraction</td>
</tr>
<tr>
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<td>Left Ventricular End-Diastolic Pressure</td>
</tr>
<tr>
<td>MACCE</td>
<td>Major Adverse Cardiac and Cerebrovascular Events</td>
</tr>
<tr>
<td>MACE</td>
<td>Major Adverse Cardiac Events</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MOCO</td>
<td>Motion Corrected</td>
</tr>
<tr>
<td>MOLLI</td>
<td>Modified Look-Locker Inversion</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSI</td>
<td>Myocardial Salvage Index</td>
</tr>
<tr>
<td>MVO</td>
<td>Microvascular Obstruction</td>
</tr>
<tr>
<td>NIAP</td>
<td>National Infarct Angioplasty Project</td>
</tr>
<tr>
<td>NAPCI</td>
<td>National Audit of Percutaneous Coronary Interventions</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NR</td>
<td>Not Reported</td>
</tr>
<tr>
<td>NSAE</td>
<td>Non-Serious Adverse Event</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous Coronary Intervention</td>
</tr>
<tr>
<td>PI</td>
<td>Principle Investigator</td>
</tr>
<tr>
<td>PIS</td>
<td>Patient Information Sheet</td>
</tr>
<tr>
<td>PP</td>
<td>Per-Protocol</td>
</tr>
<tr>
<td>PPCI</td>
<td>Primary Percutaneous Coronary Intervention</td>
</tr>
<tr>
<td>PSIR</td>
<td>Phase-Sensitive Inversion Recovery</td>
</tr>
<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
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<tr>
<td>RIC</td>
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<td>RISK</td>
<td>Reperfusion Injury Salvage Kinase</td>
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<td>Systolic Blood Pressure</td>
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<td>Standard Deviation</td>
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<td>Single-Photon Emission Computed Tomography</td>
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<td>SSFP</td>
<td>Steady-state Free Precession</td>
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<td>STEMI</td>
<td>ST-Elevation Myocardial Infarction</td>
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<tr>
<td>TIMI</td>
<td>Thrombolysis in Myocardial Infarction</td>
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<td>TTC</td>
<td>Triphenyl-Tetrazolium Chloride</td>
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<td>University College London</td>
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<td>Upper Limit of Agreement</td>
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<td>Ventricular Fibrillation</td>
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<td>Ventricular Tachycardia</td>
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<td>WHO</td>
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Chapter 1

Introduction
1.1 Background

The World Health Organisation (WHO) estimates ischaemic heart disease (IHD) to be the leading cause of mortality worldwide, accounting for about 16% of total deaths in 2016 [www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death]. In the UK, according data from the British Heart Foundation (BHF), over 66,000 deaths per year are attributed to IHD, and each year there are nearly 200,000 hospital visits due to patients suffering from an acute myocardial infarction [www.bhf.org.uk/for-professionals/healthcare-professionals/data-and-statistics/the-cvd-challenge]. Overall, cardiovascular disease costs the UK economy an estimated £19 billion per annum.

In patients suffering from an acute ST-segment elevation myocardial infarction (STEMI), reperfusion therapy with primary percutaneous coronary intervention (PPCI) aims to relieve mechanical obstruction of the occluded artery and re-establish myocardial blood supply. When undertaken in a timely manner, it has been shown to be the most effective strategy for reducing adverse outcomes including mortality\(^1\). Despite recent therapeutic advances and investment to improve call-to-balloon times, morbidity and mortality of STEMI patients remains significant. According to data from the National Audit of Percutaneous Coronary Interventions (NAPCI) in the UK, overall in-hospital mortality following PPCI for STEMI was 5.6% in 2017-18 and in the sub-group presenting with cardiogenic shock mortality was approximately 35% [www.bcis.org.uk/resources/audit-results/].

Reperfusion however can also paradoxically lead to microvascular and/or cardiomyocyte damage\(^2\) and the cumulative detrimental effects result in further cardiomyocyte death and impaired healing. Ischaemia-reperfusion injury (IRI), the term used to describe this phenomenon, therefore contributes to post-infarct left ventricular (LV) impairment, adverse remodelling and poor outcomes. There
is hence the need for new therapeutic strategies to help protect the heart against acute IRI.

Various pharmacological and mechanical strategies are currently being investigated for their potential cardioprotective effects. Remote ischaemic conditioning (RIC) is a mechanical, non-invasive process which utilises multiple short cycles (each lasting between 3-5 minutes) of blood pressure cuff inflation and deflation applied to a limb, most commonly the upper limb. It is a cost-effective strategy which can potentially further diminish infarct size, prevent the onset of heart failure symptoms and reduce adverse outcomes if utilised in addition to PPCI to treat patients suffering from STEMI.

1.2 ST-segment Elevation Myocardial Infarction (STEMI)

1.2.1 Definition of STEMI
The European Society of Cardiology (ESC) expert consensus document defines STEMI as the presence of new ST-segment elevation at the J-point in two contiguous leads with a cut-point of $\geq 1$ mm in all leads other than leads V2–V3. In leads V2-V3 the following cut-points apply:
1. $\geq 2$ mm in men $\geq 40$ years
2. $\geq 2.5$ mm in men $< 40$ years
3. or $\geq 1.5$ mm in women regardless of age

1.2.2 Management of STEMI
The management of patients suffering from STEMI changed dramatically in the late 1980s with introduction of thrombolytic therapies aimed at coronary reperfusion. Two landmark studies demonstrated significant reductions in mortality for patients treated promptly with thrombolysis. O’Neill & al.
subsequently introduced the concept of primary angioplasty as a treatment option in this cohort of patients. The small study of 56 patients concluded that both angioplasty and streptokinase resulted in similar rates of reperfusion, but coronary stenosis was significantly less in the angioplasty group. This led to larger randomised trials and a meta-analysis which demonstrated that patients treated with PPCI have significantly reduced rates of short-term mortality, non-fatal re-infarction and stroke1. Major guidelines have as a result recommended PPCI as the preferred reperfusion strategy for STEMI patients10,11.

1.3 Cardioprotective Strategies

Multiple potential cardioprotective strategies have been studied to try and identify therapies that can reduce infarct size and have a beneficial impact on short-term and long-term cardiovascular outcomes3,12. Pharmacological agents such as Metoprolol, Exenatide and Cyclosporine A and device-related strategies such as aspiration thrombectomy and therapeutic hypothermia have yielded mixed results. RIC is one of the potential strategies which has shown promising results in small scale proof of concept studies.

1.3.1 RIC Proof-of-concept Studies

The concept of ischaemic preconditioning, performed by multiple cycles of non-lethal coronary arterial occlusion and reperfusion, having cardioprotective effects in animal models was first demonstrated by Murry & al. in 198613. Furthermore, to add weight to this concept, it was demonstrated that patients with angina and a positive exercise test could exercise for longer before developing angina and the angina and electrocardiographic evidence of ischaemia were less severe during a second test when compared to a first test14. This phenomenon, previously described as “warm up”, is considered to be a clinical manifestation of myocardial preconditioning.
Remote ischaemic preconditioning was first demonstrated by Przyklenk & al. who showed that ischaemic preconditioning from one coronary vascular bed can limit infarct size in remote myocardial tissue. This small study was performed in canine models with four 5-minute cycles of left circumflex artery (LCx) occlusion and subsequent left anterior descending (LAD) artery ischaemia-reperfusion. Mean infarct size in the preconditioned group was 4% of the myocardium-at-risk as compared to 13% in the control group (p < 0.05). This finding introduced the notion of activated humoral mediators providing cardioprotection to remote tissue. Gho & al subsequently demonstrated the cardioprotective effects of a single cycle of 15 minutes of mesenteric or renal arterial occlusion in rats by measuring myocardial salvage. Ganglionic inhibition with hexamethonium abolished the beneficial effect of mesenteric arterial occlusion implying involvement of a neuronal pathway.

Birnbaum & al. investigated the concept of altering remote tissue ischaemia to precondition the myocardium by applying single 30-minute cycle of partial femoral arterial occlusion to a hind limb in combination with electrical stimulation of the gastrocnemius muscle to increase demand. This resulted in significantly smaller infarct size after 30 minutes of coronary occlusion. The two therapies in isolation did not have a significant effect suggestive of a relationship with the degree of tissue ischaemia. Kharbanda & al. subsequently demonstrated that the beneficial effects of RIC could be delivered non-invasively in pigs by applying multiple cycles of tourniquet occlusion to the hind limb. This resulted in a reduction in infarct size from 53±8% to 26±9% (p=<0.05) of the area-at-risk (AAR). A similar systemic preconditioning effect in humans could be observed by three 5-minute cycles of inflating and deflating a blood pressure cuff placed on the upper arm of healthy volunteers, introducing the concept of RIC.

The precise mechanisms underlying RIC are not entirely certain. There are interacting neuronal and humoral pathways that are involved in the signal transfer from the remote tissue to the target organ. The remote ischaemic stimulus initiated from a distant organ activates peripheral sensory nerves. It
is postulated that additional mechanisms such tissue hypoxia and shear stress play a role in activating the RIC signalling pathway\textsuperscript{21,22}. There is also a probable role for humoral factors in activating the sensory nervous system\textsuperscript{18,23}. The nervous stimulus reaches the autonomic nervous system via the spinal cord resulting in activation of the vagus nerve to release acetylcholine. These either directly activate receptors in the target organs or stimulate non-target organs to release humoral blood-borne agents which stimulate target organs\textsuperscript{24,25}. In the heart, signal transduction involves cell surface receptors, the mitochondria and intracellular signal transduction via the reperfusion injury salvage kinase (RISK) pathway\textsuperscript{21}. Overall, the exact nature of the interaction between the neuronal and humoral mediators remains unclear\textsuperscript{21,23,26}.

\textbf{Figure 1.1: Mechanism of RIC (Modified from Heusch \textit{et al.}\textsuperscript{21}).} Remote limb ischaemia-reperfusion results in activation of peripheral sensory fibres (neuronal pathway) and release of cardioprotective factors in the bloodstream with lung passage (humoral pathways). Extracellular and intracellular signal transduction at the level of the myocyte are involved in delivering the protective effect.
The potential beneficial effects of RIC have consequently been investigated in various other clinical settings such as cardiac surgery, cerebrovascular events, post-operative acute nephropathy and liver transplant\textsuperscript{27}. Some results are encouraging\textsuperscript{28–32} while others have shown that RIC provides no clinical benefit\textsuperscript{33–35}. Though the precise reasons for these conflicting findings remain unclear, there is evidence that cardiovascular risk factors such as hypertension, hyperlipidaemia and insulin resistance and pharmacological agents such as statins, nitrates and glucose-lowering drugs can potentially modify response to preconditioning\textsuperscript{36}.

In STEMI patients undergoing PPCI, multiple small proof-of-concept studies have been undertaken to demonstrate the effects of RIC on various outcome measures (Table 1.1). The first randomised study was conducted by Botker & al.\textsuperscript{37} and utilised RIC using four cycles of 5-minute upper arm cuff inflation and deflation initiated in the pre-hospital setting by paramedics \textit{en route} to the receiving tertiary centre. It showed a significant increase in the median myocardial salvage index (MSI) measured using single-photon emission computed tomography (SPECT) from 0.55 in controls to 0.75 in RIC-treated group at 30 days (n=333, p=0.033). In a post-hoc subgroup analysis of patients with anterior infarction, the difference in MSI was more pronounced (median 0.51 vs. 0.78; p=0.06). There was however no significant reduction in infarct size and improvement in LV ejection fraction at 30 days\textsuperscript{38}. Major adverse cardiac and cerebrovascular events (13.5\% vs. 25.6\%) and all-cause mortality (4\% vs. 12\%) were lower in the RIC group when compared to the control group at 3.8 years median follow-up\textsuperscript{39}. It is however important to note that this study was not powered to investigate clinical end-points and all-cause mortality was predominantly driven by non-cardiac death.

White & al.\textsuperscript{40} used a similar RIC protocol but initiated blood pressure cuff inflation upon arrival at the treating centre. As opposed to findings from Botker & al., RIC was shown to reduce mean infarct size, assessed utilising cardiac magnetic resonance imaging (CMR), as a percentage of LV by 27\% when compared with
control subjects (18.0% vs. 24.5%; p=0.009). Biochemical measures of infarct size such as 24-hour high-sensitivity troponin T levels were significantly reduced in the RIC group as compared to the control group (median 2296ng/L vs. 2736ng/L; p=0.037). Interestingly, there was also a reduction in the percentage of myocardial oedema measured by T2 parametric mapping from 35.1% in the control group to 28.5% in the RIC group (p=0.003). Mean T2 values were lower (68.7ms vs. 73.1ms; p=0.001) suggestive of a reduction in the severity of oedema in RIC treated patients.

*Liu & al.* utilised a similar RIC protocol in the pre-hospital setting and CMR to demonstrate similarly encouraging results. Assessment of early microvascular obstruction was the primary end-point. The use of RIC reduced the proportion of patients with MVO from 73.3% to 50.8% (p=0.011) and overall size of MVO as a percentage of LV from 86% to 68% (p=0.016). Various other markers of poor outcome such as infarct size (16.6% vs. 14.2%; p=0.042) and LV ejection fraction (45.4% vs. 48.0%; p=0.039) were also significantly improved by the treatment RIC along with PPCI. At 1-year follow-up, the MACE rate was 13.3% in the control arm and 5.1% in the RIC arm which did not achieve statistical significance (p=0.116). This discrepancy might be explained by a smaller sample size and a shorter follow-up period that the study by *Sloth & al.*

Using a different RIC protocol (three 4-minute cycles of upper arm cuff inflation and deflation) in a smaller study of 96 patients, *Rentoukas & al.* demonstrated that in patients treated with RIC, initiated 10 minutes prior to the estimated time of first balloon inflation, a significantly higher proportion of patients (53% vs 73%; p=0.045) showed full resolution (defined as ≥80% reduction of ST-segment deviation score) of ST-segment deviation half an hour after PCI. Peak troponin I levels were also significantly lower in patients treated with RIC as compared to controls, with an additional beneficial effect of intravenous morphine infusion noted.
Prunier & al.\textsuperscript{43} conducted a study investigating the effects of RIC alone and in combination with localised ischaemic post-conditioning (four cycles of 1-minute inflation and deflation of the angioplasty balloon positioned proximal to the implanted stented). Peak CK-MB levels were significantly lower in the RIC group as compared to controls (p=0.016). The primary end-point, levels of CK-MB area-under-curve (AUC), did not achieve statistical significance (p=0.06). The authors suggest that this might be due to 5 patients who had to be excluded from analysis. Interestingly, there was no difference between the arms receiving RIC alone and RIC in combination with post-conditioning, indicating that no added benefit is conferred by this added therapy.

Yamanaka & al.\textsuperscript{32} investigated the effect of three 5-minute cycles of RIC delivered using an automated device (FB-270, Fukuda Densi, Tokyo, Japan) in a cohort of STEMI patients undergoing PPCI. The primary aim was to look at renal end-points. Secondary cardiac outcomes were also measured. Infarct size was reduced in the RIC group as compared to the control group, demonstrated by a significant reduction in peak serum CK levels (mean 2648 vs 3653 IU/L, p=0.04). At 30-day follow-up, the MACE rate in the RIC arm was lower than the control arm but did not achieve statistical significance (4\% vs 14\%; p=0.07). This study was however designed primarily to investigate renal outcomes and was not adequately powered for cardiovascular outcomes.

Findings from another study from Verouhis & al.\textsuperscript{44} however were not as encouraging. Ninety-three patients were randomised to receiving at least 1 cycle of lower limb remote conditioning prior to arterial recanalization with 4 cycles completed after reperfusion or sham. In total, patients received between 5 to 6 cycles of 5-minute cuff inflation and deflation from an automated device (PeriVasc Cuff Unit, EBIDA, Göteborg, Sweden). There was no significant difference in the primary outcome of myocardial salvage index. Only patients with anterior STEMI were included to target patients with large infarcts. The authors concluded that RIC started in the catheter laboratory is not recommended.
The largest prospectively designed study to investigate clinical outcomes to date was conducted by Gaspar & al with 448 patients randomised\textsuperscript{45}. Three 5-minute cycles of lower limb ischaemia were used to deliver RIC. It showed that RIC, initiated 10 minutes prior to PPCI similar to Rentoukas & al.\textsuperscript{42}, improved clinical outcomes with reduced rates of cardiovascular death and HHF at up to 3.7 years follow-up as compared to standard care. Though clinical features of heart failure such as need for diuretics, inotropes or intra-aortic balloon pump were lower in the RIC arm, there was no significant difference in 48-hour AUC or peak serum troponin I levels between the two groups (p=0.078). Post-hoc echocardiographic analysis however showed that there was a larger improvement in EF at 12 months as compared to the admission scan in patients treated with RIC in a sub-group with lower EF (p<0.001). A major criticism of this study was that patients 13% of patients were excluded from analysis after randomisation predominantly because they did not meet the inclusion-exclusion criteria.

Delivery of remote conditioning after the onset of reperfusion, either in combination with conditioning prior to reperfusion or as a standalone therapy, has also been investigated. A small study of 96 STEMI patients undergoing PPCI demonstrated that RIC (three 5-minute cycles) administered using the lower limb at the onset of myocardial reperfusion resulted in a 20% reduction in enzymatic infarct size measured using 72-hour AUC of CK-MB release (median 8814 units vs 10065 units; p=0.043). CMR performed after 4 months follow-up, showed a 10% relative reduction in infarct size in the RIC group\textsuperscript{46}. Another study with similar timing of RIC delivery by Cao & al. further demonstrated the beneficial effects of RIC initiated at the time of PCI with significantly lower peak CK-MB levels (p<0.01) and increased LV ejection fraction (p=0.01) in the RIC arm as opposed to the control arm\textsuperscript{47}. There was however no significant reduction in the primary end-point of the incidence of LV remodelling at six months in a small study by Elbadawi & al. (p=0.42) in a cohort of patients randomised to receive lower limb RIC at the time of reperfusion\textsuperscript{48}.  

28
In addition to RIC, *Eitel & al.* investigated the potential combined benefits of ischaemic postconditioning with conventional therapy\(^49\). This randomised study of 696 STEMI patients had three arms: 1. RIC and postconditioning in addition to PPCI, 2. postconditioning only in addition to PPCI, and 3. the control arm receiving PPCI. RIC was delivered using three 5-minute cycles of cuff inflation to the upper limb and postconditioning was delivered locally by using four 30-second balloon inflations in the culprit vessel. CMR assessed MSI was significantly greater in the group receiving RIC combined with postconditioning when compared to the control arm (0.49 vs 0.40; \(p=0.02\)). Interestingly, postconditioning alone did not affect MSI when compared to conventional therapy (\(p=0.39\)). This finding would suggest that observed benefit in the RIC and postconditioning arm is provided mainly by RIC. At six months follow-up, no clinical benefit was observed between the three arms (\(p=0.44\)). The authors suggested that a RIC only investigative arm and a longer follow-up period would have provided more conclusive answers.

**1.3.2 Section Summary**

Preconditioning and remote preconditioning have been intensively investigated for more than 30 years in animal models and human subjects. It is now well-established that RIC has a positive impact on surrogate markers such as infarct size, myocardial oedema and LV ejection fraction. It remains however unknown whether these beneficial effects translate into better long-term clinical outcomes. The CONDI2/ERIC-PPCI study is a large multi-centre randomised trial designed to investigate the impact of RIC on clinical outcomes.
<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Details</th>
<th>Sample Size</th>
<th>RIC Protocol</th>
<th>Outcome</th>
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<td>Symptom onset &lt;12hrs</td>
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<td></td>
<td>Pre</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>4 x 5-min cycles</td>
<td></td>
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<tr>
<td>Munk</td>
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<td></td>
<td>Pre</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td>4 x 5-min cycles</td>
<td></td>
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<td>Upper</td>
<td>Increase in ST-segment resolution (53% vs 73%, p=0.045)</td>
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<td>TIMI flow NR</td>
<td></td>
<td>Pre (10 mins prior)</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>3 x 4-min cycles</td>
<td></td>
</tr>
<tr>
<td>Crimi</td>
<td>Symptom onset &lt;6hrs</td>
<td>96</td>
<td>Lower</td>
<td>Reduced AUC CK-MB levels (8814 units vs 10665 units, p=0.043)</td>
</tr>
<tr>
<td>2013</td>
<td>TIMI flow 0-I</td>
<td></td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 x 5-min cycles</td>
<td></td>
</tr>
<tr>
<td>Prunier</td>
<td>Symptom onset &lt;6hrs</td>
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<td>Upper</td>
<td>Lower peak CK-MB levels (267±168 U/L vs 415±195 U/L, p=0.016)</td>
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<td>Pre</td>
<td></td>
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<td></td>
<td></td>
<td>3 x 5-min cycles</td>
<td></td>
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<tr>
<td>Eitel</td>
<td>Symptom onset &lt;12hrs</td>
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<td>Upper</td>
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<td>Pre and ICPPost</td>
<td>compared with control (49 vs 40, p=0.02). ICPPost alone failed to</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>improve MSI (p=0.39).</td>
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<td>Pre</td>
<td></td>
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<tr>
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<td></td>
<td></td>
<td>4 x 5-min cycles</td>
<td></td>
</tr>
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<td>Pre</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>3 x 5-min cycles</td>
<td></td>
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<td>Year</td>
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<td>TIMI Flow</td>
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<td>-----------</td>
<td>------</td>
</tr>
<tr>
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<td>2016</td>
<td>&lt;12hrs</td>
<td>0-III</td>
<td>Upper limb</td>
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<td>Lower limb</td>
</tr>
<tr>
<td>Gaspar</td>
<td>2017</td>
<td>&lt;12hrs</td>
<td>0-III</td>
<td>Lower limb</td>
</tr>
<tr>
<td>Cao</td>
<td>2018</td>
<td>&lt;6hrs</td>
<td>NR</td>
<td>Upper limb</td>
</tr>
</tbody>
</table>

Table 1.1: Characteristics of RIC studies. (RIC: remote ischaemic conditioning; NR: not reported; CK-MB: creatine kinase-MB isoenzyme; ICPost: intracoronary postconditioning; LV: left ventricle; LVEF: left ventricular ejection fraction; LVEDP: left ventricular end-diastolic pressure; CV: cardiovascular; HHF: heart failure hospitalisation AUC: area-under-curve; MVO: microvascular obstruction; MSI: myocardial salvage index; CMR: cardiac magnetic resonance; TIMI: thrombolysis in myocardial infarction; SPECT: single-photon emission computed tomography).
1.4 Use of CMR in STEMI Patients

Various modalities have been used to assess myocardial injury and cardiac function as a means of predicting clinical outcomes after an acute myocardial infarction\textsuperscript{50}. Earlier studies utilised multiple different parameters such as electrocardiography, cardiac biomarkers, echocardiography and radionuclide scans. Despite being useful tools to help identify scar tissue and quantify infarct size, these techniques have serious limitations such as lack of specificity and susceptibility to coronary microvascular dysfunction\textsuperscript{51}. The pre-existing imaging modalities such as nuclear imaging also have low spatial resolution with limited ability to accurately define the extent of myocardial necrosis. More recently, CMR infarct analysis has been the favoured approach\textsuperscript{52,53}.

Magnetic resonance imaging involves hydrogen ions, a major constituent of the human body, which get disturbed by brief radiofrequency pulses in a static magnetic field, and subsequently relax to equilibrium exponentially. Recovery of the longitudinal magnetisation following inversion is termed T1 relaxation time\textsuperscript{54,55} and decay of the transverse magnetisation is termed T2 relaxation time\textsuperscript{56}. These parameters vary substantially among tissues.

1.4.1 CMR in Acute and Chronic Myocardial Infarction

CMR is the imaging modality of choice for non-invasive assessment of acute and chronic myocardial infarctions for research and clinical purposes\textsuperscript{57}.

1.4.1.1 Acute Myocardial Infarction Imaging

The presence and size of an acute infarct is assessed by T1-weighted late-gadolinium enhanced (LGE) imaging. Gadolinium-based contrast agents do not cross the intact myocardial cell membranes. Following an acute myocardial infarction, ruptured cell membranes result in an increase in extracellular space leading to prolonged contrast washout and hence accumulation\textsuperscript{58,59} (Figure 1.2). Contrast wash in is reduced in tissue with
microvascular injury resulting in a hypo-enhanced core within an infarcted area\textsuperscript{60}.

![Figure 1.2: Late-gadolinium imaging showing hyper-enhancement (arrows) due to delayed contrast wash-out following LAD territory acute myocardial infarction (Adapted from White \textit{et al.}). [LAD: left anterior descending].](image)

Acute MI size can also be quantified by measuring pre- and post-contrast T1 values on mapping to compute extra cellular volume fraction (ECV) which has been shown to correlate with infarct size\textsuperscript{61}.

T2 mapping techniques offer further insight into the pathophysiological process and enable quantification of CMR-based AAR assessment. Inflammation in the aftermath of an acute infarction results in myocardial oedema which manifests as elevated T2 values on mapping\textsuperscript{62,63}. Myocardial salvage index is calculated by subtracting the infarcted area from the AAR as a proportion of AAR (Figure 1.3). This method, though having limitations, can be used for assessing effects of investigational therapies\textsuperscript{64,65}. Recently, T1 mapping has also been shown to be a useful tool in assessing myocardial oedema and AAR\textsuperscript{66–68}. 
1.4.1.2 LGE in Chronic Myocardial Infarction

Gadolinium chelate contrast agents, which are biologically inert, of large molecular weight and highly paramagnetic, shorten tissue T1 relaxation times. Following intravenous administration, these agents (dosed at 0.1–0.2 mmol/kg of body weight) diffuse into the extracellular myocardial matrix\textsuperscript{69}. In the normal myocardium, extracellular volume is low and consequently, contrast wash-in and wash-out is swift. Chronic infarct tissue consists of collagen rich scar with a lower density of capillaries and an increased extracellular volume\textsuperscript{55,70}. It consequently exhibits slower contrast wash-in and wash-out and, when steady state is achieved, results in contrast accumulation when compared to the normal myocardium\textsuperscript{71–73} (Figure 1.4). This effect produces signal hyper-enhancement on T1-weighted imaging when acquired 15-25 minutes after contrast administration with nulling of the normal myocardium, termed late-gadolinium
enhancement (LGE) imaging. It delineates the extent of myocardial scarring resulting from a chronic infarct (Figure 1.5).

Figure 1.4: Temporal course of gadolinium contrast distribution (Modified from Arai & al.). Delayed wash-in and wash-out of the infarcted myocardium when compared to normal (or ischaemic) tissue produces a difference in signal intensity in the delayed phase.

At steady state, areas with a larger extracellular volume retain higher concentrations of gadolinium contrast and as a result experience greater shortening of T1 relaxation times. Scar tissue hence appears brighter. Conversely, in areas with chronic microvascular injury, the gadolinium agent takes much longer to reach affected tissue resulting in hypo-enhancement 75.

Figure 1.5: Late post-gadolinium contrast imaging. Short-axis acquisitions showing normal tissue (Panel A), scarring from chronic inferior infarct (Panel B) and scarring with presence of microvascular injury following an anterior infarct (Panel C).
A canine animal study by Kim & al. demonstrated that using the inversion recovery fast low-angle shot (FLASH) pulse sequence, post-contrast CMR imaging correlated strongly with histological infarct size, measured using triphenyl-tetrazolium chloride (TTC) tissue enzyme staining, both acutely (r=0.99) and at eight weeks follow-up (r=0.97). Multiple other animal studies using different LGE imaging sequences have shown similarly high degrees of correlation.

Various CMR studies have demonstrated recovery in contractility and systolic function during the convalescence phase following revascularisation. Additionally, a reduction in LGE mass during convalescence after the acute index event has also been demonstrated using sequential imaging in both animal and human studies. Presence and extent of scar tissue chronically has been shown to predict adverse LV remodelling, ventricular arrhythmia, LVEF and LV end-diastolic volumes. Crucially, CMR assessed infarct size has been shown to predict clinical outcomes (Table 1.2) and can be used as a prognostic marker to risk stratify appropriately and plan therapeutic strategies tailored to the needs of individual patients. CMR also has potential to be utilised as a reliable non-invasive surrogate end-point in smaller phase II proof-of-concept studies involving STEMI patients.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Details</th>
<th>Patient Details</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yokota 2008</td>
<td>N=86</td>
<td>Ischaemic</td>
<td>Cardiovascular events associated with presence of larger scar volume (16.8±12.4cm³ vs 11.7±12.6cm³, p=0.023)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cardiomyopathy</td>
<td>Mean FU 1.7 years</td>
</tr>
<tr>
<td>Kwon 2009</td>
<td>N=349</td>
<td>Ischaemic</td>
<td>Increased IS associated with higher mortality and need for cardiac transplant (p=0.004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cardiomyopathy</td>
<td>Mean FU 2.6 years</td>
</tr>
<tr>
<td>Petriz 2015</td>
<td>N=1959</td>
<td>Chronic infarct</td>
<td>IS &gt;21% of LV associated with significantly higher rates of cardiovascular death (p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean FU 6.4 years</td>
</tr>
<tr>
<td>Larose 2010</td>
<td>N=103</td>
<td>STEMI</td>
<td>IS &lt;23% of LV associated with higher event-free survival rates (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scanned &lt;12 hours</td>
<td>Mean FU 2.3 years</td>
</tr>
<tr>
<td>Wu 2008</td>
<td>N=122</td>
<td>STEMI</td>
<td>IS of &lt;18.5% of LV associated with significantly more even-free survival rates (p=0.007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scanned &lt;4 days</td>
<td>Mean FU 2 years</td>
</tr>
<tr>
<td>van Kranenburg 2014</td>
<td>Pooled analysis</td>
<td>STEMI</td>
<td>IS ≥25% of LV associated with increased risk of MACE at (HR:2.04 [95% CI:1.42-2.92]; p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>N=1025</td>
<td>Scanned &lt;7 days</td>
<td>Mean FU 2 years</td>
</tr>
<tr>
<td>Eitel 2014</td>
<td>N=738</td>
<td>STEMI</td>
<td>IS &gt;19% of LV associated with lower rates of event-free survival (p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scanned &lt;7 days</td>
<td>Mean FU 1 year</td>
</tr>
<tr>
<td>Bonanad 2016</td>
<td>N=546</td>
<td>STEMI</td>
<td>IS&gt;30% of LV independently doubled the risk of cardiac death and non-fatal MI (HR: 2.4; [95% CI:1.3-4.4]; p= 0.007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scanned &lt;7 days</td>
<td>Mean FU 2.3 years</td>
</tr>
</tbody>
</table>
Every 5% increase in IS was associated with increased risk of all-cause mortality (HR:1.19 [95% CI:1.18-1.20]; p< 0.0001) and HHF (HR:1.20 [95% CI:1.19-1.21]; p< 0.0001)

Higher MACE rate in patients with LGE (38.2% vs 7.4%, p<0.001)

CMR-based scoring model: LV ejection fraction ≤47% - 1 point; IS ≥19% of LV - 1 point; MVO ≥1.4% of LV - 2 points. Patients stratified into low (0 or 1 point) or high risk groups (≥2 points). MACE rates significantly higher in the high-risk cohort compared to the low-risk group (9.0% vs 2.2%; p=0.001).

Predictors of survival free of all-cause death or HHF: IS≥21% (p<0.001), MVO extent ≥2.6% (p<0.001), or LVEF <43% (p<0.001)

Median FU 5.5 years

Table 1.2: Summary of studies showing effect of infarct size on clinical end-points. (IS: infarct size; FU: follow-up; LV: left ventricle; MACE: major adverse cardiovascular events; CMR: cardiac magnetic resonance; MVO: microvascular obstruction; HR: hazard ratio; CI: confidence interval; HHF: hospitalisation for heart failure; MI: myocardial infarction; LVEF: left ventricular ejection fraction).
1.4.1.3 Post-contrast Acquisition Sequences

Standardised comparison of infarct size quantification is of vital importance for research or clinical follow-up purposes but can be difficult given the number of imaging sequences commercially available, some relatively novel, for post-contrast imaging\textsuperscript{100}.

The phase sensitive inversion-recovery FLASH sequence obtained during a single breath-hold produces the greatest increase in scarred myocardial signal intensity and is widely utilised as the reference standard\textsuperscript{72,101–103}. This sequence involves repeated emissions of radiofrequency pulses with swift readout which is subsequently followed by suppression of any residual signal to minimise degradation of ensuing acquisitions\textsuperscript{104}. It is however susceptible to artefacts due to arrhythmias or inability of patients to breath-hold. The single shot steady state free precession (bSSFP) sequence is another commonly utilised sequence which is similar to the FLASH sequence but the signal at the end of an acquisition is reused rather than spoiled\textsuperscript{104}. It has a higher contrast-to-noise ratio and the imaging process is speedier when compared to FLASH\textsuperscript{100,105}. It is however more susceptible to artefacts due to magnetic field inhomogeneities.

A novel modality using the bSSFP sequence with motion correction (BB-MOCO) averaging of multiple measurements is now widely being used\textsuperscript{106–108}. The overall acquisition time is shorter than commonly used breath-held sequences. It also has the benefit of producing images of higher diagnostic quality with less motion artefact and an increase in user confidence\textsuperscript{108–110}. Studies assessing this sequence were, however, subjective and did not assess for inter and intra observer variability.

Demarcation between the bright scarred tissue and the bright blood pool in conventional post-contrast imaging can be blurred resulting in difficulties in accurately quantifying infarct size\textsuperscript{111}. The recent dark blood motion corrected (DB-MOCO) IR sequence with T2 preparation described by Kellman & al. helps address this issue\textsuperscript{106,112}. Blood and normal myocardial relaxation curves are
shifted as a consequence of the T2 preparation so that it becomes possible to choose delays that null both components. Consequently, scar appears hyper-enhanced and the blood pool and normal myocardial tissue appear dark. Francis & al.\textsuperscript{106} demonstrated greater observer confidence and increased segments with scar in a cohort of patients (n=172) with mixed pathologies when using the DB-MOCO sequence.

Other factors which can account for discrepancies in infarct size quantification are the methodology used to delineate the infarct border with the normal myocardium\textsuperscript{113} and the dosage of contrast agent along with the timing of post-contrast image acquisition\textsuperscript{114}.

\textbf{1.4.1.4 Section Summary}

Infarct size is a useful prognostic marker in the clinical setting and a reliable surrogate marker for proof-on-concept studies. Various commercially available post-contrast imaging sequences have been used for quantification, making standardised comparison across centres and studies difficult. There is therefore the need to compare the performance of commonly used sequences with emerging ones.
1.4.2 Microvascular Obstruction and Intramyocardial Haemorrhage

Following coronary occlusion, myocytes and capillaries undergo necrosis as a result of profound and sustained ischaemia. This results in oedema of the cells lining the capillary wall with loss of wall integrity leading to extravasation of red cells\textsuperscript{115}. Additionally, there is occlusion of the capillary lumen with blood cells and debris. As a result, even after restoration of epicardial blood flow, the infarct core will not reperfuse. This results in an area of microvascular obstruction (MVO) which can be present with or without intramyocardial haemorrhage (IMH). First described in a canine models following coronary occlusion\textsuperscript{116,117}, IMH was observed in 15 out of a cohort of 30 human cadavers at autopsy following acute MI treated with intracoronary thrombolysis\textsuperscript{118}. Though an acute phenomenon, extravasation of erythrocytes followed by degradation into oxyhaemoglobin, deoxyhaemoglobin and methaemoglobin and subsequent iron deposition causes a chronic inflammatory reaction evident even weeks or months after the index event\textsuperscript{119,120}.

Using post-contrast CMR imaging techniques described earlier, MVO manifests as a dark core surrounded by a hyperenhanced infarcted zone\textsuperscript{121}. Wu & al. demonstrated that its presence on CMR (performed on 10±6 days post event) in reperfused patients following AMI was associated with a higher incidence of adverse clinical events (45% vs. 9%, p=0.016)\textsuperscript{122}. The incidence of myocardial thinning was significantly increased in patients with MVO (62.5% vs. 0%, p=0.03) when compared to patients without MVO and MVO was a better indicator of poor cardiovascular outcomes than the angiographically assessed Thrombolysis in Myocardial Infarction (TIMI) flow grade. The presence of MVO after an acute infarction has also been shown to correlate with LV dysfunction assessed by echocardiography\textsuperscript{123} and severity of heart failure\textsuperscript{124}. More recently, there is growing evidence that the presence of MVO on CMR imaging is a predictor of adverse long term clinical outcomes\textsuperscript{94,95,98,99}. 

\textsuperscript{41}
Figure 1.6: Schematic representation of development of intramyocardial haemorrhage. Ischaemia-reperfusion injury leads to myocardial oedema and intramyocardial haemorrhage which subsequently causes external compression on coronary microcirculation. This exacerbates the microvascular resistance.

1.4.2.1 Intramyocardial Haemorrhage and T2* Mapping

Following IMH, haemoglobin breakdown results in iron deposition in the myocardial tissue as haemosiderin\textsuperscript{125}. Using gradient-echo (GRE) T2 imaging, transverse relaxation influenced by the dephasing effects of magnetic field inhomogeneities is referred to as T2\* relaxation time which is shortened in the presence of iron deposition\textsuperscript{126}. O'Regan & al. first described the use of T2\* imaging for detecting IMH in reperfused STEMI patients\textsuperscript{127}.

Myocardial T2\* can be measured by acquiring bright blood or dark blood GRE sequences. The latter has numerous advantages and is therefore the method of choice for clinical applications\textsuperscript{128,129}. It involves suppression of blood pool signal using double inversion pulses. There is, as a result, a more accurate delineation of the myocardial borders. This method has also been shown to reduce susceptibility artefacts due to coronary veins and is more reproducible\textsuperscript{130}.
There are essentially two methods for evaluating myocardial T2* after image acquisition. The region-of-interest (ROI) based method involves measuring the signal intensity from myocardial region of interest, typically the mid septum due to its propensity for less artefact in iron deposition cardiomyopathies, at different echo times and manual fitting into a mono-exponential equation to yield T2* times$^{131,132}$. The pixelwise method performs automated curve fitting of the signal intensity of each pixel during post processing to produce a colour map of the entire field of view$^{126}$. This method reduces analysis time, is more reproducible and can be more accurate in identifying regions of severe iron deposition (i.e. T2* <10ms) in patients with iron deposition cardiomyopathies as compared to the ROI-based method $^{133–135}$. The mapping sequence is however noisier than the ROI-based method which affects the precision and accuracy of T2* measurements$^{136}$.

![LGE and T2* mapping](image)

**Figure 1.7**: LGE and T2* mapping in post-infarct patient with MVO and IMH (Modified from Bulluck & al.$^{119}$). (LGE: late gadolinium enhancement; MVO: microvascular obstruction; IMH: intramyocardial haemorrhage).

Another important limitation of the conventional T2* mapping sequence which is performed breath-held (BH-T2*) is the presence of motion artefacts. This is of particular relevance when imaging patients in the post-infarct convalescence period unable to breath-hold for prolonged periods. The novel free-breathing respiratory motion-corrected T2* mapping sequence (FB-T2*) has been
developed to improve the signal to noise ratio and reduce ghost artefacts. It is of particular value in the presence of respiratory motion and arrhythmia. Initial work included patients with iron-deposition disorders such as thalassaemia and haemochromatosis only.

T2* mapping is comparatively the most reliable mapping sequence for the detection of IMH as demonstrated in a canine study by Wang & al. The presence of IMH in infarcted myocardium resulted in a drop in in T1 (1148.0±49.7ms vs 1066.8±61.2ms; -7.1% [remote 988±59.8ms]) and T2 (71.7±4.6ms vs 62.2±5.3ms; -13.2% [remote 53.3±5.0ms]) mapping values as compared to the peri-haemorrhagic oedema. The fall in corresponding T2* values was comparatively larger (45.1±6.6ms vs 22.2±5.5ms; -50.7% [remote 39.8±4.0ms]).

The presence of IMH in acute post-infarct imaging is associated with adverse clinical outcomes, with a stronger association than the presence of MVO only. IMH has been shown to predict adverse LV remodelling, cardiovascular death and HHF at 6 months. Reinstadler & al. recently demonstrated that the presence of IMH on T2* mapping was independently associated with a
composite of death, reinfarction and new heart failure at 12 months after adjusting for clinical risk factors (HR 2.7; 95% CI 1.1–6.6; p=0.032).

An early canine study by Higginson & al. demonstrated that gross IMH tends to occur in reperfused myocardium but not in non-reperfused tissue, indicative of an association with reperfusion injury\textsuperscript{139}. In a rat model, Hollander & al. studied the coronary microcirculation endothelial barrier function using microspheres. It showed that ischaemia for 30 minutes followed by 60 minutes of reperfusion results in significant microvascular injury as compared to ischaemia alone for 30 or 90 minutes\textsuperscript{140}. Given these findings, a therapeutic intervention to reduce reperfusion injury can have a significant impact on reducing IMH thus positively influencing clinical outcomes. Presence and degree of IMH can therefore be used as surrogate marker in future proof-of-concept studies to assess the potential effect of a novel therapy.

1.4.2.2 Section Summary
The presence of IMH is an independent predictor of adverse cardiovascular outcome and is potentially a target for future therapeutic strategies. The BH-T2* sequence has a major limitation of producing motion artefacts and the novel FB-T2* sequence has been introduced to address this issue. I therefore aim to study the diagnostic performance of this free-breathing sequence against the BH-T2* mapping sequence in a cohort of reperfused STEMI patients.
1.5 Summary

Remote preconditioning has a beneficial effect on markers of adverse outcome. The impact on clinical outcomes has however not been investigated in a large trial. The CONDI2/ERIC-PPCI study is large multi-centre randomised trial designed to investigate the impact of RIC on clinical outcomes. CMR is commonly used to assess surrogate markers of adverse outcomes. Infarct size and, more recently, IMH have been shown to be important and measurable predictors of adverse outcomes. Established acquisition sequences for assessing these two parameters have been extensively investigated. I aim to investigate the performance of novel sequences for detecting infarct size and IMH, which have the added attribute of motion correction, against established ones.
Chapter 2

Research Objectives
2.1 Effect of Remote Ischaemic Conditioning on Clinical Outcomes in ST-segment Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention Study (CONDI-2/ERIC-PPCI)

2.1.1 Rationale
There remains significant morbidity and mortality following acute STEMI despite the introduction of PPCI\textsuperscript{141}. New adjunctive treatments are therefore needed to improve clinical outcomes. Remote ischaemic conditioning has been shown to reduce myocardial infarct size in animal models and human subjects presenting with STEMI\textsuperscript{21,38,40}. Furthermore, follow-up studies and one single-centre prospective study has shown that RIC may improve clinical outcomes post-STEMI. However, a large and sufficiently powered prospectively designed multicentre clinical study however has not been undertaken. Therefore, in chapter 3 of this thesis, we investigated the effect of RIC on clinical outcomes in STEMI patients treated by PPCI in the CONDI-2/ERIC-PPCI trial. As described in detail previously, my role was to assist in the development of the research protocol and the setup of the ERIC-PPCI arm of the study, participate in central trial management, provide day-to-day advice to various centres and help recruitment at the lead centre.

2.1.2 Hypothesis
Remote ischaemic conditioning improves long-term clinical outcomes of cardiac death and hospitalisation for heart failure at 12 months in STEMI patients undergoing PPCI.
2.1.3 Aim

The primary aim of the study is to demonstrate whether RIC affects a composite of cardiac death and heart failure hospitalisation at 1 year in STEMI patients undergoing PPCI.
2.2 Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence

2.2.1 Rationale

Following a reperfused acute STEMI, myocardial perfusion does not fully recover in some cases despite restoration of coronary flow owing to the presence of microvascular dysfunction, also known as the ‘no-reflow’ phenomenon\textsuperscript{125}. The pathophysiology is not fully understood but is thought to involve MVO and extravasation of erythrocytes leading to IMH. The presence of IMH on CMR imaging is associated with increased risk of adverse clinical outcomes and could potentially be used as a reliable surrogate marker\textsuperscript{119,137,142–144}. Identifying IMH accurately on CMR remains an issue however. Breath-held segmented T2* (BH-T2*) measurements are currently the best technique for assessing iron accumulation, a result of haemoglobin degradation following IMH, in the myocardial tissue. Pixelwise T2* mapping has the advantage of covering the entire field-of-view without additional user intervention and reducing analysis time\textsuperscript{145}. This sequence is quite noisy and produces significant motion artefacts. Sensitivity is also an issue\textsuperscript{146}. A free-breathing respiratory motion-corrected (FB-T2*) single shot sequence with averaging has been recently developed. Initial work have included patients with iron-deposition disorders such as thalassaemia and haemochromatosis only\textsuperscript{135}. In chapter 4 of this thesis, I investigate how this newly available FB-T2* detects IMH in STEMI patients treated by PPCI, when compared to the standard breath-hold sequence.
2.2.2 Hypothesis
The novel free-breathing T2* mapping sequence produces less imaging artefacts than the conventional breath-held T2* mapping sequence when used in a cohort of reperfused STEMI patients.

2.2.3 Aim
The aim is to study the diagnostic performance of this free-breathing T2* mapping sequence against the conventional breath-held T2* mapping sequence in a cohort of reperfused STEMI patients.
2.3 Comparing Infarct Size Quantification Using Four Different Post-contrast CMR Sequences

2.3.1 Rationale

The degree of myocardial injury following an infarction is an important prognostic indicator\textsuperscript{147, 148}. Quantification of MI size has been shown to predict adverse outcomes in MI and heart failure patients and hence plays a key role in prognostication and tailoring of treatment\textsuperscript{93}. Post-contrast CMR imaging is currently regarded as the gold standard method for MI size quantification and has the ability to demarcate between viable and non-viable myocardial tissue non-invasively\textsuperscript{149}. Various technical issues make imaging acquisition and analysis difficult resulting in discrepancies in MI size quantification\textsuperscript{150, 151}. In addition, different sequences have been used for image acquisition, making standardised comparison difficult\textsuperscript{100}. The FLASH sequence produces the greatest increase in LGE signal intensity and is widely utilised as the reference standard\textsuperscript{72, 101–103}. The bSSFP sequence is commonly utilised for its speediness\textsuperscript{100, 105}. The novel BB-MOCO sequence produces images with higher diagnostic quality and is now widely being used despite having longer acquisition time\textsuperscript{106, 109}. The DB-MOCO sequence addresses this issue of reduced scar contrast from the blood pool\textsuperscript{106, 111, 112}. In chapter 5 of this thesis, I investigate how these four post-contrast sequences can be utilised interchangeably for infarct size quantification.
2.3.2 **Hypothesis**

Chronic infarct size quantification as a percentage of LV mass using four LGE imaging sequences yield comparable results and can be utilised interchangeably in clinical studies.

2.3.3 **Aim**

The aim of this study is to compare the size of chronic infarct, as a percentage of LV, measured using four different LGE imaging sequences in a cohort of STEMI patients: the FLASH, bSSFP, BB-MOCO and DB-MOCO sequences. The FLASH sequence will be used as standard for comparison.
Chapter 3

The CONDI-2/ERIC-PPCI Trial
3.1 Background

Since the introduction of the concept of cardioprotection for preventing reperfusion injury by remote preconditioning, multiple small pre-clinical studies using surrogate biochemical and imaging markers have yielded positive results\textsuperscript{21,152}. A large randomised trial is therefore required to demonstrate whether or not RIC has a beneficial impact on clinical outcomes in STEMI patients treated with PPCI. The primary aim of the study is to show whether RIC affects a composite of cardiac death and heart failure hospitalisation at 1 year in STEMI patients undergoing PCI.

3.2 Methods

3.2.1 Declaration of Helsinki, Good Clinical Practice and Ethical Approval

The study conformed to the spirit and the letter of the declaration of Helsinki, and in accordance with good clinical practice guidelines. This study received the necessary ethical approvals from regional and national health service research ethics committees.

3.2.2 Study Design and Research Collaboration

The Effect of Remote Ischaemic Conditioning on clinical outcomes in ST-segment elevation myocardial infarction patients undergoing Primary Percutaneous Coronary Intervention (ERIC-PPCI) study was a blinded prospective randomised-controlled trial to investigate the effects of RIC on clinical outcomes in STEMI patients treated with PPCI (NCT02342522). It was a multi-centre study recruited across various tertiary centres in the UK. This study was conducted in collaboration the Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing PPCI (CONDI-2) trial (NCT01857414), which
was a pan-European trial led by Prof H. E. Botker (Aarhus, Denmark). Numbers of patients recruited were 2800 for the ERIC-PPCI study and 2600 for the CONDI-2 study. Combined results were published to ensure the sample size was adequately powered to evaluate the potential effects of RIC on the primary endpoint.

The ERIC-PPCI arm of the study was project managed by the Clinical Trial Units at the London School of Hygiene and Tropical Medicine, London, UK. The CONDI-2 arm of the study was project managed by Department of Cardiology and Department of Clinical Epidemiology, Aarhus University Hospital Skejby Clinical Trials Unit, Aarhus, Denmark.

3.2.3 Inclusion Criteria

The inclusion criteria for this study are as listed:

1. Onset of symptoms consistent with an AMI within 12 hours, lasting for more than 30 minutes.
2. Patients older than 18 years of age.
3. Suspected STEMI as defined by ST-elevation at the J-point in 2 contiguous leads with the cut-off points: ≥0.2 millivolt (mV) in men or ≥0.15 mV in women in leads V2-V3 and/or ≥0.1 mV in other leads.

3.2.4 Exclusion Criteria

The exclusion criteria for this study are as listed:

1. Known ineligibility for PPCI.
2. Previous coronary artery bypass graft surgery (CABG).
3. MI within the previous 30 days.
4. Treatment with thrombolysis within the previous 30 days.
5. Left bundle branch block (LBBB).
6. Patients treated with cooling.
7. Conditions precluding use of RIC such as paresis of upper limb or presence of an arterio-venous shunt
8. Life expectancy of less than 1 year due to a non-cardiac pathology
3.2.5 Recruitment Sites

In the ERIC-PPCI arm of the study, recruitment took place across 26 sites in the UK. Selected sites perform more than 200 PPCI procedures per year. The CONDI-2 arm was a multi-national study recruiting patients across four sites in Denmark, two in Spain and one in Serbia.

3.2.6 Randomisation and Blinding

A pre-allocated unblinded research investigator was responsible for the randomisation process. Patients were randomised to the RIC arm or the control arm. Randomisation, stratified by recruiting centre using random permuted blocks, was managed centrally via a secure website with strictly controlled access. In the ERIC-PPCI arm, a sham device was applied in the upper arm in the control group. The principal investigator (PI) at each site, the interventional cardiologist undertaking the procedure and other catheter laboratory staff and the researchers responsible for data collection and analysis were all blinded to the treatment allocation. In the CONDI-2 arm, sham devices were not used in the control group and as a result healthcare staff involved were not blinded to the study protocol.

Outcome assessments were performed by blinded investigators. Members of the event validation committee were blinded to treatment allocation. The non-invasive and short-term nature of the RIC therapy makes the need for emergency unblinding unlikely. Patients could be unblinded through the randomisation website if needed.

3.2.7 Trial Treatment

Upon arrival at the recruiting centre in the ERIC-PPCI arm of the study, patients satisfying the inclusion and exclusion criteria were randomised to either the RIC or sham RIC protocol. The unblinded investigator was responsible for electronic randomisation and application of the cuff devices. In the CONDI-2 arm, recruitment was performed by the ambulance crew
attending to patients who fulfil the study criteria. Randomisation was performed electronically over the phone by the receiving centre and subsequent treatment allocation instructed to ambulance crew.

3.2.7.1 Automated CellAegis autoRIC™ Devices
The CellAegis autoRIC™ cuff device is an automated blood pressure apparatus that is pre-programmed to deliver four 5-minute cycles of inflation and deflation. This device is used in conjunction with single-use disposable inner cuffs. Therapy is initiated by simple cuff application and press-of-a-button. It delivers an inflation pressure of 200mmHg, which falls to zero upon deflation. The Sham autoRIC™ device is externally visually identical to that used in to deliver therapy in the RIC arm. However, as opposed to the autoRIC™ therapy device, the sham device control unit’s pump is disconnected which means that the applicator cuff cannot be inflated. As a result, the sham device provides identical sound and vibration of the pump and identical flashing LED indicators of the control unit as the therapy device. The operation of the sham and therapy devices with respect to the procedure initiation, cycle indication and termination were identical, with the only difference being cuff inflation. An autoRIC™ device, an autoRIC™ Sham device and disposable cuffs were provided to the participating sites.

Figure 3.1: Automated CellAegis autoRIC™ device
3.2.7.2 Remote Ischaemic Conditioning (RIC)
The automated autoRIC™ cuff was applied to the upper arm and therapy initiated with four alternating 5-minute cycles of inflation and deflation.

3.2.7.3 Sham RIC
The Sham autoRIC™ cuff visually identical to that used in the RIC protocol were placed on the upper arm and the simulated protocol applied. No inflation or deflation actually occurred but the cuff vibrated in a similar manner to active device. This device was used in the ERIC-PPCI arm only.

3.2.7.4 Duration of Treatment
Thirty-five minutes of cuff application was required given that the last 5 minutes of reperfusion can be undertaken with the cuff removed. In the cases where the PPCI procedure started within 35 minutes of cuff application, the RIC/sham RIC protocol overlapped with the beginning of the procedure. Under no circumstances was the start of the procedure delayed for the purposes of this study.

3.2.8 Primary Endpoint
The primary endpoint was a composite of cardiac death and hospitalisation for heart failure (HHF) at 12 months.

3.2.8.1 Definition of Cardiac Death
All deaths where there is no clinical or post-mortem evidence of a non-cardiac aetiology were classified as cardiac death.

3.2.8.2 Definition for Hospitalisation for Heart Failure
Hospitalisation for heart failure was defined as heart failure during the index hospitalisation or re-hospitalisation for heart failure following index admission. Hospitalisation was defined as a hospital admission of at least a 24-hour stay. The presence of heart failure was based on clinical findings and imaging evidence as previously described and summarised below:
1. One of the following symptoms: new or worsening dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea or increasing fatigue or worsening exercise tolerance.

2. One of the following features: new pulmonary oedema on chest X-ray in the absence of a non-cardiac cause, crepitations believed to be due to pulmonary oedema or use of loop diuretics to treat presumed pulmonary congestion.

### 3.2.9 Secondary Endpoints

This study investigated the potential effects of RIC on the secondary endpoints listed below:

1. The rates of cardiac death and HHF within 30 days.
2. The rates of all-cause death, coronary revascularisation, re-infarction and stroke within 30 days and 12 months.
3. The rates of implantable cardioverter-defibrillator (ICD) implantation within 12 months.

#### 3.2.9.1 Definition of Stroke

Stroke was defined as a focal, central neurological deficit lasting >72 hours which resulted in irreversible brain damage or body impairment. Patients with clinical features suggestive of stroke in the absence of sufficient evidence to satisfy the definition of stroke was classed as having suffered a probable stroke. Both definite and probable stroke was included in the secondary endpoint.

#### 3.2.9.2 Definition of reinfarction

Reinfarction was defined as an acute MI that occurs within 28 days of an index event or recurrent MI, i.e., occurring 28 days after the index event\(^{154,155}\). The following ECG and enzymatic criteria were considered when assessing patients with presumed reinfarction:

1. Recurrence of ST elevation >0.1 mV in at least two contiguous leads.
2. New pathognomonic Q waves in at least two contiguous leads.
3. In patients with normal baseline troponin levels: a rise to at least one value above the upper limit of normal (based on pre-determined local healthy volunteer measurements for each centre) along with a strong pre-test likelihood.

4. In patients with baseline elevated troponin levels: a $\geq 20\%$ increase in value in the second sample.

### 3.2.10 Planned Sub-group Analysis

The following pre-specified sub-groups were analysed:

1. **Age** (<55 years; 55 to <65 years; 65 to <75 years; or $\geq 75$ years).

   Animal studies have shown that reduced gene expression of the cell proteins of the ageing myocyte could potentially contribute to loss of response to ischaemia-reperfusion stimuli\textsuperscript{156}.

2. **Presence of diabetes mellitus.**

   Patients suffering from diabetes are have shown to be more susceptible to ischaemia-reperfusion injury, the mechanisms of which are not fully understood\textsuperscript{157}. In addition, preconditioning appears to be less effective in diabetic subjects. This is thought to be due to alterations in various individual components of the cardioprotective pathways\textsuperscript{158,159}. Another confounding factor in this patient group is that anti-diabetic therapies such as metformin and exenatide have been shown to display cardioprotective properties\textsuperscript{160–162}.

3. **LAD artery or other.**

   The LAD artery is the most important of the three major coronary vessels as its proximal occlusion results in the largest AAR, around 45\% of LV on average, and potentially the largest infarct size\textsuperscript{163,164}. Given the association between infarct size and outcome\textsuperscript{53,99,165,166}, cardioprotection after LAD occlusion can potentially deliver the most substantial clinical benefits.

4. **TIMI flow grade at presentation.**

   Pre-procedural TIMI flow grade 0/1 is associated with an increased infarct size, presence of MVO, reduced MSI and significantly worse clinical outcomes at 30 days\textsuperscript{167–170}. Additionally, cardioprotection has been shown to be ineffective in STEMI patients with a TIMI flow grade 2/3 at presentation\textsuperscript{171}.
whereas those with a flow grade of 0/1 benefit the most\textsuperscript{37,49}. A third of STEMI patients present with a TIMI flow grade 2/3\textsuperscript{172} and can potentially affect the efficacy of RIC.

5. First-medical-contact to balloon time (<90 minutes; 90 to <120 minutes; or 120 to 720 minutes).

Time-to-reperfusion is an independent predictor for transmurality and reversibility of acute myocardial injury with longer than 90-minute delays resulting in reduced MSI\textsuperscript{173,174}. Preconditioning has therefore the potential to have a positive impact on patients experiencing the longest delays to reperfusion.

\subsection*{3.2.11 Endpoint Validation}

The primary combined endpoint, stroke, MI, reinfarction and revascularisation were validated by an independent event validation committee (EVC). The EVC were blinded to the randomised treatment allocation.

\subsection*{3.2.12 Power Calculations}

Figures from various published studies were analysed to predict the expected event rates for STEMI patients undergoing PPCI. In the UK National Infarct Angioplasty Project (NIAP) dataset published in 2008, the cardiac mortality rate for reperfused STEMI patients, stratified according to call-to-balloon time, at 12 months was between 5.8\% and 16.7\%. The overall 12-month mortality rate was 8.7\% for all patients. Terkelsen \textit{et al.} observed that in a Danish cohort of patients (n=7952) treated with PPCI, at the 1 year, the rate of death was 9.2\% and of hospital re-admission or outpatient contact due to congestive heart failure (CHF) was 7.4\%\textsuperscript{175}. Another more recent European study demonstrated that the rate of HHF was up to 12.7\% at 1 year post PPCI\textsuperscript{176}.

Using data from these published studies and taking into account recent advancements in clinical outcomes from novel therapeutic strategies, we opted for significantly more conservative event rates. We estimated that the combined primary end-point of cardiac death and HHF event rate was 11.0\%
at 12 months for all-comer STEMI patients following PPCI. Based on proof-of-concept clinical studies which have reported a 30% reduction in infarct size\textsuperscript{37-40,42,43,46}, the RIC effect size is estimated to result in a 25% relative reduction in the primary outcome event rate from 11.0% to 8.25%. The estimated sample size with 80% power and at 5% significance level was calculated at 3610, requiring 1805 patients for each treatment arm. Allowing for a 15% drop-out rate after randomisation and during the follow-up period, 4300 patients in total were initially planned for recruitment (2000 patients in the UK and 2300 patients in Denmark). An independent blinded review of the data was performed roughly halfway through the recruitment process by the Clinical Trial Units at the London School of Hygiene and Tropical Medicine, London, UK. Given the actual event rates were lower than first anticipated, the sample size was adjusted to 5400 patients to achieve adequate power (2800 patients in ERIC-PPCI and 2600 in CONDI-2).

3.2.13 Statistical Analysis

The primary and secondary time-to-event endpoints were analysed and comparative evaluation between the RIC and control groups was performed. Cox proportional hazards modelling was used to evaluate hazard ratios and confidence intervals. Kaplan-Meier survival curves for the 2 study arms were produced. Data was analysed on an intention-to-treat basis.

A negative binomial model was used to compare the total number of outcomes experienced within the two arms at 12 months after randomisation. This was performed to account for recurrent HHF and competing mortality risk. The proportion of patients with an ICD implant in each arm at 12 months was compared using a generalised linear model for a binary outcome with a log link. Prespecified sub-group analyses on the primary outcome were performed by including an interaction term between the treatment group and sub-group in the Cox regression model. Results were considered statistically significant if the 2-sided p value was $<0.05$. 
Blinded statistical analysis was performed by the Clinical Trial Units at the London School of Hygiene and Tropical Medicine, London, UK.

3.2.14 Consent and Ethical Considerations

3.2.14.1 The ERIC-PPCI Arm
Patients were approached at the time the STEMI was confirmed and information about the trial was provided to their level of capacity. A patient leaflet was provided for this purpose. Patients had the option to sign the leaflet if they agreed to participate. A verbal agreement however was also acceptable. Agreement to take part in the trial was recorded in the patient's notes. A signed patient leaflet was not mandatory and was not considered as equivalent to informed consent. After the PPCI procedure, patients were given sufficient time to read the Patient Information Sheet (PIS), evaluate participation in the trial and ask questions. If they were agreeable to proceed, informed consent was taken.

3.2.14.2 The CONDI-2 arm
All patients provided informed written consent prior to randomisation. Given that patients did not have adequate time for reflection before consenting, additional oral information was provided after the acute phase.

3.2.14.3 Withdrawal
Patients had the right to withdraw from the trial at any time without prejudice to their future care.
3.2.15 Data Collection
Patients had a full medical history taken and various clinical examinations as part of usual care. The information listed below where available was recorded electronically.

3.2.15.1 Basic Information
The following basic patient details were collected:
1. Weight, height and body mass index (BMI)
2. Blood Pressure
3. Heart Rate
4. Gender
5. Ethnicity
6. Date of birth
7. NHS number - mortality data was tracked for up to 10 years after randomisation (for ERIC-PPCI arm only).

3.2.15.2 Medical and Other History
The following data on each patient’s medical and other related background was collected:
1. Diabetes Mellitus
2. Hypercholesterolaemia
3. Hypertension
4. Previous myocardial infarction
5. Previous PCI
6. Previous CABG
7. Previous stroke
8. Atrial fibrillation
9. Peripheral arterial disease
10. Smoking history
11. Family history of IHD
12. NYHA class
13. CCS class
3.2.15.3 Admission and Procedural Data

The following data regarding patient’s initial presentation and angioplasty procedure was collected:

1. Analgesia use including morphine doses
2. Culprit vessel
3. Time of onset of symptoms to ballooning
4. ECG at admission and prior to discharge
5. Call to balloon time, door to balloon time and symptoms to balloon time
6. Angiographic data (TIMI flow pre and post-PPCI)
7. Use of thrombectomy
8. Details of the PPCI procedure
9. Procedural drugs
10. LV Ejection fraction

3.2.15.4 Medication at Admission and Discharge

The list of medications on prior to admission and at discharge was collected with particular attention to the following:

1. Aspirin
2. Beta-blocker
3. Calcium-channel blocker
4. Nitrates
5. Cholesterol-lowering drug
6. ACE inhibitor/ angiotensin receptor blocker
7. Insulin
8. Sulphonylurea
9. Metformin
10. Other medications

3.2.16 Follow-up

The patients were followed up at 6-8 weeks and at 12 months after the index event. Data was collected in order to determine whether the clinical requirements for primary and/or secondary endpoints have been fulfilled.
Follow-up was either be planned to coincide with existing clinical appointments or conducted by telephone.

3.2.17 Compliance and Loss to Follow-up

Compliance to trial therapy was monitored and given its brief and non-invasive nature, it was not a major issue. Loss to follow-up was expected to be low and was accounted for in the power calculations (up to 15%). Patients were encouraged to allow data and samples collected prior to withdrawal to be used in the analyses but these were discarded if consent to use data and samples was also withdrawn.

3.2.18 Safety Reporting

3.2.18.1 Definition

Unexpected events that did not fulfil the definition of the primary and secondary endpoints, expected complications of the RIC stimulus or expected complications of usual clinical care were reported as either serious adverse events (SAE) or non-serious adverse events (NSAE), depending on overall severity. Safety reporting for each patient was done from randomisation to completion of follow up at one year.

3.2.18.2 Data and Safety Monitoring Committee (DSMC)

The DSMC was tasked to meet periodically to determine whether there are any unforeseen and adverse effects of RIC.

3.2.18.3 Expected Adverse Events (recognised potential adverse effects of providing RIC stimulus)

Expected serious adverse events from the RIC were thought to be unlikely given the benign and non-invasive nature of the trial intervention. Skin petechiae, transient pain and paraesthesia caused by cuff inflation is an expected non-serious event and were recorded.
3.2.18.4 Expected Serious Adverse Events Related to Usual Clinical Care

The following are recognised complications of STEMI or angioplasty and were recorded but not be included in the adverse events analysis:

1. Death.

2. Acute renal failure which may require haemodialysis, peritoneal dialysis or haemofiltration.

3. Ventricular tachycardia (VT) or ventricular fibrillation (VF) requiring direct-current cardioversion (DCCV).

4. Significant heart block requiring temporary or permanent cardiac pacing.

5. Tamponade requiring urgent surgical intervention.

6. Cardiogenic shock requiring intra-aortic balloon pump or other LV assist devices.

The following events are recognised complications of routine clinical care and for the purposes of this trial were not reported:

1. Atrial fibrillation.

2. Acute mitral valve chordal rupture or ventricular septal rupture requiring surgical intervention.

3. Persistent complete heart block requiring permanent pacemaker implantation.

4. Aspiration pneumonia following VF arrest.

5. Rib fracture following chest compression.

3.2.18.5 Unexpected Serious Adverse Events

Adverse events which lead to any of the following were included:

1. Death.

2. Threat to life: defined as a serious adverse event in which the patient was at risk of death at the time of event but not an event which hypothetically might have caused death if more severe.

3. New episode of hospitalisation or prolongation of existing hospitalisation.

4. Persistent or significant disability or incapacity.
3.2.18.6 Unexpected Non-Serious Adverse Events

Unexpected non-serious adverse events were assessed for causality and intensity. Reports were reviewed to consider intensity, causality and expectedness.

3.2.18.7 Reporting Unexpected Adverse Events

All unexpected adverse events were reported regardless of severity of clinical features.

3.2.18.8 Assessment of Intensity

The intensity of adverse events was assessed as detailed below (Table 3.1).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Noticeable but easily tolerated event or symptom</td>
</tr>
<tr>
<td>Moderate</td>
<td>Cases sufficient discomfort to interfere with or reduce patient’s usual level of activity</td>
</tr>
<tr>
<td>Severe</td>
<td>Significant impairment of functioning; patient unable to carry out usual activities; and/or risk to life</td>
</tr>
</tbody>
</table>

Table 3.1: Guidance on assessment of severity of adverse event
### 3.2.18.9 Assessment of Causality

The likelihood that a particular adverse event is cause by RIC was assessed as detailed below (Table 3.2).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable</td>
<td>Causal relationship clinically and/or biologically highly plausible; plausible time sequence between onset of the adverse event and RIC procedure</td>
</tr>
<tr>
<td>Possible</td>
<td>Causal relationship clinically and/or biologically plausible; plausible time sequence between onset of the adverse event and RIC procedure</td>
</tr>
<tr>
<td>Unlikely</td>
<td>Causal relationship improbable; another documented cause of the adverse event is most plausible</td>
</tr>
<tr>
<td>Unrelated</td>
<td>Causal relationship definitely excluded; another documented cause of the adverse event most plausible</td>
</tr>
</tbody>
</table>

Table 3.2: Guidance on assessment of causality of adverse event

### 3.3 Results

#### 3.3.1 CONDI-2 and ERIC-PPCI Data

Pooled data from the ERIC-PPCI study (n=2788) and the CONDI-2 study (n=2613) were used for analysis. The data below was produced with input from the Clinical Trial Units, London School of Hygiene and Tropical Medicine, London, UK and the Department of Cardiology and Department of Clinical Epidemiology, Aarhus University Hospital Skejby Clinical Trials Unit, Aarhus, Denmark.

#### 3.3.2 Study Population

Between 6\(^{th}\) of November 2013 and 31\(^{st}\) of March 2018, 5401 patients were randomised to either the RIC arm (n=2700) or the control arm (n=2701) and of those, 5115 were included in the intention-to-treat (ITT) analysis: 2569 in the control arm and 2546 in the RIC arm (Figure 3.2). Baseline characteristics
and procedural details for patients included in ITT analysis were well balanced between the control and RIC groups (Tables 3.3 and 3.4). Per-protocol analysis was separately undertaken (n=2205 in control arm and n=2008 in RIC arm). Reasons for non-adherence to study protocol are described in detail in Figure 3.2.

3.3.3 Primary Outcomes

There was no significant difference in the combined primary end point of cardiac death or HHF between the control arm (8.6% [n=220]) and the RIC arm (9.4% [n=239]) at 12 months follow-up (HR, 1.10; 95% CI, 0.91 to 1.32; p=0.32; Table 3.5, Figure 3.3). There was similarly no difference between the control and RIC groups in the individual components of cardiac death or HHF at 12 months (Table 3.5, Figures 3.4 and 3.5). Pre-specified subgroup analyses demonstrated no evidence that RIC had an effect on the primary outcome by age, presence of diabetes, pre-PPCI TIMI flow, ischaemia time, or infarct location (Figure 3.6). Yielding similar results to the ITT analysis, the PP analysis showed 9.0% (n=179) of participants in the RIC arm experienced the primary combined end point within 12 months compared to 8.1% (n=178) in the control arm (hazard ratio, 1.11; 95% CI, 0.90 to 1.36; p=0.35).
Figure 3.2: Consort diagram of the ERIC-PPCI/CONDI2 study.

*Full screening log data not available.
**ERIC-PPCI had ethics approval to collect data on patients who died before written consent could be obtained and were hence included in subsequent analysis (n=20 in the control group and n=22 in RIC group).

(ITT: Intention-to-treat; STEMI:ST-elevation myocardial infarction; PPCI: Primary percutaneous coronary intervention; RIC: Remote ischaemic conditioning)
Table 3.3: Baseline Characteristics of the Patients in the ERIC-PPCI/CONDI-2 trial (ITT Analysis). Data are in mean (standard deviation) or median (interquartile range). (RIC: remote ischaemic conditioning; SD: standard deviation; IQR: interquartile range; BMI: body mass index; MI: myocardial infarction; IHD: ischaemic heart disease; PPCI: primary percutaneous coronary intervention; BP: blood pressure).
<table>
<thead>
<tr>
<th></th>
<th>Control (n=2569)</th>
<th>RIC (n=2546)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPCI procedure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMI flow grade at admission, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMI 0</td>
<td>1513 (67.9)</td>
<td>1478 (67.1)</td>
</tr>
<tr>
<td>TIMI 1</td>
<td>154 (6.9)</td>
<td>145 (6.6)</td>
</tr>
<tr>
<td>TIMI 2</td>
<td>218 (9.8)</td>
<td>225 (10.2)</td>
</tr>
<tr>
<td>TIMI 3</td>
<td>342 (13.4)</td>
<td>355 (16.1)</td>
</tr>
<tr>
<td>Symptom to balloon time (min)</td>
<td>177 (128 to 279)</td>
<td>178 (130 to 278)</td>
</tr>
<tr>
<td>FMC to balloon time (min)</td>
<td>102 (82 to 126)</td>
<td>103 (83 to 128)</td>
</tr>
<tr>
<td>Infarct-related coronary artery, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>974 (43.1)</td>
<td>911 (40.9)</td>
</tr>
<tr>
<td>Circumflex</td>
<td>297 (13.2)</td>
<td>298 (13.4)</td>
</tr>
<tr>
<td>Right coronary</td>
<td>985 (43.6)</td>
<td>1014 (45.6)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (0.1)</td>
<td>3 (0.1)</td>
</tr>
<tr>
<td>Vessels with clinically significant disease, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (0.0)</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>1</td>
<td>1314 (58.2)</td>
<td>1274 (57.2)</td>
</tr>
<tr>
<td>2</td>
<td>659 (29.2)</td>
<td>641 (28.8)</td>
</tr>
<tr>
<td>3</td>
<td>284 (12.6)</td>
<td>310 (13.9)</td>
</tr>
<tr>
<td>Stenting of culprit lesion, n (%)</td>
<td>2104 (93.2)</td>
<td>2080 (93.4)</td>
</tr>
<tr>
<td>Aspiration thrombectomy, n (%)</td>
<td>560 (24.8)</td>
<td>553 (24.8)</td>
</tr>
<tr>
<td>Supplementary staged PPCI, n (%)</td>
<td>291 (12.9)</td>
<td>261 (11.7)</td>
</tr>
<tr>
<td>Supplementary staged CABG, n (%)</td>
<td>52 (2.3)</td>
<td>62 (2.8)</td>
</tr>
<tr>
<td>TIMI flow grade after procedure, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMI 0</td>
<td>27 (1.2)</td>
<td>26 (1.2)</td>
</tr>
<tr>
<td>TIMI 1</td>
<td>20 (0.9)</td>
<td>9 (0.4)</td>
</tr>
<tr>
<td>TIMI 2</td>
<td>112 (5.0)</td>
<td>86 (3.9)</td>
</tr>
<tr>
<td>TIMI 3</td>
<td>2064 (92.8)</td>
<td>2079 (94.5)</td>
</tr>
<tr>
<td><strong>Medications given in relation to PPCI, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opioids*</td>
<td>549 (52.1)</td>
<td>522 (50.2)</td>
</tr>
<tr>
<td>Heparin</td>
<td>1904 (84.4)</td>
<td>1893 (85.1)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>2147 (95.2)</td>
<td>2129 (95.9)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>610 (27.0)</td>
<td>573 (25.7)</td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>1551 (68.7)</td>
<td>1554 (69.8)</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>103 (4.6)</td>
<td>97 (4.4)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>1758 (79.1)</td>
<td>1693 (77.5)</td>
</tr>
<tr>
<td>Glycoprotein IIb/IIa inhibitor</td>
<td>450 (20.0)</td>
<td>404 (18.2)</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>505 (22.4)</td>
<td>489 (22.0)</td>
</tr>
<tr>
<td>Cangrelor*</td>
<td>134 (12.3)</td>
<td>127 (11.7)</td>
</tr>
</tbody>
</table>

Table 3.4: Procedural Details in the ERIC-PPCI/CONDI-2 trial (ITT Analysis). Data are in mean (standard deviation) or median (interquartile range). (RIC: remote ischaemic conditioning; PPCI = primary percutaneous coronary intervention; TiMi: thrombolysis in myocardial infarction; FMC: first medical contact; CABG: coronary artery bypass grafting). *Data collected only in the CONDI-2 study.
Table 3.5: Primary and Secondary Outcomes in intention-to-treat population. *Data are Kaplan-Meier estimates of the n (%) of patients with the outcome at the specified timepoint (for time-to-event outcomes) or mean (SD; for event frequency outcomes). **Data are hazard ratios (for time-to-event outcomes) or ratio of means (for event frequency outcomes), for treatment group versus control group. (RIC = remote ischaemic conditioning; CI = confidence interval; HHF = hospitalisation for heart failure; MACCE = composite of all-cause death, re-infarction, unplanned revascularisation and stroke; ICD = implantable cardioverter-defibrillation).
Figure 3.3: Cumulative Incidence of Cardiac Death or HHF in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis).

Figure 3.4: Cumulative Incidence of Cardiac Death in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis).
Figure 3.5: Cumulative Incidence of HHF in the ERIC-PPCI/CONDI-2 trial at 12 Months (ITT Analysis).

Figure 3.6: Forest plot for prespecified subgroup analyses of the primary endpoint in the intention-to-treat population.
3.3.4 Secondary Outcomes

There was no difference seen between the control group (7.8% [n=197]) and the RIC group (8.4% [n=212]) with respect to MACCE within 12 months of follow-up [HR: 1.09; 95%; CI: 0.90 to 1.32; p=0.38] (Table 3.5). Furthermore, there was no difference between the control and RIC groups in the individual components of MACCE (Table 3.5). For combined cardiac death or HHF within 30 days and the individual components of cardiac death or HHF, there were no evidence for a difference between the control group and the RIC group (Table 3.5). Similarly, no differences between the study groups were observed for MACCE or the individual components after 30 days, for rates of ICD implantation within 12 months, for repeat episodes of HHF, or for episodes of repeat HHF plus cardiac death (Table 3.5).

3.3.5 Adverse Events

No unexpected adverse events relating to the RIC cuff application were reported. Skin petechiae, transient pain or paraesthesia are regarded as expected adverse events of RIC. Skin petechiae were reported in 72 patients (2.8% of the intervention arm), and transient pain or paraesthesia were reported in 147 patients (5.8% of the intervention arm). There were no withdrawals from the study due to adverse events.

3.4 Discussion

The CONDI-2/ERIC-PPCI study has shown that adjunctive RIC therapy conferred no incremental benefit to a composite of cardiac death and HHF at 12 months when compared to standard care alone, consisting of PPCI, in STEMI patients\textsuperscript{177}. This contradicts the evidence from previous small proof-of-concept studies and meta-analyses which have demonstrated significant improvements in infarct size, myocardial salvage index, MVO and LV ejection fraction in reperfused STEMI patients receiving adjunctive RIC.
therapy[37,178,179]. However, there were also some studies which were equivocal with respect to the effect of RIC on markers of adverse outcomes[44,48].

Various other cardioprotective strategies designed to reduce IRI have successfully demonstrated benefit when studied in the pre-clinical setting but clinical trials have failed to show a positive impact[34,35]. This could be explained by patient characteristics such as age, co-morbidity or drug therapy which can reduce the potential beneficial effects of preconditioning[156,159,180–183]. One such drug class which has been shown to impact on the cardioprotective action of RIC is P2Y12 receptor antagonists[183,184]. In our study cohort, the P2Y12 receptor antagonist ticagrelor was administered to 69.8% of patients in the RIC arm and this did not have any significant impact on clinical outcomes of RIC.

Another factor requiring consideration which can potentially influence the effect of RIC is pre-dilatation prior to angioplasty. Zhou & al have postulated that the potential beneficial effect of ischaemic postconditioning using repeated balloon inflations within the infarct-related artery can be reduced by planned balloon pre-dilatation and as a consequence patients undergoing direct-stenting benefit the most[185]. Procedural details regarding direct-stenting and balloon pre or post-dilatation were not collected as part of our study. Whether or not these procedural steps could explain a lack of benefit remains unknown.

Cuff application and initiation of the first inflation in relation to the timing of coronary reperfusion during the PPCI procedure could be a significant factor influencing the impact of RIC. This theory is however not supported by pre-clinical studies which have shown beneficial effects of RIC when completed prior to PPCI[37,41] or initiated prior to PPCI but with procedural overlap[40] or applied after onset of infarct-related coronary arterial reperfusion[47]. These findings are consistent with results from sub-group analyses of the ERIC-PPCI/CONDI-2 study data. There was no significant difference in outcomes.
between patients who completed the 4 cycles of RIC prior to arrival at the receiving PPCI centre and those who had RIC applied upon hospital arrival with onset of reperfusion in some patients occurring prior to completion of the RIC protocol. Patients who completed 4 cycles of RIC prior to coronary reperfusion did not have improved clinical outcomes as compared to those who did not.

A different approach of delivering RIC using the lower limbs has been suggested and investigated in multiple studies with mixed results\textsuperscript{44,46}. Data regarding the cardioprotective potential of RIC using larger peripheral muscle masses is equally inconclusive. There is evidence from studying cutaneous blood flow that upper limb RIC has greater potency in providing cardioprotection than lower limb RIC in humans\textsuperscript{186}. Rat models of IRI have shown that using both hindlimbs for remote preconditioning rather than one results in significantly smaller infarct mass\textsuperscript{187}. Another animal study by Johnsen \textit{& al.} highlights the complexities related to the optimal RIC application protocol\textsuperscript{188}. It found that tissue mass had no effect on cardioprotective potential. Variables such as number of RIC cycles and duration of ischaemic phase had an impact on overall degree of cardioprotection. For instance, using 6 cycles of RIC resulted in the largest reduction in infarct size, but this was not statistically significant as compared to 4 cycles. Such phase II studies in humans however are lacking.

Even though there was no benefit for clinical end-points at 12 months follow-up, there is data which indicates that RIC might have some benefit in reducing adverse cardiovascular outcomes with long-term follow-up. Despite not being prospectively designed nor powered to investigate clinical end-points, Sloth \textit{& al.} were able to show significant benefits with adjunctive RIC with lower rates of MACE and all-cause mortality more than a year after the index event. A similar improvement in clinical outcomes with reduced rates of cardiovascular death and HHF was also demonstrated by Gaspar \textit{& al.} This suggests that a prolonged follow-up period of more than 12 months might be required to
assess the potential benefit of RIC on clinical outcomes. It is interesting to note that the two studies which showed improved clinical outcomes at long-term follow-up (up to 4 years) did not show any reduction in acute enzymatic infarct size in the RIC arm\textsuperscript{37,39,41}. An additional strategy of applying daily RIC on day 3 following an acute STEMI has not shown to confer any significant benefit.

There is strong evidence that patients with smaller infarct size following a STEMI have a better clinical outcomes\textsuperscript{85,91}. The question that remains unresolved however is what degree of reduction of infarct size from an investigational therapy in smaller phase II studies with a selected group of patients will eventually translate into clinical benefit for the larger population in phase III trials. It is also worth noting that, conceptually, translation from smaller studies with acute infarct size quantification as endpoint to larger trials with long-term clinical outcomes as endpoint covers two phases: 1. the acute phase with focus on size of MI, MSI and presence of MVO, and 2. a long-term phase which involves, without or with infarct size reduction, infarct healing, repair, remodeling, presence of chronic inflammation and progression to heart failure. Even though it raises the possibility of a type 1 error in the previously published proof-of-concept studies, it remains plausible that RIC has a beneficial effect on longer term outcomes rather than markers of infarct size in the acute phase. This theory requires further consideration given that a recent study into the effects of post-conditioning with repeated angioplasty balloon inflation/deflation in reperfused STEMI patients demonstrated no beneficial effect on infarct size assessed using CMR but found improved LV remodeling, particularly in patients with MVO, at 1 year follow-up\textsuperscript{189}. This is however contradictory to various previous animal studies which describe a reduction in acute infarct size and the signaling pathways involved to help achieve it\textsuperscript{190}.

An emerging pattern is that patients with the largest area of ischaemia, such as those with TIMI 0 or I prior to angioplasty, benefit the most from RIC\textsuperscript{37,49}. It is therefore interesting to note that the proof-of-concept RIC studies did not
exclude patients with TIMI II/III prior to PCI. However, there was no difference in outcomes in pre-specified sub-groups of patients with TIMI 0/1 flow at admission or with LAD occlusion.

RIC has been regarded as a particularly attractive cardioprotective intervention, as there have been a number of positive pre-clinical and clinical proof-of-concept studies and it is an easily feasible, non-invasive, safe and inexpensive procedure. The translation from promising pre-clinical and clinical proof-of-concept studies on infarct size reduction to clinical trials with clinical outcome endpoints has however been largely disappointing. The conclusions from the CONDI-2/ERIC-PPCI dataset are similar to the findings from the ERICCA and RIPHeart studies in patients undergoing cardiac surgery whereby previous reports suggested that RIC does have significant benefit in reducing infarct size in phase II trials. In this instance, the use of peri-operative Propofol was thought to have nullified the effect of RIC.

3.5 Limitations

There were trial protocols variations between the CONDI2 and the ERIC-PPCI arms of the study. The most notable differences were that the CONDI2 arm did not have a sham procedure in the control group and that RIC was initiated in the ambulance (Denmark and Spain only) rather than upon arrival at the receiving centre. The primary outcome did not differ when patients were analysed in either arm of the study.

Despite being an all-comers study, only 0.8% of the patients recruited were in cardiogenic shock with the UK average in 2017-18 being 3.0% [British Cardiovascular Intervention Society Audit 2017-18; http://www.bcis.org.uk/wp-content/uploads/2019/02/BCIS-Audit-2017-18-data-for-web-ALL-excl-TAVI-as-27-02-2019.pdf]. Recruitment of this high-risk cohort has been an issue in
other large trials involving STEMI patients\textsuperscript{194}. This represents an ongoing difficulty in assembling a sample which is truly representative of the real-life STEMI population.

### 3.6 Future Directions

One major criticism of this and other equivocal RIC studies is that confounding factors such as co-morbidities and drug therapy have not been sufficiently addressed in the study design. The overall effect of these factors in affecting the value of RIC in human studies is unclear. Pre-specified subgroup analyses in our study showed that elevated age and diabetes had no impact on clinical outcomes of cardioprotective potential of RIC. A more selective approach to patient selection however could be considered in future studies, with emphasis on inclusion and exclusion criteria formulated to reflect findings of pre-clinical studies. Another proposed strategy could be to utilise more than 4 cycles of RIC in a pre-defined cohort of patients, though this would need validating in humans.

Although RIC has not shown any added benefit in the setting of PPCI and cardiac surgery, it remains to be seen whether it can be of value in various other clinical contexts such as renal transplantation, chemotherapy-related cardio-toxicity, cerebrovascular events or STEMI patients treat with thrombolysis which is common practice in many developing countries.

Given clinical trials in STEMI patients tend to recruit low-risk patients and only a have limited number of patients in cardiogenic shock, the effect of various cardioprotective strategies on the latter remains uncertain. This is of particular value as these patients have high in-patient mortality rates. RIC could also prove to be beneficial in cases of septic and haemorrhagic shock.
Further insight into the effects of RIC on the ischaemic myocardium will be available after completion of our CMR sub-study of this trial. This will enable understanding of the effects of RIC on myocardial oedema and microvascular damage. Enzymatic infarct size was not affected by RIC in published data from our study suggesting that focus on infarct size, which many recent CMR proof-of-concept studies have done, might not enable us to identify potentially effective therapies at an early stage.
Chapter 4
Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence
4.1 Introduction

Although timely reperfusion with primary angioplasty restores epicardial flow in most patients suffering from an acute STEMI, myocardial perfusion does not fully recover in some cases owing to the presence of microvascular injury, known commonly as the ‘no-reflow’ phenomenon\textsuperscript{125}. The pathophysiology of this is not fully understood but it is associated with MVO with disruption of the inter-endothelial junctions and, following reperfusion, extravasation of erythrocytes leading to IMH. Recent studies have shown that the presence of IMH on CMR is associated with increased risk of adverse outcomes\textsuperscript{119,137,142–144}, and could be used as a reliable surrogate marker. Additionally, therapeutic strategies aimed at preventing endothelial damage and IMH could be beneficial in the post-infarct phase.

Identifying the presence IMH accurately on CMR, however, can be technically challenging. Breath-held segmented T2* (BH-T2*) measurement is currently the best technique for assessing iron accumulation in the myocardial tissue, which is a consequence of haemoglobin degradation following IMH. More recent pixel-wise T2* mapping has the advantage of covering the entire field-of-view without the need for manual post-processing and reducing analysis time\textsuperscript{145}. This sequence is however quite noisy and produces significant respiratory motion artefacts. Sensitivity of the existing sequences is also an issue. \textit{Kumar \\& al.} showed that of the 40 segments positive for haemorrhage on histological triphenyltetrazoliumchloride (TTC) staining, T2*-weighted imaging positively identified only 37 segments on day 3 post-infarct imaging\textsuperscript{146}.

A free-breathing respiratory motion-corrected (FB-T2*) single shot sequence with averaging has been recently developed to improve the signal to noise ratio and reduce ghost artefacts as compared to the breath-held sequence. Initial work included patients with iron-deposition disorders such as thalassaemia and haemochromatosis only\textsuperscript{135}. We therefore aim to study the
diagnostic performance of this free-breathing sequence against the conventional breath-held T2* mapping sequence in a cohort of reperfused STEMI patients.

4.2 Methods

4.2.1 Study Population
Forty-three STEMI patients undergoing PPCI were prospectively recruited (as part of the ERIC-PPCI study, The Barts Heart Centre, St Bartholomew’s Hospital, London, UK) between November 2015 and March 2018. The main inclusion criteria, in addition to those of the ERIC-PPCI study, were TIMI 0 flow in the infarct-related artery on diagnostic coronary angiography at presentation. The main exclusion criteria, in addition to those of the ERIC-PPCI study, were as follows: significant claustrophobia; allergy to gadolinium chelate contrast; severe renal insufficiency with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m²; presence of MRI contraindications such as implanted devices (e.g. pacemaker or other implanted cardiac devices, cochlear implant); embedded metal objects such as shrapnel or any other contraindication for CMR. The study was approved by the UK Research Ethics Committee as part of the ERIC-PPCI study.

4.2.2 CMR Acquisition
CMR imaging was performed six months after a STEMI utilising a 32-channel cardiac coil at 1.5-Tesla (Magnetom Aera, Siemens Healthineers, Germany) and were electrocardiogram gated. The entire LV short-axis stack (with 2mm gaps between slices, 8-10 acquisitions in total) was acquired for the following sequences: T2 map, FB-T2* map, T1 map, and early (EGE) and late (LGE) gadolinium enhancement. Immediately ensuing the FB-T2* stack, at base, mid and apical levels, three images were chosen for repeat acquisition using the BH-T2* sequence. The order of acquisition was therefore not random. The
choice of the FB-T2* image selected for repeat acquisition and comparison was at the discretion of the operator.

4.2.2.1 BH-T2* Mapping
The dark-blood preparation based multi-echo gradient recalled echo T2* sequence with curve fitting and integrated image reconstruction yielding a colour pixel-wise map was used$^{145,195}$. The following imaging parameters were utilised: bandwidth 814 Hz/pixel; echo times (ms) - 2.2, 4.1, 6.0, 8.0, 9.9, 11.9, 13.8, 15.7; flip angle 20°; matrix size 256x115; and slice thickness 8 mm. Three LV short-axis images were acquired at base, mid and apical levels.

4.2.2.2 FB-T2* Mapping
The FB-T2* sequence uses multi-gradient GRE similar to the BH sequence and is described in detail by Kellman & al$^{135}$. In brief, the acquisition is based on respiratory motion correction and single-shot multiple repetition imaging with selective averaging (doubling the number of acquisitions and discarding 50% of images) to enhance signal-to-noise ratio. Curve fitting is performed in a pixel-wise manner by the scanner using the Gadgetron framework$^{196}$. Typical imaging parameters were as follows: bandwidth 1080Hz/pixel; echo times (ms) - 1.2, 2.9, 4.6, 6.2, 7.9, 9.6, 11.3, 13.0; flip angle 18°; matrix size 160x92; and slice thickness 8mm. A full LV short-axis stack was acquired.

4.2.2.3 T2 Mapping
Three images were acquired using different T2 preparation times, with motion correction and processing to fit the T2 decay curve at each pixel producing a T2 map. Typical imaging parameters were as follows: flip angle 70°; pixel bandwidth 1184Hz/pixel; matrix size128x92; echo time 1.35ms; repetition time 3 x RR-interval; and slice thickness 8mm. A full LV short-axis stack was acquired.
4.2.2.4 Native T1 Mapping

Native T1 maps were acquired with a motion-corrected Modified Look-Locker Inversion (MOLLI) recovery sequence during a single breath-hold using a 5(3)3 sampling protocol\textsuperscript{197}. Typical imaging parameters were as follows: flip angle 35°; pixel bandwidth 1085Hz/pixel; matrix size 256x144; echo time 1.12ms; and slice thickness 8mm. Curve fitting is performed on a pixel-wise basis using the inversion times measured at various time points on the recovery curve to produce a pixel-map of T1. A full LV short-axis stack was acquired.

4.2.2.5 Gadolinium Enhanced Imaging

Early gadolinium enhanced images were acquired 5 minutes after injection of 0.1mmol/kg of gadobutrol (Gadovist, Bayer AG, Leverkusen, Germany). Two different sequences were used for post-contrast image acquisition. A phase-sensitive inversion recovery (PSIR) sequence with respiratory motion correction and single shot steady state free precession (SSFP) readout was used for EGE in most patients. Typical imaging parameters were as follows: bandwidth 1184Hz/pixel; echo time 1.06ms; repetition time 700ms; flip angle 40°; matrix size 116x192; and slice thickness 8 mm. The second sequence involved acquisition of free-breathing PSIR images using a single shot balanced SSFP sequence with MOCO averaging of multiple measurements\textsuperscript{106}. Typical imaging parameters were as follows: bandwidth 975Hz/pixel; echo time 1.14 ms; repetition time 918ms; flip angle 50°; matrix size 144x256; and slice thickness 8 mm. Inversion time was set between 400-450ms. Late gadolinium enhanced image acquisition was performed 15 minutes following contrast administration using the MOCO sequence in all patients. Inversion time was set between 300-350ms. Full LV short-axis stacks were acquired for both EGE and LGE imaging.
4.2.3 Quality Analysis

The BH and FB-MOCO T2* sequences were independently analysed for image quality and for presence of IMH. Quality assessment was performed using the 5-point scale previously described by Kellman & al.\textsuperscript{135}: 1 - very poor image quality; 2 - average image quality, not all of segment clearly seen and a lot of artefact; 3 - good image quality with moderate artefact; 4 - very good quality with minimal artefacts; 5 - excellent image quality with no significant artefact. A quality score of 1 was deemed to be of poor diagnostic quality and unusable. A quality score of 2 was deemed be usable but of moderate diagnostic quality. A quality score of $\geq$3 was deemed to be of good quality and high diagnostic value. T2* images from a randomly selected cohort of ten patients were analysed by a second experienced observer (Rohin Francis) to assess for inter-observer variability and were re-analysed by the first observer for intra-observer variability. Both observers were unblinded to the sequences being analysed.

4.2.4 Intramyocardial Haemorrhage

Each segment was assessed for presence or absence of IMH. Areas of hypointensity were manually demarcated. The mean and minimum T2* measurements of each ROI were noted. A mean T2* value $<$20ms was used for the diagnosis IMH. The size of any IMH present was not measured.

4.2.5 Statistical Analysis

Statistical analysis was carried out using SPSS Version 25 (IBM Corporation, USA). T2* map quality score data was expressed as mean $\pm$ standard deviation. Sample means for the two protocols were compared using the Mann-Whitney U test. Chi-square test was used to assess the significance of differences in frequencies for scores between the 2 sequences. The strength of linear association between two variables was assessed by Spearman's rank-order correlation.
4.3 Results

Forty-three STEMI patients were successfully scanned at 2±1 days following presentation and reperfusion therapy. Baseline characteristics (Table 4.1) and procedural features (Table 4.2) are detailed below. T2* maps were acquired for all patients using the 2 different protocols along with T1 maps, T2 maps, and EGE and LGE images. The FB-T2* apical slice (4 segments) was missing for 1 patient from the data set and hence not included for analysis. Using the American Heart Association (AHA) 17-segment LV model\(^{198}\) (excluding the 4 apical segment), 684 segments of BH-T2* and FB-T2* images were analysed.
### Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.0 ± 10.5</td>
</tr>
<tr>
<td>Male Gender (%)</td>
<td>90.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 4.4</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7.1</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>45.2</td>
</tr>
<tr>
<td>Previous</td>
<td>21.4</td>
</tr>
<tr>
<td>Non</td>
<td>33.4</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>39.0</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>33.3</td>
</tr>
<tr>
<td>Positive Family History (%)</td>
<td>38.4</td>
</tr>
</tbody>
</table>

Table 4.1: Baseline patient characteristics. (BMI: body mass index).

#### 4.3.1 T2* Mapping Quality Score

The mean quality score for the BH-T2* and FB-T2* protocols were 4.1±1.3 and 4.9±0.6 respectively (p<0.0001). Across the BH-T2* segments, 62 (9.0%) were found to be unusable (quality score 1) as compared to 4 (0.6%) of the FB-T2* segments (p<0.0001). FB-T2* mapping produced a significantly higher proportion of excellent images with no significant artefact (86.2% vs 57.7%; p<0.0001).

Mean quality score analysis for each segment indicates that image quality was variable across various segments for both sequences (Figure 4.2). This difference was more pronounced when using BH-T2* sequences for image acquisition. The inferolateral segments had a higher tendency to produce artefacts as compared to the septal segments for both the BH and FB sequences. The mean quality score for the basal and mid inferolateral
segments was 3.5 as compared to 4.4 for the basal and mid anterolateral segments for the BH-T2* sequence (p<0.0001) and 4.6 and 4.9 respectively for the FB-T2* sequence (p<0.0001).
### Procedural Characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMC-balloon time (mins)</td>
<td>99.0 ± 35.8</td>
</tr>
<tr>
<td>Culprit vessel (%)</td>
<td></td>
</tr>
<tr>
<td>LMS</td>
<td>0</td>
</tr>
<tr>
<td>LAD</td>
<td>41.9</td>
</tr>
<tr>
<td>LCx</td>
<td>20.9</td>
</tr>
<tr>
<td>RCA</td>
<td>37.2</td>
</tr>
<tr>
<td>TIMI flow pre-procedure</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>95.8</td>
</tr>
<tr>
<td>I</td>
<td>2.1</td>
</tr>
<tr>
<td>II</td>
<td>2.1</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
</tr>
<tr>
<td>Peri-procedural drugs (%)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>97.7</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>4.6</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>0</td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>86.0</td>
</tr>
<tr>
<td>Heparin</td>
<td>93.0</td>
</tr>
<tr>
<td>Gp IIb/IIIa</td>
<td>34.9</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>0</td>
</tr>
<tr>
<td>Stent deployment (%)</td>
<td>97.6</td>
</tr>
<tr>
<td>TIMI flow post-procedure (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>9.3</td>
</tr>
<tr>
<td>III</td>
<td>92.7</td>
</tr>
<tr>
<td>By-stander disease requiring staged procedure (%)</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Table 4.2: Procedural details. (FMC: first medical contact; TIMI: thrombolysis in myocardial infarction; LMS: left mains stem; LAD: left anterior descending; LCx: left circumflex; RCA: right coronary artery).
Figure 4.1: Quality score for BH-T2* mapped and FB-T2* mapped (n=684) segments.

Figure 4.2: Mean quality scores for each segment for BH-T2* (Panel A) and FB-T2* (Panel B) sequences.
4.3.2 Inter and Intra Observer Variability

Quality score analysis for the BH-T2* and FB-T2* sequences were performed by a second observer in ten patients to assess for inter-observer variability. In this cohort, mean quality scores for observer 1 were $3.3 \pm 1.5$ and $4.6 \pm 0.9$ for the BH-T2* and the FB-T2* protocols respectively. Scores for observer 2 were $3.2 \pm 1.2$ and $4.4 \pm 0.9$ for the BH-T2* and the FB-T2* protocols respectively. There was very good correlation between the two sets of mean quality scores for each segment between the two observers for both the BH-T2* ($r=0.92$) and FB-T2* ($r=0.89$) sequences (Figure 4.4). Observer 1 scored the FB-T2* method by an average of 1.3 higher than the BH-T2* method and observer 2 by an average of 1.2 higher. For the BH images, 30 segments out of 160 were scored 1 (poor diagnostic quality and unusable) by observer 1. Observer 2 allocated a score of 1 to 16 segments only. Fifteen of these were also allocated a score of 1 by the observer 1. For the FB images, both observers allocated a score of 1 to 7 segments out of 160. Both observers found the same 7 segments to be unusable.

The same sample was reassessed by observer 1 at a later date for intra-observer variability. There was good correlation between the two sets of
scores from for both the BH-T2* (3.3 ± 1.5 vs 3.2 ± 0.4; r=0.98) and the FB-T2* (4.6 ± 0.9 vs 4.5 ± 0.2; r=0.96) sequences.
4.3.3 Acquisition Time

The mean time taken to plan and acquire three short-axis BH-T2* images was 152.0 seconds (SD: 66.8 seconds). The mean time taken to plan and acquire a stack covering the entire LV was 169.1 seconds (SD: 103.4 seconds). This difference was not statistically significant (p=0.992).

4.3.4 Pick-up Rate of IMH using FB-T2* and BH-T2* Mapping

The presence of IMH was identified in 16 patients (37.2%) using the BH-T2* sequence. The FB-T2* sequence identified IMH in 15 patients only (34.8%). One patient had a small area of IMH in the basal inferior segment on the BH sequence (Figure 4.5). There was a corresponding area of MVO on early post-contrast imaging and T2 mapping showed a hypo-intense core surrounded by
an area of elevated T2 values. Mean and minimum BH-T2* readings in the IMH area were 19.6ms and 15.8ms respectively. The corresponding mean and minimum T2* readings using the FB sequence were 27.5ms and 20.3ms respectively. No evidence of IMH was identified on any of the short-axis slices using the novel FB-T2* mapping for this patient.

On a segmental basis, IMH was identified in 48 segments using the BH-T2* sequence and 43 segments using FB-T2* mapping. Comparing the T2* measurements of the hypo-intense core in the 48 segments, the mean BH-T2* readings were significantly lower than the mean FB-T2* readings (14.6 vs 16.8, p=0.01). On average, BH-T2* measurements were 2.2ms lower than the corresponding FB measurements (Figure 4.6). The FB-T2* sequence has a tendency to overestimate the T2* mapping values. There was moderate correlation between the 2 sets of measurements (r=0.61, p<0.0001).
Figure 4.5: Patient with IMH on BH sequence only. EGE imaging shows features of MVO in mid inferior segment (Panel A). T2 mapping shows a hypo-intense core in the corresponding segment (Panel B). IMH (red arrow) is present in the BH T2* sequence (Panel C) but not in the FB sequence (Panel D). Signal artefacts (blue arrow) are present in both the BH and FB sequences. (EGE: early gadolinium enhanced; MVO: microvascular obstruction; IMH: intramyocardial haemorrhage; BH: breath-held; FB: free-breathing)
Figure 4.6: Bland-Altman plot of mean BH and FB T2* measurements against the difference (BH minus FB). The mean and the upper and lower limits of agreement are represented by the dotted lines. (ULOA: upper limit of agreement; LLOA: lower limit of agreement).
Figure 4.7: Scatter plot of BH-T2* versus FB-T2* measurements of areas with intramyocardial haemorrhage (BH-T2* measurement of <20ms). The dotted line on the left represents the best fit with intercept set at the origin (0,0). The dotted line on the right represents identity line. (BH: breath-held; FB: free-breathing; r=0.61 using Spearman’s Rho analysis).
Figure 4.8: Segmental analysis of diagnostic performance of BH (A1-D1) and FB (A2-D2) T2* sequences. The BH-T2* sequence identified more segments with IMH than the FB-T2* sequence.
4.3.5 Post-contrast Imaging and MVO

EGE images were missing for 1 patient at mid and apical ventricular levels (10 segments). LGE images were missing for another patient at base and mid ventricular levels (12 segments). Early MVO was present in 118 out of 678 segments. Of those, 92 segments also showed presence of late MVO. Seven segments with late MVO were identified in the patient with missing EGE dataset. In total, 99 out of 676 (14.4%) segments showed presence of late MVO. The patient with missing LGE dataset did not show any evidence of MVO in any of the acquired EGE or LGE images. Overall, 60.5% of patients were found to have MVO on LGE imaging.

4.3.6 T1 and T2 Mapping

T2 mapping data was missing for 1 patient at apical short-axis level (4 segments). Elevated T1 values with a hypointense core was identified in 135 segments analysed. T2 mapping identified 113 segments (n=684) with a hypointense core. Twenty-eight segments on T1 mapping (4.9%) and 13 segments on T2 mapping (2.3%) with a hypointense core hid not have MVO on LGE. Five segments (5.1%) with late MVO had a hypointense core on T1 mapping but not T2 mapping. None of these segments demonstrated the presence of IMH on T2* mapping.
4.4 Discussion

The FB-T2* mapping sequence consistently yielded diagnostic quality images with less than 1% of images discarded for poor quality. Data acquisition and interpretation is more reliable. There were fewer artefacts than the current BH-T2* maps which produced 9% unusable images. This is comparable to data from Carrick & al. which reported that, using a similar sequence, 14% of a cohort of 286 reperfused STEMI patients had unusable T2* maps due to severe motion artefacts\textsuperscript{137}. Bulluck & al. excluded 24% of acquisitions due to motion and off-resonance artefacts out of a sample of 40 patients\textsuperscript{119}. It is noteworthy that 25% of breath-held mapping images were found to be unusable in patients with iron overload cardiomyopathies\textsuperscript{135}. Both observers agreed that 7 segments from the FB-T2* sequences were unusable. For the BH-T2* images however, agreement on poor quality was reached with 15 segments only and either observer deemed 16 segments unusable. The process is subjective, but such a significant discrepancy suggests that artefacts on BH-T2* maps are more difficult to assess.

Inferolateral segments were found to be more susceptible to artefacts. To my knowledge, this finding has not been previously reported in T2* mapping in literature. This could be due to chemical shift artefacts secondary to the presence adipose tissue adjacent to the lung-liver interface which more prominently affects T2 imaging\textsuperscript{199,200}. It is nonetheless interesting to note that interregional variability assessment using a conventional T2* mapping sequence from the same vendor demonstrated that inferolateral segments in healthy volunteers have a significantly lower T2* readings than the anteroseptal segments (31±6 vs 37±4, p<0.01)\textsuperscript{201}. This finding could help explain an increased predisposition of the inferolateral segments to artefacts as, in my study, the latter tended to exhibit themselves as regions with low T2* (<20ms) measurements (Figure 4.3). In addition, investigators assessing regional variations in other mapping techniques have suggested that the
segmental differences are unlikely to represent a genuine regional disparity and are rather a related to numerous confounding factors such as magnetic susceptibility artefacts and the distances of the heart segments from the magnetic coils\textsuperscript{202,203}. These factors could play a part in explaining variations in quality score of the different segments on T2* mapping.

The entire LV can be scanned using the FB-T2* sequence with little added time rather than only acquiring 3 short-axis images with the BH-T2* sequence. The latter is currently common practice in various clinical studies\textsuperscript{119,137,138} as many post-infarct patients are unable to tolerate prolonged episodes of repeated breath-hold. This raises questions about the accuracy of IMH detection and quantification. Studies assessing prognosis and treatment effect could yield different findings if the presence of IMH within the entire ventricular mass is assessed using the FB-T2* sequence.

The ability of these two mapping techniques to identify IMH could be a factor which helps explain a pick up rate towards the lower end of the reported range. MVO was present in 60.5% of patients which is consistent with rates of 44-65% reported in previous studies\textsuperscript{99,119,137}. Overall, IMH was present in 36.0% of cases. Recent studies using T2* mapping have identified IMH in 23-58% of patients scanned following an acute infarction\textsuperscript{119,137,138,204}. T2-weighted imaging, commonly used in previous studies, could positive identify IMH in 25-54% of reperfused STEMI patients with a sensitivity of 82% compared to 100% for T2* imaging\textsuperscript{204}. Animal studies also support the notion that T2* mapping is more suitable for IMH imaging that T2-based modalities\textsuperscript{120,146,205}.

My study demonstrated that there is a discrepancy in IMH pick-up rate when using the FB-T2* sequence as compared to the BH-T2* sequence in contrast to the findings from the Kellman & al.\textsuperscript{135} study which did not investigate MI patients and included thalassaemia patients only.
The FB-T2* sequence has a tendency to underestimate the presence and severity of IMH as compared to the BH-T2* sequence. One possible explanation for the difference in the presence of IMH on segmental analysis could be positional as the BH sequence is acquired on expiration and the FB sequence is acquired throughout the respiratory cycle. A potential solution to mitigate this issue could be using a higher cut-off, a T2* value of 25ms for instance, when utilising the FB-T2* sequence for defining the presence of IMH. This would however need validating.

The T2* readings using the two different sequences are mostly within the upper and lower limits of agreement with moderate correlation. These findings suggest that the FB sequence can be utilised for assessment of the presence of IMH. The operator however needs to be aware that despite more diagnostic quality images, there is a potential for under diagnosing the extent and severity of IMH.

4.5 Limitations and Future Directions

Image acquisition was performed at median 2 days following presentation, as opposed to day 3 post-infarct, which is not optimal for T2* imaging in MI patients as suggested by Bulluck & al65. This could be the reason why 52% of segments with late MVO and hypointense core on T2 mapping did not show the presence of IMH on T2* mapping.

The patients included in the analysis were randomised to RIC or sham procedure and this could have potentially affected the findings.

The order of acquisition of the FB and BH sequences was not random. The choice of the FB-T2* image selected for repeat acquisition and comparison was at the discretion of the operator. Given the obvious differences in images
produced, quality score analysis was not blinded. These factors could have introduced bias.

The matrix sizes utilised for the two sequences were different (BH-T2*: 256x115 and FB-T2*: 160x92). This would have impacted on signal-to-noise ratio and basic resolution. These parameters were selected as they were used in standard practice at The Barts Heart Centre.

The susceptibility of T2* mapping for signal drop-outs (Figure 4.5) could also explain some of the discrepancies in segmental pick-up rate of IMH.

This study did not correlate MRI findings with histological analysis for obvious reasons. This is of particular importance given a significant difference in the mean T2* measurements using the BH and FB sequences. Future studies can aim to correlate with histological findings in animal infarct models. Using higher reference T2* values for the diagnosis of IMH by the FB sequence will improve sensitivity and, given the higher quality of the FB-T2* maps, diagnostic accuracy can be improved. This would need to be validated in animal models and correlated with outcome in clinical studies.
Chapter 5
Comparing Infarct Size Quantification
Using Four Different Post-contrast
CMR Sequences
5.1 Introduction

The degree of myocardial injury following acute myocardial infarction is an important prognostic indicator\(^{147,148}\). Quantification of MI size has been shown to predict adverse outcomes in MI and heart failure patients and hence have a key role in prognostication and for tailoring treatment\(^93\). Various biochemical and imaging modalities exist to enable quantification of myocardial scarring\(^206\). Post-contrast CMR imaging is currently regarded as the gold standard method for MI size quantification and has the ability to accurately demarcate between viable and non-viable myocardial tissue non-invasively\(^{111,149,207}\). Animal studies have demonstrated good correlation between the extent of LGE and fibrosis on histocytological analysis\(^{208,209}\). In humans however, various technical issues make imaging acquisition and analysis difficult resulting in discrepancies in MI size quantification\(^{150,151}\).

One of the factors affecting infarct size analysis is the pulse sequence utilised to delineate between infarcted and normal myocardium. The wide variety of sequences studied since the introduction of CMR imaging have shown significant differences in overall volume of late enhancement measured\(^{100}\). The contrast-enhanced inversion recovery turbo fast low angle shot (FLASH) sequence has been shown to produce the greatest increase in infarcted myocardial signal intensity in both animal and human models in early studies and hence been widely utilised as the reference standard\(^{72,101–103}\).

Another commonly used sequence is the IR prepared balanced single shot steady state free precession (bSSFP) sequence which has a higher contrast-to-noise ratio than FLASH imaging and hence a single image is acquired without the need for averaging\(^{100,105}\). This makes the process speedy, being useful when imaging uncooperative patients who struggle to hold their breaths, and provides a reasonable compromise between spatial and contrast resolution. A novel sequence using a single shot balanced SSFP sequence
with motion correction (BB-MOCO) averaging of multiple measurements is now widely being used\textsuperscript{106}. The acquisition time is shorter than the standard breath-held sequences with the added benefit of producing images of higher diagnostic quality and of improving user confidence\textsuperscript{109,110}.

Conventional bright blood (BB) LGE imaging provides very good delineation between the bright infarcted tissue, with reduced contrast wash-out, and the normal dark myocardial tissue\textsuperscript{111}. Demarcation between the bright infarcted tissue and the bright blood pool can however be limited in many circumstances resulting in difficulties in accurately quantifying infarct size. The novel dark blood IR sequence with T2 prep described by Kellman & al. helps address this issue\textsuperscript{106,112}.

The aim of this study is to compare the size of chronic infarct measured using four different LGE imaging sequences in a cohort of STEMI patients, using the FLASH as standard for comparison.

### 5.2 Methods

#### 5.2.1 Study Population

Twelve STEMI patients undergoing PPCI were prospectively recruited (as part of the ERIC-PPCI study at St Bartholomew’s Hospital, London) between November 2015 and March 2018. The main inclusion criteria were TIMI 0 flow in the infarct-related artery on diagnostic coronary angiography at presentation. The main exclusion criteria were as follows: significant claustrophobia; allergy to gadolinium chelate contrast; severe renal insufficiency with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m\textsuperscript{2}; presence of MRI contraindications such as implanted devices (e.g. pacemaker or other implanted cardiac devices, cochlear implant); embedded metal objects such as shrapnel or any other contraindication for CMR. The
CMR scans was performed at 191±20 days post infarct. The study was approved by the UK Research Ethics Committee.

5.2.2 CMR Acquisition

CMR imaging was performed utilising a 32-channel cardiac coil at 1.5-Tesla (Magnetom Aera, Siemens Healthineers, Germany) and was electrocardiogram gated. Single-slice LGE LV short-axis images, selected at the discretion of the operator, were acquired in random order at mid-level 15-25 minutes after injection of 0.1mmol/kg of gadobutrol (Gadovist, Bayer AG, Leverkusen, Germany) using each of the four sequences described below. Where required, inversion time to null the myocardium was set between 300-400ms. An inversion time (TI) scout was not performed.

5.2.2.1 Balanced Single Shot Steady State Free Precession (bSSFP) Imaging

A phase-sensitive inversion recovery (PSIR) sequence with respiratory motion correction and single shot steady state free precession readout was used. Typical imaging parameters were as follows: bandwidth 1184Hz/pixel; echo time 1.06ms; repetition time 700ms; flip angle 40°; matrix size 116x192; and slice thickness 8 mm.

5.2.2.2 Fast Low-angle Shot (FLASH) Imaging

A standard segmented fast low-angle shot (FLASH) two-dimensional inversion recovery gradient echo sequence was used. Typical imaging parameters were: bandwidth 140 Hz/pixel; echo time 3.16 ms; repetition time 946 ms; flip angle 23°; acquisition matrix 125x256; and slice thickness 8 mm.

5.2.2.3 Bright-blood Motion Corrected (BB-MOCO) Imaging

This sequence consisted of acquisition of free-breathing PSIR images using a single shot balanced SSFP sequence with MOCO averaging of multiple measurements. Typical imaging parameters were as follows: bandwidth...
975Hz/pixel; echo time 1.14 ms; repetition time 918ms; flip angle 50°; matrix size 256x144; number of averages 8; and slice thickness 8 mm.

**5.2.2.4 Dark-blood Motion Corrected (DB-MOCO) Imaging**

Detailed parameters of this novel LGE sequence has been described in previous articles\textsuperscript{106,112}. In brief, a T2 preparation was added between the IR preparation and the readout with subsequent respiratory motion correction averaging. As a result, myocardial null time relative to blood is altered. Delay parameters can therefore be selected to null both the myocardium and blood and following PSIR reconstruction, blood appears darker than myocardium. Unlike standard post-contrast imaging modalities which require one inversion time parameter to null the myocardium, the DB-MOCO sequence needs 3 parameters. These are calculated by the sequence after input of myocardial and blood T1 values measured using a motion corrected modified look-locker inversion recovery (MOLLI) sequence. Typical imaging parameters were as follows: bandwidth 1085Hz/pixel; flip angle 50°; matrix size 256x144; number of averages 16 and slice thickness 8 mm.

**5.2.3 Quantitative Image Analysis**

The sequences were independently analysed for the presence and size of subendocardial LGE in the infarcted myocardium using CVI42 Version 5.10 (Circle, Calgary, Canada). Epicardial and endocardial borders were manually traced. Manual contouring was used to delineate the infarct region identified by increased signal intensity as compared to the remote unaffected myocardium. Hypo-enhanced core within area of hyperenhancement, indicative of MVO, were included for the infarct size calculation. The infarct area was quantified as the LGE mass expressed as a percentage of the LV mass for each segment. Myocardial mass (expressed in grams of tissue) was calculated as: measured area (cm\(^2\)) x slice thickness (cm) x myocardial density (1.05 g/cm\(^3\)). Special care was taken to avoid including papillary muscle enhancement particularly when interpreting DB-MOCO images.
5.2.4 Statistical Analysis

A pilot study of 12 chronic infarct patients was performed to provide further information for sample size calculations for future studies. Statistical analysis was carried out using SPSS Version 25 (IBM Corporation, USA). Sample means for the sequences were compared using the Wilcoxon signed-rank test. The strength of linear association between two variables was assessed by Spearman’s rank-order correlation. Bland-Altman plots will be used to compare values from the various measurement modalities. The FLASH sequence was used as the gold standard method. A repeat analysis of MI was performed to assess for intra-observer variability.

5.3 Results

Twelve STEMI patients were successfully scanned at 191±20 days following presentation and reperfusion therapy. Mid-level LGE images were acquired using the four sequences. The mean infarct size using the reference FLASH sequence was 18.5±16.2% of LV. There was no statistical difference in the mean infarct sizes measured using the bSSFP (17.9±16.0% vs 18.5±16.2%; p=0.885), BB-MOCO (18.7±16.4% vs 18.5±16.2%; p=0.977) and DB-MOCO (20.6±16.1% vs 18.5±16.2%; p=0.544) sequences as compared to FLASH sequence (Figure 5.1). In 11 out of 12 patients, the DB-MOCO sequence identified a numerically larger infarct mass as compared to the FLASH sequence (Figure 5.2). In 2 patients, the presence of high signal intensity was identified in the non-remote myocardium using the DB-MOCO sequence which were not identified using the bSSFP, FLASH or BB-MOCO sequences. There was strong correlation between infarct mass measured using the FLASH sequence and bSSFP (r=0.993), BB-MOCO (r=1.000) and DB-MOCO (r=0.954) sequences (Figure 5.4). The manual contouring method of infarct size measurement was reproducible for all four sequences with no significant intra-observer variability (bSSFP: 17.9±16.0% vs 17.7±16.1%, p=0.331;
FLASH: 18.5±16.2% vs 18.6±16.1%, p=0.878; BB-MOCO: 18.7±16.4% vs 18.5±16.2%, p=0.382; DB-MOCO: 20.6±16.1% vs 20.5±16.1%, p=0.132).
Figure 5.1: Mean percentage infarct size as measured using the four different sequences with manual contouring. Using FLASH (18.5±4.7%) as standard for comparison, no significant difference was identified as compared to bSSFP (17.9±4.6%, p=0.885), BB-MOCO (18.7±4.8%, p=0.977) and DB-MOCO (20.6±4.7%, p=0.544). Percentage LV mass reported as mean±standard error of mean.
Figure 5.2: Late-gadolinium enhancement and method of manual contouring used for the bSSFP (A1-2), FLASH (B1-2), BB-MOCO (C1-2) and DB-MOCO (D1-2) sequences.
Figure: 5.3: Infarct size for the 12 patients as measured using the four different sequences. In 11 patients, DB-MOCO identified a larger infarct mass as compared to FLASH. In 2 patients, the presence of high signal intensity was identified in the non-remote myocardium using the DB-MOCO sequence which were not identified using the bSSFP, FLASH or BB-MOCO sequences.
Figure 5.4: Relationship between infarct size measured using various modalities using the FLASH sequence as standard. There is good correlation between infarct size measured using FLASH as compared to bSSFP BB-MOCO and DB-MOCO. Bland-Altman analysis shows the degree of agreement between the FLASH sequence and the bSSFP (ULA 3.0%; LLA -1.8%), BB-MOCO (ULA 0.8%; LLA -1.2%) and DB-MOCO (ULA 1.7%; LLA -5.9%) sequences. (----- mean; ----- upper [ULA] and lower [LLA] limits of agreement)
5.4 Discussion

The conventional FLASH post-contrast method is the most routinely used sequence, but it is susceptible to artefacts due to arrhythmias and inability of patients to breath-hold adequately\textsuperscript{72,102,103,107}. In many such cases, images are of non-diagnostic quality and patients may require repeat scanning. In this study, infarct size was assessed using four different sequences (FLASH, bSSFP, BB-MOCO and DB-MOCO) with the FLASH method as reference standard. To our knowledge, this is the first study which compares infarct size using these four sequences in patients with previous ST-elevation myocardial infarction. This is of particular relevance with the increasing availability of free-breathing sequences in clinical practice\textsuperscript{106–108}.

This is primarily a pilot study which demonstrated that the four different sequences used for infarct size analysis yielded similar readings. There is therefore a possibility that these sequences can be used interchangeably for clinical and research purposes. Good correlation between LGE size using FLASH and the BB-MOCO sequences has recently been reported by Fan \textit{et al.} in study of 40 patients with mixed pathologies ($r^2=0.984$, $p<0.001$)\textsuperscript{107}. Similar findings between these two sequences were previously reported by Piehler \textit{et al.}\textsuperscript{210} in a study where 41 patients with mixed pathologies had LGE quantification.

The DB-MOCO sequence had a tendency to identify larger areas of hyperenhancement than any of the other three sequences studied. It is worth noting that this difference was not statistically significant, and this may have been because the study was performed with a small sample size. In two patients, the DB-MOCO sequence identified subendocardial LGE in an area supplied by the infarct-related artery which were absent in images acquired using the other three sequences. These findings are consistent with previously reported data by Francis \textit{et al.}\textsuperscript{106} who demonstrated that the DB-MOCO
sequence identified significantly more segments with LGE than the BB-MOCO sequence (12.8% vs 9.0%; p< 0.05) in a cohort of 172 patients with mixed pathologies. There are various clinical scenarios in which detection of subendocardial LGE could potentially lead to a change in management, one of those being the long-term management of patients with acute coronary syndromes in the presence of unobstructed coronary arteries. The DB-MOCO sequence however requires an extra T1 mapping sequence to be performed for the measurement of myocardial and blood T1 values meaning that acquisition time is increased. It is also worth noting that LGE acquisition more 20 minutes after contrast administration is optimal for detecting small subendocardial infarcts as the blood-pool appears less enhance than if images are acquired sooner.

Accurate and reproducible infarct size quantification after image acquisition can be challenging. The two major issues are detection of the subendocardial border and delineating the normal myocardium in the peri-infarct zone. There are automated and semi-automated infarct size measuring software that help with the latter, but these still rely on manually tracing the endocardial and epicardial borders. The merits of these various tools have been investigated elsewhere but consensus on the optimal technique is lacking113. This study presented a unique challenge as the existing semi-automated infarct measuring tools, such as FWHM (full-width half max) and 5-SD (5-standard deviation), were developed for analysis of bright blood images and none have been validated using the DB-MOCO sequence. Their use for analysis of DB-MOCO images yielded ambiguous measurements and hence cannot be recommended at present.
5.5 Limitations

There is currently no set gold standard for the technical aspects of measuring infarct size using CMR. This includes the optimal sequence and method of delineating the area of high signal intensity. This is a clinical study with human subjects and hence direct comparison with histopathological measurements is unfeasible. The accuracy and specificity of the various sequences cannot therefore be assessed, particularly with regards to the small areas of high signal intensity are identified on DB-MOCO sequences absent using the other modalities. Manual contouring method for infarct size quantification is susceptible to bias. A small cohort of patients were investigated in this study and only one slice per patient was acquired and analysed. Infarct size was calculated using manual contouring which can be subjective.

5.6 Conclusion and Future Directions

FLASH, bSSFP, BB-MOCO and DB-MOCO yield comparable results when used to measure infarct size in post-contrast imaging. The DB-MOCO sequence however can be used to demonstrate areas of scar absent on images acquired using either of the other three modalities. Future studies could involve histopathological analysis of such tissue areas to help clarify the significance of such findings. Comparing infarct mass of the entire LV rather than one slice would provide more definitive conclusion to the degree of correlation between the various sequences.
Chapter 6
Discussion and Conclusion
6.1 Summary of Results

6.1.1 The CONDI-2/ERIC-PPCI Trial
The randomised CONDI-2/ERIC-PPCI trial showed that in reperfused STEMI patients adjunctive RIC therapy had no clinical benefit as compared to standard therapy\textsuperscript{177}. As with various other cardioprotective strategies designed to reduce IRI, RIC demonstrated significant benefit when studied in the pre-clinical setting but large clinical trials have failed to show a positive impact\textsuperscript{34,35,37,178,179}. Our findings on the clinical benefit of RIC mirror those from the ERICCA\textsuperscript{34} and RIPHeart\textsuperscript{35} studies in patients undergoing cardiac surgery\textsuperscript{40,191}.

6.1.2 Comparative Study of Free-breathing T2* Mapping Sequence for Detection of Intramyocardial Haemorrhage as Compared to Standard Breath-held Sequence
The novel FB-T2* mapping sequence consistently yielded diagnostic quality images with fewer artefacts than the current BH-T2* maps making data interpretation more reliable. The entire LV can be scanned with little added time rather than only acquiring 3 short-axis images. The FB-T2* sequence however has a tendency to underestimate the presence and severity of IMH, in contrast to the findings from Kellman & al.\textsuperscript{135}. The T2* readings using the two different sequences are mostly within the upper and lower limits of agreement with moderate correlation. These findings suggest that the FB sequence can be utilised for assessment of the presence of IMH.

6.1.3 Comparing Infarct Size Quantification Using Four Different Post-contrast CMR Sequences
This study demonstrated that the four different sequences used for infarct size analysis yielded similar measurements and that they can be used interchangeably for clinical and research purposes. The DB-MOCO sequence
6.2 Discussion and Conclusion

The results of the CONDI-2/ERIC-PPCI study have provided evidence that RIC confers no additional benefit to standard therapy in STEMI patients undergoing PPCI. There was no beneficial impact on primary or secondary clinical outcomes at 30 days or 12 months follow-up. Sub-group analysis showed that confounding factors such as age and diabetes did not have any significant impact on the primary outcome. These findings contradict smaller proof-of-concept studies which have investigated surrogate markers of adverse clinical outcomes such as infarct size, LV ejection fraction or presence of microvascular obstruction. A longer follow-up period may be required to fully evaluate the impact of this therapy given that heart failure related adverse events can take time to emerge. This would provide conclusive evidence of the potential benefits of RIC.

This study also raises the question as to whether surrogate markers can be reliably utilised to predict which therapy is likely to improve long-term clinical outcomes. One aspect that impacts on the reliability of such markers is lack of standardised analytical tools. Using CMR, our work demonstrated that analysis of infarct size and microvascular obstruction can be performed reliably using different available sequences and though some differences exist, these do not reach statistical significance. More work is however required with larger sample sizes to be conclusive. Another aspect for further study would to use histopathological correlation in animal models. Ultimately, the aim for future studies would be to identify a reliable surrogate marker which
can reproducibly predict the effect of a particular investigative therapy on long-term clinical outcomes in STEMI patients undergoing PPCI.
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