The effect of neprilysin inhibition on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

Kieran Francis Docherty

BSc (Hons), MB ChB (Hons), MRCP

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

BHF Cardiovascular Research Centre

Institute of Cardiovascular and Medical Sciences

College of MVLS

University of Glasgow

January 2022

Abstract

Background

The development of heart failure and reduced ejection fraction (HFrEF) in survivors of myocardial infarction occurs as a result of progressive left ventricular dilatation and a reduction in systolic function, a process commonly referred to as adverse left ventricular remodelling. One of the earliest advances in the management of myocardial infarction was the finding that the angiotensin converting enzyme (ACE) inhibitor captopril which inhibits the maladaptive activation of the renin-angiotensin system (RAS) promoting the process of adverse remodelling, reduced the risk of heart failure and mortality by attenuating progressive ventricular enlargement. Subsequently, the angiotensin receptor blocker valsartan (in a dose of 160 mg twice daily) was shown to be as effective as captopril in preventing adverse clinical outcomes after myocardial infarction. Beta-blockers are believed to have similar benefits as a result of attenuating the harmful actions of excessive activation of the sympathetic nervous system (SNS).

Not all neurohumoral activation following myocardial infarction (and in heart failure) is harmful. The natriuretic peptides are released in response to increased left atrial and ventricular wall stress and counteract the harmful effects of RAS and SNS activation through natriuretic, vasodilatory, anti-fibrotic and sympatholytic effects. Endogenous levels of the natriuretic peptides (along with a range of other potentially cardioprotective peptides) can be increased by preventing their breakdown by the enzyme neprilysin.

In patients with symptomatic HFrEF, the combined angiotensin receptor-neprilysin inhibitor sacubitril/valsartan (dosed 97/103mg twice daily), compared with the gold-standard ACE inhibitor enalapril, has been demonstrated to reduce the risk of worsening heart failure and cardiovascular death. It may be that part of the clinical benefits of sacubitril/valsartan (i.e., the addition of neprilysin inhibitor), relate to a favourable reverse remodelling effect. Therefore, the addition of a neprilysin inhibition to a RAS inhibitor in high-risk patients following myocardial infarction may result in greater attenuation of adverse left

ventricular remodelling than RAS inhibition alone, and potentially reduce the attendant risk of the development of HFrEF

Aim

To examine the effect of neprilysin inhibition on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction using the gold-standard method, cardiac magnetic resonance imaging (MRI).

Methods

I performed a prospective, randomised, double-blind, active-comparator trial comparing sacubitril/valsartan 97/103mg twice daily with valsartan 160mg twice daily in patients at least 3 months following an acute myocardial infarction with a left ventricular ejection fraction (LVEF) less than, or equal to 40% who were taking a RAS inhibitor (equivalent dose of ramipril ≥2.5mg twice daily), and a beta-blocker unless contraindicated or intolerant. Patients in New York Heart Association (NYHA) functional classification II or greater were excluded. The primary endpoint was change from baseline to 52-weeks in left ventricular end-systolic volume index (LVESVI) measured using cardiac MRI. Secondary endpoints included other MRI measurements of left ventricular remodelling, change in NT-proBNP (a marker of left ventricular wall stress) and hs-TnI (a marker of myocardial injury), and a patient global assessment of change questionnaire. In exploratory analyses, I also examined the effect of neprilysin inhibition on a range of circulating biomarkers relating to substrates for neprilysin and myocardial fibrosis.

Results

In the 93 randomised patients, mean age was 60.7±10.4 years, median time from myocardial infarction 3.6 years (interquartile range [IQR] 1.2-72), mean LVEF 36.8%±7.1, median NT-proBNP 230pg/ml (IQR 124-404) and a beta-blocker was taken by 94% of patients.

Sacubitril/valsartan, compared with valsartan, did not significantly reduce LVESVI; between-group difference -1.9ml/m^2 (95%CI -4.8, 1.0); p=0.19. A reduction in LVESVI was seen with sacubitril/valsartan in those with NT-proBNP levels greater than or equal to the median than those below (interaction p=0.036). There were no significant between-group differences in NT-proBNP, hs-TnI, left ventricular end-diastolic volume index, left atrial volume index, LVEF, left ventricular mass index, or patient global assessment of change.

Sacubitril/valsartan, compared with valsartan, significantly increased levels of atrial natriuretic peptide (ANP) (p=0.013), a substrate for neprilysin, and its intracellular secondary messenger urinary cyclic guanosine monophosphate (cGMP) (P=0.001), indicating increased natriuretic peptide bioactivity. Midregional pro-atrial natriuretic peptide (MR-proANP), which is not a substrate for neprilysin, was significantly reduced with sacubitril/valsartan (P=0.009) and may reflect a reduction in left ventricular filling pressures. No significant increase in B-type natriuretic peptide (BNP) was observed which was consistent with the greater affinity neprilysin has for ANP relative to BNP. Midregional proadrenomedullin (MR-proADM) (P<0.001), glucagon-like peptide-1 (GLP-1) (P<0.001) and galectin-3 (P=0.045) were increased with sacubitril/valsartan, as compared with valsartan. No significant favourable changes were seen with the addition of a neprilysin inhibitor in biomarkers of profibrotic processes.

Conclusion

In patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction, treatment with sacubitril/valsartan compared with valsartan alone (i.e., the addition of a neprilysin inhibitor) did not have a significant reverse remodelling effect and did not reduce biomarkers of left ventricular wall stress (NT-proBNP) or myocardial injury (hs-TnI) despite augmenting natriuretic peptide activity.

Table of Contents

ABSTRACT **2**

TABLE OF CONTENTS **5**

LIST OF TABLES **11**

LIST OF FIGURES **12**

PUBLICATIONS RELATING TO THIS WORK **15**

PRESENTATIONS RELATING TO THIS WORK **16**

ACKNOWLEDGEMENTS **17**

AUTHOR'S DECLARATION **18**

ABBREVIATIONS **19**

CHAPTER 1 INTRODUCTION **23**

1.1 Background **23**

1.2 Left ventricular remodelling **25**
1.2.1 Left ventricular remodelling following myocardial infarction 26
1.2.1.1 Early remodelling following myocardial infarction 26
1.2.1.2 Late remodelling following myocardial infarction 28
1.2.2 Epidemiology of left ventricular remodelling and heart failure following myocardial infarction 30
1.2.3 Preventing adverse left ventricular remodelling following myocardial infarction 32
1.2.3.1 Coronary reperfusion therapy 33
1.2.3.2 Renin angiotensin system 34
1.2.3.3 Sympathetic nervous system 35
1.2.3.4 Mineralocorticoid receptor antagonists 35

1.3 Natriuretic peptides **36**

1.4 Neprilysin **39**
1.4.1 Substrates for neprilysin 40
1.4.1.1 Neprilysin and the natriuretic peptides 41
1.4.2 Pharmacological inhibition of neprilysin 41
1.4.3 Neprilysin inhibition and left ventricular remodelling 45

CHAPTER 2 TRENDS IN THE RISK OF HEART FAILURE HOSPITALISATION FOLLOWING FIRST MYOCARDIAL INFARCTION IN SCOTLAND BETWEEN 1991-2015 50

2.1 Introduction **50**

2.2 Methods **51**
2.2.1 Data sources 51
2.2.2 Study funding 52

2.2.3 Statistical analysis 52

2.3 Results 55
2.3.1 Trends in age-standardised incidence of first acute myocardial infarction 55
2.3.2 Heart failure hospitalisation following acute myocardial infarction 57
2.3.3 Trends in incidence of first hospitalisation for heart failure 61
2.3.4 Myocardial infarction subclassification 65
2.3.5 Mortality 66

2.4 Discussion 68

2.5 Limitations 72

2.6 Conclusions 73

CHAPTER 3 METHODS OF A TRIAL EXAMINING THE EFFECT OF NEPRILYSIN INHIBITION ON LEFT VENTRICULAR REMODELLING IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 74

3.1 Introduction 74

3.2 Trial design 76

3.3 Trial Aims 78
3.3.1 Primary Aim 78
3.3.2 Secondary Aims 78

3.4 Trial Outcomes 78
3.4.1 Primary Outcome 78
3.4.2 Secondary Outcomes 79
3.4.3 Exploratory Outcomes 79

3.5 Trial population 79
3.5.1 Identification of patients 79
3.5.2 Inclusion criteria 81
3.5.3 Exclusion criteria 82
3.5.4 Consent 83
3.5.5 Screening 84
3.5.5.1 Screening echocardiography protocol 84

3.6 Trial procedures 85
3.6.1 Pre-randomisation investigations 85
3.6.2 Randomisation 85
3.6.3 Follow-up visits 86
3.6.4 End-of-trial 89
3.6.5 Cardiac MRI protocol 89
3.6.6 Cardiac MRI analysis 90
3.6.7 Biomarker sampling 92
3.6.8 Patient global assessment of change 92

3.7 Study drug 93
3.7.1 Patients taking an ACE inhibitor at baseline 93
3.7.2 Dose adjustment 93
3.7.3 Monitoring of potential side effects 95
3.7.3.1 Monitoring of hypotension 95
3.7.3.2 Monitoring of renal function 95
3.7.3.3 Monitoring of hyperkalaemia 96
3.7.3.4 Management of angioedema 97

3.7.4 Development of heart failure and study drug/study discontinuation during follow-up 98

3.8 Withdrawal criteria 98

3.9 Funding of the trial 98

3.10 Pharmacovigilance 99
3.10.1 Definitions 99
3.10.2 Recording and reporting of Adverse Events 100
3.10.3 Assessment of adverse events 101
3.10.4 Reporting to sponsor (Pharmacovigilance Office) 103
3.10.5 Reporting to the MHRA and REC 103

3.11 Statistical analysis 104
3.11.1 Sample size 104
3.11.2 Statistical analysis 104

3.12 Covid-19 pandemic mitigation 105

3.13 Rationale for key aspects of the trial design 106

3.14 Conclusion 108

CHAPTER 4 THE EFFECT OF NEPRILYSIN INHIBITION ON LEFT VENTRICULAR REMODELLING IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 110

4.1 Introduction 110

4.2 Methods 110
4.2.1 Recruitment and screening 110
4.2.2 Cardiac MRI techniques and analysis 115
4.2.3 Statistical analysis 115

4.3 Results 117
4.3.1 Baseline characteristics 117
4.3.2 Completeness of Follow-Up and Adherence 120
4.3.3 Effect of neprilysin inhibition on left ventricular end-systolic volume index 121
4.3.4 Effect of neprilysin inhibition on left ventricular end-diastolic volume index 125
4.3.5 Effect of neprilysin inhibition on left atrial volume index 127
4.3.6 Effect of neprilysin inhibition on left ventricular ejection fraction 129
4.3.7 Effect of neprilysin inhibition on left ventricular mass index 131
4.3.8 Effect of neprilysin inhibition on left ventricular global function index 133
4.3.9 Effect of neprilysin inhibition on patient global assessment of general well-being 135

4.4 Safety and adverse events 136

4.5 Discussion 138
4.5.1 Study population 138
4.5.2 The effect of neprilysin inhibition on left ventricular remodelling 141
4.5.3 Limitations 145
4.5.4 Conclusions 147

CHAPTER 5 THE EFFECT OF NEPRILYSIN INHIBITION ON NATRIURETIC PEPTIDE LEVELS IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 148

5.1 Introduction 148

5.2 Methods 150
5.2.1 Study patients and protocol 150
5.2.2 Statistical methods 151

5.3 Results 152
5.3.1 Baseline levels 152
5.3.2 Correlation between baseline natriuretic peptide levels and urinary cGMP 153
5.3.3 Correlation between baseline natriuretic peptides and left ventricular and atrial volumes 154
5.3.4 Correlation between change in natriuretic peptides and left ventricular and atrial volumes 159
5.3.5 Effect of neprilysin inhibition on natriuretic peptide levels 165
5.3.5.1 Mid-regional prohormone of atrial natriuretic peptide (MR-proANP) 165
5.3.5.2 Atrial natriuretic peptide (ANP) 167
5.3.5.3 N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) 169
5.3.5.4 B-type natriuretic peptide (BNP) 171
5.3.5.5 C-type natriuretic peptide (CNP) 173
5.3.5.6 Urinary cyclic guanosine monophosphate (cGMP) 175

5.4 Discussion 178
5.4.1 Limitations 183
5.4.2 Conclusion 183

CHAPTER 6 THE EFFECT OF NEPRILYSIN INHIBITION ON POSTULATED CIRCULATING SUBSTRATES FOR NEPRILYSIN IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 185

6.1 Introduction 185

6.2 Methods 186
6.2.1 Study patients and protocol 186
6.2.2 Statistical methods 187

6.3 Results 188
6.3.1 Baseline values 188
6.3.2 MR-proADM 189
6.3.3 GLP-1 190
6.3.4 Apelin 191
6.3.5 Endothelin-1 192

6.4 Discussion 193
6.4.1 Limitations 198
6.4.2 Conclusion 199

CHAPTER 7 THE EFFECT OF NEPRILYSIN INHIBITION ON CARDIAC TROPONIN LEVELS IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 200

7.1 Introduction 200

7.2 Methods 202
7.2.1 Study population 202
7.2.2 Measurement of troponin 202
7.2.3 Statistical methods 203

7.3 Results 205
7.3.1 Baseline troponin values and correlation with degree of cardiac remodelling and natriuretic peptide levels 205

7.3.2 Baseline troponin and natriuretic peptide levels as predictors of the degree of adverse remodelling 208
7.3.3 Correlation between change in troponin and change in cardiac MRI measures of left ventricular remodelling 210
7.3.4 Correlation between change in troponin and change in natriuretic peptide levels 212
7.3.5 Effect of neprilysin inhibition on troponin levels 213
7.3.6 Remodelling effect of neprilysin inhibition according to baseline troponin concentration 215

7.4 Discussion 216
7.4.1 Limitations 220
7.4.2 Conclusions 221

CHAPTER 8 THE EFFECT OF NEPRILYSIN INHIBITION ON BIOMARKERS OF MYOCARDIAL FIBROSIS IN PATIENTS WITH ASYMPTOMATIC LEFT VENTRICULAR SYSTOLIC DYSFUNCTION LATE AFTER MYOCARDIAL INFARCTION 222

8.1 Introduction 222

8.2 Methods 223
8.2.1 Study patients and protocol 223
8.2.2 Statistical methods 224

8.3 Results 226
8.3.1 Baseline values 226
8.3.2 Galectin-3 227
8.3.3 GDF-15 228
8.3.4 sST2 229
8.3.5 MMP-9 230
8.3.6 TIMP-1 231
8.3.7 PIIINP 232
8.3.8 Effect of neprilysin inhibition on left ventricular remote zone extracellular volume fraction 233

8.4 Discussion 235
8.4.1 Limitations 238
8.4.2 Conclusion 238

CHAPTER 9 DISCUSSION 239

9.1 Summary of findings 239

9.2 Strengths 244

9.3 Limitations 244

9.4 Future areas of research 245

9.5 Conclusions 246

APPENDIX 1: PATIENT INFORMATION SHEET 247

APPENDIX 2: CONSENT FORMS 255

APPENDIX 3: CARDIAC MRI IMAGING ACQUISITION PARAMETERS 257

APPENDIX 4: PATIENT GLOBAL ASSESSMENT OF CHANGE QUESTIONNAIRE 259

REFERENCES 260

List of Tables

Table 1-1 Substrates for neprilysin and the potential physiological effects of neprilysin inhibition. 40
Table 1-2 Summary of data regarding the remodelling effect of sacubitril/valsartan in heart failure and following myocardial infarction 48
Table 2-1 Baseline characteristics according to subsequent heart failure hospitalisation and year of acute myocardial infarction 58
Table 2-2 Baseline characteristics according to development of heart failure .. 60
Table 2-3 Crude case incidence rates of heart failure following discharge from first myocardial infarction..... 62
Table 2-4 Adjusted risk of first heart failure hospitalization 64
Table 3-1 Total daily doses of commonly used ACE inhibitors or ARB corresponding to ramipril 2.5mg twice daily 82
Table 3-2 Schedule of assessments 87
Table 3-3 Pharmacovigilance definitions..... 99
Table 4-1: Baseline characteristics 118
Table 4-2 Change in primary and secondary MRI outcomes with sacubitril/valsartan or valsartan from baseline to week 52 124
Table 4-3 Response at 12 months relating to the patient's global assessment of any change in their general well-being since the start of the trial 135
Table 4-4 Adverse events of interest 137
Table 5-1 Baseline levels of natriuretic peptides and urinary cGMP.....152
Table 5-2 Effect of neprilysin inhibition on natriuretic peptide levels.....177
Table 6-1 Baseline levels of circulating neprilysin substrates 188
Table 7-1 Multivariable predictors of left ventricular end-systolic volume 209
Table 7-2 Change in hs-TnI with sacubitril/valsartan or valsartan from baseline to week 52 214
Table 7-3 Effect of neprilysin inhibition on left ventricular remodelling according to baseline troponin 215
Table 8-1 Baseline levels of myocardial fibrosis biomarkers.....226

List of Figures

Figure 1-1 Neurohumoral activation in response to a reduction in cardiac output and the effect of pharmacological neurohumoral antagonists 29
Figure 2-1 Study population 55
Figure 2-2 Trends in age-standardised incidence of first myocardial infarction 1991-2015 56
Figure 2-3 Cumulative incidence of first hospitalisation for heart failure following first acute myocardial infarction 63
Figure 2-4 Cumulative incidence of first hospitalisation for heart failure by type of acute myocardial infarction 65
Figure 2-5 Risk of mortality in patients following first hospitalisation for heart failure compared to those never hospitalised 67
Figure 3-1 Trial overview 77
Figure 3-2 Outline of cardiac MRI protocol 89
Figure 3-3 Study drug up-titration criteria 94
Figure 4-1: Trial recruitment timeline 112
Figure 4-2 Consort diagram 113
Figure 4-3 Summary of reasons for ineligibility on screening. 114
Figure 4-4 Change in left ventricular end-systolic volume index from baseline to week 52 122
Figure 4-5 Change in left ventricular end-systolic volume index by baseline NT-proBNP level 123
Figure 4-6 Change in left ventricular end-diastolic volume index from baseline to week 52 126
Figure 4-7 Change in left atrial volume index from baseline to week 52 128
Figure 4-8 Change in left ventricular ejection fraction from baseline to week 52 130
Figure 4-9 Change in left ventricular mass index from baseline to week 52 132
Figure 4-10 Change in left ventricular global function index from baseline to week 52 134
Figure 5-1 Pearson correlation coefficients for baseline natriuretic peptides and urinary cGMP 153
Figure 5-2 Correlations between baseline natriuretic peptides levels and baseline left ventricular end-systolic volume index 155

Figure 5-3 Correlations between baseline natriuretic peptides levels and baseline left ventricular end-diastolic volume index 156
Figure 5-4 Correlations between baseline natriuretic peptides levels and baseline left ventricular ejection fraction..... 157
Figure 5-5 Correlations between baseline natriuretic peptides levels and baseline left atrial volume index..... 158
Figure 5-6 Correlation between change in MR-proANP and cardiac MRI measurements of left ventricular remodelling at 52 weeks..... 160
Figure 5-7 Correlation between change in ANP and cardiac MRI measurements of left ventricular remodelling at 52 weeks 161
Figure 5-8 Correlation between change in NT-proBNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks..... 162
Figure 5-9 Correlation between change in BNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks 163
Figure 5-10 Correlation between change in CNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks 164
Figure 5-11 165
Figure 5-12 Change in ANP with sacubitril/valsartan or valsartan from baseline to week 52 168
Figure 5-13 Change in NT-proBNP with sacubitril/valsartan or valsartan from baseline to week 52 170
Figure 5-14 Change in BNP with sacubitril/valsartan or valsartan from baseline to week 52 172
Figure 5-15 Change in CNP with sacubitril/valsartan or valsartan from baseline to week 52 174
Figure 5-16 Change in cGMP with sacubitril/valsartan or valsartan from baseline to week 52 176
Figure 6-1 Change in MR-proADM with sacubitril/valsartan or valsartan from baseline to week 52 189
Figure 6-2 Change in GLP-1 with sacubitril/valsartan or valsartan from baseline to week 52 190
Figure 6-3 Change in apelin with sacubitril/valsartan or valsartan from baseline to week 52 191
Figure 6-4 Change in endothelin-1 with sacubitril/valsartan or valsartan from baseline to week 52 192

Figure 7-1 Correlations between baseline troponin I levels and cardiac volumes, function and mass at baseline 206
Figure 7-2 Correlation between baseline troponin I and MR-proANP and NT-proBNP concentrations 207
Figure 7-3 Correlations between change in troponin I levels and cardiac volumes, function and mass 211
Figure 7-4 Correlation between change in troponin and change in natriuretic peptide levels 212
Figure 7-5 Change in hs-TnI with sacubitril/valsartan or valsartan from baseline to week 52 213
Figure 8-1 Change in galectin-3 with sacubitril/valsartan or valsartan from baseline to week 52 227
Figure 8-2 Change in GDF-15 with sacubitril/valsartan or valsartan from baseline to week 52 228
Figure 8-3 Change in sST2 with sacubitril/valsartan or valsartan from baseline to week 52 229
Figure 8-4 Change in MMP-9 with sacubitril/valsartan or valsartan from baseline to week 52 230
Figure 8-5 Change in TIMP-1 with sacubitril/valsartan or valsartan from baseline to week 52 231
Figure 8-6 Change in PIIINP with sacubitril/valsartan or valsartan from baseline to week 52 232
Figure 8-7 Change in remote zone extracellular volume fraction from baseline to week 52 234

Publications relating to this work

Docherty KF, Campbell RT, Brooksbank KJM, Dreisbach JG, Forsyth P, Godeseth RL, Hopkins T, Jackson AM, Lee MMY, McConnachie A, Roditi G, Squire IB, Stanley B, Welsh P, Jhund PS, Petrie MC, McMurray JJV. Effect of Neprilysin Inhibition on Left Ventricular Remodeling in Patients With Asymptomatic Left Ventricular Systolic Dysfunction Late After Myocardial Infarction. Circulation. 2021 Jul 20;144(3):199-209.

Docherty KF, Campbell RT, Brooksbank KJM, Godeseth RL, Forsyth P, McConnachie A, Roditi G, Stanley B, Welsh P, Jhund PS, Petrie MC, McMurray JJV. Rationale and methods of a randomized trial evaluating the effect of neprilysin inhibition on left ventricular remodelling. ESC Heart Fail. 2021 Feb;8(1):129-138.

Docherty KF, Vaduganathan M, Solomon SD, McMurray JJV. Sacubitril/Valsartan: Neprilysin Inhibition 5 Years After PARADIGM-HF. JACC Heart Fail. 2020 Oct;8(10):800-810.

Docherty KF, Ferreira JP, Sharma A, Girerd N, Gregson J, Duarte K, Petrie MC, Jhund PS, Dickstein K, Pfeffer MA, Pitt B, Rossignol P, Zannad F, McMurray JJV. Predictors of sudden cardiac death in high-risk patients following a myocardial infarction. Eur J Heart Fail. 2020 May;22(5):848-855.

Docherty KF, McMurray JJV. PIONEER-HF: a new frontier in the role of neprilysin inhibition in the management of heart failure with reduced ejection fraction. Cardiovasc Res. 2019 Nov 1;115(13):e136-e139.

McMurray JJV, **Docherty KF**. Phosphodiesterase-9 Inhibition in Heart Failure: A Further Opportunity to Augment the Effects of Natriuretic Peptides? J Am Coll Cardiol. 2019 Aug 20;74(7):902-904.

Dewan P, **Docherty KF**, McMurray JJV. Sacubitril/Valsartan in Asian Patients with Heart Failure with Reduced Ejection Fraction. Korean Circ J. 2019 Jun;49(6):469-484.

Docherty KF, McMurray JJV. Angiotensin receptor-neprilysin inhibitors: A new paradigm in heart failure with reduced ejection fraction. Int J Cardiol. 2019 Apr 15;281:179-185.

Presentations relating to this work

Heart Failure Society of American Annual Meeting October 2020 (Late-breaking clinical trial – oral presentation). A randomized trial comparing the effect of sacubitril/valsartan to valsartan on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction after myocardial infarction.

European Society of Cardiology Congress August 2019 (Oral presentation and moderated poster - *Winner of best poster in Real-World Heart Failure Populations session*). Declining risk of heart failure hospitalisation following first acute myocardial infarction in Scotland between 1990-2015.

Acknowledgements

I would like to thank my supervisors, Professor John McMurray, Professor Mark Petrie, and Professor Pardeep Jhund for their ever-present support and encouragement throughout this project. My ongoing involvement in academic cardiology is in no small part due to the inspiration, guidance, and opportunities that they have provided me with over the last decade and continue to do so.

This project would not have been possible without the advice, friendship and time which Dr Ross Campbell provided – for this, I am ever grateful.

I would like to express my gratitude to the British Heart Foundation and Novartis for funding this project and to the Institute of Cardiovascular and Medical Sciences who provided funding for my role as a Clinical Research Fellow.

I am grateful to the staff of the Imaging Centre of Excellence at the Queen Elizabeth University Hospital, Glasgow for their assistance in the running of this study. My thanks to the team of the Glasgow Biomarker Laboratory and Dr Paul Welsh for their patience with my numerous questions and in particular to Philip Stewart for braving the Glasgow weather on a near-daily basis to ensure timely processing of the samples. I am extremely grateful to Sister Barbara Meyer and Dr Katriona Brooksbank for their help in the day-to-day running of this study and for guiding me through the challenges of a clinical trial.

This study would not have been possible without the trial participants, and I am extremely appreciative of the time and effort which they selflessly contributed.

This thesis is dedicated to my family. My parents' and sister Sarah's support has been ever-present for as long as I can remember, and I am indebted to them for the opportunities and love which they have afforded me. My wife Emma has had to endure my focus on this project for several years – that she agreed to marry me during the running of this study is a testament to her infinite patience. I would be a lesser man without her, and I am eternally grateful for her love and encouragement. Finally, to our son Patrick – you arrived into the world during the writing of this thesis and provided the necessary inspiration to get it finished.

Author's declaration

The work presented in this thesis was performed during my employment as a Clinical Research Fellow in the Institute of Cardiovascular and Medical Sciences at the BHF Glasgow Cardiovascular Research Centre, University of Glasgow. I was supervised by Professor John McMurray, Professor Mark Petrie and Professor Pardeep Jhund.

I designed and wrote the study protocol and obtained regulatory and ethical approval for the study. I performed the screening and recruitment, including obtaining informed consent, of all patients who participated in the study. I, with the assistance of Sister Barbara Meyer, performed all study follow-up visits.

I organised and was present for all cardiac magnetic resonance scans. Analysis of these scans for the purposes of the primary and secondary trial outcomes was performed in a blinded fashion by Dr Ross Campbell. Any additional analyses were performed by me under his supervision. Biomarker sample processing and analysis were performed by the Staff of the Glasgow Biomarker Laboratory in the Institute of Cardiovascular and Medical Sciences at the BHF Glasgow Cardiovascular Research Centre, University of Glasgow under the supervision of Dr Paul Welsh. Statistical analyses for the primary and secondary outcomes of the trial were performed by Ms. Bethany Stanley and Dr Alex McConnachie of the Robertson Centre for Biostatistics according to a pre-specified Statistical Analysis Plan. Any additional analyses were performed by me.

I confirm that this thesis has been composed by me solely and that it has not been submitted for any other degree at the University of Glasgow or any other institution. The writing of this thesis is entirely my own work. All sources of information within this thesis are specifically acknowledged.

Kieran F. Docherty

December 2021

Abbreviations

ACE	Angiotensin-Converting Enzyme
ADM	Adrenomedullin
AIRE	Acute Infarction Ramipril Efficacy
ALT	Alanine transaminase
AMI	Acute Myocardial Infarction
ANP	Atrial Natriuretic Peptide
ARB	Angiotensin Receptor Blocker
ARIC	Atherosclerosis Risk in Communities
ARNI	Angiotensin Receptor-Neprilysin Inhibitor
ASPIRE	Safety and Efficacy of Aliskiren in Post Myocardial Infarction Patients
AST	Aspartate aminotransferase
AT1R	Angiotensin II type 1 receptor
BHF	British Heart Foundation
BNP	B-type Natriuretic peptide
CABG	Coronary artery bypass graft
CAPRICORN	Carvedilol Post-Infarct Survival Control in LV Dysfunction
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
CKD	Chronic Kidney Disease
CNP	C-type natriuretic peptide
COX	Cyclo-oxygenase
eCRF	Electronic case report form
DAPA-MI	Dapagliflozin Effects on Cardiovascular Events in Patients With an Acute Heart Attack
DPP-IV	Dipeptidyl peptidase-IV
EACVI	European Association of Cardiovascular Imaging
ECG	Electrocardiogram
ECV	Extracellular volume
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EMPACT-MI	The Effect of EMPAgliflozin on Hospitalisation for Heart Failure and Mortality in Patients With aCuTe Myocardial Infarction
EMPEROR-Reduced	EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Reduced Ejection Fraction
EMPRESS-MI	EMpagliflozin to PREvent worSening of left ventricular volumes and Systolic function after Myocardial Infarction
EPHESUS	Eplerenone Post-AMI Heart Failure Efficacy and Survival Study
EVALUATE-HF	Effect of Sacubitril-Valsartan versus Enalapril on Aortic Stiffness in Patients With Heart Failure and Reduced Ejection Fraction
GALACTIC-HF	Global Approach to Lowering Adverse Cardiac Outcomes Through Improving Contractility in Heart Failure
GCP	Good Clinical Practice

GDF-15	Growth differentiation factor-15
eGFR	Estimated glomerular filtration rate
GISSI	Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico
GLP-1	Glucagon-like peptide 1
cGMP	Cyclic guanosine monophosphate
GTP	Guanosine triphosphate
HCT	Haematocrit
HEAAL	High-dose versus low-dose losartan on clinical outcomes in patients with heart failure
HEART	Healing and Early Afterload Reducing Therapy study
HFrEF	Heart failure with reduced ejection fraction
HORIZONS-AMI	Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction
HR	Hazard ratio
ICAMS	Institute of Cardiovascular and Medical Sciences
ICD	Implantable cardioverter defibrillator
IMP	Investigational medicinal product
IMPRESS	Inhibition of Metalloprotease by Omapatrilat in a Randomized Exercise and Symptoms Study in Heart Failure
IQR	Interquartile range
ISD	Information Service Division
ISIS	International Study Of Infarct Survival
LAVI	Left atrial volume index
LBBB	Left bundle branch block
LFT	Liver function tests
LVEDD	Left ventricular end-diastolic dimension
LVEDVI	Left ventricular end-diastolic volume index
LVEF	Left ventricular ejection fraction
LVESVI	Left ventricular end-systolic volume index
LVGFI	Left ventricular global function index
LVMI	Left ventricular mass index
MACE	Major Adverse Cardiovascular Events
MESA	Multi-Ethnic Study of Atherosclerosis
MHRA	Medicines and Healthcare Products Regulatory Agency
MMP	Matrix metalloproteinase
MOLLI	Modified look-locker inversion recovery
MR-proADM	Mid-regional pro-adrenomedullin
MRA	Mineralocorticoid receptor antagonist
MRI	Magnetic resonance imaging
MYBPC3	Myosin Binding Protein C3
MYH7	Myosin Heavy Chain 7
NEP	Neprilysin
NHS	National Health Service
NPR	Natriuretic peptide receptor
NSAID	Non-steroidal anti-inflammatory drug
NSTEMI	Non-ST elevation myocardial infarction

NT-proBNP	N-terminal prohormone of B-type natriuretic peptide
NYHA	New York Heart Association
OCTAVE	Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril
OPTIMAAL	Optimal Treatment in Myocardial infarction with the Angiotensin II Antagonist Losartan
OVERTURE	Omapatrilat versus Enalapril Randomized Trial of Utility in Reducing Events
PAMP	Proadrenomedullin N-terminal 20 peptide
PARADIGM-HF	Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure
PARAMOUNT	Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction
PCI	Percutaneous coronary intervention
PEACE	Prevention of Events with Angiotensin Converting Enzyme
PIIINP	Procollagen III N-terminal peptide
PIONEER-HF	Comparison of Sacubitril–Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode
PPCI	Primary percutaneous coronary intervention
PREDICTS	PREDiction of ICd Treatment Study
PRIME	Pharmacological Reduction of Functional, Ischemic Mitral Regurgitation
PROVE-HF	Prospective Study of Biomarkers, Symptom Improvement, and Ventricular Remodeling During Sacubitril/Valsartan Therapy for Heart Failure
RAS	Renin angiotensin system
REC	Research ethics committee
RESOLVED	Randomized Evaluation of Strategies for Left Ventricular Dysfunction Pilot Study
REVERT	REversal of VEntricular Remodeling with Toprol-XL
RSI	Reference Safety Information
SAR	Serious adverse reaction
SAVE	Survival and Ventricular Enlargement
SD	Standard deviation
SGLT2	Sodium-glucose cotransporter 2
SIMD	Scottish index of multiple deprivation
SNS	Sympathetic nervous system
SOLVD	Studies of Left Ventricular Dysfunction
SSFP	Steady-state free-precession
SST	Serum separating tube
STEMI	ST elevation myocardial infarction
SUSAR	Serious unexpected adverse reaction
TIMP	Tissue inhibitor of metalloproteinase
TRACE	TRAndolapril Cardiac Evaluation
TTE	Transthoracic echocardiogram
UK	United Kingdom
ULN	Upper limit of normal

VALIANT	Valsartan in Acute Myocardial Infarction Trial
VIP	Vasoactive intestinal peptide

Chapter 1 Introduction

1.1 Background

The last fifty years have seen a substantial reduction in deaths due to cardiovascular disease in the United Kingdom (UK) due to improvements in both primary prevention (i.e., better management of cardiovascular risk factors including hypertension and hypercholesterolaemia, along with a reduction in smoking rates) and secondary prevention. In 1961 more than half of all deaths in the UK were attributable to cardiovascular disease, and in the following sixty years this proportion has approximately halved, with most recent estimates reporting that 27% of all deaths in the UK are due to cardiovascular disease.[1] Furthermore, improvements in life expectancy mean that the annualised rate of death from cardiovascular diseases has declined by more than three-quarters over the last fifty years.[1] Similar trends have been reported across the world.[2] The most common cause of cardiovascular death in the UK is ischaemic heart disease, which is second only to Alzheimer's disease/dementia as the most common cause of death.[3] However, despite these improvements in mortality rates in the United Kingdom and elsewhere, ischaemic heart disease remains the most common cause of death in adults worldwide, accounting for an estimated 9.1 million deaths per year (1 in 6 of all deaths).[4]

Myocardial infarction, the most common acute presentation of ischaemic heart disease, accounts for over 100 000 admissions to hospitals in the UK each year.[1] In the United States, it is estimated that an individual has a myocardial infarction approximately every forty seconds.[2] The incidence of myocardial infarction has been reported to have declined over the last 40 years; in the US, the incidence of hospitalisation for myocardial infarction in Medicare beneficiaries fell between 2002 and 2011 from 1485 to 1122 per 100 000 person-years.[5] Along with improvements in secondary preventative pharmacological therapy, increased access to and use of emergent coronary reperfusion therapy, initially with thrombolysis and most recently with percutaneous coronary intervention, has reduced the risk of mortality associated with acute myocardial infarction.[6–11] Furthermore, changes in the clinical presentation of myocardial infarction with a reduction in the proportion of ST-elevation myocardial infarction (STEMI), which is associated with a higher risk of early mortality, and

an increase in the rates of non-ST-elevation myocardial infarction (NSTEMI) are also thought to have contributed to the observed decline in mortality rates from myocardial infarction.[12] As a result of this reduction in the risk of death, a greater proportion of patients are surviving myocardial infarction with a degree of left ventricular damage (i.e. left ventricular systolic dysfunction) who previously may not have survived. Indeed, it is estimated that around 1.4 million people alive in the UK currently have survived a myocardial infarction.[1]

The presence of left ventricular systolic dysfunction after myocardial infarction is associated with a higher risk of the subsequent development of heart failure with reduced ejection fraction (HFrEF).[13,14] Following MI, a series of haemodynamic and structural changes occur in response to a reduction in stroke volume secondary to impaired systolic function in the area of myocardium subtended by the infarct-related artery. This process is referred to as "left ventricular remodelling".[15–17] Initially protective, these changes, which are driven by activation of the body's neurohumoral systems, become maladaptive over time and promote progressive dilatation of the left ventricle, further reductions in the left ventricular ejection fraction (LVEF) and, ultimately, the development of the signs and symptoms of the syndrome of HFrEF.

Along with more survivors of MI, an increasing elderly population and growing prevalence of comorbidities such as diabetes, obesity, and chronic kidney disease, all of which are associated with a greater risk of the development of heart failure following MI, have led to concerns that the prevalence of heart failure may increase in the coming years. Indeed, in the UK, despite a modest decline in the overall age and sex standardised incidence of heart failure between 2002 to 2014, the incidence in patients aged 85 years or older increased, which, alongside a growing elderly population and improved survival following a diagnosis of HF, resulted in a 23% increase in the absolute number of patients with HF.[18] As well as having a significant impact on patients health and well-being, the rising prevalence of heart failure presents a significant socioeconomic burden; heart failure hospitalisations are estimated to account for approximately 2% of all UK National Health Service (NHS) inpatient bed days, 5% of all emergency admissions and approximately 2% (£2 billion) of the total NHS budget.[19]

Therefore, the prevention of the development of heart failure in high-risk patients following myocardial infarction (i.e., those with evidence of left ventricular systolic dysfunction) is a key therapeutic target. This thesis will examine the temporal trends in the development of heart failure following myocardial infarction in Scotland and discuss the potential role of pharmacological inhibition of the enzyme neprilysin in attenuating the process of adverse left ventricular remodelling in high-risk patients following myocardial infarction. The experimental data presented will also provide novel insights into the mechanisms of action of neprilysin inhibition.

1.2 Left ventricular remodelling

Cardiac remodelling has been defined as the process of genomic, molecular, cellular, extracellular and neurohumoral changes which result in changes in the size, shape, and function of the heart in response to cardiac injury.[20] Cardiac remodelling is a physiological response that aims to maintain or increase cardiac output and can be a normal physiological adaptation (e.g. in athletes or as part of normal growth) or a pathological response to increased cardiac afterload (e.g. hypertension or aortic stenosis), volume overload (e.g. aortic regurgitation), myocardial infarction or ischaemia, inflammation (myocarditis) or as a result of inherited cardiomyopathies (e.g. hypertrophic cardiomyopathy due to MYH7 or MYBPC3 genetic mutations among others, and dilated cardiomyopathy secondary to mutations in the LMNA gene among others) or idiopathic dilated cardiomyopathy.

Traditionally, three different remodelling phenotypes have been proposed: 1) concentric remodelling, in which cardiac myocytes thicken and expand into the left ventricular cavity in response to an increase in pressure or afterload (e.g. hypertension or aortic stenosis); 2) eccentric remodelling, where the cardiac myocytes lengthen due to volume overload of the left ventricle (e.g. aortic regurgitation); and 3) mixed load remodelling where there is an increase in both cardiac preload and afterload, as occurs in post-myocardial infarction remodelling.[21] Remodelling of all cardiac chambers can occur, but the focus of this thesis is the process of pathological remodelling of the left ventricle which is the key driver in the development of HFrEF following myocardial infarction.

1.2.1 Left ventricular remodelling following myocardial infarction

Acute myocardial infarction, most often due to the acute occlusion of an epicardial coronary artery, begins a series of changes in both the infarct zone and non-infarct zone myocardium which result in pathological remodelling of the left ventricular myocardium.[17,22] This process of adverse left ventricular remodelling can be separated into the early remodelling phase occurring in the minutes, hours and days following acute infarction, and the late remodelling phase which takes place during the weeks, months and years following infarction.

Key to understanding the remodelling process is an appreciation of the basic structure of the myocardium which can be separated into three main components: cardiac myocytes, the extracellular matrix, and the capillary microcirculation.[23] Cardiac myocytes are terminally differentiated cells whose primary function are to produce tension by shortening and thereby provide the contractile function of the heart. The extracellular matrix consists of predominantly type I and III collagen fibres which act as a scaffold between cardiomyocytes and their blood supply which is provided by a capillary microcirculation derived from the coronary arteries.

1.2.1.1 Early remodelling following myocardial infarction

The early phase of pathological left ventricular remodelling following myocardial infarction occurs due to the loss of functional cardiac myocytes in both the infarct and peri-infarct zones, a reduction in cardiac function secondary to changes in loading conditions, and the subsequent activation of the body's neurohumoral systems.

Interruption of the blood supply to the area of myocardium subtended by the infarct-related artery results in a reduction in oxygen supply, apoptosis and necrosis of the infarct zone cardiac myocytes resulting in an acute reduction in myocardial contraction and left ventricular systolic function.[24] An increase in macrophages, neutrophils, monocytes and fibroblasts in the infarct zone results in an inflammatory response that activates the matrix metalloproteinases (MMPs).[25] The MMPs are a group of proteases that breakdown the collagen struts

holding together cardiomyocytes and their activation, along with the mechanical stress of collagen fibres due to elevated ventricular wall stress, results in expansion of the infarct due to myocyte slippage in the hours following infarction with subsequent thinning of the myocardium.[26] As well as altered intra-cardiac loading conditions (increased left ventricular volumes and wall tension), this loss of functioning myocytes results in a reduction in stroke volume (the proportion of the left ventricular end-diastolic volume ejected from the heart during systole) and therefore, cardiac output.

A reduction in cardiac output results in activation of the sympathetic nervous system (increasing plasma norepinephrine concentrations) and the renin-angiotensin system (RAS) (Figure 1-1). Increased norepinephrine aims to preserve cardiac output by increasing heart rate and contractility (i.e., increased chronotropy and inotropy). It also has deleterious effects by increasing RAS activation and the release of endothelin 1, a powerful vasoconstrictor that increases cardiac afterload, as well as promoting further activation of the MMPs.[27] Activation of the RAS results in increased production of angiotensin II and aldosterone, which aim to preserve organ perfusion via vasoconstriction and increased sodium reabsorption increasing the circulating blood volume and systemic blood pressure.

These responses are initially protective with cardiac output maintained through the Frank-Starling mechanism, however progressive myocyte death and ventricular dilation can ultimately result in a reduction in cardiac output with the subsequent development of the signs and symptoms of HFrEF. In response to the maladaptive activation of the sympathetic system and RAS, the body aims to counteract the harmful increase in cardiac preload and afterload through release of the natriuretic peptides, a group of cardioprotective vasoactive peptides that promote natriuresis, vasodilatation and have sympatholytic, anti-hypertrophic and anti-fibrotic effects – their role in protecting against adverse cardiac remodelling will be discussed further in the following sections.[28]

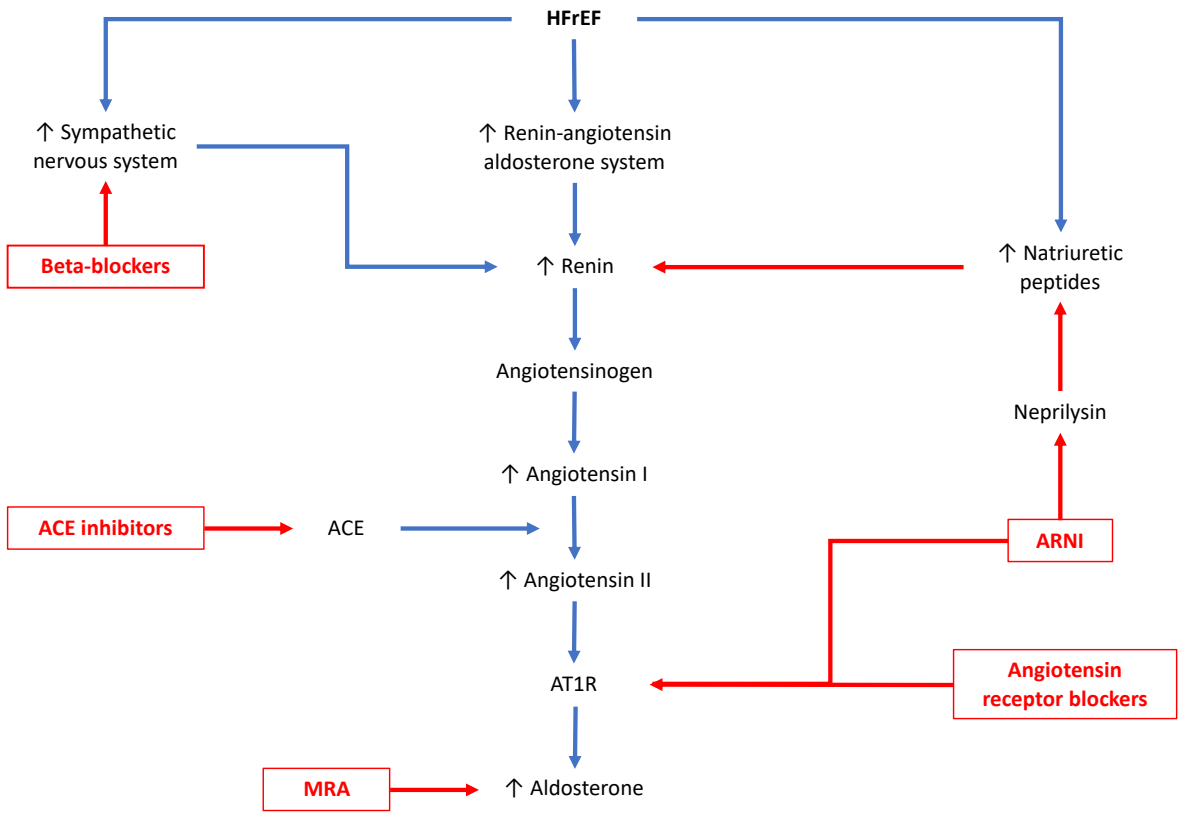
However, it is also important to highlight that not all left ventricular dysfunction following myocardial infarction is secondary to myocardial necrosis; a degree of reversible dysfunction can be secondary to the phenomenon known as “myocardial stunning”.[29] This explains why a significant proportion of patients

with left ventricular systolic dysfunction measured early following myocardial infarction can experience complete recovery within days and weeks following infarction.[30]

1.2.1.2 Late remodelling following myocardial infarction

Late ventricular remodelling refers to global ventricular dilatation, myocyte hypertrophy and the formation of a collagen scar in the infarct and peri-infarct zone which occurs in the weeks, months and years following acute infarction.[22] The law of Laplace states that ventricular wall tension is directly proportional to intra-ventricular pressure and size.[31] Therefore, as the ventricle dilates following myocardial infarction as described above, in an effort to maintain intraventricular pressure, hypertrophy of the non-infarct zone myocytes occurs. As well as this mechanical trigger, chronic neurohumoral activation of both the sympathetic nervous system and RAS stimulate myocyte hypertrophy and increase ventricular loading which furthers dilatation of both the infarct and non-infarct zone myocardium. After the initial breakdown of collagen in the hours following infarction by the MMPs, the tissue inhibitors of metalloproteinases (TIMP), prevent further collagen degradation with evidence of increased circulating TIMP within hours of infarction, peaking by 48 hours and returning to normal levels within 14 days.[25,32] The cytokine transforming growth factor beta 1 (TGF-β1) is released shortly after infarction and promotes the proliferation of fibroblasts which begin the process of tissue repair and reparative fibrosis.[33] Myofibroblasts synthesise type I and III collagen which adheres to a fibrin-fibronectin matrix and this process of collagen deposition and the creation of myocardial scar is stimulated by aldosterone and angiotensin II.[34] The effect of the inhibition of these neurohormones in the post-myocardial infarction setting will be discussed in the following sections. The non-tensile collagen scar which replaces the infarcted, necrotic myocardium aims to provide structural integrity to the infarct zone, however ongoing neurohumoral activation results in remote non-infarct zone fibrosis, further worsening systolic function and promoting ventricular dilatation.[17,22,35] Ultimately, this progressive deterioration in left ventricular systolic function can lead to the development of the signs and symptoms of the syndrome of HFrEF with the resultant elevated risk of hospitalisation and death.

Figure 1-1 Neurohumoral activation in response to a reduction in cardiac output and the effect of pharmacological neurohumoral antagonists



Blue lines indicate activating pathways and red lines indicated an inhibitory effect.

Abbreviations: ACE, angiotensin converting enzyme; AT1R, angiotensin type 1 receptor; ARNI, angiotensin receptor-neprilysin inhibitor.

1.2.2 Epidemiology of left ventricular remodelling and heart failure following myocardial infarction

The degree of adverse left ventricular remodelling measured in both the early and late time periods is one of the key determinants of outcome following acute myocardial infarction.[13,36–38] The majority of data on the epidemiology of left ventricular systolic dysfunction following myocardial infarction is from the pre-primary percutaneous coronary intervention (PPCI) era when patients were treated with thrombolytics and did not receive contemporary pharmacotherapy with RAS inhibitors, beta-blockers and, where indicated, mineralocorticoid receptor antagonists.[37] The incidence of left ventricular systolic dysfunction was much higher then, than that seen in contemporary populations, where the majority of patients received reperfusion therapy. In a substudy of patients who received reperfusion therapy in the Healing and Early Afterload Reducing Therapy (HEART) which studied patients following an anterior Q-wave myocardial infarction, 65% of patients received thrombolysis alone, 15% had PPCI alone, and 8% had both.[30] At day 1 following myocardial infarction, only 3.4% of patients had a normal left ventricular ejection fraction as measured by echocardiography (>55%). Of patients with a depressed left ventricular ejection fraction at baseline, 36% of patients had a partial recovery and 22% a complete recovery in left ventricular function after 90 days following infarction (i.e., over half of patients with depressed function had some degree of improvement). At the same time point, 16% of patients had a deterioration in left ventricular ejection fraction of 5% or greater.[30]

In a contemporary Dutch registry population of almost 2000 patients presenting with a STEMI and treated with PPCI, 95% and 97% of whom were treated with a beta-blocker and a RAS inhibitor, respectively, approximately one-half of patients were seen to adversely remodel within the first year after infarction with an increase in left ventricular end-diastolic volume (LVEDV) of ≥20%.[39] Of those who did have a significant increase in ventricular volume, this increase was evident in the majority (69%) by 3 months following infarction. Compared to those who did not remodel, remodellers had a higher incidence of heart failure hospitalisation during 10 years of follow-up with a cumulative incidence of 9% compared with 4% in those who did not have evidence of adverse remodelling. The findings of this registry were similar to an Italian study of acute myocardial

infarction patients who received PPCI.[40] In another contemporary study using cardiac magnetic resonance imaging (MRI) in Glasgow, 25% of revascularised STEMI patients had evidence of adverse remodelling at 6 months, a similar proportion to that seen in the two registries described above.[41] In the PREDiction of ICd Treatment Study (PREDICTS), of those with an ejection fraction of ≤35% at the time of MI, at 90-days 43% of patients had persistent ejection fraction ≤35%, 31% had an ejection fraction of 36% to 49%, and 26% had an ejection fraction ≥50%.[42]

The data presented above show that approximately between 25-50% of patients have evidence of adverse left ventricular remodelling on serial imaging studies performed within the first year following myocardial infarction. However, the process of adverse remodelling does not stop after one year as demonstrated in the Survival and Ventricular Enlargement (SAVE) trial, where progressive ventricular dilatation was seen to continue in the second year following infarction and was associated with a higher risk of adverse outcomes including heart failure and cardiovascular death.[37]

The prevalence of asymptomatic left ventricular systolic in long-term survivors of myocardial infarction and risk of progression to heart failure is not well documented. In a meta-analysis of studies reporting community prevalence of asymptomatic left ventricular systolic dysfunction, patients with a depressed left ventricular ejection fraction were at an almost 5-fold higher risk of the development of symptomatic heart failure compared to those without, however most of these studies excluded patients with a history of coronary artery disease including myocardial infarction.[43] Perhaps the best evidence with regards to the risk of progression to symptomatic heart failure in patients with asymptomatic left ventricular systolic dysfunction measured late after a myocardial infarction is provided by the Prevention arm of the Studies of Left Ventricular Dysfunction (SOLVD) program.[44] The SOLVD-Prevention trial compared the angiotensin-converting enzyme (ACE) inhibitor enalapril with placebo in 4228 patients with a left ventricular ejection fraction of 35% or less, 80% of whom had had a prior myocardial infarction (but not within the 30 days prior to screening). It is worth highlighting however that it can be assumed the use of reperfusion therapy in this population was minimal as the trial enrolled in the late 1980s and

furthermore, just under a quarter of patients were taking a beta-blocker at baseline. Also of note was that although these patients were “asymptomatic”, a third were classed to be in New York Heart Association functional limitation class II (i.e., slight limitation of physical activity with ordinary physical activity resulting in fatigue, palpitation, or dyspnoea). During a mean follow-up of 37 months, 24.5% and 20.6% of patients in the placebo and enalapril groups, respectively, were hospitalised for heart failure.[44] The equivalent data for death from cardiovascular causes were 14.1% and 12.6%.

Therefore, the available data show that a significant proportion of patients develop progressive adverse left ventricular remodelling following an acute myocardial infarction despite emergent coronary reperfusion and modern pharmacological secondary prevention. The development of adverse left ventricular remodelling is associated with worse outcomes and the prevention of progressive adverse left ventricular remodelling should therefore be a focus for improving outcomes for patients following myocardial infarction. In Chapter 2, I will describe the epidemiological trends in heart failure following myocardial infarction in Scotland between 1991 and 2015, a period covering the pre-reperfusion era, the introduction of emergency reperfusion therapy and modern-day clinical practice.

1.2.3 Preventing adverse left ventricular remodelling following myocardial infarction

One of the first major therapeutic advances in the management of acute myocardial infarction was the demonstration that inhibition of the RAS with the ACE inhibitor captopril reduced the risk of mortality and the development of heart failure in patients with left ventricular systolic dysfunction as a result of acute myocardial infarction.[45,46] This beneficial effect of captopril was related to its ability to attenuate the process of adverse left ventricular remodelling.[38,45,47] Following this, a range of interventions aiming at minimising infarct size along with pharmacological inhibitors of the neurohumoral activation which drives the process of adverse left ventricular remodelling have been shown to improve outcomes in patients at high risk of heart failure as a result of acute myocardial infarction (Figure 1-1). Furthermore, the same neurohumoral antagonists are beneficial in patients with established chronic HFrEF, a finding

which is of no surprise, given the common pathophysiological process of progressive left ventricular remodelling underpins both the development of HFrEF and the worsening of established HFrEF.

1.2.3.1 Coronary reperfusion therapy

The occlusion of an epicardial coronary artery and resulting impairment of coronary blood flow is the first step in the sequence of events that lead to the development of heart failure as a result of progressive ventricular dilatation and impaired systolic function, i.e., adverse left ventricular remodelling. One of the earliest developments in our understanding of the pathophysiology of left ventricular remodelling following acute infarction was the demonstration by Maroko and Braunwald in 1973, that in a canine model of myocardial infarction the reperfusion of an occluded infarct-related artery resulted in a reduction in the size of infarct.[48] Subsequently, the finding by Pfeffer and colleagues that a smaller infarct size was associated with a lesser degree of systolic dysfunction and lower risk of mortality in rat models of infarction, led to the development of the "open artery hypothesis" which theorised that early reperfusion of the infarct-related artery would minimise infarct size and thereby reduce the associated risk of mortality and the development of heart failure.[49,50]

Subsequently, the early administration of intravenous thrombolytic agents to patients with an acute STEMI was shown to reduce the risk of mortality in the GISSI (Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico) and ISIS-2 (Second International Study Of Infarct Survival) randomised-controlled trials.[51,52] This finding was replicated in a series of later randomised-controlled trials using a range of other thrombolytic agents as well as the finding that early, pre-hospital thrombolysis, compared with in-hospital thrombolysis, significantly reduced mortality by 17%, highlighting the importance of the early opening of the occluded infract-related artery.[53] Furthermore, thrombolysis which resulted in complete opening of the infarct-related artery resulted in a greater improvement in the left ventricular ejection fraction than in patients with partial or no reperfusion.[54]

Following the widespread adoption of thrombolysis into clinical practice, advances in technology and clinical experience led clinicians to explore a

percutaneous method of restoring coronary artery flow, firstly with balloon angioplasty and latterly with coronary stents. In a meta-analysis of 23 trials, Keeley et al. demonstrated that PPCI was superior to thrombolytic therapy in reducing the risk of death, non-fatal reinfarction and stroke.[55] Furthermore, PPCI resulted in a reduction in infarct size, and less adverse remodelling than thrombolysis.[56] Accordingly, PPCI is now the standard care for patients presenting with ST-elevation myocardial infarction.

1.2.3.2 Renin angiotensin system

As described above, activation of the RAS is a key driver of infarct enlargement and progressive ventricular dilation and remodelling which portends the development of heart failure following myocardial infarction. Building on experimental preclinical work in animal models of infarction, Pfeffer et al. were the first to demonstrate in the SAVE trial that inhibition of activation of the RAS with the ACE inhibitor captopril reduced morbidity and mortality in survivors of acute myocardial infarction with left ventricular systolic dysfunction.[46] This finding was replicated with two other ACE inhibitors in the AIRE (ramipril) and TRACE (trandolapril) trials and with the angiotensin II type 1 receptor (AT1R) inhibitor (ARB) valsartan in the Valsartan in Acute Myocardial Infarction Trial (VALIANT).[46,57–59] In the SAVE trial, the benefit of captopril was related to its effect in attenuating ventricular dilatation following MI, i.e., attenuation of adverse remodelling.[38] Furthermore in the VALIANT trial, the remodelling effect of the ARB valsartan was equivalent to that of captopril.[13]

In patients with asymptomatic left ventricular systolic dysfunction (approximately 80% of who had a prior myocardial infarction), the ACE inhibitor enalapril was shown to reduce the risk of heart failure hospitalisation in the Prevention arm of the Studies Of Left Ventricular Dysfunction (SOLVD) trial.[44] The degree of left ventricular dilatation and dysfunction was less in the Prevention (asymptomatic) arm of the trial as compared with the Treatment (symptomatic) arm, however enalapril had a beneficial effect on attenuating progressive left ventricular dilatation in both asymptomatic and symptomatic patients.[60]

Activation of the RAS can also be inhibited by blocking the proximal rate-limiting step in the production of angiotensin II with a direct renin inhibitor. In the Safety and Efficacy of Aliskiren in Post Myocardial Infarction Patients (ASPIRE) placebo-controlled trial, the addition of the direct renin inhibitor aliskiren to standard therapy with an ACE inhibitor or ARB, and a beta-blocker did not have any additional beneficial remodelling effect despite evidence of additional RAS blockade (increased frequency of hypotension and increases in creatinine and serum potassium).[61]

1.2.3.3 Sympathetic nervous system

Beta-blockers inhibit the sympathetic nervous system activation which occurs in response to a reduction in cardiac output and activation of the RAS as a result of left ventricular systolic dysfunction following myocardial infarction and in patients with chronic symptomatic HFrEF. The Carvedilol Post-Infarct Survival Control in LV Dysfunction (CAPRICORN) trial examined the effect of the beta-blocker carvedilol compared with placebo in patients with left ventricular systolic dysfunction following acute myocardial infarction. When added to a RAS inhibitor, carvedilol significantly reduced the risk of mortality by 23% with no significant effect on the incidence of heart failure hospitalisation.[62] In a substudy of 127 patients enrolled in the CAPRICORN trial, carvedilol had a significant beneficial effect on preventing adverse remodelling as measured using echocardiography with significantly lower left ventricular end-systolic volumes and higher ejection fraction in patients treated with carvedilol as compared with placebo.[63] In patients with asymptomatic left ventricular systolic dysfunction, 94% of whom were taking an ACE inhibitor or ARB at baseline, the beta-blocker metoprolol succinate has been shown to have a significant reverse remodelling effect as compared with placebo in the REversal of VEntricular Remodeling with Toprol-XL (REVERT) Trial.[64]

1.2.3.4 Mineralocorticoid receptor antagonists

As described previously, increased aldosterone production plays a key role in infarct expansion and the progression of left ventricular dilatation and myocardial fibrosis following myocardial infarction. Following the demonstration that inhibition of the effect of aldosterone with the mineralocorticoid receptor

antagonist (MRA) spironolactone reduced mortality and prevented heart failure hospitalisation in HFrEF patients when added to a RAS inhibitor, the potential role of an MRA in reducing adverse outcomes in high-risk survivors of acute myocardial infarction was examined in the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS).[65] In acute myocardial infarction patients with left ventricular systolic dysfunction and heart failure or diabetes, the MRA eplerenone, compared with placebo, significantly reduced mortality and cardiovascular hospitalisations.[65] The additive remodelling effect of eplerenone, however, appears to be less than that of RAS inhibitors or beta-blockers; in a small trial of non-diabetic patients with asymptomatic left ventricular systolic dysfunction immediately following myocardial infarction, eplerenone had a minimal effect on attenuating adverse remodelling which was only statistically significant after covariate adjustment.[66] A similar minimal reverse remodelling effect with MRA in HFrEF patients has also been reported suggesting that the predominant benefits of this class of drugs may relate to other mechanisms of action such as a reduction in fibrosis which can reduce the risk of fatal ventricular arrhythmias, reduced sympathetic drive and a reduction in oxidative stress.[67,68]

1.3 Natriuretic peptides

Not all neurohumoral activation following myocardial infarction is harmful. The natriuretic peptides are a group of hormones the most important of which are thought to be atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide.

The first of the natriuretic peptides to be described by De Bold and colleagues in 1981 was ANP which is encoded by the NPPA gene located on chromosome 1.[69] The other main circulating natriuretic peptide, BNP, is encoded by the NPPB gene.[70] Translation of NPPA and NPPB results in the production of the precursor molecules, preproANP and preproBNP, respectively. PreproANP, a 151 amino acid polypeptide is stored in atrial cardiomyocyte granules as proANP (1-126) which is created after the removal of a 25-peptide signal sequence from preproANP. On release from atrial granules, proANP (1-126) is rapidly degraded by the enzyme corin to form the biologically active C-terminal ANP (99-126) and an N-terminal prohormone of ANP (1-98) which is biologically inactive.[71] ProANP

is also processed in the kidney which results in an extended 32 peptide bioactive ANP molecule known as urodilatin.[72] PreproBNP, a 134 amino acid peptide, undergoes similar processing to ANP with the removal of a 26 amino acid signal peptide resulting in proBNP (1-108). ProBNP undergoes proteolysis by the enzymes corin and furin to form bioactive BNP (77-108) and the inactive N-terminal prohormone of BNP (NT-proBNP [1-76]).[73] Unlike proANP, the majority of proBNP is not stored in granules but is transcribed in response in increased ventricular wall stress, although a small amount of BNP is stored in atrial granules.[74] A third natriuretic peptide, C-type natriuretic peptide (CNP) was also discovered circulating at 10 to 1000-fold lower concentrations than the other natriuretic peptides.[75] CNP is not stored in cardiac tissue but is found in chondrocytes, where it stimulates long-bone growth and in the endothelium where it causes vasodilation. Unlike ANP and BNP, CNP lacks a C-terminal extension which means it does not have the same natriuretic properties as the other peptides in this family.

Release of stored ANP or increased transcription of BNP is stimulated by increased cardiac wall stress, such as that seen in the setting of HFrEF or following myocardial infarction.[76] The three natriuretic peptide binding receptors (NPR-A, NPR-B and NPR-C) are cell-membrane bound guanylyl cyclases. NPR-A is the predominant receptor for ANP and BNP with a binding potency for ANP≥BNP>>CNP, and CNP binds to the NPR-B.[76] Binding of the natriuretic peptides to their receptor results in guanylyl cyclase mediated conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Increased intracellular cGMP activates cGMP-dependent protein kinases, cGMP-regulated cyclic nucleotide phosphodiesterases and cGMP-gated ion channels, resulting in a range of physiological effects which will be discussed below. CNP acts predominantly via binding to NPR-B. NPR-C, which does not have a guanylyl cyclase component, is predominantly a clearance receptor for the natriuretic peptides, acting in conjunction with the enzyme neprilysin to breakdown and clear the natriuretic peptides from circulation.

The natriuretic peptides have a wide range of biological effects, many of which aim to alleviate the elevated cardiac wall stress which stimulates their production and release. Activation of the NPR-A results in smooth muscle

relaxation and vasodilatation, increased natriuresis, diuresis and endothelial permeability as well as inhibition of the RAS and sympathetic nervous system.[77,78] ANP and BNP are also thought to prevent adverse cardiac remodelling with anti-fibrotic and anti-hypertrophy effects.[79]

As well as being a diagnostic tool in the identification of patients with heart failure, elevated natriuretic peptide levels are predictors of outcomes in patients with HFrEF and following myocardial infarction. In patients with HFrEF, elevated natriuretic peptide concentrations are a powerful independent predictor of outcome, and a treatment's effect on reducing NT-proBNP is correlated with its treatment effect on reducing the risk of heart failure hosptialisation.[80] Similarly, when measured in the acute phase following myocardial infarction, elevated BNP and NT-proBNP are independent predictors of an elevated risk of mortality, heart failure and recurrent myocardial infarction.[81–83] Furthermore, in the SOLVD registry in which approximately 40% of patients were asymptomatic of their systolic dysfunction, elevated ANP concentrations were significantly correlated with a greater degree of ventricular dilatation and a lower left ventricular ejection fraction.[84]

It is important to highlight that elevated levels of natriuretic peptides in these settings are a sign of the body's own cardioprotective efforts to maximise the beneficial physiological effects of increased levels of bioactive natriuretic peptides. In an effort to maximise these benefits, several pharmacological strategies have been examined to augment the body's endogenous natriuretic peptides. The administration of recombinant BNP (nesiritide) or urodilatin (ularitide) have not been shown to improve outcomes or symptoms in patients with acutely decompensated HFrEF.[85,86] In small studies, administration of recombinant ANP has been shown to reduce infarct size and attenuate adverse left ventricular remodelling following myocardial infarction.[87] Another method to augment endogenous natriuretic peptide levels and potentially harness their cardioprotective effects is to prevent their breakdown by the enzyme neprilysin, which will be discussed in the following section.

1.4 Neprilysin

Neprilysin, a zinc-dependant membrane-bound metalloendopeptidase, is an endogenous enzyme which is responsible for the breakdown of the natriuretic peptides as well as a wide range of substrates including other vasoactive peptides such as angiotensin-II, endothelin, adrenomedullin and bradykinin (Table 1-1). Since its discovery in rabbits in 1973 in the brush border of renal tubular microvilli, neprilysin has been known as a variety of names including NEP EC 3.4.24.11, neutral endopeptidase 24.11, endoprotease 24.11, common acute lymphoblastic leukaemia antigen (CALLA), neutrophil antigen cluster differentiation antigen 10 (CD10), membrane metalloendopeptidase EC 3.4.24.11, vasopeptidase, atriopeptidase and enkephalinase.[88,89] Neprilysin has since been discovered to be widely distributed in the body and has a wide range of substrates, some of which have important roles in cardiovascular physiology. Due to their potential cardioprotective effects, the augmentation of endogenous levels of some of the substrates for neprilysin, by inhibiting their breakdown, may have benefits in preventing adverse left ventricular remodelling following myocardial infarction.

Neprilysin synthesis

Neprilysin is coded for by the membrane metalloendopeptidase (MME) gene on chromosome 3 (3q25.2).[90] It is an integral type II zinc-dependant membrane-bound metalloendopeptidase which is composed of 749 amino acids.[91,92] It is a member of the M13 family of peptidases and is composed of a short N-terminal cytoplasmic domain, a single transmembrane helix, and a large C-terminal extracellular domain with 2 alpha-helical structures, one of which contains a solitary zinc atom that is necessary for its catalytic activity. It hydrolyses substrates at the amino side of hydrophobic amino acids and can only catabolise peptides at a molecular weight of approximately 3000 kDa or below, a limit imposed by the size of the catalytic crypt.[93–95]

Neprilysin is a ubiquitous enzyme having been found in the brain, thyroid, lungs, heart, gastrointestinal tract, adrenal glands, placenta, synovium, kidneys and genital tract, as well as in a soluble form in the cerebrospinal fluid, blood, and

urine.[96–103] It is not known how soluble neprilysin is created, however it retains catalytic activity, albeit at a lower level of activity than membrane-bound neprilysin.[97,98]

1.4.1 Substrates for neprilysin

Over 50 peptides have been identified as substrates for neprilysin. Those thought to be relevant to heart failure include the natriuretic peptides, adrenomedullin, glucagon-like peptide 1 (GLP-1), apelin, substance P, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), angiotensin II, endothelin-I, and bradykinin (Table 1-1).

Table 1-1 Substrates for neprilysin and the potential physiological effects of neprilysin inhibition.

Substrate	Potential Effects of Neprilysin Inhibition
Natriuretic peptides (ANP, BNP, CNP, urodilatin)	↑ Natriuresis ↓ Renin and aldosterone secretion ↓ SNS activation ↓ Cardiac hypertrophy and fibrosis
Substance P	↑ Vasodilation
Bradykinin	↑ Vasodilation
Adrenomedullin	↑ Vasodilation ↓ Cardiac hypertrophy and fibrosis
Apelin	↑ Vasodilation ↑ Inotropy ↑ Aquaresis ↓ Cardiac hypertrophy and fibrosis
Glucagon-like peptide-1 (GLP-1)	↑ Glycaemic control ↑ Vasodilation ↑ Chronotropy ↑ Weight loss ↓ Atherosclerosis ↓ Cardiac hypertrophy and fibrosis
Calcitonin gene-related peptide (CGRP)	↑ Vasodilation
Vasoactive intestinal peptide (VIP)	↑ Vasodilation
Angiotensin II	↑ Vasoconstriction
Endothelin-1	↑ Vasoconstriction

Abbreviations; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; SNS, sympathetic nervous system.

1.4.1.1 Neprilysin and the natriuretic peptides

The natriuretic peptides are cleared from the circulation by two processes. Firstly, clearance by the NPR-C, which has been described above, and secondly, enzymatic degradation by neprilysin.[104] Under normal conditions, clearance by NPR-C and neprilysin occurs equally, however in the elevated natriuretic peptide conditions of heart failure when the NPR-C is saturated, neprilysin is thought to account for a greater proportion of natriuretic peptide clearance.[105]

Neprilysin catabolises all forms of natriuretic peptides but with varying affinity for the individual peptides owing to their ability to fit in and be correctly orientated within the enzyme's catalytic cleft.[93] For this reason, neprilysin has a greater affinity for ANP and CNP than BNP, the longer amino and carboxy tails of which curtails the ability of the 17-amino acid ring structure to orientate correctly with neprilysin's catalytic cleft.[106–108] The different affinities that neprilysin has for the natriuretic peptides explains their different plasma half-life's; ANP and CNP have half-life's which are less than four minutes, whereas BNP is relatively more stable with a half-life of over twenty minutes.[74,108]

1.4.2 Pharmacological inhibition of neprilysin

Interest in neprilysin inhibition as a potential pharmacological target was initially focused on its potential role in lowering blood pressure in patients with hypertension as well as being a possible treatment for heart failure. The first synthetic neprilysin inhibitor, thiorphan, was demonstrated by Roque and colleagues in 1980 to have an antinociceptive action in mice, an action which was secondary to inhibition of the breakdown of enkephalins by neprilysin.[109] Subsequently, thiorphan and other synthetic neprilysin inhibitors were demonstrated to have favourable haemodynamic (reduction in arterial blood pressure and left atrial pressure, and increased cardiac output, diuresis and natriuresis) and hormonal responses (increased ANP and cGMP, and reduced renin and aldosterone) in animal models of hypertension and heart failure.[110–115] Furthermore, this diuretic response was not accompanied by deleterious activation of the renin-angiotensin aldosterone system, as is the case with loop diuretics.[116]

Trials examining the potential efficacy of extended treatment with neprilysin inhibitors failed to show any benefit in terms of sustained blood pressure-lowering effect. In a randomised, placebo-controlled trial in 40 patients with essential hypertension and elevated diastolic blood pressure, despite a significant increase in plasma ANP and a non-significant trend to increased urinary cGMP, the oral pro-drug candoxatril did not significantly lower blood pressure.[117] This result was subsequently explained by the finding that the potential anti-hypertensive effects of augmented natriuretic peptide and other vasodilatory peptide levels secondary to neprilysin inhibition was neutralised by the accompanied inhibition of breakdown, and therefore increased levels of, the vasoconstrictors angiotensin II and endothelin-1 (as these are also substrates for neprilysin).[118-120]

Combined neprilysin and ACE inhibitors

In an effort to mitigate the lack of antihypertensive effect of sole neprilysin inhibition, the focus then moved to examine the potential efficacy of combining a neprilysin inhbitor and an ACE inhibitor (which would provide RAS blockade thereby neutralising the augmentation of angiotensin II secondary to neprilysin inhibition). This class of medications was known as vasopeptidase inhibitors.[121,122] The most studied vasopeptidase inhibitor was omapatrilat, which was initially shown to have a promising anti-hypertensive effect and as well as increasing levels of ANP and cGMP (signalling increased natriuretic peptide bioactivity) in both experimental models and small human studies.[123-127]

The Inhibition of Metalloprotease by Omapatrilat in a Randomized Exercise and Symptoms Study in Heart Failure (IMPRESS) trial reported a trend towards improved survival and reduced risk of admission for worsening heart failure with omapatrilat, as compared with the ACE inhibitor lisinopril, in 573 patients with HFrEF.[128] Subsequently, the larger (n=5770) Omapatrilat versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) reported that the primary composite endpoint of all-cause death or hospitalisation for heart failure requiring intravenous treatment was not significantly reduced with omapatrilat.[129] There was, however, a significant 9% lower risk of the composite endpoint of cardiovascular death or hospitalisation, and furthermore, in a *post hoc* analysis including all hospitalisations for heart failure (i.e. not just those

requiring intravenous treatment) there was a significant 11% reduction in the risk of death from any cause or heart failure hospitalisation.[129] In the OVERTURE trial, omapatrilat was administered once daily and further analyses of the cohort indicating early post-dose hypotension with omapatrilat suggested that once-daily dosing may have been insufficient, and potentially resulted in a lesser degree of ACE inhibition than that of the active-comparator enalapril which was administered at the 10mg twice daily dose used in the SOLVD-Treatment trial.[130]

However, in both the OVERTURE trial and in a larger 25 000 patient trial in hypertension (Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril [OCTAVE]), angioedema occurred more frequently with omapatrilat than with enalapril. In addition to the inhibition of bradykinin breakdown by both ACE and neprilysin inhibition (i.e. both pharmacological effects of omapatrilat), omapatrilat was also discovered to inhibit the breakdown of aminopeptidase P, a third enzyme involved in bradykinin catabolism.[131,132]

Therefore, given the signal of potential efficacy despite potentially inadequate dosing, the OVERTURE investigators concluded that combined RAS and neprilysin inhibition warranted further study, but due to the increased risk of angioedema, further development of vasopeptidase inhibitors was halted.

Angiotensin receptor-neprilysin inhibitors

The use of an AT1R inhibitor (also known as an ARB) along with a neprilysin inhibitor in place of an ACE inhibitor (as with vasopeptidase inhibitors), offered, in theory, the additional benefits of RAS blockade but without the attendant risk of angioedema seen with combined ACE-neprilysin inhibition.

Sacubitril/valsartan (formerly known as LCZ696), is the first-in-class combined angiotensin receptor-neprilysin inhibitor (ARNI) and is a combination of the AT1R inhibitor valsartan and a neprilysin inhibitor pro-drug, sacubitril (AHU377).[133] Upon ingestion, sacubitril is rapidly metabolised by enzymatic cleavage of the ethyl ester into its active form, sacubitrilat (LBQ657). Pharmacokinetic and pharmacodynamic studies of the target dose of 97/103 mg twice daily, reported equivalent plasma concentrations of valsartan as valsartan 160 mg twice daily (the dose studied in the Valsartan Heart Failure Trial [Val-HeFT]) and rises in

cGMP representing an observed increase in nariuretic peptide bioactivity secondary to effective neprilysin inhibition.[134] Furthermore, the risk of angioedema with sacubitril/valsartan was expected to be lower as, unlike ompatrilat, sacubitrilat does not inhibit aminopeptidase P (as well as the use of valsartan as the RAS inhibitory component rather than an ACE inhibitor).[135] In addition, the twice-daily dosing of sacubitril/valsartan led to sustained RAS and neprilysin inhibition over a 24-hour period, overcoming one of the issues with omapatrilat at the dose used in OVERTURE.[134,136]

The landmark Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial examined the efficacy and safety of sacubitril/valsartan (target dose 97/103mg twice daily) compared with the gold-standard ACE inhibitor in HFrEF, enalapril, at the target dose of 10mg twice daily (i.e., that used in the SOLVD-Treatment trial) in 8744 patients with HFrEF.[137] To be eligible for inclusion, patients had to have a left ventricular ejection of 35% or less (this was changed by protocol amendment from ≤40%), be in New York Heart Association (NYHA) functional class II-IV, have elevated natriuretic peptide levels, be treated with a stable dose of an ACE inhibitor or an ARB equivalent to enalapril 10 mg/day for 4 weeks prior to screening and be treated with a stable dose of a beta-blocker for 4 weeks prior to screening (unless contraindicated or not tolerated).[137] Key exclusion criteria included a known history of angioedema, symptomatic hypotension and/or a systolic blood pressure <100mmHg at screening and <95 during the active run-in period or at randomisation, an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2 , serum potassium >5.2 mmol/L at screening or >5.4 mmol/L during the run-in or at randomisation. Following a run-in period, during which patients were exposed to both study drugs at their target doses, patients who tolerated the run-in period were randomised 1:1 to either double-blind treatment with sacubitril/valsartan or enalapril, and matched placebo.[137]

The primary endpoint of PARADIGM-HF was the composite of time to first hospitalisation for heart failure or cardiovascular death.[137] Compared with enalpril, sacubitril/valsartan reduced the occurrence of the primary endpoint by 20% (hazard ratio [HR] 0.80, 95% confidence interval [95% CI] 0.73-0.87; p<0.001).[138] The risk of both individual components of the primary endpoint

were also significantly reduced; heart failure hospitalisation by 21% and cardiovascular death by 20%. Death from any cause was reduced by 16% and the effect of sacubitril/valsartan was consistent across the range of prespecified age groups including age, sex, LVEF and natiuretic peptide levels.[139] Overall, as compared with enalapril, sacubitril/valsartan was well tolerated; there was no significant difference in the rate of angioedema between the groups and symptomatic hypotension occurred significantly more frequently with sacubitril/valsartan, however there was no difference between the groups in discontinuations due to this.[138]

Subsequent to the publication of the results of PARADIGM-HF in 2014, sacubitril/valsartan has been afforded a Class 1, level of evidence B indication for the treatment of symptomatic HFrEF in international guidelines. Further evidence for the efficacy and safety of sacubitril/valsartan in HFrEF has been provided by the PIONEER-HF (Comparison of Sacubitril–Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode) trial in which three important groups of patients who were not studied in the PARADIGM-HF trial were studied: those who were RAS blocker naïve, those who were hospitalised with worsening heart failure at the time of initiation following haemodynamic stabilisation and finally, patients with de-novo presentations of HFrEF.[140] In PIONEER-HF, sacubitril/valsartan significantly reduced NT-proBNP (the primary outcome), as compared with enalapril.

1.4.3 Neprilysin inhibition and left ventricular remodelling

As described previously, the benefits of neurohumoral antagonists (ACE inhibitors/ARB, beta-blockers and mineralocorticoid receptor antagonists) in patients at high risk of the development of heart failure following myocardial infarction and those with established chronic HFrEF are, in part, related to their ability to attenuate the process of adverse left ventricular remodelling. Given the vasodilatory, anti-hypertrophic, anti-fibrotic, and sympatholytic effects of natriuretic peptides, along with the clinical benefits observed in patients with HFrEF, it is reasonable to propose the mechanism of benefit of sacubitril/valsartan may be, in part, a beneficial effect on left ventricular

remodelling secondary to the augmentation of natriuretic peptides and other substrates for neprilysin.

Initial data regarding a potential positive remodelling effect of neprilysin inhibition were from pre-clinical experimental models of MI, ischaemia-reperfusion injury, and heart failure.[141–146] Neprilysin inhibition was reported to have positive effects in terms of attenuating ventricular dilation, reducing left ventricular hypertrophy, improving cardiac function as measured by left ventricular ejection fraction and reducing myocardial fibrosis, one of the key processes underlying adverse left ventricular remodelling. Following the results of PARADIGM-HF, a series of observational studies were published reporting improvements in left ventricular function and a reduction in left ventricular volumes in patients following initiation of sacubitril/valsartan. The results of these observational studies are summarised in Table 1-2. Most of these studies were in patients with HFrEF and not in patients at high risk of heart failure following myocardial infarction (i.e., those with symptomless left ventricular systolic dysfunction). Furthermore, the observational nature of these studies limits their ability to make conclusions regarding treatment effect.

Two randomised-controlled trials have examined the effect of sacubitril/valsartan on left ventricular remodelling in patients with HFrEF along with a trial in patients with hypertension (Table 1-2). In the Pharmacological Reduction of Functional, Ischemic Mitral Regurgitation (PRIME) trial, in patients with significant functional mitral regurgitation and LVEF between 25% and <50%, 12 months of treatment with sacubitril/valsartan, compared with valsartan (i.e. the effect of the addition of neprilysin inhibition), reduced left ventricular end-diastolic volume index (LVEDVI) by 7.0 mL/m^2 with no significant effect on left ventricular end-systolic volume index (LVESVI) or LVEF.[147] In the Effect of Sacubitril-Valsartan versus Enalapril on Aortic Stiffness in Patients With Heart Failure and Reduced Ejection Fraction (EVALUATE-HF) trial, sacubitril/valsartan, in comparison with enalapril, did not have a significant effect on the primary endpoint of central aortic stiffness but significantly reduced the secondary echocardiography endpoints of LVESVI by 1.6 mL/m^2, LVEDVI by 2.0 mL/m^2 and left atrial volume index (LAVI) by 2.8 mL/m^2, with no difference in LVEF after 12 weeks in patients with HFrEF.[148] In a randomised active-comparator trial of

sacubitril/valsartan compared with the ARB olmesartan in patients with hypertension and elevated pulse pressure, sacubitril/valsartan significantly reduced left ventricular mass index (LVMI) from baseline to 12 and 52 weeks.[149] This positive remodelling effect appeared to be independent of the anti-hypertensive effect of sacubitril/valsartan suggesting that other mechanisms may have contributed to the effect on LVMI.

The remodelling effect of neprilysin inhibition has also been examined in patients with heart failure with preserved ejection fraction (HFpEF), the other major phenotype of heart failure accounting for approximately half of all cases of heart failure. The Phase II Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction (PARAMOUNT) trial, compared 36-weeks of treatment with sacubitril/valsartan with valsartan, and reported a significant reduction in NT-proBNP, the trial's primary endpoint, as well as a significant reduction in left atrial volume, indicating a potential reduction in left ventricular filling pressures.[150]

The limited data available suggest that one of the mechanisms of action underlying the observed clinical benefits of sacubitril/valsartan in HFrEF may be, in part, a reverse remodelling effect. Given that the process of adverse left ventricular remodelling is the common link in the development of heart failure in patients at high risk following myocardial infarction and worsening of established HFrEF, it follows that similarly to RAS inhibitors, beta-blockers and MRA, the addition of a neprilysin inhibitor to pharmacological therapy in patients at high risk of heart failure following myocardial infarction may offer an additional benefit in terms of preventing, delaying or even reversing adverse left ventricular remodelling, thereby reducing the attendant risk of HF.

Table 1-2 Summary of data regarding the remodelling effect of sacubitril/valsartan in heart failure and following myocardial infarction

	n=	Follow-up	Patient characteristics	LVESVI (ml/m^2)	LVEDVI (ml/m^2)	LVEF (%)	LAVI (ml/m$^{2)}$
Randomised controlled trials							
Desai et al. 2019[148] Sacubitril/valsartan vs. enalapril	464	12 weeks	HFrEF NYHA II-III LVEF ≤40%	-1.6 (-3.1, -0.03)	−2.0 (−3.7, −0.3)	+0.6 (−0.4, 1.7)	−2.8 (−4.0, −1.6)
Kang et al. 2019[147] Sacubitril/valsartan vs. valsartan	118	52 weeks	Functional MR LVEF ≥25% to ≤50%	-4.2 (-10.1, 1.7)	-7.01 (-13.83, -0.19)	-0.2 (-2.0, 1.6)	-8.9 (-14.6, -3.3)
Solomon et al. 2012[150] Sacubitril/valsartan vs. valsartan	301	36 weeks	HFpEF NYHA II-IV LVEF ≥45%	N.S	N.S	N.S	-0.9; P=0.007
Observational studies							
Martens et al. 2018[151]	125	Median 16 weeks	HFrEF NYHA II-IV LVEF <35%	-18.4; p<0.001	-10.2; p=0.27	+5.2; p<0.001	-
Romano et al. 2019[152]	205	6 months	HFrEF NYHA II-III LVEF ≤35%	-	N.S.	+3.0; p>0.001	N.S.
Januzzi et al. 2019[153]	794	52 weeks	HFrEF NYHA II-IV LVEF≤40%	−15.29 (−16.03, −14.55)	−12.25 (−12.92, −11.58)	+9.4 (8.8, 9.9)	−7.57 (−7.98,−7.15)

Liu et al. 2020[154]	93	6 Months	HFrEF NYHA II-IV LVEF <40%	Significant reduction in LVESD	Significant reduction in LVEDD	+15; p<0.001	Significant reduction in LA dimension
Castrichini et al. 2020[155]	77	Median 9 months	HFrEF NYHA II-IV LVEF <40%	-11; p<0.001	-8; p=0.02	+7; p<0.001	-9; p<0.001
Landolfo et al. 2020[156]	49	12 months	HFrEF NYHA II-IV LVEF <40%	-53.3; p<0.05*	-40; p<0.05*	+16.5; p<0.05	Significant reduction in LA dimension
Paolini et al. 2021[157]	52	24 months	HFrEF NYHA II-IV LVEF ≤35%	−51.3±61.4; p=0.003*	−40.7±58.1; p=0.003*	+9.8±10.2; p<0.001	-
Guerra et al. 2021[158]	226	6 months	HFrEF NYHA I-IV LVEF ≤35%	-10.4; p=0.038	-13.3; p=0.04	+3.9; p<0.001	-
Rezq et al. 2021[159]	200	6 months	ST-elevation MI	Significant reduction in LVESD	Significant reduction in LVEDD	+4.7%; p=0.012	-

* Non-indexed value

Data presented as mean value ± standard deviation or mean (95% confidence interval).

Abbreviations: HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; LAVI, left atrial volume index; LVEDD, left ventricular end-diastolic dimension; LVEDVI, left ventricular end-diastolic volume index; LVESD, left ventricular end-systolic dimension; LVESVI, left ventricular end-systolic volume index; LVEF, left ventricle ejection fraction; NYHA, New York Heart Association; N.S., not significant (p≥0.05)

Chapter 2 Trends in the risk of heart failure hospitalisation following first myocardial infarction in Scotland between 1991-2015

2.1 Introduction

The last 3 decades have seen a substantial decline in the risk of mortality following acute myocardial infarction.[160] Reports have suggested that the increasing pool of myocardial infarction survivors and an ageing population may be contributing to a growing prevalence of heart failure (HF) in the general population.[18,161,162] However, the widespread implementation of emergency coronary reperfusion services and improvements in secondary preventative therapy may act to offset the purported increase in risk of developing heart failure secondary to improvements in survival. Further complicating this issue are the changing demographics of patients with myocardial infarction. The proportion of myocardial infarction presenting as STEMI has declined and the proportion of NSTEMI has increased.[163–165] Although STEMI is typically associated with greater myocardial damage than NSTEMI, patients presenting with NSTEMI are frequently older and have a higher prevalence of co-morbidities such as diabetes mellitus and chronic kidney disease which may contribute to an increased risk of developing HF.[166] A further consideration contributing to the uncertainty about current trends is the increased availability of high-sensitivity cardiac troponin assays which allow diagnosis of myocardial infarction with lesser degrees of myocardial injury. The complex interplay between these competing influences and their effect on trends in the long-term risk of heart failure following myocardial infarction over the last quarter of a century has not been described.

To describe long-term trends in incident heart failure hospitalisation after first myocardial infarction, I examined the rates of first hospitalisations for heart failure in Scotland in patients who were discharged alive from a hospitalisation for a first myocardial infarction between 1991 and 2015.

2.2 Methods

2.2.1 Data sources

Routinely collected clinical data on all discharges (including in-hospital deaths and those patients discharged alive) from National Health Service (NHS) hospitals in Scotland are collated by the Information and Statistics Division (ISD) and the electronic Data Research and Innovation Service (eDRIS) of the NHS in Scotland. Care is free at the point of delivery for all residents in Scotland therefore this data represents virtually all hospitalisations in the country. The 2017 mid-year population estimate in Scotland was 5 424 800. For each admission, information on discharge diagnoses (a principal diagnosis and up to 5 secondary diagnoses), procedures performed, demographics, prior admission diagnoses, postcode of residence and length of stay are recorded. Diagnoses are coded using the International Classification of Diseases (ICD) system (ICD 9th revision until April 1996 and ICD 10th revision thereafter). Record linkage is obtained through probability matching (with an accuracy of ≈98%) facilitating the analysis of data at the level of the individual patient and episode of care.[167] Data on mortality is derived from linkage to the Registrar General's Death Certificate Data with an accuracy of 98%. When compared to adjudicated events in the setting of a clinical trial, the accuracy of discharge diagnoses in Scotland has been reported as >95%.[168]

For the purposes of this study, I identified individuals aged 20 years or above with a first discharge from hospital with a principal diagnosis of myocardial infarction (ICD 9th revision 410 or ICD 10th revision I21 or I22). A first discharge was defined as one with a myocardial infarction code in the primary diagnostic position, with no prior hospitalisation for myocardial infarction (in any diagnostic position) since 1981 (a minimum "look back" of 10-years), the time-point at which routine discharge coding was first available in Scotland. Patients with a history of heart failure recorded prior to their index myocardial infarction admission were excluded for the purposes of this analysis, as were patients who died during their index myocardial infarction admission. A subsequent first heart failure hospitalisation was defined as one occurring after discharge from the index

myocardial infarction with a heart failure code (ICD 9th revision 425, 428, 402 or ICD 10th revision I50, I42, I11.0) in the primary diagnostic position.

Patients were allocated by postcode of residence into deprivation categories using the Scottish Index of Multiple Deprivation (SIMD) 2016 release, which takes into account seven measures of deprivation; current income, employment, health, education, skills and training, housing, geographic access and crime.[169] Comorbid diagnoses were defined as those which were coded as a secondary diagnosis during a hospitalisation or as the principal diagnosis during a prior hospitalisation within 5-years of the index hospitalisation. The following comorbidities of interest were included in this analysis: coronary heart disease, hypertension, heart failure recorded during index myocardial infarction admission, atrial fibrillation, cerebrovascular disease, diabetes, peripheral vascular disease, chronic kidney disease, cancer, and respiratory disease. Information on procedures (percutaneous coronary intervention [PCI] and/or coronary artery bypass grafting [CABG]) were collected for those performed within 30-days of the index myocardial infarction.

2.2.2 Study funding

This study was funded by an NHS Greater Glasgow and Clyde Endowment fund award (GN17CA406) awarded to Dr Kieran F. Docherty.

2.2.3 Statistical analysis

Baseline demographics are presented grouped by the year of first admission with a myocardial infarction and whether patients had a subsequent hospitalisation for HF. Normally distributed continuous variables are reported as means with standard deviations and skewed continuous variables as medians with interquartile ranges. Categorical variables are presented as counts and percentages.

Time-to-first hospitalisation for heart failure was calculated as the time from discharge from a first myocardial infarction to a first admission with HF, or time to death from any cause or censoring at December 31st 2016 if never hospitalised

for HF. In order to ensure a minimum of one-year follow-up for all patients, survival analysis was performed only on those patients with a first myocardial infarction from January 1st 1991 through to December 31st 2015. Time to death was calculated as the time from discharge from first admission with a myocardial infarction to death from any cause or censoring on December 31st 2016.

A joinpoint regression model was fitted to explore points of significant change in the trend of the incidence of admissions with myocardial infarction and provide estimated annual percentage change (Joinpoint Software, Version 4.6).[170] The Bayesian information criterion was used to select the best-fitting model. A maximum of 5 joinpoints were allowed for estimations and 95% confidence intervals were calculated for each estimate.

Crude incidence rates per 1000-patient years were calculated for first hospitalisation for heart failure at 1, 5 and 10-years following discharge from index myocardial infarction and stratified by age, sex, deprivation (SIMD 2016 quintile), co-morbidity, procedures performed and year of admission with myocardial infarction. In order to take into account temporal trends in the competing risk of death, the cumulative incidence rates of first heart failure hospitalisation, stratified by year of index myocardial infarction, were calculated and are presented using cumulative incidence curves, with the use of the nonparametric cumulative incidence function of Fine and Gray with death from any cause treated as a competing risk.[171] Competing risk regression models were used to explore the association of age, sex, deprivation (SIMD 2016 quintile), co-morbidity, procedures performed and year of admission with myocardial infarction with heart failure hospitalisation at 1, 5 and 10-years.

To examine the relative hazard for death following a first heart failure hospitalisation, a Cox proportional hazards model was created in which a variable indicating heart failure hospitalisation was entered into the model as a time-updated covariate (with follow-up time starting at discharge from index MI) and adjusted for age, sex, deprivation (SIMD 2016 quintile), co-morbidity, procedures performed and year of admission with myocardial infarction. The

period at risk prior to a first heart failure hospitalisation was attributed to the group with no heart failure hospitalisation in order to calculate incidence rates that reflect patients' time-updated event status. This was presented graphically using Kaplan-Meier estimates. The rate of death was calculated per 1000 patient-years of follow-up, with follow-up starting on the day of the first heart failure hospitalisation (or discharge from index myocardial infarction if the individual did not have a heart failure hospitalisation event). Temporal trends in mortality at 1-year following a first heart failure hospitalisation were examined in a cox-proportional hazards model adjusting for the same variables as above with the exceptions that year of myocardial infarction was replaced by year of first heart failure hospitalisation and age at time of heart failure hospitalisation was included. Time-to-event was calculated as the time from admission with a first heart failure hospitalisation to death or censoring at 1-year. Only those heart failure hospitalisations occurring up to the 31st of December 2015 were included to ensure one-year follow up for all patients.

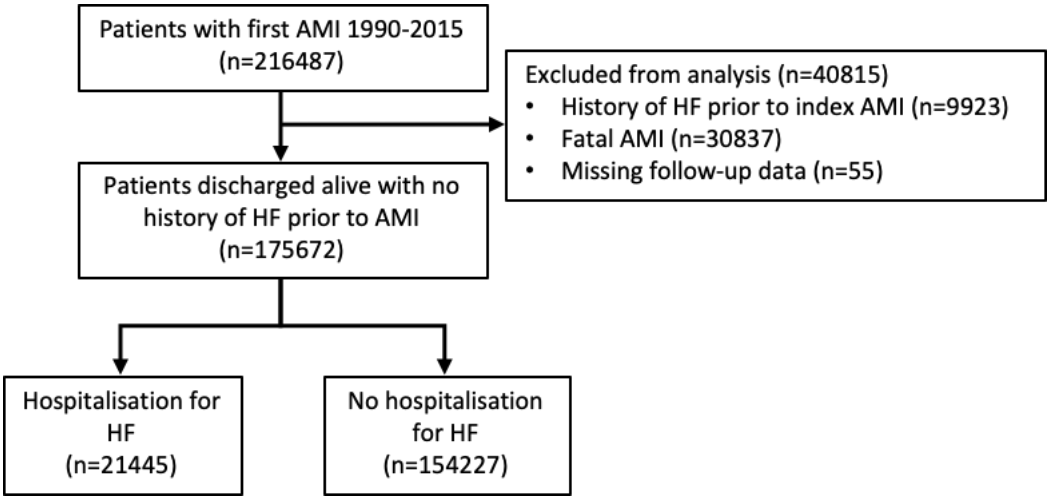
For patients who presented with a first myocardial infarction from 2012-2016, additional analyses using the methods described above were performed according to the classification of myocardial infarction presentation denoted by discharge coding (STEMI, NSTEMI, or unspecified).[172]

All analyses were performed using Stata 16 (StataCorp LP, College Station, Texas).

2.3 Results

There were 216 487 patients admitted to hospital in Scotland with a first diagnosis of myocardial infarction between 1991 to 2015. After excluding those who had a history of heart failure prior to index admission (n=9923), those who died during index admission (n=30 837), and those with missing follow-up data (n=55), 175 672 patients were included in the cohort for analysis, providing 1.5 million patient-years of follow-up (Figure 2-1).

Figure 2-1 Study population



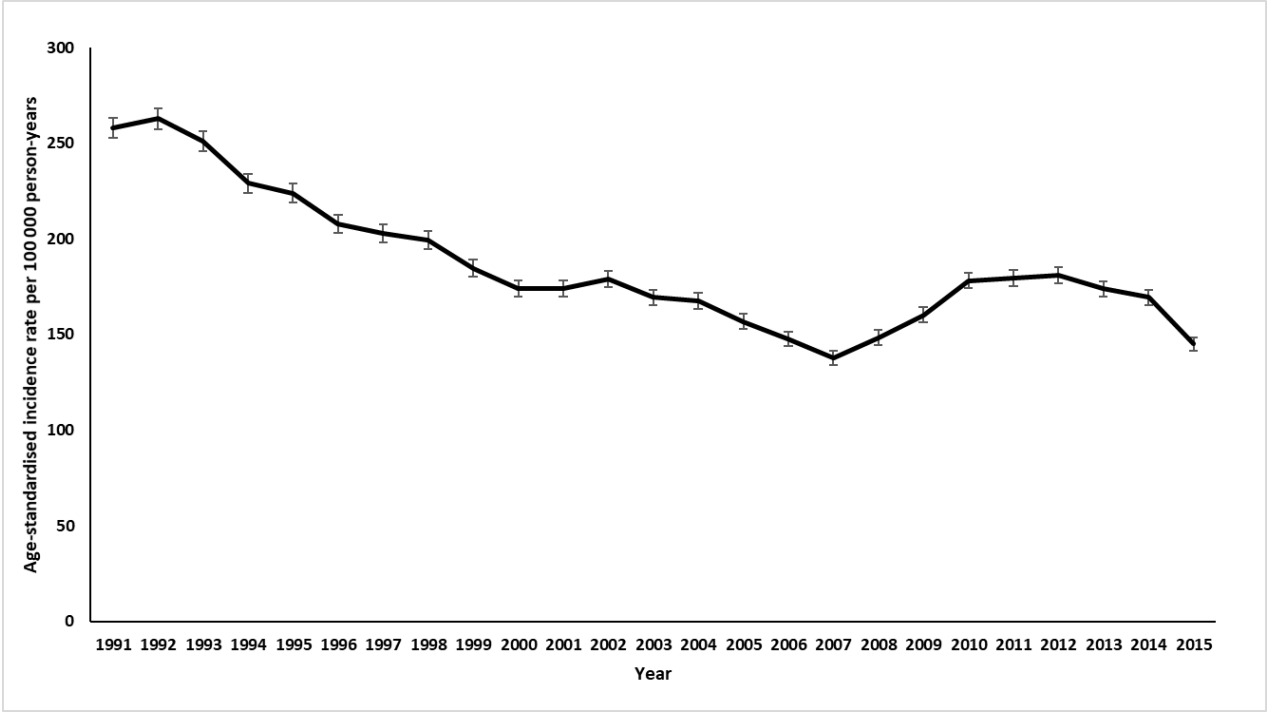
Legend: AMI, acute myocardial infarction; HF, heart failure.

2.3.1 Trends in age-standardised incidence of first acute myocardial infarction

Figure 2-2 shows the age-standardised incidence of first myocardial infarction from 1991-2015. Overall, the annual rate decreased by 2.3% (95% CI 1.3-3.2; p<0.001). There were significant differences in the trends in incidence of myocardial infarction over the time period examined; between 1991-2007 the rate of myocardial infarction decreased by 3.7% per year (95%CI 3.3-4.1; p<0.001). The rate of myocardial infarction then increased between 2007 and 2012 by 5.9% per year (95% CI 2.1-9.7; p=0.004). Subsequently, between 2012 and 2015, the trend was again for a decline in the rate of myocardial infarction with an annual decrease of 7.6% (2.5-12.4; p=0.007). The adjusted risk of death

at one year after myocardial infarction fell by 46% (95%CI 40-52; P<0.001) between 1991 and 2015. The risk of death at 5 and 10 years after myocardial infarction fell by 37% (95%CI 33-41; P<0.001) and 36% (95%CI 33-39; P<0.001), respectively.

Figure 2-2 Trends in age-standardised incidence of first myocardial infarction 1991-2015



2.3.2 Heart failure hospitalisation following acute myocardial infarction

Of those patients who were discharged alive from a first myocardial infarction with no history of heart failure prior to index admission, 21 445 (12.2%) were subsequently hospitalised for heart failure over a median follow-up time of 6.7 years (interquartile range [IQR] 2.8-12.9) (Table 2-1 and Table 2-2). The median time from myocardial infarction to development of heart failure was 2.6 years (IQR 0.4-7.7) In those who were subsequently admitted with HF, age at time of myocardial infarction rose from 67.4 ± 10.9 in 1991-1995 to 74.7 ± 12.0 in 2011-2015; a similar pattern was not observed in those who did not develop heart failure (64.9 ± 12.2 [1991-1995] vs. 65.6 ± 13.6 [2010-2015]) – Table 2-1. The mean age of patients at time of first heart failure hospitalisation within one year of discharge rose from 70.8 ± 10.7 to 76.2 ± 11.9 (1991 vs. 2015); in men, age rose from 68.1±10.6 to 73.6±12.6 and in women from 74.0±9.8 to 79.5±10.0. Similar increases were observed overall for heart failure occurring within 5 and 10 years; 71.6 ± 10.7 to 76.8 ± 11.9 (1991 vs. 2011) and 72.3 ± 10.4 to 76.0 ± 11.8 (1991 vs. 2006), respectively.

Compared to those patients who were never admitted for HF, those who had a first heart failure hospitalisation were older at the time of myocardial infarction, more frequently women and from a lower deprivation category (Table 2-2). A history of heart failure during index myocardial infarction admission, atrial fibrillation, diabetes, peripheral arterial disease and chronic kidney disease was more prevalent in those who were subsequently hospitalised for heart failure and patients were less likely to have undergone a revascularisation procedure within 30 days of their index myocardial infarction. Percutaneous revascularisation within 30 days was performed in 82.0% of STEMI, 40.9% of NSTEMI and 40.3% of unspecified myocardial infarction (data available from 2012-2015 only). The prevalence of comorbidities also increased over time both in those who were subsequently hospitalised for heart failure and those who were not.

Table 2-1 Baseline characteristics according to subsequent heart failure hospitalisation and year of acute myocardial infarction

	1991-1995 (n=43592)		1996-2000 (n=34773)		2001-2005 (n=31032)		2006-2010 (n=29997)		2010-2015 (n=36278)	
	No HF (n=35189)	HF (n=8403)	No HF (n=29359)	HF (n=5414)	No HF (n=27463)	HF (n=3569)	No HF (n=27646)	HF (n=2351)	No HF (n=34570)	HF (n=1708)
Age - years	64.9 ± 12.2	67.4 ± 10.9	65.0 ± 12.7	69.6 ± 11.2	65.5 ± 13.4	71.6 ± 11.7	65.5 ± 13.6	73.4 ± 11.8	65.6 ± 13.6	74.7 ± 12.0
Age group – no. (%)										
<55	7265 (20.7)	1048 (12.5)	6344 (21.6)	577 (10.7)	6107 (22.2)	333 (9.3)	6543 (23.7)	174 (7.4)	8062 (23.3)	115 (6.7)
55-65	9343 (26.6)	2130 (25.4)	7360 (25.1)	1051 (19.4)	6489 (23.6)	550 (15.4)	6510 (23.6)	313 (13.3)	8295 (24.0)	204 (11.9)
65-74	10543 (30.0)	2908 (34.6)	8500 (29.0)	1822 (33.7)	7187 (26.2)	1068 (29.9)	6583 (23.8)	631 (26.8)	8270 (23.9)	420 (24.6)
75-84	6428 (18.3)	1946 (23.2)	5430 (18.5)	1545 (28.5)	5651 (20.6)	1214 (34.0)	5700 (20.6)	861 (36.6)	6947 (20.1)	591 (34.6)
≥85	1610 (4.6)	371 (4.4)	1725 (5.9)	419 (7.7)	2029 (7.4)	404 (11.3)	2310 (8.4)	372 (15.8)	2996 (8.7)	378 (22.1)
Men – no. (%)	22015 (62.6)	4902 (58.3)	18519 (63.1)	3092 (57.1)	17391 (63.3)	1934 (54.2)	17840 (64.5)	1251 (53.2)	22210 (64.3)	937 (54.9)
Median length of stay – days (IQR)	7 (6-10)	8 (7-12)	7 (5-9)	8 (6-12)	6 (5-9)	8 (6-13)	5 (4-9)	8 (5-14)	4 (3-7)	7 (4-13)
Deprivation category* – no. (%)										
1 (most deprived)	9926 (28.5)	2508 (30.1)	7947 (27.2)	1550 (28.7)	7236 (26.5)	987 (27.7)	6657 (24.3)	597 (25.5)	8360 (24.5)	441 (25.9)
2	8813 (25.3)	2121 (25.5)	7177 (24.6)	1382 (25.6)	6609 (24.2)	875 (24.5)	6209 (22.7)	579 (24.7)	7843 (23.0)	444 (26.1)
3	6836 (19.6)	1698 (20.4)	5741 (19.7)	1100 (20.4)	5351 (19.6)	763 (21.4)	5589 (20.4)	457 (19.5)	7043 (20.6)	362 (21.3)
4	5091 (14.6)	1100 (13.2)	4594 (15.7)	785 (14.6)	4416 (16.2)	523 (14.7)	4956 (18.1)	404 (17.2)	5905 (17.3)	264 (15.5)
5 (least deprived)	4181 (12.0)	906 (10.9)	3727 (12.8)	579 (10.7)	3690 (13.5)	418 (11.7)	4001 (14.6)	308 (13.1)	5028 (14.7)	192 (11.3)
Co-morbidity – no. (%)										
Pre-exisiting documented coronary heart disease	4870 (13.8)	1367 (16.3)	5762 (19.6)	1361 (25.1)	7435 (27.1)	1148 (32.2)	12153 (44.0)	1138 (484)	18251 (52.8)	921 (53.9)
Hypertension	2645 (7.5)	800 (9.5)	4060 (13.8)	967 (17.9)	7162 (26.1)	1186 (33.2)	8997 (32.5)	1041 (44.3)	11277 (32.6)	789 (46.2)

Heart failure during index MI admission	3211 (9.1)	1512 (18.0)	3296 (11.2)	1291 (23.9)	4293 (15.6)	1130 (31.7)	3758 (13.6)	732 (31.1)	3804 (11.0)	553 (32.4)
Atrial fibrillation	1530 (4.4)	537 (6.4)	1807 (6.2)	577 (10.7)	2238 (8.2)	549 (15.4)	2426 (8.8)	474 (20.2)	3328 (9.6)	429 (25.1)
Cerebrovascular disease	1367 (3.9)	371 (4.4)	1377 (4.7)	314 (5.8)	1393 (5.1)	262 (7.3)	1286 (4.7)	189 (8.0)	1509 (4.4)	132 (7.7)
Diabetes	2092 (6.0)	874 (10.4)	2446 (8.3)	832 (15.4)	2987 (10.9)	752 (21.1)	3376 (12.2)	618 (26.3)	5102 (14.8)	517 (30.3)
Peripheral arterial disease	1520 (4.3)	449 (5.3)	1455 (5.0)	369 (6.8)	1584 (5.8)	299 (8.4)	1639 (5.9)	274 (11.7)	2161 (6.3)	200 (11.7)
Chronic kidney disease	380 (1.1)	117 (1.4)	604 (2.1)	178 (3.3)	998 (3.6)	252 (7.1)	1410 (5.1)	318 (13.5)	2634 (7.6)	361 (21.1)
Cancer	1350 (3.8)	307 (3.7)	1329 (4.5)	242 (4.5)	1532 (5.6)	207 (5.8)	1593 (5.8)	152 (6.5)	2128 (6.2)	139 (8.1)
Respiratory disease	1992 (5.7)	526 (6.3)	2260 (7.7)	476 (8.8)	2842 (10.4)	415 (11.6)	3225 (11.7)	348 (14.8)	4870 (14.1)	335 (19.6)
Index MI procedures – no. (%)										
PCI during admission	1190 (3.4)	278 (3.3)	2049 (7.0)	300 (5.5)	4382 (16.0)	366 (10.3)	11672 (42.2)	552 (23.5)	18183 (52.6)	482 (28.2)
PCI within 30 days	1241 (3.5)	284 (3.4)	2237 (7.6)	319 (5.9)	4803 (17.5)	399 (11.2)	12132 (43.9)	580 (24.7)	19243 (55.7)	497 (29.1)
CABG during admission	121 (0.3)	35 (0.4)	192 (0.7)	40 (0.7)	387 (1.4)	53 (1.5)	642 (2.3)	69 (2.9)	861 (2.5)	41 (2.4)
CABG within 30 days	155 (0.4)	44 (0.5)	260 (0.9)	51 (0.9)	514 (1.9)	66 (1.8)	800 (2.9)	83 (3.5)	995 (2.9)	52 (3.0)

* Data missing in 1403 (0.8%) of patients. Abbreviations: IQR, interquartile range; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting

Table 2-2 Baseline characteristics according to development of heart failure

	No Heart Failure (n=154277)	Heart Failure (n=21445)
Age - years	65.3 ± 13.1	69.9 ± 11.6
Age group – no. (%)		
<55	34321 (22.3)	2247 (10.5)
55-64	37997 (24.6)	4248 (19.8)
65-74	41083 (26.6)	6849 (31.9)
75-84	30156 (19.6)	6157 (28.7)
≥85	10670 (6.9)	1944 (9.1)
Men – no (%)	97975 (63.5)	12116 (56.5)
Median length of stay – days (IQR)	6 (4-9)	8 (6-12)
Deprivation category* – no. (%)		
1 (most deprived)	40126 (26.2)	6083 (28.5)
2	36651 (24.0)	5401 (25.3)
3	30560 (20.0)	4380 (20.5)
4	24962 (16.3)	3076 (14.4)
5 (least deprived)	20627 (13.5)	2403 (11.3)
Co-morbidity – no. (%)		
Coronary heart disease	48471 (31.4)	5935 (27.7)
Hypertension	34141 (22.1)	4783 (22.3)
Heart failure during index MI admission	18362 (11.9)	5218 (24.3)
Atrial fibrillation	11329 (7.4)	2566 (12.0)
Cerebrovascular disease	6932 (4.5)	1268 (5.9)
Diabetes	16003 (10.4)	3593 (16.8)
Peripheral arterial disease	8359 (5.4)	1591 (7.4)
Chronic kidney disease	6026 (3.9)	1226 (5.7)
Cancer	7932 (5.1)	1047 (4.9)
Respiratory disease	15189 (9.9)	2100 (9.8)
Index MI procedures		
PCI during admission	37476 (24.3)	1978 (9.2)
PCI within 30 days	39656 (25.7)	2079 (9.7)
CABG during admission	2203 (1.4)	238 (1.1)
CABG within 30 days	2724 (1.8)	296 (1.4)

*Data missing in 1403 (0.8%) of patients. Abbreviations: IQR, interquartile range; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting

2.3.3 Trends in incidence of first hospitalisation for heart failure

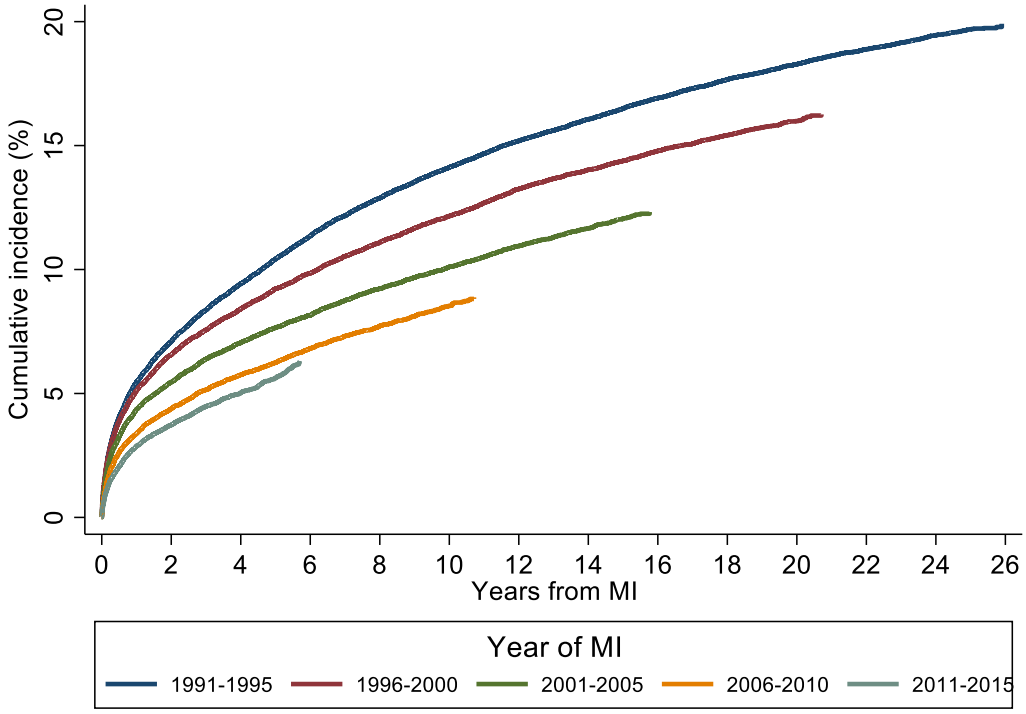
Crude case incidence (per 1000 patient-years) for heart failure at 1 year following discharge from a first myocardial infarction fell from 59.3 (95%CI 54.2-64.7) in 1991 to 31.3 (27.3-35.8) in 2015 (Figure 2-3). Similar trends were observed for heart failure occurring within 5 years (27.9 [26.2-29.7] to 14.1 [12.8-15.5]– 1991 vs. 2011) and 10 years (21.8 [20.6-23.0] to 12.5 [11.4-13.6] – 1991 vs. 2006). Rates of heart failure hospitalisation after 5 and 10 years in patients aged ≥85 years were 10-fold higher than those aged <55 years (Table 2-3). Rates were higher in women compared to men (10-year rate: 22.1 [21.6-22.6] vs. 14.0 [13.7-14.2]). The rate of heart failure hospitalisation increased with increasing socioeconomic deprivation – the rate of 10 -year heart failure hospitalisation in the most deprived was 18.2 (17.7-18.7) compared to 14.1 (13.5-14.7) in the least deprived.

Accounting for the competing risk of death, the cumulative incidence of first heart failure hospitalisation at 1-year, fell between 1991 and 2015 from 5.3% to 2.9%; the 5-year risk fell from 10.4% to 5.8% (1991 vs. 2011); 10-year risk from 14.4% to 9.0% (1991 vs. 2006) - Figure 2-3. After adjustment for age, sex, socioeconomic deprivation, comorbidities and revascularisation procedures, and accounting for the competing risk of death, the risk of heart failure hospitalisation at 1-year after discharge fell by 53% (95%CI 45-60%) – Table 2-4. The adjusted 5-year risk of heart failure hospitalisation fell by 57% (95%CI 52-61%) and 10-year risk fell by 48% (95%CI 44-53%). The 10-year risk of heart failure following myocardial infarction was higher in older individuals (<55 years vs. ≥85 years HR 3.14; 95%CI 2.89-3.41), if there was heart failure complicating the index admission (HR 2.08; 95%CI 2.00-2.16), in patients with diabetes (HR 1.72; 95%CI 1.64-1.81), CKD (HR 1.24; 95%CI 1.14-1.35), atrial fibrillation (HR 1.29; 95%CI 1.22-1.36), and in those not undergoing coronary revascularisation within 30 days of myocardial infarction (HR 1.18; 95%CI 1.10-1.27). Similar results were seen at 1 and 5-years (Table 2-3).

Table 2-3 Crude case incidence rates of heart failure following discharge from first myocardial infarction

	HF incidence per 1000 patient-years (95% confidence intervals)		
	1 year	**5 year**	**10 year**
All patients	47.0 (45.9-48.0)	20.7 (20.3-21.0)	16.8 (16.5-17.0)
Age – years			
<55	13.7 (12.6-15.0)	6.0 (5.6-6.4)	5.0 (4.8-5.3)
55-65	27.4 (25.9-29.1)	11.7 (11.2-12.2)	9.8 (9.5-10.2)
65-74	51.1 (49.1-53.3)	22.9 (22.2-23.6)	19.5 (19.0-20.0)
75-84	84.9 (81.7-88.2)	42.0 (40.8-43.2)	36.9 (36.0-37.9)
≥85	109.8 (103.5-116.5)	61.0 (58.2-63.9)	55.2 (52.8-57.8)
Sex			
Men	38.5 (37.3-39.7)	17.1 (16.7-17.5)	14.0 (13.7-14.2)
Women	61.7 (59.8-63.8)	27.3 (26.6-27.9)	22.1 (21.6-22.6)
Deprivation category			
1 (most deprived)	51.0 (48.9-53.2)	22.7 (22.0-23.4)	18.2 (17.7-18.7)
2	48.3 (46.1-50.5)	21.7 (21.0-22.5)	17.8 (17.3-18.3)
3	47.4 (45.1-49.8)	21.0 (20.2-21.8)	17.1 (16.6-17.7)
4	42.7 (40.3-45.3)	18.7 (17.9-19.5)	15.2 (14.6-15.8)
5 (least deprived)	42.7 (40.0-45.6)	17.6 (16.8-18.5)	14.1 (13.5-14.7)
Co-morbidity			
Coronary heart disease	46.9 (45.1-48.9)	22.0 (21.4-22.7)	18.8 (18.3-19.3)
Hypertension	55.2 (52.8-57.7)	25.5 (24.7-26.4)	21.3 (20.6-21.9)
Heart failure during index MI admission	132.3 (127.3-137.5)	56.3 (54.6-58.0)	44.5 (43.3-45.8)
Atrial fibrillation	100.3 (94.7-106.3)	50.7 (48.5-52.9)	43.8 (42.1-45.6)
Cerebrovascular disease	81.9 (75.4-89.0)	41.6 (39.1-44.3)	35.0 (33.0-37.0)
Diabetes	83.6 (79.4-88.0)	41.4 (39.9-43.0)	35.3 (34.1-36.5)
Peripheral arterial disease	82.9 (77.0-89.3)	40.6 (38.3-42.9)	34.0 (32.3-35.8)
Chronic kidney disease	119.3 (110.7-128.5)	62.6 (58.9-66.4)	54.2 (51.2-57.4)
Cancer	64.5 (58.9-70.6)	31.1 (29.0-33.4)	26.4 (24.8-28.2)
Respiratory disease	60.2 (56.5-64.3)	28.8 (27.4-30.2)	24.3 (23.3-25.4)
Index MI procedures			
PCI or CABG within 30 days	20.5 (19.2-21.9)	9.0 (8.6-9.5)	8.2 (7.9-8.6)
No PCI or CABG within 30 days	56.7 (55.3-58.0)	24.9 (34.3-25.3)	19.4 (19.1-19.7)

Figure 2-3 Cumulative incidence of first hospitalisation for heart failure following first acute myocardial infarction



Legend: Cumulative incidence curves of first heart failure hospitalisation accounting for the competing risk of death from any cause. Data are presented by year of index myocardial infarction. MI, acute myocardial infarction; HF, heart failure.

Table 2-4 Adjusted risk of first heart failure hospitalization

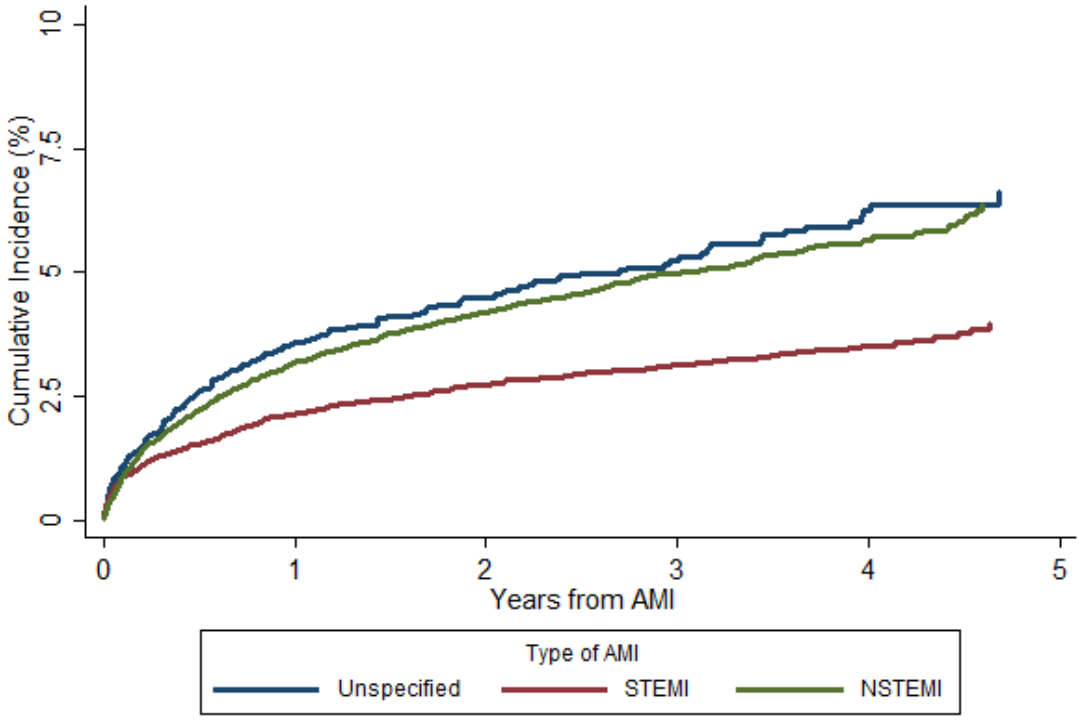
	1-year	5-year	10-year
Year of MI			
1991-1992	1.00	1.00	1.00
1993-1994	0.95 (0.87-1.04)	0.93 (0.87-0.99)	0.94 (0.89-0.99)
1995-1996	0.86 (0.78-0.94)	0.86 (0.81-0.92)	0.85 (0.80-0.90)
1997-1998	0.81 (0.74-0.90)	0.77 (0.72-0.83)	0.77 (0.72-0.82)
1999-2000	0.76 (0.69-0.84)	0.71 (0.66-0.76)	0.70 (0.65-0.74)
2001-2002	0.61 (0.55-0.68)	0.57 (0.53-0.62)	0.57 (0.53-0.61)
2003-2004	0.61 (0.55-0.68)	0.55 (0.50-0.59)	0.55 (0.51-0.59)
2005-2006	0.52 (0.46-0.58)	0.49 (0.45-0.53)	0.52 (0.48-0.56)
2007-2008	0.52 (0.46-0.58)	0.45 (0.41-0.49)	-
2009-2010	0.47 (0.42-0.53)	0.43 (0.39-0.48)	-
2011-2012	0.43 (0.38-0.48)	-	-
2013-2014	0.44 (0.38-0.49)	-	-
2015	0.47 (0.40-0.55)	-	-
Women vs. men	1.13 (1.08-1.18)	1.09 (1.05-1.13)	1.07 (1.04-1.11)
Age (years)			
<55	1.00	1.00	1.00
55-64	1.71 (1.54-1.90)	1.65 (1.52-1.78)	1.60 (1.50-1.72)
65-74	2.63 (2.39-2.91)	2.58 (2.39-2.78)	2.48 (2.33-2.65)
75-84	3.76 (3.40-4.15)	3.76 (3.49-4.06)	3.36 (3.14-3.60)
≥85	4.32 (3.86-4.84)	3.98 (3.65-4.35)	3.14 (2.89-3.41)
Deprivation category			
1 (most deprived)	1.00	1.00	1.00
2	0.90 (0.84-0.96)	0.91 (0.86-0.95)	0.92 (0.88-0.96)
3	0.89 (0.83-0.95)	0.88 (0.84-0.93)	0.92 (0.88-0.97)
4	0.81 (0.76-0.87)	0.81 (0.77-0.86)	0.83 (0.78-0.87)
5 (least deprived)	0.80 (0.74-0.87)	0.77 (0.72-0.82)	0.77 (0.73-0.82)
Co-morbidity			
Coronary heart disease	1.11 (1.06-1.17)	1.17 (1.12-1.22)	1.16 (1.11-1.21)
Hypertension	1.09 (1.03-1.16)	1.12 (1.07-1.17)	1.11 (1.06-1.16)
Heart failure during index MI admission	2.70 (2.57-2.84)	2.31 (2.22-2.41)	2.08 (2.00-2.16)
Atrial fibrillation	1.36 (1.28-1.45)	1.34 (1.27-1.42)	1.29 (1.22-1.36)
Cerebrovascular disease	1.04 (0.95-1.14)	1.04 (0.97-1.12)	1.01 (0.94-1.09)
Diabetes	1.67 (1.57-1.77)	1.76 (1.68-1.85)	1.72 (1.64-1.81)
Peripheral arterial disease	1.26 (1.17-1.37)	1.23 (1.15-1.31)	1.16 (1.09-1.24)
Chronic kidney disease	1.58 (1.45-1.71)	1.44 (1.34-1.55)	1.24 (1.14-1.35)
Cancer	0.98 (0.89-1.07)	0.87 (0.81-0.95)	0.85 (0.78-0.92)
Respiratory disease	1.14 (1.06-1.22)	1.11 (1.05-1.18)	1.07 (1.01-1.13)
Index MI procedures			
No PCI or CABG within 30 days	1.45 (1.34-1.57)	1.41 (1.32-1.50)	1.18 (1.10-1.27)

Multivariable analysis adjusted for age, co-morbidities, socioeconomic deprivation, and year of admission
Data presented as hazard ratio (95% confidence intervals). Abbreviations as per Table 2-1.

2.3.4 Myocardial infarction subclassification

From 2012 to 2015 the rate of STEMI and NSTEMI could be calculated using specific codes introduced in Scotland for the coding of subtypes of myocardial infarction. Of the 29011 myocardial infarctions occurring during this period, 10148 (35.0%) were classified as STEMI, 16456 (56.7%) as NSTEMI and 2407 (8.3%) were unspecified. During follow-up from January 1st 2012 to December 31st 2016 (minimum one-year and maximum 5-years), the incidence of hospitalisation for heart failure per 1000 person-years was 11.3 (95%CI 10.1-12.6) following STEMI, 19.3 (18.0-20.6) after NSTEMI and 20.4 (17.2-24.1) after unspecified MI; the cumulative incidence of first hospitalisation for heart failure is displayed by type of myocardial infarction in Figure 2-4. Accounting for the competing risk of death from any cause and adjusting for age, deprivation, comorbidities, year of myocardial infarction and revascularisation, compared to STEMI, the hazard ratio of heart failure hospitalisation was 1.01 (95% CI 0.87-1.16) for NSTEMI and 1.04 (95% CI 0.83-1.29) for unspecified myocardial infarction.

Figure 2-4 Cumulative incidence of first hospitalisation for heart failure by type of acute myocardial infarction

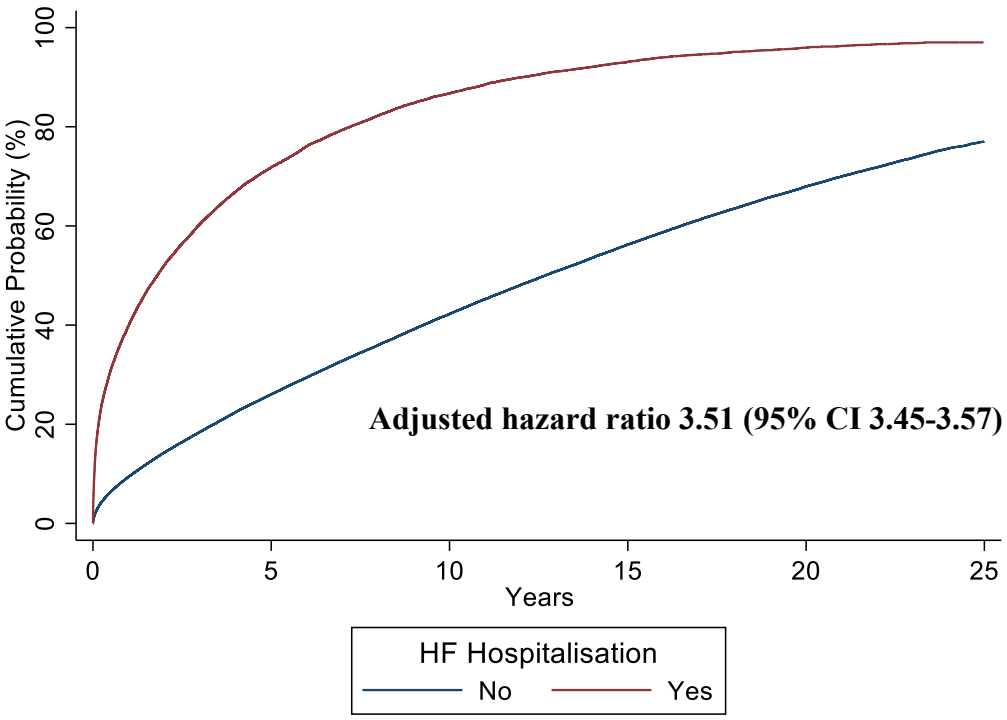


Cumulative incidence curves of first heart failure hospitalisation accounting for the competing risk of death from any cause. Data are presented by type of myocardial infarction. AMI, acute myocardial infarction; HF, heart failure.

2.3.5 Mortality

Annualised mortality was 5-fold greater in those after a first hospitalisation for heart failure compared to those who were never hospitalised for HF; the fatality rate per 1000 patient-years was 254.2 (95% CI 250.5-258.0) for patients following a first heart failure hospitalisation and 53.7 (53.3-54.1) for those never hospitalised for heart failure following a first myocardial infarction. When considered as a time-updated covariate, the occurrence of a first hospitalisation for heart failure increased the risk of mortality by over 3-fold compared to those who never had this event (adjusted hazard ratio 3.51; 95% CI 3.45-3.57) – Figure 2-6. Following a first hospitalisation for HF, median survival did not change significantly between 1991-95 and 2011-16 (1.7 years [95%CI 1.5-1.8] vs. 1.8 years [1.7-1.9]). However, after covariate adjustment, the risk of death at 1-year following a first hospitalisation for heart failure fell by 30% (95% CI 15%-43%) between 1991 and 2015. This represented a yearly decrease in the risk of mortality following admission with heart failure by 1.3% (95% CI 1.0%-1.6%; $p<0.001$).

Figure 2-5 Risk of mortality in patients following first hospitalisation for heart failure compared to those never hospitalised



Legend: Kaplan–Meier curves of death from any cause following a first heart failure hospitalisation or time from myocardial infarction discharge if no heart failure hospitalisation occurred. HF, heart failure.

2.4 Discussion

In this nationwide study of a population of 5.4 million individuals with a single healthcare provider, the incidence of a first hospitalisation for heart failure following discharge after a first myocardial infarction was found to have decreased over the last quarter of a century. This is despite a progressive reduction in the risk of mortality following myocardial infarction, increasing the potential pool of survivors at risk of developing heart failure and increasing age at time of myocardial infarction along with increasing prevalence of comorbidities such as diabetes.

Both the short and long-term risk of mortality following myocardial infarction has fallen over the last quarter of century, likely as a result of an increase in the availability of coronary reperfusion services, improvements in secondary prevention, and increasing sensitivity of troponin assays and diagnosis of myocardial infarction with lesser degrees of myocardial injury (and lower subsequent risk of mortality).[160] In the present study, along with these improvements in survival, three trends were identified which could potentially drive an increasing incidence of heart failure following a first myocardial infarction. Firstly, age at time of myocardial infarction (and at time of first heart failure hospitalisation) increased and secondly, the prevalence of comorbidities associated with a higher risk of developing heart failure following myocardial infarction such as diabetes and chronic kidney disease rose in myocardial infarction patients.[166,173,174] Another trend that might have led to an increase in the rate of incident heart failure was the increase in proportion of NSTEMI to STEMI, as heart failure was more likely to develop after NSTEMI than STEMI.[15]

Despite these observations, the crude incidence of heart failure following myocardial infarction decreased over the period of study. Previous studies have reported similar findings in Olmsted County, United States of America (1990-2010)[175], Sweden (1993-2004 and 2004-2013)[176,177], Western Australia (1996-2007)[178], Denmark (1997-2010)[179], England (1998-2010)[180], in Medicare beneficiaries in the United States of America (1998-2010)[181], New Jersey, United States of America (2000-2015)[182], and in Norway (2001-2009)[183]. The main

strength of the present study is that the follow-up covers more than quarter-of-a-century, including the pre- and post-primary percutaneous coronary intervention eras, with 1.5 million patient-years of follow-up. Therefore, I was able to examine the influence of changes in patient characteristics, diagnostic criteria, myocardial infarction phenotype, therapy and practice, and in long-term survival following myocardial infarction on the subsequent risk of heart failure at a country-wide level and in a single healthcare provider system.

What may explain this decrease? Firstly, the use of emergent coronary reperfusion procedures has increased exponentially, thereby reducing infarct size and the subsequent risk of HF. This is supported by reports of a reduction in the incidence of heart failure complicating the index myocardial infarction admission, particularly in patients with STEMI, a factor which is greatly influenced by the degree of acute ventricular damage sustained at time of myocardial infarction.[184] Additionally, the increased uptake of secondary preventative therapies may have contributed to a decreased long-term risk of heart failure in two ways. Firstly, improvements in coronary stent technology along with the use of more effective antiplatelet agents and statins, promote infarct artery patency and reduce the risk of both acute and remote reinfarction, thereby limiting myocardial damage.[185–187] Secondly, RAS inhibitors and beta-blockers reduce the risk of adverse left ventricular remodelling and therefore, the development of HF.[13,38,63] The implementation of these secondary preventative measures has increased over time and has contributed to reducing the risk of developing heart failure in both the acute period following myocardial infarction and in the longer term.[174,188,189]

Elderly patients, as well as being at a greater risk of developing HF, also have a higher competing risk of death from any cause. This may, in part, explain why I have found that an increase in the proportion of elderly patients with myocardial infarction has not translated into an increase (or plateauing) in the overall crude incidence of first hospitalisation for HF. A further factor that should be considered in the decreasing incidence of heart failure is the changes in the definition of myocardial infarction and increased use of high sensitivity troponin assays, resulting in a greater proportion of myocardial infarction representing

relatively small infarcts with a subsequently low risk of developing HF.[190,191] I found evidence of an increase in age-standardised incidence of myocardial infarction from 2007 following the release of the Universal Definition of Myocardial Infarction[192]; I believe this reflects the increased use of troponin and high sensitivity assays to diagnose myocardial infarction, along with the introduction of the definition of type 1 and type 2 myocardial infarction. Following this, the rate of myocardial infarction declined again, consistent with an overall downward trend in the rate of myocardial infarction observed in Scotland and globally. I report a higher cumulative incidence of heart failure following discharge for NSTEMI and unspecified myocardial infarction compared to STEMI, however, after adjustment for prognostic variables (including age, gender and co-morbidities) I found no significant differences in the risk of heart failure between types of myocardial infarction. This finding likely reflects the different phenotypes of patients presenting with NSTEMI and STEMI, in that patients with NSTEMI are older with more frequent co-morbidities and therefore more frequently develop heart failure following myocardial infarction. It may also reflect a degree of misdiagnosis with small troponin elevations related to myocardial injury in the setting of heart failure presentations being incorrectly classified as NSTEMI.

Over the time period examined, median survival following a first heart failure hospitalisation did not change significantly. However, after covariate adjustment, including year of heart failure hospitalisation and age at time of hospitalisation, the risk of death at one year has fallen by 27% (95% CI 10-41%) between 1990 and 2015. Advances in both pharmacological and device therapy for heart failure with reduced ejection fraction (HFrEF) have contributed to the finding of a reduction in the risk of mortality.[193,194] The 5-fold greater annualised rate of mortality for patients following a first hospitalisation for heart failure following myocardial infarction compared to those never hospitalised for heart failure should act as a reminder to physicians that prevention of this event should be one of the key priorities in care following myocardial infarction. This study has highlighted that particular sub-groups of patients are at a relatively increased risk of heart failure and require close attention to ensure appropriate use of reperfusion service resources and implementation of secondary

preventative measures. As already mentioned, elderly patients are at increased risk of developing heart failure but often do not receive reperfusion therapy and are less likely to be prescribed secondary preventative medications.[195,196] Similarly, patients with diabetes or chronic kidney disease (CKD) are less likely to receive evidence-based treatments, in particular revascularisation, however patients with advanced CKD are frequently excluded from clinical trials therefore evidence of benefit in this group is scarce.[197–199]

Women were 12% more likely than men to develop heart failure at one year following myocardial infarction and this difference persisted out to 10-years (adjusted HR:1.07 [95%CI 1.04-1.11]). Similar findings were reported in an analysis of the HORIZONS-AMI (harmonizing outcomes with revascularization and stents in acute myocardial infarction) trial where women were more likely to develop heart failure at two years following myocardial infarction (multivariable-adjusted odds ratio 1.34 [1.10-1.51]) and in an analysis of high-risk myocardial infarction patients (those in whom myocardial infarction was complicated by heart failure, left ventricular systolic dysfunction or both) in the VALIANT.[200,201] Potential reasons for this include that women are more likely to have a delayed presentation with non-chest pain symptoms of myocardial infarction (potentially increasing infarct size) and are less likely to receive evidence-based treatments (including revascularisation) and effective secondary prevention than men.[202–206] In the present study women, compared with men, were older at the time of myocardial infarction, had a greater prevalence of hypertension, atrial fibrillation, diabetes, renal impairment, more frequently had heart failure complicating index myocardial infarction admission and were less likely to have percutaneous revascularisation within 30 days of myocardial infarction (18.3% versus 27%). Despite adjustment for these factors, women remained at higher risk relative to men which may reflect gender imbalances in myocardial infarction care along with other unmeasured confounders. Finally, in the setting of a universal, single healthcare provider, I have identified persisting differences in outcomes by level of socioeconomic deprivation. This finding is not novel, however highlights that focused efforts are needed to ensure equal provision of resources and robust follow-up for those patients at greatest risk of developing heart failure.

2.5 Limitations

As with all analyses of this nature there are limitations. I did not have information on ejection fraction or levels of biomarkers of cardiac injury which may influence the risk of development of heart failure. Data regarding other predictive factors including body mass index, blood pressure and natriuretic peptide levels were not available I did not have information on secondary preventative therapies which may influence these trends. I only examined mortality from any cause as information regarding cause-specific death from death certificates is often unreliable. The analysis of outcomes by type of myocardial infarction is limited to the recent time period following implementation of specific sub-classification coding in Scotland and I was unable to distinguish between type 1 and type 2 myocardial infarction. I did not have information regarding the community-based diagnosis of heart failure or was unable to differentiate between presentations with heart failure with a reduced or preserved ejection fraction.

2.6 Conclusions

Despite an increasing pool of survivors of myocardial infarction at risk of heart failure, the incidence of heart failure hospitalisation following myocardial infarction in Scotland has consistently decreased since 1990. These trends suggest that better treatment of myocardial infarction and secondary prevention are having an impact on the risk of heart failure at a population level. Furthermore, changes in the diagnostic criteria of myocardial infarction, a decreasing incidence of STEMI and rising NSTEMI incidence may have resulted in a population at risk with less myocardial damage and a subsequently lower risk of heart failure. The risk of mortality following a first hospitalisation for heart failure has also fallen in the period examined, likely reflecting advances in the treatment of heart failure with reduced ejection fraction.

Chapter 3 Methods of a trial examining the effect of neprilysin inhibition on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

3.1 Introduction

As described in Chapter 1, the development of left ventricular systolic dysfunction as a result of myocardial infarction increases the subsequent risk of developing heart failure.[13,36] Progressive dilation of the left ventricle and reduction in stroke volume, the process known as adverse left ventricular remodelling, precedes the development of heart failure and can occur in the days, weeks and even years following myocardial infarction.[17] Indeed, patients can experience a latent asymptomatic period prior to the development of symptomatic heart failure despite a significantly reduced LVEF and dilated left ventricle.[44] The process of adverse left ventricular remodelling following myocardial infarction can be attenuated by pharmacological inhibition of the maladaptive neurohumoral system activation which occurs in response to the reduction in stroke volume.[38] Four different neurohumoral antagonists (ACE inhibitors or ARBs, beta-blockers, and an MRA have been shown to reduce the risk of developing heart failure and death in patients at high risk of developing heart failure following myocardial infarction and the benefit of these drugs are, in part, due to an attenuation of adverse left ventricular remodelling.[46,59,62,65]

The natriuretic peptides, which are secreted by the heart in response to increased wall stress, aim to counteract the adverse effects of activation of the renin-angiotensin aldosterone system and sympathetic nervous system by promoting vasodilation, natriuresis and diuresis, along with inhibiting pathological hypertrophy and fibrosis (Chapter 1-3).[207] Endogenous levels of the natriuretic peptides can be augmented by inhibition of neprilysin, the enzyme responsible for their breakdown along with the catabolism of a range of other vasoactive peptides including adrenomedullin, glucagon-like peptide 1 (GLP-1), apelin, and bradykinin.[208] The addition of a neprilysin inhibitor to an angiotensin II type 1 receptor blocker, in the form of sacubitril/valsartan, has been shown to reduce the risk of cardiovascular death or heart failure hospitalisation in

patients with established HFrEF when compared to RAS inhibition alone with the ACE inhibitor enalapril.[138] Given their vasodilatory, anti-hypertrophic, anti-fibrotic, and sympatholytic effects, along with the clinical benefits observed in patients with HFrEF, the augmentation of natriuretic peptides and other substrates for neprilysin with a neprilysin inhibitor may offer additional protection against progressive adverse left ventricular remodelling following myocardial infarction, thereby reducing the attendant risk of developing heart failure.

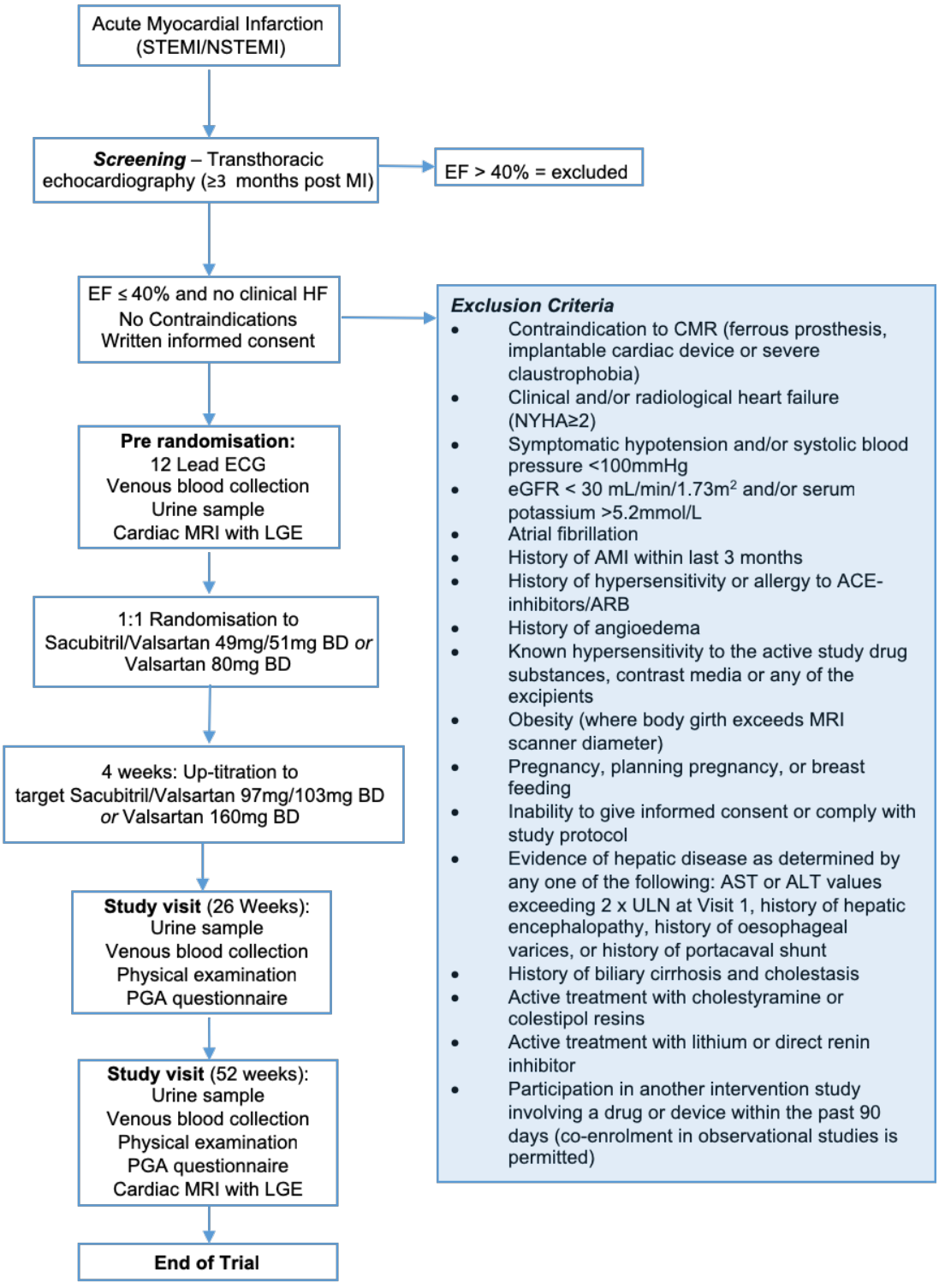
Consequently, I designed a prospective, multicentre, randomised, double-blind, active-comparator trial powered to investigate the effects of the addition of neprilysin inhibition to RAS inhibition on left ventricular volumes in patients with symptomless left ventricular systolic dysfunction late after myocardial infarction.

3.2 Trial design

I performed a multi-centre, prospective, randomised, double-blind, double-dummy active-comparator trial designed to evaluate the effect of the combined angiotensin receptor-neprilysin inhibitor (ARNI) sacubitril/valsartan at a target dose of 97/103mg twice daily, compared with the angiotensin II type 1 receptor blocker (ARB) valsartan at a target dose of 160mg twice daily, on left ventricular volumes in patients with asymptomatic left ventricular systolic dysfunction following myocardial infarction.

The trial was approved by the East of Scotland Research Ethics Committee and was registered as ClinicalTrials.gov Identifier: NCT03552575. A summary of the trial is displayed in Figure 3-1.

Figure 3-1 Trial overview



Abbreviations: AMI, acute myocardial infarction; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; EF, ejection fraction; HF, heart failure; LGE, late gadolinium enhancement; MRI, magnetic resonance imaging; NSTEMI, Non-ST elevation myocardial infarction; NYHA, New York Heart Association; PGA, patient global assessment; STEMI, ST-elevation myocardial infarction;ULN, upper limit of normal.

3.3 Trial Aims

3.3.1 Primary Aim

The primary aim of this trial was to investigate the effect of sacubitril/valsartan compared with the current standard of care valsartan (i.e., the addition of neprilysin inhibition), on attenuating adverse left ventricular remodelling in high-risk asymptomatic patients post-myocardial infarction as a result of residual left ventricular systolic dysfunction (i.e., a reduced left ventricular ejection fraction without symptoms or signs of heart failure).

3.3.2 Secondary Aims

To provide an understanding of the cardiac effects and mechanisms of action of sacubitril/valsartan in patients with asymptomatic left ventricular systolic dysfunction, namely:

- The effect of neprilysin inhibition on NT-proBNP, a marker of left ventricular wall stress.
- The effect of neprilysin inhibition on high sensitivity cardiac troponin-I (hs-TnI), a marker of myocardial injury.
- The effect of neprilysin inhibition on circulating levels of a selection of its substrates (ANP, CNP, adrenomedullin, apelin, GLP-1, and endothelin-I).
- The effect of neprilysin inhibition on biomarkers of myocardial fibrosis and inflammation.

3.4 Trial Outcomes

3.4.1 Primary Outcome

The primary outcome was the change in left ventricular end-systolic volume indexed for body surface area (LVESVI), from baseline to 12 months, based on cardiac MRI measurements.

3.4.2 Secondary Outcomes

The prespecified secondary outcomes were:

- Change in N-terminal pro-B-type natriuretic peptide (NT-proBNP)
- Change in high sensitivity troponin I (hs-TnI)
- Change in other cardiac MRI-based metrics of left ventricular remodelling:
    - Left ventricular end-diastolic volume indexed for body surface area (LVEDVI)
    - Left atrial volume indexed for body surface area (LAVI)
    - Left ventricular ejection fraction (LVEF)
    - Left ventricular mass index (LVMI)
- Change in patient well-being, assessed using a patient global assessment questionnaire

3.4.3 Exploratory Outcomes

- Change in neurohormonal levels and substrates for neprilysin: BNP, MR-proANP, ANP, CNP, MR-proADM, cGMP, endothelin-1, apelin, and GLP-1
- Change in biomarkers of left ventricular remodelling, fibrosis and inflammation: sST2, Galectin 3, TIMP-1, MMP-9, Type III procollagen Peptide and GDF-15
- Change in left ventricular global function index (LVGFI) and left ventricular remote zone extracellular volume (ECV) as measured using cardiac MRI.

3.5 Trial population

3.5.1 Identification of patients

Patients were considered if they had suffered a myocardial infarction at least 3 months prior to randomisation.

The definition of myocardial infarction was in line with the guidelines provided by the European Society of Cardiology and the third Universal definition of myocardial infarction.212,213 Patients were required to meet the following criteria:

- Detection of rise and/or fall of cardiac biomarker values (preferably troponin) with at least one value above the 99th percentile of the upper reference limit and with at least one of the following:
    - Symptoms of ischaemia;
    - New or presumably new significant ST-T changes or new LBBB;
    - Development of pathological Q waves in the electrocardiogram (ECG);
    - Imaging evidence of new loss of viable myocardium, or new regional wall motion abnormality;
    - Identification of an intracoronary thrombus by angiography or autopsy.

All patients without obvious contraindications to enrolment based on review of case notes were approached with regards to taking part in trial-screening.

Potential participants were approached in one of two ways:

(1) Approach in person by the clinical care team at a routine outpatient appointment. Those who demonstrated interest were given the patient information sheet (PIS) (Appendix 1) and asked for verbal consent for their details to be passed to the research team. The PIS contained a contact email address and telephone number to allow patients to “opt in” to the study or get in contact for further information. If there had been no contact from a patient 48 hours after being given the PIS the patient was telephoned to ascertain interest in participation in the study.

(2) Letter drop: letters were posted to the patient with information about the study. The same contact information as above was provided for patients contacted by letter drop to allow them to opt into the study. These patients were also contacted 5-7 days by telephone after posting the letter to assess interest in participating in the study; Initial screening was based on a telephone discussion with the potential patient who has expressed an interest in participating in the study supported by a review of their health records. Where possible, any missing biochemical data required as an inclusion criterion was

ordered ahead of screening as part of standard of care if unrecorded in the last 6 months. This was to ensure appointments for screening echocardiography were only made for patients considered suitable to enter the study and avoid unnecessary inconvenience if they were not thought to be eligible based on routine information. Telephone discussions with the patient aimed to ensure their ability to adhere to the trial requirements, diagnostic tests, outcomes measures, and their ability to give informed consent. If the potential participant wished to discuss any aspect of the study, they were given the opportunity to do so with a member of the research team either over the phone or at the screening study visit and before consent is taken. An independent contact was also provided on the PIS if the patient wished further discussion with an independent party.

3.5.2 Inclusion criteria

This trial recruited male and female patients aged ≥18 years with asymptomatic left ventricular systolic dysfunction (i.e. no evidence of clinical and/or radiological heart failure) ≥ 3 months following acute myocardial infarction. Patients were required to satisfy the following criteria. Eligibility waivers to the inclusion / exclusion criteria were not permitted.

- Acute myocardial infarction ≥3 months prior to randomisation
- Left ventricular ejection fraction ≤40% as measured by transthoracic echocardiography
- Ability to provide written, informed consent
- Age ≥18 years
- Tolerance of a minimum dose of ACE inhibitor/ARB (ramipril 2.5mg twice daily or equivalent – Table 3-1)
- Treatment with a beta-blocker unless not tolerated or contraindicated.

Table 3-1 Total daily doses of commonly used ACE inhibitors or ARB corresponding to ramipril 2.5mg twice daily

ACE inhibitor	Dose	ARB	Dose
Captopril	100mg	Candesartan	16mg
Enalapril	10mg	Irbesartan	150mg
Lisinopril	10mg	Losartan	50mg
Perindopril	4mg	Valsartan	160mg
Trandolapril	2mg		

3.5.3 Exclusion criteria

- Contraindication to cardiac MRI (ferrous prosthesis, implantable cardiac device or severe claustrophobia)
- Clinical (NYHA functional class ≥II) and/or radiological heart failure
- Symptomatic hypotension and/or systolic blood pressure <100 mmHg
- eGFR < 30 mL/min/1.73m^2 and/or serum potassium >5.2mmol/L
- Persistent/permanent atrial fibrillation
- History of acute myocardial infarction within last 3 months
- History of hypersensitivity or allergy to ACE-inhibitors/ARB
- History of angioedema
- Known hypersensitivity to the active study drug substances, contrast media or any of the excipients
- Obesity (where body girth exceeds MRI scanner diameter)
- Pregnancy, planning pregnancy, or breast feeding
- Inability to give informed consent or comply with study protocol
- Evidence of hepatic disease as determined by any one of the following: AST or ALT values exceeding 2 x ULN at Visit 1, history of hepatic encephalopathy, history of oesophageal varices, or history of portacaval shunt
- History of biliary cirrhosis and cholestasis
- Active treatment with cholestyramine or colestipol resins
- Active treatment with lithium or direct renin inhibitor
- Participation in another intervention study involving a drug or device within the past 90 days (co-enrolment in observational studies is permitted)

3.5.4 Consent

Assenting patients who met the eligibility criteria and without any contraindications to taking part in the study were given information regarding the trial as detailed in the PIS and in the prior sections. If agreeable to taking part in the trial, then written, informed consent was sought (Appendix 2). Enrolled patients were allocated a unique patient identifier number which lasted for the duration of the entire trial. The assessment of eligibility and the informed consent process was undertaken by a member of the research team who was qualified by training and experience in taking informed consent to good clinical practice (GCP) standards. Informed, written consent was necessary prior to randomisation. A copy of the consent form was given to the patient, and another uploaded to the patient's online case notes. The original consent form was filed in the study file and a copy of the consent forms was uploaded into the secure study database for each consented patient.

A log of all patients screened for eligibility was completed. Anonymised information was collected including:

- Age
- Gender
- Ethnicity
- Whether the patient was recruited or not recruited to the study

This information was collated in the study database. Screened patients who were not recruited either because they were ineligible or because they declined participation also had the following information recorded:

- The reason not eligible for study participation OR
- Where eligible, reason declined

However, the right of the patient to refuse consent without giving reasons was respected.

3.5.5 Screening

If agreeable and eligible on review of their medical case notes, assenting patients proceeded to screening echocardiography. Patients were consented for screening echocardiography and routine biochemical blood tests (urea and electrolytes [U&Es] and liver function tests [LFTs]) if unrecorded in the last 6 months to assess trial eligibility.

3.5.5.1 Screening echocardiography protocol

Transthoracic echocardiography (TTE) was performed in the left lateral decubitus position. Images were taken from the standard parasternal, apical and subcostal windows. A standard exam was performed, and images were stored for off-line measurements. left ventricular ejection fraction was calculated from apical 4 and 2 chamber views using Simpson's biplane rule.[214] A cut-off of less than or equal to 40% was used for inclusion in the trial. Contrast agents were not used. Patients with insufficient endocardial definition to allow accurate planimetry were excluded.

After screening echocardiography, suitable patients were approached for consent to take part in the trial as described above.

3.6 Trial procedures

3.6.1 Pre-randomisation investigations

The following investigations were performed prior to randomisation and all results were recorded in a study-specific electronic case report form (eCRF):

- Full physical examination – including measurement of height, weight and resting heart rate and blood pressure
- A record of the patients past medical history was obtained. Details of the index myocardial infarction were recorded including date, type of myocardial infarction (STEMI, NSTEMI or unknown), treatment for myocardial infarction (PCI, CABG, thrombolysis, or medical therapy). A history of diabetes mellitus (Type-1 or Type-2), paroxysmal atrial fibrillation, hypertension, hyperlipidaemia, cerebrovascular disease, peripheral arterial disease, chronic obstructive pulmonary disease, asthma, and smoking history was recorded. Prescribed medication pre-randomisation was also recorded.
- 12-lead ECG
- Spot urine collection
- Venepuncture – 50ml of venous blood was withdrawn for biochemical, biomarker and hormonal analysis and baseline renal/liver function for trial eligibility
- Cardiac MRI with gadolinium contrast

3.6.2 Randomisation

Following baseline measurements, participants were randomly assigned to sacubitril/valsartan or valsartan and matched placebo in a 1:1 ratio. Randomisation was stratified by baseline LVESVI measured using cardiac MRI ($\leq 45ml/m^2$ / $>45ml/m^2$) and by use of diuretics. The randomisation schedule was generated by a computer using permuted blocks, with block lengths of 4 and 6 (at random). All participants and trial staff were blind to treatment allocation.

3.6.3 Follow-up visits

Table 3-2 details all of the visits and indicates with an “x” when assessments or procedures were performed. Patients were seen for all visits at the designated time or as close to as possible.

Table 3-2 Schedule of assessments

Study Procedure	**Screening (>3months post myocardial infarction)**	**Visit 1 - Randomisation (Week 0)**	**Visit 2 (Week 1 ± 3 days)**	**Visit 3 (Week 2± 3 days)**	**Visit 4 (Week 4± 3 days)**	**Visit 5 (Week 5± 3 days)**	**Visit 6 (Week 14 ± 7 days)**	**Visit 7 (Week 26 ± 7 days)**	**Visit 8 (Week 39 ± 7 days)**	**Visit 9 (Week 52 ± 3 months)**
Review Inclusion/Exclusion Criteria	X									
Echocardiogram	X									
Obtain informed consent	X	X								
Cardiac MRI		X								X
Physical examination		X	X		X			X		X
Medical history	X	X								
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Vital Signs (Blood pressure/Heart rate)	X	X	X	X	X	X	X	X	X	X
12 Lead ECG		X								
Spot urine collection		X						X		X
Venepuncture (Urea and Electrolytes/FBC and LFTs)	X[&]	X	X	X	X	X	X	X	X	X
Venepuncture (Biochemical/hormonal/ biomarker analysis)		X						X		X
Pregnancy testing in WoCBP		X			X		X	X	X	X

Study Procedure	**Screening (>3months post myocardial infarction)**	**Visit 1 - Randomisation (Week 0)**	**Visit 2 (Week 1 ± 3 days)**	**Visit 3 (Week 2± 3 days)**	**Visit 4 (Week 4± 3 days)**	**Visit 5 (Week 5± 3 days)**	**Visit 6 (Week 14 ± 7 days)**	**Visit 7 (Week 26 ± 7 days)**	**Visit 8 (Week 39 ± 7 days)**	**Visit 9 (Week 52 ± 3 months)**
Patient global assessment questionnaire								X		X
IMP Dispensing*		X			X		X	X		
Up-titrate IMP#					X					
IMP Administration+		X	X	X	X	X	X	X	X	X
Adverse event reporting			X	X	X	X	X	X	X	X
Study completion										X

* Study drug was introduced at equivalent dose to existing ACE-i/ARB treatment (Dose level 2 or 3) at investigator's discretion

#Up-titration not required if patient already on dose level 3 but down titration to dose level 2 permitted in response to COVID-19

&If not done in the preceding 6 months

+Emergency IMP dispensing permitted during COVID-19 pandemic

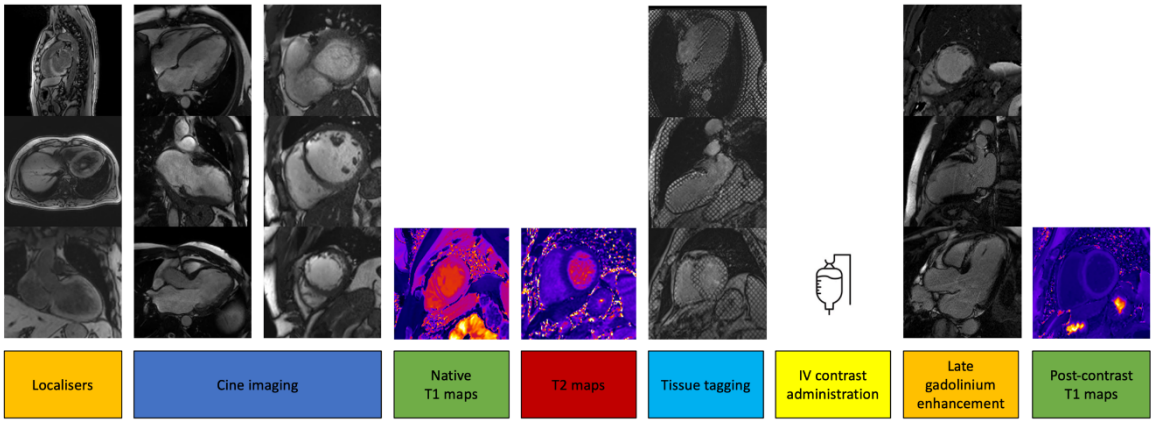
3.6.4 End-of-trial

The end of the trial was defined as the date of the last patient's 52-week (+/- 3 months) study visit plus an additional 30 days of event reporting.

3.6.5 Cardiac MRI protocol

Cardiac Magnetic Resonance Imaging was performed prior to randomisation and at 52 weeks following randomisation with a single 3-Tesla Siemens MAGNETOM Prisma scanner at the Queen Elizabeth University Hospital Glasgow Imaging Centre of Excellence. Images were obtained with a phased-array chest coil, during breath-hold, and gated to the electrocardiogram. The MRI protocol is outlined in Figure 3-2.

Figure 3-2 Outline of cardiac MRI protocol



A steady-state free-precession (SSFP) sequence was used to acquire long-axis (2, 3, and 4-chamber) images and a short-axis cine stack of the left ventricular from the mitral valve plane base to the left ventricular apex, consisting of 7mm thick slices with a 3mm interslice gap. T1 mapping images were created using a modified look-locker inversion recovery (MOLLI) sequence in the short axis view of the left ventricle at the base, mid, and apical ventricle. T1 mapping sequences were performed pre- and post-gadolinium administration. T2-mapping short-axis images (base, mid and apical) were created using a fast low-angle shot (FLASH) sequence pre-gadolinium administration. Myocardial tissue tagging sequences were used to acquire 3 short-axis (base, mid and apex) and three long-axis (2, 3, and 4-chamber) views.

Late gadolinium enhancement images (3 long axis views, and short axis slices at the base, mid and apex of the left ventricle) were acquired 10-15 minutes after intravenous injection of 0.15 mmol/kg of gadolinium diethyltriaminepenta-acetic acid (Gd-DTPA, Magnevist, Bayer Healthcare) using segmented phase-sensitive inversion recovery turbo fast low-angle shot. Full details of the technical parameters of each sequence are presented in Appendix 3.

3.6.6 Cardiac MRI analysis

A single operator (Dr Ross T. Campbell), accredited by the European Association of Cardiovascular Imaging (EACVI) in cardiac MRI analysis with >5 years of experience, analysed all scans blinded to treatment assignment. The baseline and 12-month follow-up scans were analysed in pairs to reduce intra-observer variability, using the methods detailed below and in accordance with the Society for Cardiovascular Magnetic Resonance and European Society of Cardiovascular Imaging guidelines for reporting cardiovascular magnetic resonance examinations.[212,213] A random selection of scans (10%) was analysed by a second operator blinded to treatment assignment for assessment of inter-operator variability and quality assurance and no significant inter-observer variation was seen. All scans were reviewed by a third operator (Dr Giles Roditi) for the purposes of a clinical report, review of non-cardiac elements and the presence of any incidental findings.

For the purposes of the measurement of the primary and secondary MRI endpoints, measurements were performed offline using the commercially available software package (Circle CVI42, Circle Cardiovascular Imaging, Canada) using standard techniques according to the Society for Cardiovascular Magnetic Resonance and European Society of Cardiovascular Imaging guidelines for reporting cardiovascular magnetic resonance examinations.

SSFP short-axis cine images were used to calculate ventricular volumes. Specifically, ventricular volumes were calculated by manually tracing the endocardial border (excluding papillary muscles and trabeculations) in end-systole and end-diastole. The basal left ventricular slice was defined as the most basal slice with >50% myocardium present. Left ventricular outflow tract volume

was included in volumetric analysis. End-diastole was defined as the frame in which the blood pool of the mid-ventricular slice was at its largest, and end-systole was defined as the frame in which the blood pool of the mid ventricular slice was at its smallest. Values for both volumes were indexed by body surface area (BSA) calculated using the Mosteller formula, measured at the time of the scan. Left ventricular mass was calculated as the total difference between the inner and outer circumferences of the left ventricular myocardium in end-diastole, multiplied by the myocardial density (1.05g/cm^3), indexed to body surface area. Left atrial volume was calculated by manually tracing the left atrium endocardial volume in end-systole. This was performed in both the 2-chamber (vertical long-axis view) and 4-chamber (horizontal long-axis view). A left atrial biplane volume was then calculated using the biplane area-length method. T1 areas of interest were drawn using the aforementioned software package. Regions of interest were drawn in myocardium remote to the area of infarction (defined as myocardium 180 degrees from the infarct area site) and left ventricular blood pool. Haematocrit (HCT) was measured at the time of scanning. Extracellular volume (ECV) was calculated as a ratio of corresponding T1 values measured pre- and post-contrast in each of the regions of interest. ECV was calculated using ECV = (1-HCT) × λ, where Lambda (λ)= ΔR1 myocardium /ΔR1 blood, ΔR1= R1 post-contrast - R1 pre-contrast and R1=1/T1. Infarct size, measured in mass and as a percentage of myocardium, was calculated by manually drawing around the epicardial and endocardial border of the late enhancement short axis images, then drawing an area of interest in normal myocardium (180 degrees from the area of infarction). An auto-threshold of 5 standard deviations from this normal myocardium was used to identify areas of late enhancement. Left ventricular global function index (LVGFI) was calculated using the formula:

$$LVGFI = \frac{LVEDV - LVESV}{\frac{LVEDV + LVESV}{2} + \frac{LVmass}{1.05}} \times 100\%$$

Data relating to other cardiac MRI sequences performed, including myocardial strain, are not presented as part of this thesis and will be analysed at a later date.

3.6.7 Biomarker sampling

Venous blood and spot urine samples were collected at baseline and at 6 and 12-months following randomisation (Table 3-2). Patients rested in a supine or seated position for 10-15 minutes prior to sample collection. Venesection was performed and venous blood samples were collected in chilled tubes (2* SST serum, 3* ethylenediaminetetraacetic acid (EDTA) plasma [1* including p800 protease inhibitor] and 1* plasma LiHep) and centrifuged immediately at 1500g at 4°C for 10 minutes before aliquoting and storage at -80°C. A protease inhibitor (Aprotinin, Abcam Cambridge UK) was added to EDTA plasma to minimise degradation of labile peptides such as ANP. A randomly voided sample of urine was collected as the same visit and transferred chilled prior to being aliquoted into 4*samples which were stored at -80°C. All samples were analysed after one freeze-thaw cycle. Individual biomarker assays used for measurement of trial outcomes are detailed in Chapter 5, 6, 7 and 8

3.6.8 Patient global assessment of change

At the 52-week visit patients were asked to complete a patient reported outcome global assessment of change questionnaire as detailed in Appendix 4.

3.7 Study drug

Following randomisation, patients were provided with two packs of tablets – valsartan or matching placebo and sacubitril/valsartan or matching placebo – and instructed to take one pill from each pack (i.e., one active treatment and one placebo pill) twice daily. All participants and trial staff were blind to treatment allocation.

3.7.1 Patients taking an ACE inhibitor at baseline

To minimise the risk of angioedema due to overlapping ACE and neprilysin inhibition, all patients taking an ACE inhibitor at baseline underwent a 36-hour "washout" period following randomisation, prior to the first dose of study drug. For the same reason, use of open-label ACE inhibitor or ARB in addition to the randomised study drug was strictly prohibited for the duration of the trial.

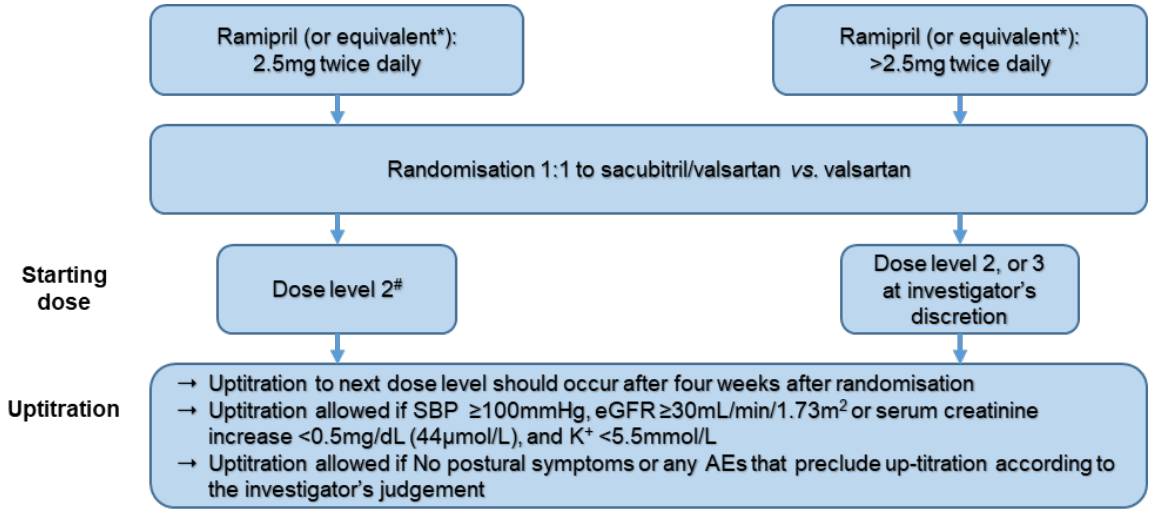
3.7.2 Dose adjustment

Three dose levels of study medication were available, with planned stepwise up-titration (Figure 3-3). Study drug was started at dose level 2 (sacubitril/valsartan 49mg/51mg twice-daily or valsartan 80mg twice-daily) and up-titrated after 4 weeks to dose level 3 (sacubitril/valsartan 97mg/103mg twice-daily or valsartan 160mg twice-daily) if tolerated as assessed by clinical review (systolic blood pressure and symptomatic hypotension) and laboratory evaluation (potassium and renal function). Patients already on a high dose of ACE-inhibitor/ARB could start at dose level 3 at the investigator's discretion.

Alternatively, patients could be started at dose level 1 (sacubitril/valsartan 24mg/26mg twice-daily), with a two-step titration to target dose over visits 3 and 4, if systolic blood pressure at visit 1 was 100 to 110mmHg or if eGFR 30-60 ml/min/1.73 m^2. Down-titration was possible during follow-up, but the goal was to maintain patients on dose level 3 for as much of the trial as possible. Initial up-titration was only halted (or dose subsequently decreased) because of safety or tolerability concerns related to a) symptomatic hypotension, b) a clinically significant decline in renal function or c) hyperkalaemia (Figure 3-3).

Patients continued standard background therapy as prescribed prior to their involvement in the trial.

Figure 3-3 Study drug up-titration criteria



Dose Level	Sacubitril/valsartan arm	Valsartan arm
1	24/26mg twice daily	40mg twice daily
2	49/51mg twice daily	80mg twice daily
3	97/103mg twice daily	160mg twice daily

***** Equivalent doses detailed in Table 3-1

Dose level 1 was considered as an option for patients with systolic blood pressure ≥100 to 110mmHg and/or moderate renal impairment (eGFR 30-60 ml/min/1.73 m^2) at time of randomisation.

Abbreviations: AE, adverse event; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

3.7.3 Monitoring of potential side effects

3.7.3.1 Monitoring of hypotension

Both study drugs can have the potential side effect of symptomatic hypotension and/or postural symptoms. Prior to any dose change, blood pressure was checked and up-titration only occurred if there was no symptomatic hypotension and systolic blood pressure was ≥ 100mmHg. If appropriate, adjustments could be made to doses of diuretic and/or concomitant antihypertensive agents to facilitate the patient's ability to tolerate the target dose of trial treatment.

3.7.3.2 Monitoring of renal function

Both study drugs can lead to changes in renal function and potassium concentrations therefore close monitoring of renal function (serum urea, creatinine and electrolytes) for the duration of the trial was mandatory. This included baseline measurements along with measurements prior to and after the introduction of the study drug and after any dose change. Measurements were taken at regular intervals for the duration of the trial as per the Schedule of Assessments detailed in Table 3-2 and below. Additional measurements were also taken if clinically indicated. All results were reviewed in a timely manner once available and if action is required this was expedited immediately. Each patient had their renal function (along with liver function tests) checked at the following time-points:

- Visit 1: Week 0 (pre-randomisation)
- Visit 2: Week 1 (± 3 days)
- Visit 3: Week 2 (± 3 days)
- Visit 4: Week 4 (± 3 days - pre up-titration of study drug)
- Visit 5: Week 5 (± 3 days - 1 week post up-titration of study drug)
- Visit 6: Week 14 (± 7 days)
- Visit 7: Week 26 (± 7 days)
- Visit 8: Week 39 (± 7 days)
- Visit 9: Week 52 (± 7 days)

If, at any time after randomisation, eGFR decreased by ≥ 25% from baseline (or if serum creatinine concentration increased to 221 µmol/L), potentially reversible cases of renal dysfunction were sought including: non-steroidal anti-inflammatory drug intake, antibiotics, or other nephrotoxic medications; hypovolaemia; and urinary tract infection. If felt to be appropriate, the study drug dose could be reduced and continued with regular monitoring of renal function. If a patient's eGFR decreased by ≥ 40% from baseline (or if serum creatinine concentration rose above 265 µmol/L, then this was an indication to stop the study drug. Thereafter, serum creatinine assessments were repeated at least weekly until levels returned to acceptable values. Re-challenging with the study drug at a lower dose level could be thereafter considered if deemed clinically safe to do so.

3.7.3.3 Monitoring of hyperkalaemia

Patients with elevated potassium value were managed according to the corrective actions outlined below.

Serum potassium > 5.3 and less than or equal to 5.5 mmol/L

- Confirm potassium concentration in a non-haemolysed sample
- Reinforce low potassium diet and restriction of food/drinks with a high potassium content
- (e.g. orange juice, melon, bananas, low-salt substitutes etc.)
- Review medical regimen (including dietary supplements and over-the-counter medications) for agents known to cause hyperkalaemia. Consider reduction in dose or discontinuation of these agents:
    - Potassium-sparing diuretics (e.g. amiloride and triamterene) including in combination products with thiazide or loop diuretics
    - Potassium supplements, e.g., potassium chloride
    - Salt substitutes
    - Non-steroidal anti-inflammatory drugs (NSAIDs)
    - Cyclo-oxygenase-2 (COX-2) inhibitors
    - Trimethoprim and trimethoprim-containing combination products
    - Herbal Supplements: For example, Noni juice, alfalfa (Medicago sativa), dandelion (Taraxacum officinale), horsetail (Equisetum

arvense), nettle (Urtica dioica), milkweed, lily of the valley, Siberian ginseng, hawthorn berries

- Repeat serum potassium measurement within 3 to 5 days
- If serum potassium remained > 5.3 and ≤ 5.5 mmol/L, regular monitoring of serum potassium levels to ensure stability (suggested once monthly)
- Consider down-titration of study medication, according to the investigator's medical judgment.

Serum potassium > 5.5 and < 6.0 mmol/L

- Confirm potassium concentration in a non-haemolysed sample
- Consider down-titration or temporarily discontinue study drug according to the investigator's medical judgment.
- Apply all measures outlined for serum potassium > 5.3 and ≤ 5.5 mmol/L
- Repeat serum potassium measurement after 2-3 days
- If serum potassium < 5.5 mmol/L, consider resumption of study drug at lower dose with repeat potassium within 5 days

Serum potassium greater than or equal to 6.0 mmol/L

- Immediately discontinue study drug
- Confirm potassium concentration in a non-haemolysed sample
- Urgently evaluate patient and treat hyperkalaemia as clinically indicated
- Apply all measures outlined for serum potassium > 5.3 and < 6.0 mmol/L
- Resumption of study drug was decided on a case-by-case basis by the investigators.

3.7.3.4 Management of angioedema

Angioedema is a potential side effect of combined RAS and neprilysin inhibition. Angioedema has been reported in patients treated with sacubitril/valsartan. The trial protocol mandated that if angioedema occurred, then study drug was immediately discontinued, and appropriate therapy and monitoring provided until complete and sustained resolution of signs and symptoms occurred. Study drug was not re-administered. Angioedema occurring during the trial was an automatic withdrawal criterion.

3.7.4 Development of heart failure and study drug/study discontinuation during follow-up

The trial protocol mandated that if a patient developed symptomatic heart failure during follow-up then they were offered open-label sacubitril/valsartan as per current clinical care guidelines. Patients who started open-label sacubitril/valsartan or withdrew from study medication (or study follow-up) ≥6 months after randomisation were asked to undergo an "end-of-study" cardiac MRI examination (patient withdrawing before 6 months were not asked to have a second cardiac MRI as an effect of left ventricular remodelling was unlikely to be detected before that time point).

3.8 Withdrawal criteria

Any patient enrolled in the trial was free to withdraw from the study at any time without giving reason and without prejudicing any further treatment/care. The trial protocol stipulated that following their involvement in the trial any patient who withdrew was converted to an ACE-inhibitor or ARB as per local practice guidelines at an equivalent dose to their final study drug dose level.

3.9 Funding of the trial

This trial was funded by the British Heart Foundation (PG/17/23/32850). Trial medication and funding for trial drug packaging, labelling, distribution, storage and destruction was supplied by Novartis Pharmaceuticals UK Limited who had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation or decision to submit the trial results for publication.

3.10 Pharmacovigilance

3.10.1 Definitions

The following definitions were used as part of the trials monitoring and pharmacovigilance procedures:

Table 3-3 Pharmacovigilance definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other events that are considered medically significant may also be considered serious if they jeopardise the

	participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product. • In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

3.10.2 Recording and reporting of Adverse Events

All AEs which occurred during the trial that were observed by the Investigator or reported by the participant were recorded in the participant's medical records whether or not attributed to trial medication.

Details of the following adverse reactions were collected within the eCRF at all follow-up visits from randomisation until the participants' final visit.

- Clinically relevant hypotension (syncope, dizziness, etc)
- Worsening renal function
- Acute kidney injury
- Hyperkalaemia

- Occurrence of angioedema

The seriousness of each adverse reaction was collected along with the outcome of the event at the time of the visit i.e. whether the reaction was ongoing at the time of the visit or the participant had recovered.

SAEs were documented within the eCRF and were collected from the date of randomisation until 30 days post-cessation of trial treatment to allow for reporting of any ongoing adverse reactions associated with the use of the trial drug.

The following events were excluded from the need for expedited reporting:

- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- Treatment which was elective or pre-planned, for a pre-existing condition not associated with any deterioration in condition, e.g. pre-planned hip replacement operation which does not lead to further complications.
- Any admission to hospital or other institution for general care where there was no deterioration in condition.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious as given above and not resulting in hospital admission.

3.10.3 Assessment of adverse events

All adverse events had to be assessed for seriousness. SAEs occurring between the date of consent and 30 days post-treatment were assessed for causality, expectedness and severity and notified to the Sponsor. This was the responsibility of the Chief Investigator (Professor John McMurray) or designee (Professor Mark Petrie).

Seriousness

AEs were assessed for seriousness as per the definitions in section 10.1; those meeting the criteria for an SAE were subject to expedited reporting to the sponsor.

Assessment of causality

SAEs were assessed for their causality i.e. did the event have a reasonable causal relationship with the trial medication.

Assessment of expectedness

If an SAE was considered to be related (possibly, probably or definitely) to the study medication, an assessment was made of the expectedness of the reaction i.e. is the reaction a recognised adverse effect of the medication.

The expectedness of an adverse reaction was assessed against the Reference Safety Information (RSI) i.e. the list of expected reactions detailed in the Summary of Produce Characteristics (SmPC) for the Investigational Medicinal Product approved during Clinical Trial Authorisation process.

Expected: consistent with the relevant product information documented in the RSI.

Unexpected: not consistent with the relevant product information documented in the RSI.

Assessment of Severity

This was assessed and described using the following categories:

- Mild-awareness of event but easily tolerated
- Moderate-discomfort enough to cause some interference with usual activity
- Severe-inability to carry out usual activity.

3.10.4 Reporting to sponsor (Pharmacovigilance Office)

All SAEs arising during the study were reported by the Principal Investigator (or designee) to the sponsor (Pharmacovigilance Office) by completion of the trial eCRF as soon as reasonably practicable and in any event within 24 hours of first becoming aware of the event. Any follow up information was also reported.

3.10.5 Reporting to the MHRA and REC

All SAEs assigned by the Chief Investigator (on behalf of the sponsor), as both suspected to be related to IMP-treatment and unexpected were classified as Suspected Unexpected Serious Adverse Reactions (SUSARs). These were subject to expedited reporting to the Medicines and Healthcare Products Regulatory Agency (MHRA). The Sponsor Pharmacovigilance office had responsibility to inform the MHRA, the REC and the Sponsor of SUSARs within the required expedited reporting timescales:

- Fatal or life threatening SUSARs: not later than 7 days after the sponsor had information that the case fulfilled the criteria for a fatal or life threatening SUSAR, and any follow up information within a further 8 days.

- All other SUSARs: not later than 15 days after the sponsor had information that the case fulfilled the criteria for a SUSAR.

3.11 Statistical analysis

3.11.1 Sample size

The target study size was 100 patients, based on the calculation that 45 patients in each treatment group provided >90% power (α level=0.05) to detect a difference of 6mL/m^2 in LVESVI (standard deviation=7.8mL/m^2)[214], accounting for a discontinuation rate of 10% (lost to follow up, development of heart failure or death). A 6mL/m^2 difference in LVESVI was selected as it is believed to represent a minimally important difference.[215,216] Prior remodelling studies have reported a 9.2mL (i.e., approximately 5-6mL/m^2) with the beta-blocker carvedilol, 7-11ml/m^2 with the ACE inhibitor captopril and 9mL with enalapril; therefore, the proposed treatment-effect difference is of a magnitude similar to that observed with these established medications with a favourable remodelling effect post-myocardial infarction.[60,63,217,218] In a systematic review of HF remodelling studies a 10 mL (i.e., approximately 6mL/m^2) was the minimal difference associated with a discernible difference in mortality: OR 0.96, 95%CI 0.93-0.98.[219]

3.11.2 Statistical analysis

Statistical analyses were conducted at the study data centre (Clinical Trials Unit, Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow) according to a prespecified Statistical Analysis Plan. All analyses were performed according to the intention-to-treat principle, including all randomly assigned participants with post-randomisation data available for the outcome of interest at any given timepoint, irrespective of their subsequent participation in the study and their adherence to randomised treatment. No imputation for missing data was performed. Data were summarized descriptively for each randomised treatment group, using counts and percentages for categorical variables and mean, standard deviation (SD), or median, 25th and 75th percentiles (IQR), depending on the distribution of the data. Each outcome was analysed using a linear regression analysis model adjusted for randomised treatment, the baseline value of the outcome in question and use of diuretic at baseline. MRI outcomes also included adjustment for the time from baseline to

follow-up MRI. The regression model coefficients for the treatment indicators variable are reported as adjusted between-treatment group mean differences for outcomes at 52 weeks. Log-transformations were performed where required to satisfy modelling assumptions, with regression coefficients back transformed, and interpretable as relative differences. Between-treatment group difference in the patient global assessment of change questionnaire was assessed by means of a Fisher's exact text. For all analyses, a two-sided p-value of <0.05 was considered statistically significant. All analyses for the trial's primary, secondary and exploratory outcomes were conducted by Ms. Bethany Stanley (Clinical Trials Unit, Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow) using R Studio and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). These analyses were verified by me using Stata 16.1 (Statacorp, College Station, Texas, United States). Any further exploratory statistical analyses were performed by me using Stata 16.1, R Studio and R version 4.0.0.

3.12 Covid-19 pandemic mitigation

In March 2020, the COVID-19 pandemic meant that public health measures had to be put in place to limit the spread of the SARS-CoV19 virus and protect the population. Prior to the "stay-at-home" order which was instigated on the 23rd of March 2020 the trial management group made the following plans to assess patient safety with regards to the COVID-19 pandemic and their involvement in the study.

In order to be able to obtain the primary outcome for this clinical trial, and ensure patient participation has been worthwhile, the following amendments were made to the study protocol:

1. An extension to the windows in which the week 52 MRI scan could be done to obtain the primary endpoint by 3 months either side.

2. If the week 52 visit was carried out early (depending on MRI availability) then the IMP was discontinued with conversion to ACE inhibitor or ARB as trial protocol and biomarker samples obtained at this time.

3. The potential to continue IMP provision for this new extended period (i.e., an additional 3 months).

4. Included the option to down titrate patients from dose level 3 to dose level 2 to ensure continued provision of IMP if required due to supply issues.

5. Extend the study finish date to October 2020.

Of the patients scheduled for the 52-week visit after March 23, 2020, 4 had this visit at an earlier (earliest=48 weeks) and 16 at a later time point (latest=62 weeks). All patients remained on the study drug until the end-of-trial visit. No patients were lost to follow-up or did not complete the study due to the COVID-19 pandemic.

3.13 Rationale for key aspects of the trial design

Current guidelines advocate the use of ACE-inhibitors (or angiotensin receptor blockers if ACE-inhibitor contraindicated/not tolerated) along with beta-blockers and mineralocorticoid receptor antagonists to reduce morbidity and mortality after myocardial infarction.[220] The role of ACE-inhibitors/ARBs in preventing adverse remodelling after myocardial infarction is well established. A meta-analysis of 16 trials examining ACE-inhibition after myocardial infarction demonstrated a significant improvement in reduction of left ventricular volumes at both short and long-term follow up in patients with reduced left ventricular ejection fraction who were administered an ACE-inhibitor.[221] Similarly, in patients with asymptomatic left ventricular systolic dysfunction, usually as a result of remote myocardial infarction, ACE inhibitor therapy attenuates progressive left ventricular remodelling.[60]

To date, the remodelling effect of neprilysin inhibition has not been studied in patients after myocardial infarction. Sacubitril/valsartan is a first-in-class ARNI, which has been studied in the pivotal PARADIGM-HF trial in the setting of chronic HF-REF in patients with left ventricular ejection fractions of ≤40%.[138] Sacubitril/valsartan (at a target dose of 97mg/103mg twice daily) when compared to the gold standard ACE-inhibitor, enalapril (target dose 10mg twice

daily), was associated with a significant reduction in cardiovascular death and hospitalisation for heart failure. Reductions in NT-proBNP (indicating a reduction in left ventricular wall stress) and troponin (indicating a reduction in myocardial injury) were also reported in PARADIGM-HF.[222] Sacubitril/valsartan has been shown to have favourable effects on left ventricular remodelling in experimental pre-clinical models of acute myocardial infarction, although in some of these studies, sacubitril/valsartan was compared to control rather than an ACE-inhibitor or ARB.[223]

The choice of an ARB rather than an ACE inhibitor as the comparator agent in this trial was intentional. Unlike PARADIGM-HF and PARADISE-MI which used an ACE-inhibitor (enalapril and ramipril, respectively), in the present trial, the use of valsartan at the dose shown to be as efficacious as captopril in the VALIANT trial, allowed me to precisely define the effects of neprilysin inhibition *per se* without the uncertainty about comparing RAS blockade with an ACE-inhibitor, compared with an ARB.

The target dose (97mg/103mg twice daily) of sacubitril/valsartan was based on the clinical benefit and safety results seen with this dose in PARADIGM-HF. In this trial there was a high prevalence of ischaemic cardiomyopathy (60%), with 43% of patients having had a prior myocardial infarction.[138] This target dose delivers equivalent valsartan exposure (assessed by AUC) as valsartan 160mg twice daily and biomarker analysis (increase in ANP and cGMP) indicates that this dose delivers approximately 90% of its maximal NEP inhibition.[134] The target dose of valsartan (160mg twice daily) was shown to be as effective as the ACE inhibitor captopril (target dose 50mg three times daily) in the VALIANT trial at reducing mortality, recurrent myocardial infarction and hospitalisation for heart failure as well as attenuating adverse remodelling in patients with left ventricular systolic dysfunction and/or heart failure following myocardial infarction. [13,59]

Cardiac MRI is the gold standard means of assessment of left ventricular mass, volumes and ejection fraction. In addition to not requiring the use of ionising radiation, compared with other modalities including transthoracic non-contrast echocardiography, contrast angiography during left heart catheterisation and

radionucleotide ventriculography, cardiac MRI has superior spatial resolution and reproducibility when measuring ventricular volumes and function.[224] This means that smaller samples sizes are required to detect treatment effect differences in cardiac volumes.[225] It also has the additional benefit of allowing assessment of myocardial viability, myocardial fibrosis and regional dysfunction.[226] The choice of the primary endpoint for this trial LVESVI has been shown to be a major determinant of survival after myocardial infarction.[36,227] Furthermore, the short-term effects of established HFrEF therapies on left ventricular volumes and function have been shown to closely correlate with their long-term effect on mortality in patients with left ventricular systolic dysfunction.[219] The degree of left ventricular remodelling and effect of treatment will be measured by the primary endpoint of the change in LVESVI from baseline to 12 months. I will also examine the effect of the addition of neprilysin inhibition on LVGFI, a novel remodelling metric incorporating left ventricular function (volumes) and structure (left ventricular mass) which has been demonstrated to have incremental prognostic value to the measurement of LVEF in patients following STEMI.[228] The benefits of neprilysin inhibition may be in part, related to a prevention of progressive myocardial fibrosis; cardiac MRI has the ability to quantify the degree of myocardial interstitial fibrosis as expressed by the extracellular volume (ECV) fraction.[229,230] This study will provide novel data on the effect of neprilysin inhibition on non-infarct remote zone fibrosis measured using ECV.

I did not consider it ethical to carry out a trial like the one described in patients with symptomatic HFrEF as sacubitril/valsartan has already been shown to be definitively superior to RAS blockade alone in those patients.

3.14 Conclusion

This trial was designed to provide detailed insight into the effects of neprilysin inhibition, added to standard care, in patients at high risk of developing heart failure as a result of residual left ventricular systolic dysfunction following myocardial infarction. It was the first adequately powered, randomised and long-term examination of the effect of sacubitril/valsartan on left ventricular remodelling, and the only comparison with valsartan i.e. the only study to

ensure identical background RAS blocking therapy in both randomised treatment groups. The use of multi-parametric cardiac magnetic resonance imaging provided high-fidelity information about the effects of neprilysin inhibition on cardiac structure and function and, along with comprehensive biomarker profiling of patients, provided further understanding of the mechanisms of action underlying the clinical benefits observed with sacubitril/valsartan in patients with HFrEF.

Chapter 4 The effect of neprilysin inhibition on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

4.1 Introduction

This chapter will describe the results of the primary and secondary remodelling cardiac MRI outcomes of the study described in Chapter 3. This study was designed to examine the effect of the addition of neprilysin inhibition to standard therapy including a RAS inhibitor and beta-blocker on left ventricular remodelling in patients with symptomless left ventricular systolic dysfunction resulting from a previous myocardial infarction. The primary outcome was the change in LVESVI from baseline to 52-weeks measured using the gold-standard method of assessing cardiac volumes, cardiac MRI. Secondary outcomes discussed in this chapter included the change from baseline to 52 weeks in LVEDVI, LAVI, LVEF and LVMI.

4.2 Methods

I designed a prospective, multicentre, randomised, double-blind, active-comparator trial powered to investigate the effects of the addition of neprilysin inhibition to RAS inhibition on left ventricular volumes as measured by cardiac MRI in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction. Full details of the study protocol including randomisation and the study drug are detailed in Chapter 3. The trial protocol and any subsequent substantial amendments were approved by the East of Scotland Research Ethics Committee. All patients provided written consent. The trial is registered (URL: http://www.clinicaltrials.gov. Unique identifier: NCT03552575).

4.2.1 Recruitment and screening

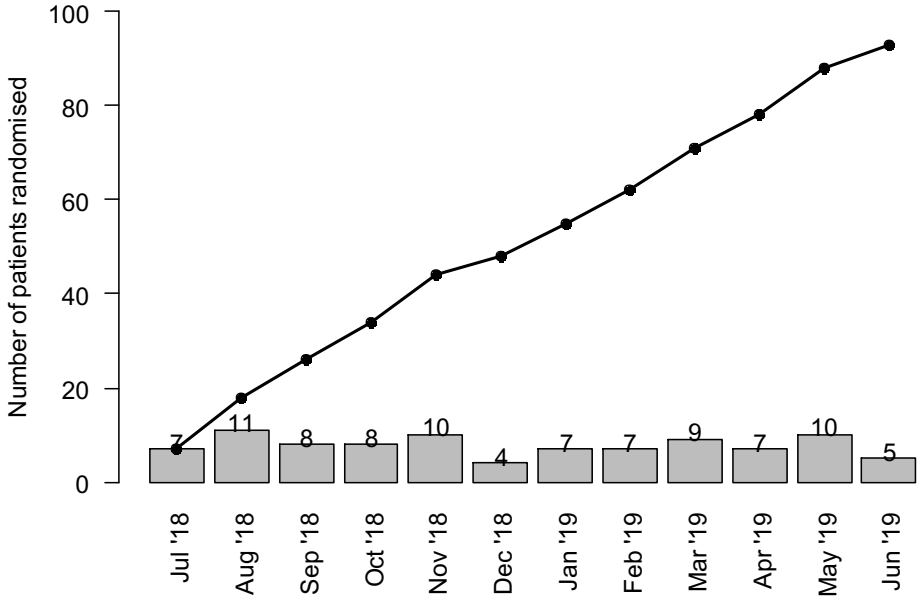
Eligible patients were those who had suffered an acute myocardial infarction at least 3 months prior to screening, had a left ventricular ejection fraction as measured by screening transthoracic echocardiography of 40% or less (see section 3-4-5 for details of screening procedures), were taking or able to

tolerate a minimum dose of ACE inhibitor or ARB (ramipril 2.5mg twice daily or equivalent Table 3-1) and were taking a beta-blocker unless not tolerated or contraindicated. Key exclusion criteria included signs or symptoms of chronic heart failure (i.e., New York Heart Association (NYHA) functional classification ≥II), permanent or persistent atrial fibrillation, an estimated glomerular filtration rate of <30 mL/min per 1.73 m^2, or a serum potassium level of >5.2 mmol/L. Full inclusion and exclusion criteria are detailed in Chapter 3-5.

Following screening echocardiography, eligible and assenting patients underwent a series of baseline investigations including cardiac MRI and blood and urine collection for biomarker analysis. Patients were then randomised 1:1 to sacubitril/valsartan (target dose 97/103mg twice daily) or valsartan (target dose 160mg twice daily plus matching placebo (see Chapter 3.6 for full details of the randomisation procedure). Both investigators and patients were blind to study drug allocation for the full duration of the trial.

Target recruitment was n=100 and screening began on July 9th 2018 and ended on 28th June 2019 (Figure 4-1). Follow-up was completed on June 19th 2020 (See Chapter 3-12 for details of the COVID-19 mitigation strategy). A total of 158 patients were screened of whom 93 were randomised, 47 to sacubitril/valsartan and 46 to valsartan (Figure 4-2). Of the 158 patients screened, 65 (41%) were not randomised: 2 (3.1% of 65) patients met eligibility criteria but did not tolerate baseline MRI pre-randomisation; 2 (3.1%) patients underwent baseline MRI, however review of their imaging pre-randomisation demonstrated relatively preserved left ventricular systolic function and, therefore, it was felt that they did not represent the desired patient population (i.e. those with left ventricular systolic dysfunction) and they were excluded from further involvement; 3 patients (4.6%) met eligibility criteria but declined further involvement following screening; 58 (89%) patients did not meet eligibility criteria and the reasons for this are detailed in Figure 4-3. The most common reason was a left ventricular ejection fraction >40% on screening echocardiography (n=36 [62%]), followed by 17 patients (29%) who were taking a dose of an ACE inhibitor or ARB which was less than a total daily dose equivalent to ramipril 2.5mg twice daily. All 17 of these patients subsequently underwent a period of observed uptitration and were eligible for the study on rescreening.

Figure 4-1: Trial recruitment timeline



Number of subjects randomised (grey bars) and cumulative number (solid black line) randomised per month, by calendar month and year.
Total number randomised = 93.

Figure 4-2 Consort diagram

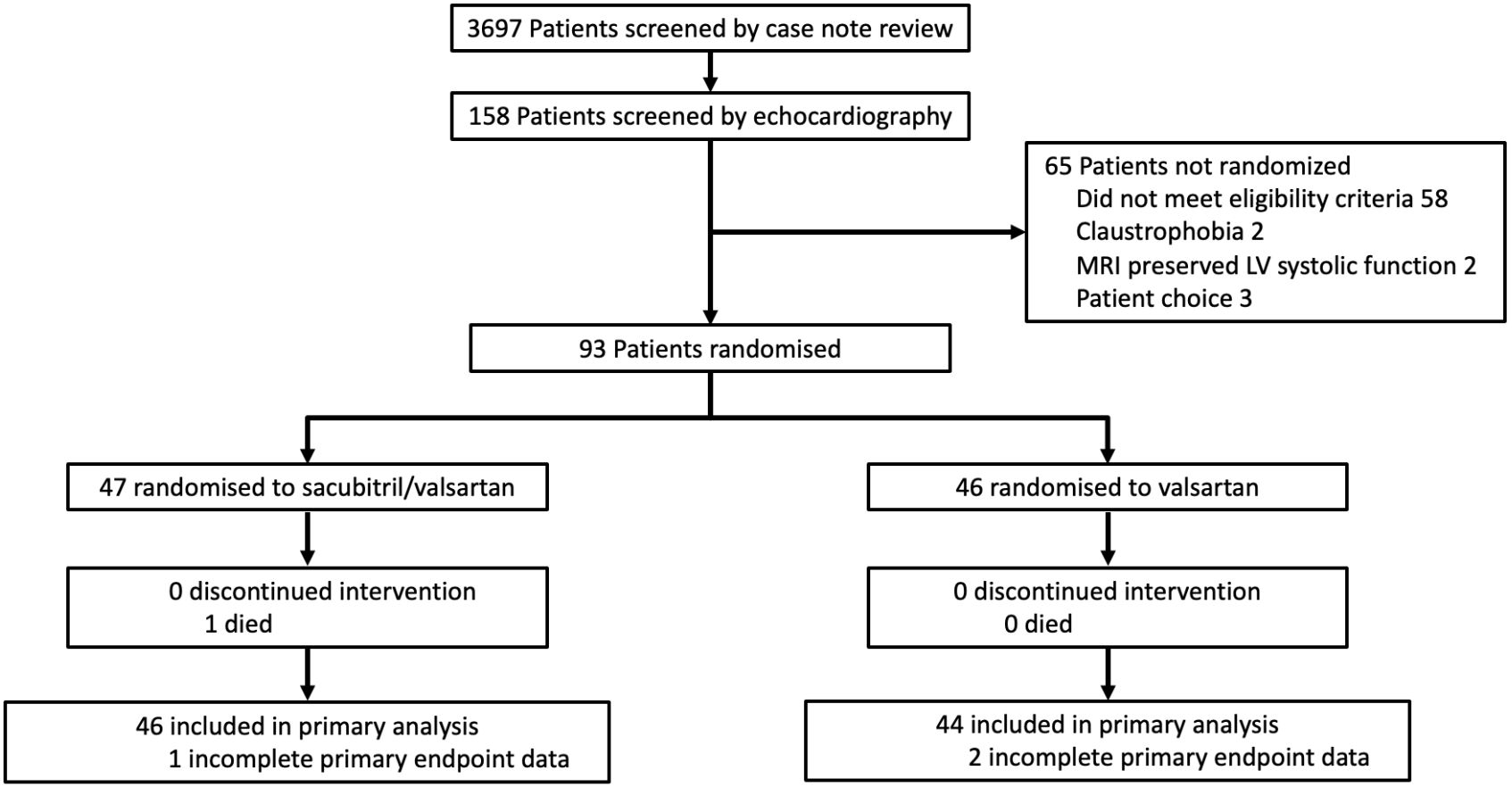
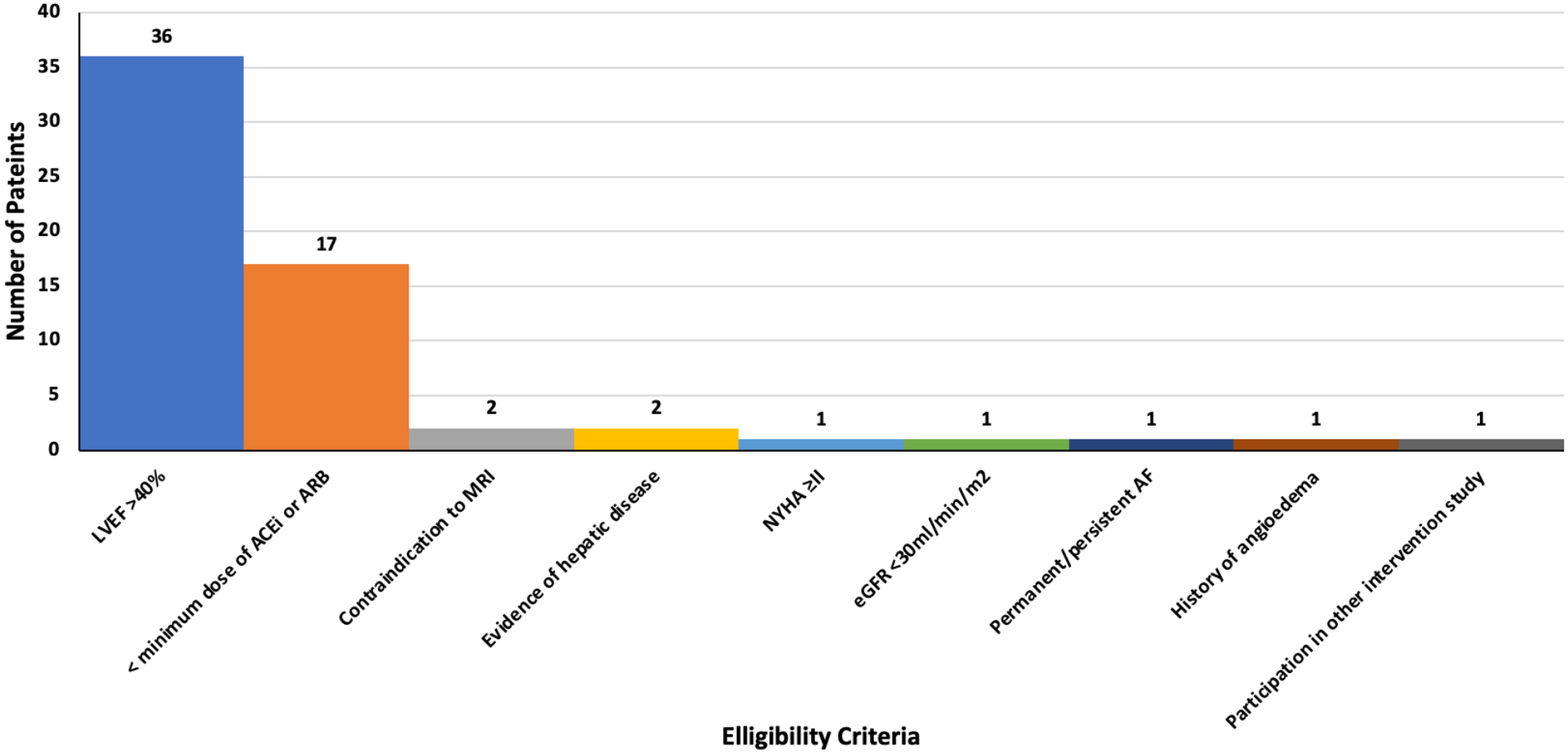


Figure 4-3 Summary of reasons for ineligibility on screening.



N=58 patients but some patients had >1 reason for inellibility

4.2.2 Cardiac MRI techniques and analysis

ECG-gated cardiac MRI was performed at the Glasgow Clinical Research Imaging Facility, Queen Elizabeth University Hospital) at baseline pre-randomisation and week 52 using a 3.0 Tesla scanner (MAGNETOM Prisma, Siemens Healthcare). The imaging protocol is detailed in Chapter 3.6. All scans were reported by 1 European Association of Cardiovascular Imaging cardiac MRI–certified observer (Dr Ross Campbell) blinded to treatment allocation. Measurements of left ventricular and atrial volumes were performed according to standard techniques as detailed in Chapter 3.6.

4.2.3 Statistical analysis

All statistical analyses were conducted by Ms. Bethany Stanley at the study data centre (Clinical Trials Unit, Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow) and replicated by myself according to a prespecified Statistical Analysis Plan as detailed in Chapter 3-11. All outcomes were performed according to the intention-to-treat principle, including all randomly assigned participants with post-randomisation data available for the outcome of interest at any given time point, irrespective of their subsequent participation in the study and their adherence to randomised treatment.

As described in Chapter 3-11, the MRI outcomes reported in this chapter were analysed using a linear regression analysis model adjusted for randomised treatment, the baseline value of the outcome in question, use of diuretic at baseline and the time from baseline to follow-up MRI. The regression model coefficients for the treatment indicators variable are reported as adjusted between-treatment group mean differences for outcomes at 52 weeks

In a *post hoc* analysis, I examined for the effect of any modification of treatment effect on the primary outcome by baseline NT-proBNP level using a linear regression model with interaction between treatment group and baseline NT-proBNP (examined as a categorical variable below or at and above the median baseline level [230 pg/mL]), adjusted for randomised treatment, baseline LVESVI, use of diuretics at baseline and time from randomisation to cardiac MRI.

The primary outcome of the study was the change from baseline to 52 weeks in LVESVI, measured using cardiac MRI, and indexed for body surface area. A sample size of n=100 was proposed based on the calculation that 45 patients in each treatment group provided>90% power (α level=0.05) to detect a mean between-group difference in change in LVESVI from baseline of 6 mL/m^2 (SD of change=7.8 mL/m^2), accounting for a discontinuation rate of 10% (lost to follow-up, development of heart failure, or death). A 6 mL/m^2 change in LVESVI was felt to represent a minimum clinically meaningful difference based on prior reports that this was the minimal difference associated with a discernible difference in mortality.[219]

4.3 Results

4.3.1 Baseline characteristics

The baseline characteristics of patients summarised by randomised treatment allocation are displayed in Table 4-1 and baseline cardiac MRI outcome measurements in Table 4-2. Mean age was 60.7 (SD 10.4) years, and 85 patients (91.4%) were male. A history of hypertension was recorded in 20 (21.5%), paroxysmal AF in 4 (4.3%), previous stroke in 3 (3.2%), chronic obstructive pulmonary disease in 1 (1.1%), asthma in 6 (6.5%), previous cancer in 6 (6.5%), diabetes in 15 (16.1%) and 57 (61.3%) were current (14 [24.6%] or ex-smokers (43 [46.2%]).

The median time from myocardial infarction was 3.6 years (IQR 1.2-7.2), the index myocardial infarction was an ST-elevation MI in 90 (96.8%) patients, in the anterior location in 88 (94.6%) patients, and most patients (89 [95.7%]) had received percutaneous (n=86 [92.5%]) or surgical revascularisation (n=3 [3.2%]) as treatment for the myocardial infarction. Of the randomised patients, 6 (6.5%) had a myocardial infarction prior to the index MI, of whom 5 had percutaneous revascularisation prior to the index myocardial infarction.

By very nature of the inclusion criteria, all patients were taking an ACE-inhibitor (84.9%) or ARB (15.1%) prior to enrolment and a beta-blocker was taken by 87 (93.5%) patients, an MRA by 40 (43%), and a loop diuretic by 11 (11.8%).

Mean time from randomisation to 52-week cardiac MRI was 371 days (SD 17.2); range 342-440.The mean (SD) cardiac MRI LVEF was 36.8% (7.1%) and mean infarct size was 28.4% (10.9%). Mean (SD) LVESVI, LVEDVI, LAVI, and LVMI were 74.8 (19.6) ml/m^2, 117.2 ml/m^2 (21.0), 46.7 ml/m^2 (14.0), and 51.9 g/m^2 (8.4), respectively. Median NT-proBNP was 230 pg/mL (IQR 124–404). There were significant correlations between infarct size and the degree of ventricular remodelling at baseline; LVESVI (Pearson r=0.38; p=0.0003, LVEDVI r=0.29; p=0.005, LVEF r=-0.37, p=0.0004).

Table 4-1: Baseline characteristics

Characteristic	Sacubitril/valsartan (n=47)	Valsartan (n=46)
Age, mean (SD), years	61.8 (10.6)	59.7 (10.1)
Male, n (%)	42 (89.4)	43 (93.5)
Body mass index, mean (SD),kg/m^2	28.8 (4.2)	28.0 (5.0)
Systolic blood pressure, mean (SD), mmHg	124 (14)	123 (13)
Heart rate, mean (SD), beats/min	60.2 (7.6)	59.7 (9.4)
Estimated glomerular filtration rate, mean (SD), mL/min/1.73m^2	87.3 (15.4)	88.2 (15.0)
NT-proBNP, median (IQR), ng/L	216 (128-394)	242 (124-426)
Cardiac MRI left ventricular ejection fraction, mean (SD), %	36.0 (6.4)	37.7 (7.6)
Infarct size, %	27.7 (10.9)	29.1 (10.9)
Myocardial infarction history		
Time since MI, median (IQR), years	3.6 (1.5-6.5)	4.0 (1.2-7.2)
MI type:		
STEMI, n (%)	46 (97.9)	44 (95.7)
NSTEMI, n (%)	1 (2.1)	2 (4.3)
Infarct location:		
Anterior, n (%)	44 (93.6)	44 (95.7)
Inferior, n (%)	2 (4.3)	1 (2.2)
Lateral, n (%)	1 (2.1)	1 (2.2)
Treatment for MI:		
PCI, n (%)	46 (97.9)	40 (87.0)
CABG, n (%)	1 (2.1)	2 (4.3)
Thrombolytic, n (%)	0 (0)	1 (2.2)
Medical history		
Hypertension, n (%)	12 (25.5)	8 (17.4)
Diabetes, n (%)	9 (19.1)	6 (13.0)
Stroke, n (%)	1 (2.1)	2 (4.3)
Medications		
Antiplatelet, n (%)	46 (97.9)	42 (91.3)
Anticoagulant, n (%)	5 (10.6)	6 (13.0)
Statin, n (%)	42 (89.4)	46 (100.0)
ACE inhibitor*, n (%)	41 (87.2)	38 (82.6)
Angiotensin receptor blocker*, n (%)	6 (12.8)	8 (17.4)
Beta-blocker, n (%)	45 (95.7)	42 (91.3)
Mineralocorticoid receptor antagonist, n (%)	18 (38.3)	22 (47.8)
Loop diuretic, n (%)	6 (12.8)	5 (10.9)

Baseline characteristics are presented for all randomised patients.

Estimated glomerular filtration rate was calculated using the CKD-EPI formula.

*Prior to enrolment

Abbreviations: ACE, angiotensin-converting enzyme; CABG, coronary artery bypass graft; IQR, interquartile range; NSTEMI, non-ST elevation myocardial infarction; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST elevation myocardial infarction.

4.3.2 Completeness of Follow-Up and Adherence

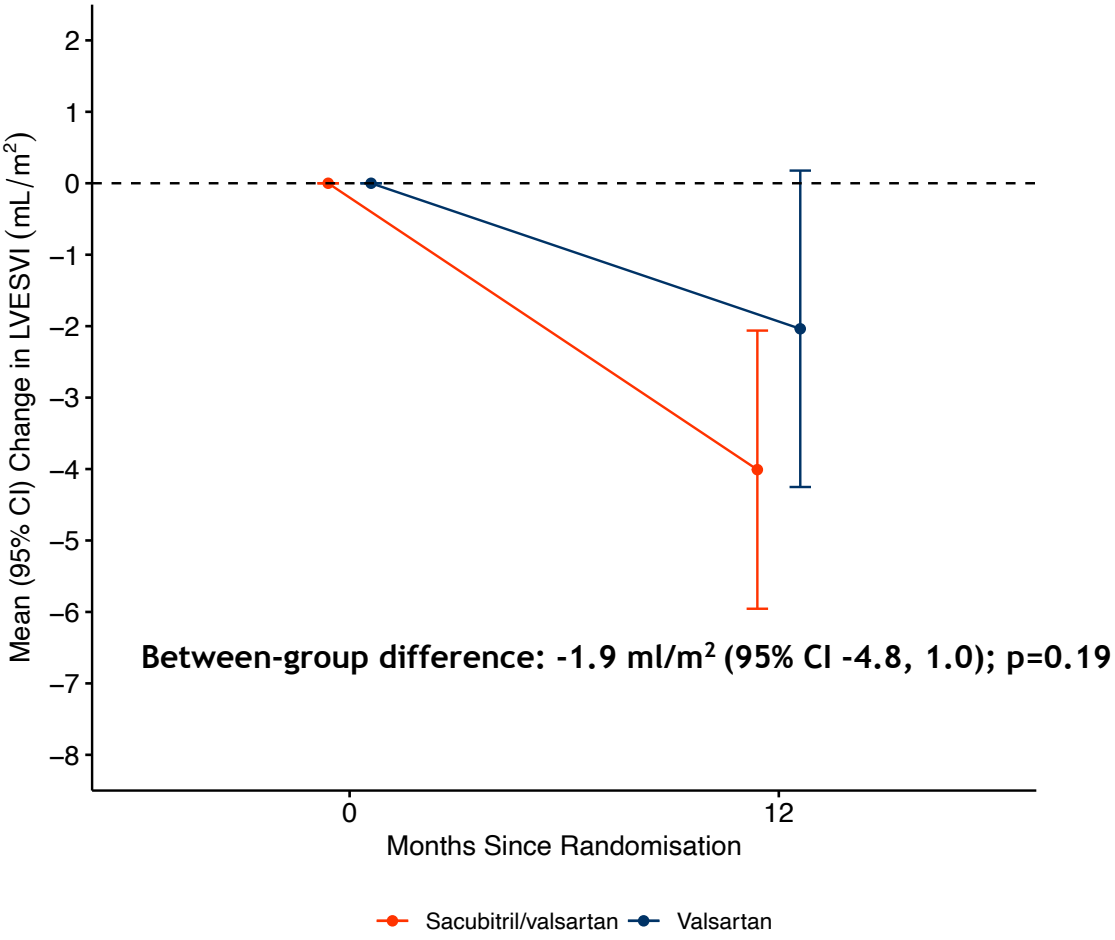
Of the 47 patients randomised to sacubitril/valsartan, 46 remained on randomised treatment and had complete primary outcome data at baseline and week 52 (Figure 4-2). Of the 46 patients randomly assigned to valsartan, 46 remained on randomised therapy and 44 had complete primary outcome data at baseline and week 52. There was 1 death (sudden cardiac death) in the sacubitril/valsartan group. There were no deaths in the valsartan group. At the end of the trial, of patients alive, 42 of 46 (91.3%) patients were taking the target dose of sacubitril/valsartan (97/103mg twice daily) and 46 of 46 (100%) patients were taking the target dose of valsartan (160mg twice daily).

4.3.3 Effect of neprilysin inhibition on left ventricular end-systolic volume index

Mean (SD) LVESVI at baseline was 74.7 (18.2) mL/m^2 in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 75.3 (21.3) mL/m^2 in those randomised to valsartan (n=44). LVESVI decreased by 4.0 (SD 6.6) mL/m^2 between baseline and 52 weeks in the sacubitril/valsartan group and by 2.0 (SD 7.3) mL/m^2 in the valsartan group: adjusted between-group difference –1.9 (95% confidence interval [CI], –4.8, 1.0) mL/m^2; p=0.19 (Table 4-2 and Figure 4-4).

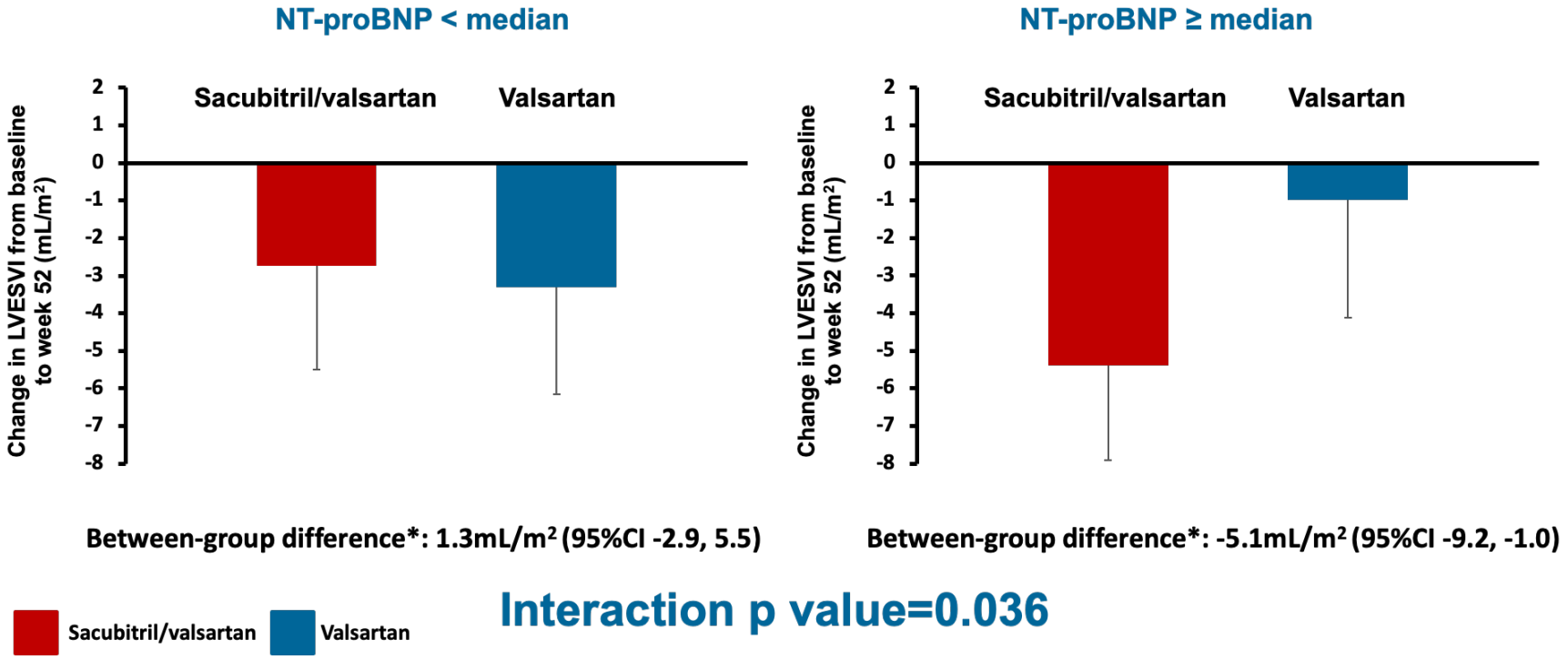
In a *post hoc* subgroup analysis of patients below and at or above the median NT-proBNP level at baseline (230 pg/mL), there was a nominally significant interaction between baseline NT-proBNP and randomised treatment effect (interaction p value=0.036). The adjusted mean between-treatment group difference in LVESVI from baseline was -5.1 (95% CI -9.2, -1.0) mL/m^2 in those with NT-proBNP ≥230 pg/mL at baseline, and 1.3 (95% CI -2.9, 5.5) mL/m^2 in those with baseline NT-proBNP <230 pg/mL (Figure 4-5).

Figure 4-4 Change in left ventricular end-systolic volume index from baseline to week 52



Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LVESVI, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LVESVI = left ventricular end-systolic volume index.

Figure 4-5 Change in left ventricular end-systolic volume index by baseline NT-proBNP level



Data presented as mean and error bars represent 95% confidence intervals.
This analysis was *post-hoc*.
Median NT-proBNP was 230 pg/mL
LVESVI, left ventricular end-systolic volume index.
* Treatment effect calculated using a linear regression model with interaction between treatment group and baseline NT-proBNP, adjusted for randomised treatment, baseline value of the outcome, use of diuretics at baseline and time from randomisation to cardiac MRI.

Table 4-2 Change in primary and secondary MRI outcomes with sacubitril/valsartan or valsartan from baseline to week 52

	Sacubitril/valsartan				Valsartan				Between-group difference (95% CI) *	P Value
	n	Baseline	Week 52	Change	n	Baseline	Week 52	Change		
LVESVI, mL/m^2	46	74.7 (18.2)	70.7 (17.3)	-4.0 (6.6)	44	75.3 (21.3)	73.3 (24.1)	-2.0 (7.3)	-1.9 (-4.9, 1.0)	0.19
LVEDVI, mL/m^2	46	115.4 (20.6)	111.0 (19. 8)	-4.4 (8.8)	44	119.3 (21.9)	118.1 (26.5)	-1.2 (8.6)	-3.1 (-6.8, 0.6)	0.10
LAVI, mL/m^2	46	46.2 (13.6)	43.4 (14.2)	-2.8 (9.0)	43	47.4 (14.6)	46.5 (16.6)	-0.8 (11.7)	-2.3 (-6.6, 2.0),	0.29
LVEF, %	46	35.8 (6.4)	36.9 (6.6)	1.1 (3.4)	44	37.7 (7.6)	39.1 (7.3)	1.4 (3.6)	-0.5 (-2.0, 0.9),	0.46
LVMI, g/m^2	46	51.9 (9.0)	49.4 (9.4)	-2.4 (4.9)	44	52.1 (8.0)	51.0 (9.5)	-1.1 (5.0)	-1.5 (-3.5, 0.6)	0.16

Data presented as mean (SD).

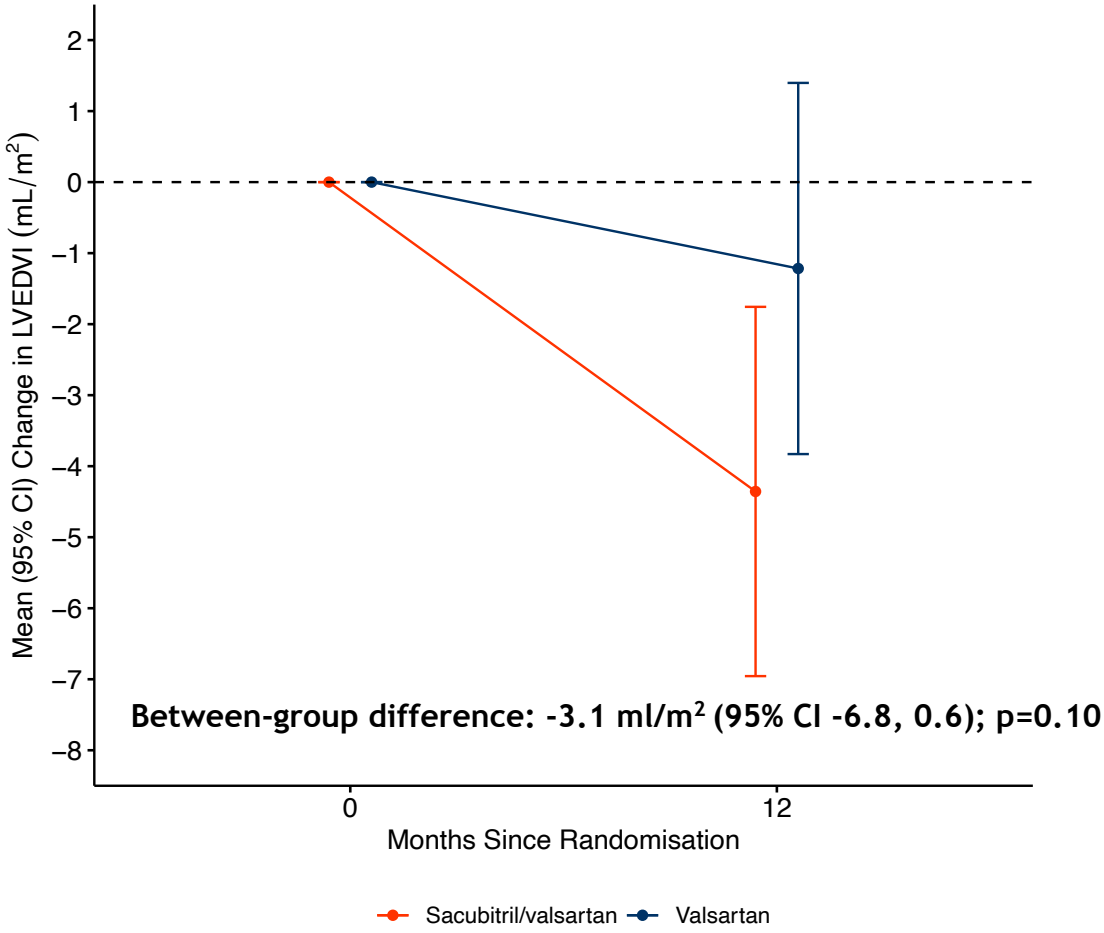
Results reported for those with data available at baseline and 52-weeks.

Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline value of the outcome, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging.

4.3.4 Effect of neprilysin inhibition on left ventricular end-diastolic volume index

Mean (SD) LVEDVI at baseline was 115.3 (20.6) mL/m^2 in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 119.3 (21.9) mL/m^2 in those randomised to valsartan (n=44). LVEDVI decreased by 4.4 (SD 8.8) mL/m^2 between baseline and 52 weeks in the sacubitril/valsartan group and by 1.2 (SD 8.6) mL/m^2 in the valsartan group: adjusted between-group difference –3.1 (95% CI, –6.8, 0.6) mL/m^2; p=0.10 (Table 4-2 and Figure 4-6). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in LVEDVI from baseline was -6.1 (95% CI -11.4,-0.8) mL/m^2 in those with NT-proBNP ≥230 pg/mL at baseline, and 0.1 (95% CI -5.3, 5.5) mL/m^2 in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.11).

Figure 4-6 Change in left ventricular end-diastolic volume index from baseline to week 52

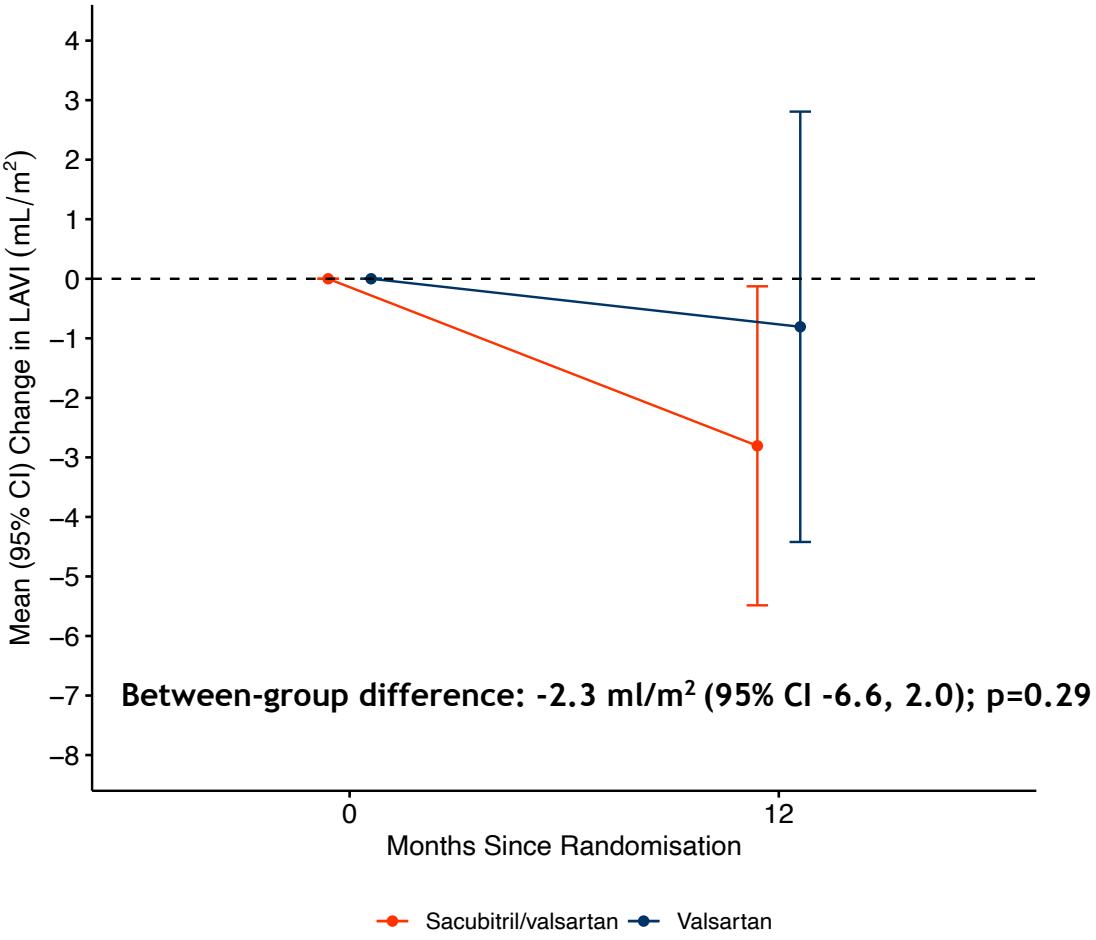


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LVEDVI, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LVEDVI = left ventricular end-diastolic volume index.

4.3.5 Effect of neprilysin inhibition on left atrial volume index

Mean (SD) LAVI at baseline was 46.2 (13.6) mL/m^2 in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 47.5 (14.6) mL/m^2 in those randomised to valsartan (n=43). LAVI decreased by 2.8 (SD 9.0) mL/m^2 between baseline and 52 weeks in the sacubitril/valsartan group and by 0.8 (SD 11.7) mL/m^2 in the valsartan group: adjusted between-group difference –2.3 (95% CI, –6.6, 2.0) mL/m^2; p=0.29 (Table 4-2 and Figure 4-7). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in LAVI from baseline was -2.0 (95% CI -8.1, 4.1) mL/m^2 in those with NT-proBNP ≥230 pg/mL at baseline, and -2.3 (95% CI -8.5, 3.9) mL/m^2 in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.95).

Figure 4-7 Change in left atrial volume index from baseline to week 52

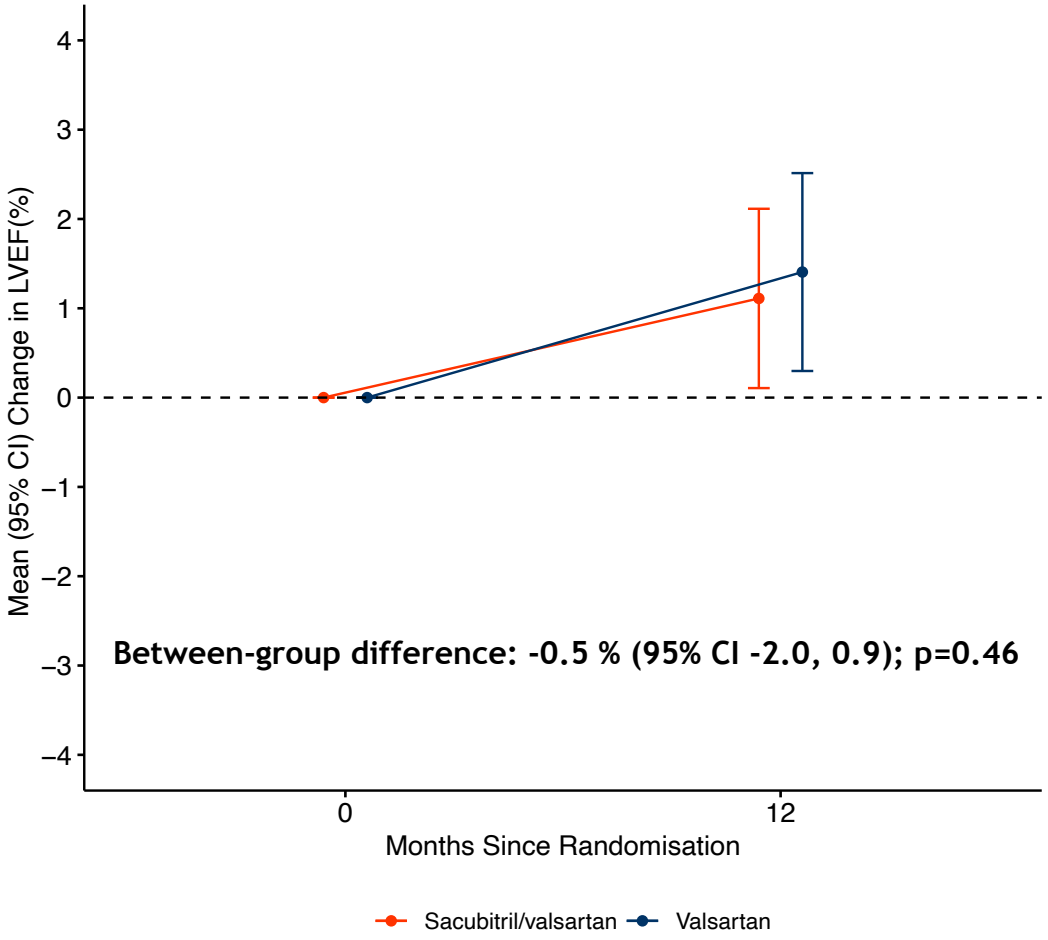


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LAVI, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LAVI = left atrial volume index.

4.3.6 Effect of neprilysin inhibition on left ventricular ejection fraction

Mean (SD) LVEF at baseline was 35.8 (6.4) % in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 37.7 (7.6) % in those randomised to valsartan (n=44). LVEF increased by 1.1 (SD 3.4) % between baseline and 52 weeks in the sacubitril/valsartan group and by 1.4 (SD 3.6) % in the valsartan group: adjusted between-group difference –0.5 (95% CI, –2.0, 0.9) %; p=0.46 (Table 4-2 and Figure 4-8). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in LVEF from baseline was 0.52 (95% CI -1.5,2.6) % in those with NT-proBNP ≥230 pg/mL at baseline, and -1.7 (95% CI -3.8, 0.5) % in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.15).

Figure 4-8 Change in left ventricular ejection fraction from baseline to week 52

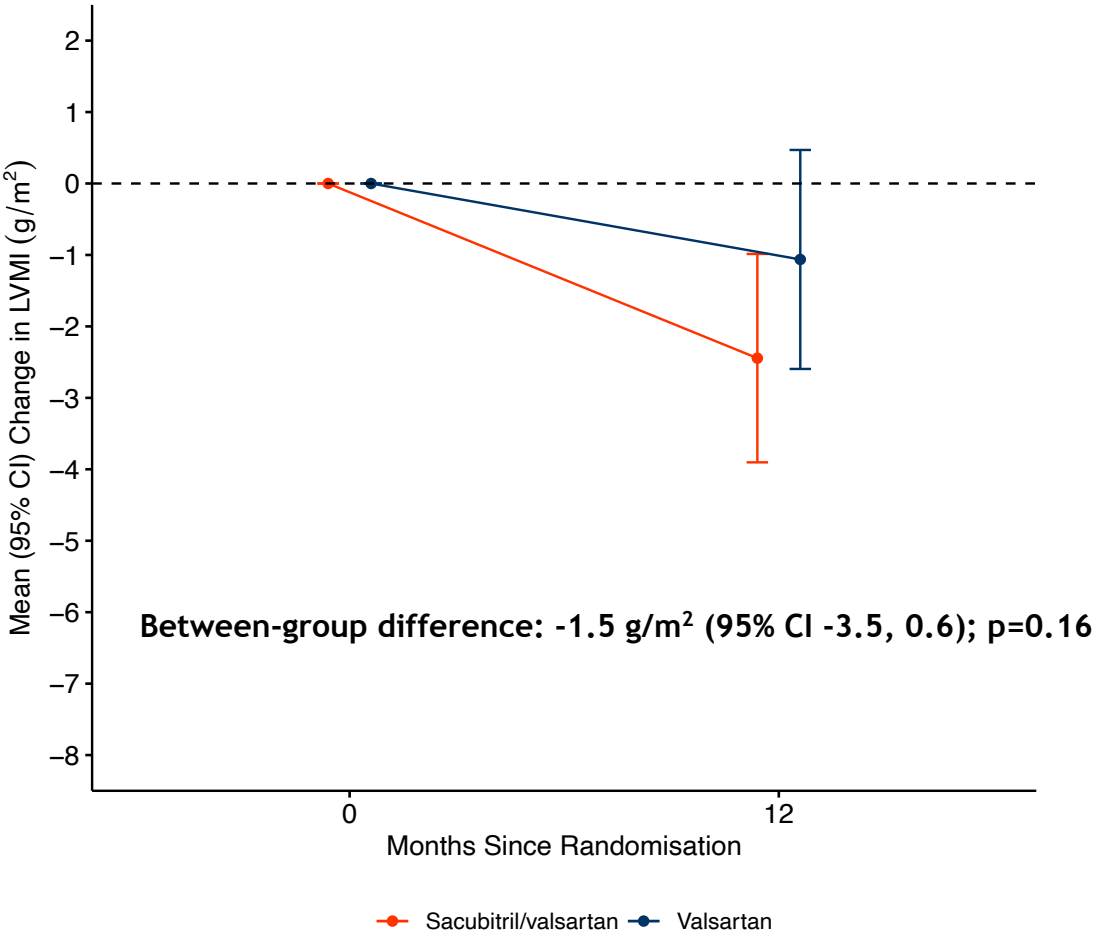


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LVEF, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LVEF = left ventricular ejection fraction.

4.3.7 Effect of neprilysin inhibition on left ventricular mass index

Mean (SD) LVMI at baseline was 51.9 (9.0) g/m^2 in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 52.1 (8.0) g/m^2 in those randomised to valsartan (n=44). LVMI decreased by 2.4 (SD 4.9) g/m^2 between baseline and 52 weeks in the sacubitril/valsartan group and by 1.1 (SD 5.0) g/m^2 in the valsartan group: adjusted between-group difference –1.5 (95% CI, –3.5, 0.6) g/m^2; p=0.46 (Table 4-2 and Figure 4-9). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in LVMI from baseline was -3.1 (95% CI -6.1,-0.2) g/m^2 in those with NT-proBNP ≥230 pg/mL at baseline, and 0.2 (95% CI -2.8, 3.2) g/m^2 in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.13).

Figure 4-9 Change in left ventricular mass index from baseline to week 52

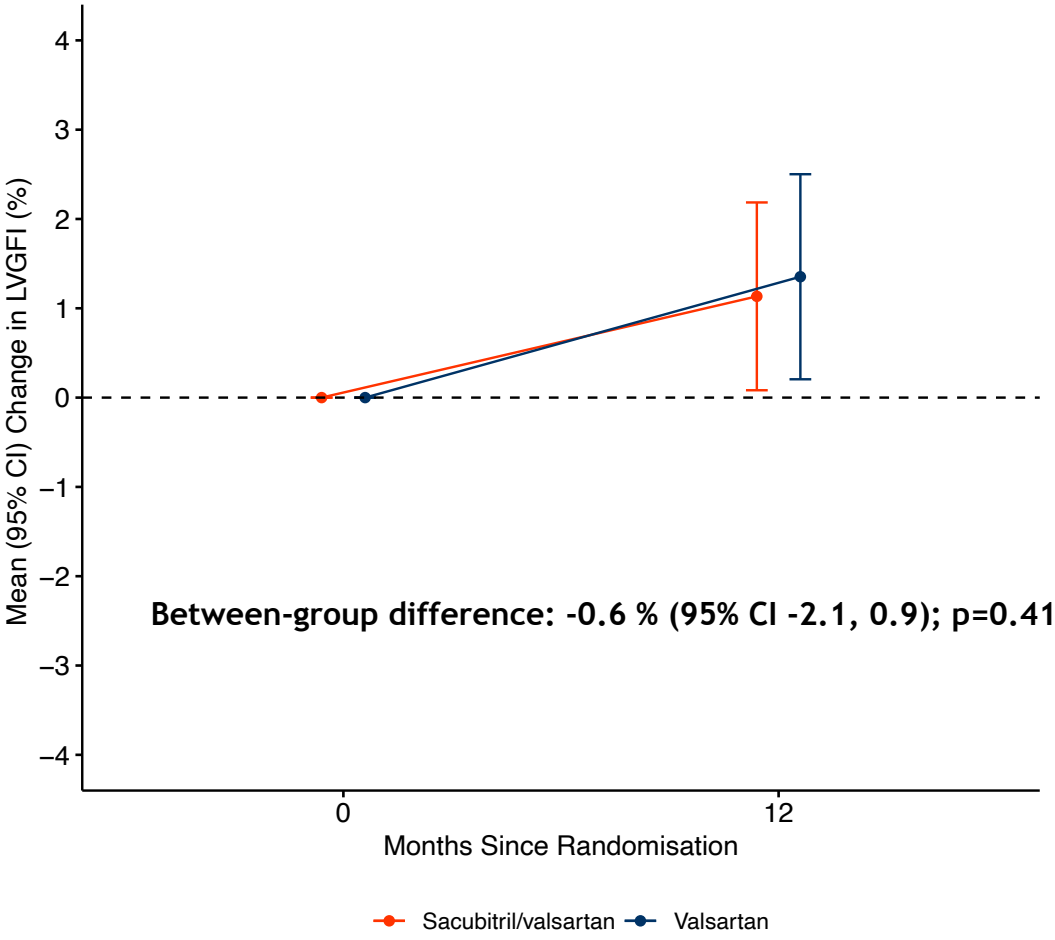


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LVMI, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LVMI = left ventricular mass index.

4.3.8 Effect of neprilysin inhibition on left ventricular global function index

Mean (SD) LVGFI at baseline was 28.6 (5.4) % in those randomised to sacubitril/valsartan with follow-up MRI data (n=46) and 30.6 (6.9) % in those randomised to valsartan (n=44). LVGFI increased by 1.1 (SD 3.5) % between baseline and 52 weeks in the sacubitril/valsartan group and by 1.4 (SD 3.8) % in the valsartan group: adjusted between-group difference –0.6 (95% CI, –2.1, 0.9) %; p=0.41 (Figure 4-10). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in LVGFI from baseline was 0.2 (95% CI -2.1, 2.4) % in those with NT-proBNP ≥230 pg/mL at baseline, and -1.9 (95% CI -4.2, 0.4) % in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.21).

Figure 4-10 Change in left ventricular global function index from baseline to week 52



Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline LVGFI, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging. LVGFI = left ventricular global function index.

4.3.9 Effect of neprilysin inhibition on patient global assessment of general well-being

Data on the patient global assessment of general well-being (Appendix 4) were available for 92 patients (46 in both treatment groups) at 52 weeks. An improvement in general well-being from baseline was reported by 22 (47.8%) and 25 (54.3%), no change in 24 (52.2%) and 21 (45.7%) in the sacubitril/valsartan and valsartan groups, respectively, with no significant between-group difference (p=0.56) (Table 4-3).

Table 4-3 Response at 12 months relating to the patient's global assessment of any change in their general well-being since the start of the trial

	Sacubitril/valsartan (n=46)	**Valsartan (n=46)**	**P-Value**
Markedly improved	8 (17.4%)	8 (17.4%)	**p=0.56**
Moderately improved	9 (19.6%)	7 (15.2%)	
Slightly improved	5 (10.9%)	10 (21.7%)	
Unchanged	24 (52.2%)	21 (45.7%)	
Slightly worsened	0 (0.0%)	0 (0.0%)	
Moderately worsened	0 (0.0%)	0 (0.0%)	
Markedly worsened	0 (0.0%)	0 (0.0%)	

4.4 Safety and adverse events

Study treatment was very well tolerated with no permanent discontinuations for reasons other than death (n=1 in the sacubitril/valsartan group) during follow-up. Adverse events of interest by randomised treatment arm are summarised in Table 4-4.

Compared with baseline, change in systolic blood pressure at 52 weeks was –5.8 (16.5) mm Hg in the sacubitril/valsartan group and +0.17 (16.8) mm Hg in the valsartan group; between-group adjusted mean difference –5.3 mm Hg (95% CI, –11.5, 1.0); P=0.10. There were numerically more cases of symptomatic hypotension with sacubitril/valsartan than valsartan (n=7 versus n=1). No cases of symptomatic hypotension required permanent discontinuation of study treatment.

There were no cases of significant worsening renal function and 3 cases of hyperkalaemia (serum potassium >5.5mmol/L) (2 in the sacubitril/valsartan group and 1 in the valsartan group). There were no cases of severe hyperkalaemia (serum potassium >6.0mmol/L).

Table 4-4 Adverse events of interest

Adverse event	Sacubitril/valsartan (n=47)	Valsartan (n=46)
Serum creatinine ≥2.5mg/dl	0	0
Serum potassium >5.5mmol/L	2 (4)	1 (2)
Serum potassium >6.0mmol/L	0	0
Symptomatic hypotension	7 (15)	1 (2)
Symptomatic hypotension with systolic blood pressure <90mmHg	1 (2)	0
Angioedema	0	0
Cough	0	0

n= number of patients with event (%)

Due to the small number of events no tests for statistical significance were performed.

4.5 Discussion

4.5.1 Study population

The population enrolled in the present study were specifically targeted to represent a group of patients who were at an elevated risk of the future development of heart failure due to the development and persistence of left ventricular systolic dysfunction following myocardial infarction. Several characteristics of the population enrolled are worthy of further discussion.

Firstly, the mean age (60.7 years) and the very high proportion of men (91%) are consistent with previous reports in similar populations; a contemporary post myocardial infarction study in patients with left ventricular systolic dysfunction (cardiac MRI LVEF <45%) enrolled immediately following anterior ST elevation myocardial infarction reported almost identical results with a mean age of 58 years and 88% of participants were male.[231] In the 2016 OMEGA-REMODEL trial which recruited a broad selection of patients with acute myocardial infarction, mean age was approximately 60 years and around 80% were male.[232] In the High-Risk Myocardial Infarction Initiative pooled dataset of 4 trials enrolling patients with left ventricular systolic dysfunction with or without heart failure as a result of acute myocardial infarction, the mean age at the time of myocardial infarction was 65.0 years and 70% of patients were male.[233] In this cohort of approximately 29000 patients, the proportion of patients with diabetes (26%) and hypertension (54%) were significantly higher than those observed in the present cohort (16% and 22%, respectively). This difference could represent that both diabetes and hypertension are independent risk factors for heart failure both at the time of acute myocardial infarction (an inclusion criterion for some of the trials) and later after myocardial infarction (thereby rendering the patient ineligible for the present trial).[234,235] Comparison with the most contemporary large post-myocardial infarction left ventricular systolic dysfunction/heart failure trial, the PARADISE-MI trial, is not possible as this trial utilised a range of enrichment criteria for eligibility which limits the generalisability of any comparisons made.[236,237]

Several characteristics relating to sub-types of myocardial infarction have been identified as being predictors of persisting left ventricular systolic dysfunction:

consistent with these reports the majority of patients in the present trial had experienced a STEMI in the anterior anatomical territory.[238] Furthermore, the mean infarct size, an established independent predictor of the risk of heart failure following myocardial infarction, was 28.4% of left ventricular mass in the present study, significantly higher than the 18.7% reported at 3 months following infarct in a recent population of patients with STEMI.[239] These infarcts had developed despite very high use of reperfusion therapy with 92.5% of patients having undergone percutaneous coronary intervention. This figure is in keeping with the 89.4% who were reported as having had acute reperfusion therapy in the recently reported PARADISE-MI trial.[237] This is significantly higher than the number of patients who had reperfusion therapy in the High-Risk Myocardial Infarction Initiative database, the constituent trials of which were completed over 15 years ago, prior to the widespread adoption of emergent coronary reperfusion therapy.[233] Patients in the present study were also well treated with medical therapies which are known to attenuate adverse left ventricular remodelling and reduce the risk of heart failure and death following myocardial infarction; all patients were taking an ACE inhibitor or ARB prior to enrolment, 93.5% were taking a beta-blocker, and 43% an MRA.

The primary outcome of this study was the treatment effect of the addition of neprilysin inhibition on attenuating adverse left ventricular remodelling as measured by the LVESVI. Mean LVESVI at baseline was 74.8 (SD 19.6) mL/m^2; the upper limit of the normal reference range for men in the UK Biobank project was 49 mL/m^2 and the equivalent numbers for LVEDVI were 117.2 (21.0) mL/m^2 and 110 mL/m^2 indicating that the patients enrolled in the present study had evidence of significant ventricular dilatation.[213] Mean MRI measured left ventricular ejection fraction (36.8 [7.0] %), by merit of the study inclusion criteria, was lower than the lower limit of the normal MRI reference range (48-69% for men).[213] It is of interest to place these values in context of other post-myocardial infarction and heart failure populations. In a contemporary population of patients with an anterior ST-elevation MI, all of whom had a cardiac MRI ejection fraction of 45% or less at baseline immediately following myocardial infarction (mean 43%), the mean LVESVI at 3 months was 40 ml/m^2, confirming that the population enrolled in our study represented a population who had evidence of adverse left ventricular remodelling.[231] When compared to

cardiac MRI measured volumes in patients with established HFrEF, the degree of ventricular dilation and reduction in systolic function was less in this cohort than in those with symptomatic HFrEF. In the cardiac MRI substudy of the Metoprolol CR/XL Randomized Intervention Trial in Heart Failure (MERIT-HF) trial, mean placebo group ejection fraction 32.0%, LVESVI 111 mL/m^2 and LVEDVI 156 mL/m^2.[240]

Few studies have examined the remodelling effect of neurohumoral antagonists in patients with asymptomatic left ventricular systolic dysfunction. Several factors in the enrolled population suggest that the patients in the present study differed than those with established chronic heart failure. The use of loop diuretics was low at 12% and this small group of patients likely reflects those who had transient heart failure at the time of myocardial infarction and were commenced (and continued) on an oral loop diuretic. The cardiac volumes in the present trial are similar to other asymptomatic populations such as in the REversal of VEntricular Remodeling with Toprol-XL (REVERT) Trial where the mean echocardiographic measured LVESVI and LVEDVI in the placebo group were 82.5 mL/m^2 and 110.7 ml/m^2, respectively.[241] Arguably, the most objective evidence of difference between this symptomless cohort and patients with HFrEF was the median NT-proBNP level of 230 pg/ml, a level below the 400 pg/ml cut-off used in the diagnosis of heart failure.[19] Further discussion of the natriuretic peptide levels in the present cohort will follow in Chapter 5.

In summary, the cohort enrolled represented a group of patients with evidence of established adverse left ventricular remodelling (i.e., ventricular dilatation and reduced systolic function) as a result of prior myocardial infarction. Therefore, this was a population who are at a higher risk of the development of symptomatic HFrEF. It is also worth highlighting the very high use of evidence-based therapies which have been shown to prevent adverse left ventricular remodelling following myocardial infarction, therefore the effect of neprilysin inhibition was examined in addition to these therapies.

4.5.2 The effect of neprilysin inhibition on left ventricular remodelling

In this study which was designed to assess the potential additional reverse remodelling effect of a neprilysin inhibitor when added to a RAS inhibitor and beta-blocker in patients with evidence of persisting left ventricular systolic dysfunction late after myocardial infarction, sacubitril/valsartan, compared with valsartan, did not have any significant favourable effects on left ventricular or atrial volumes, left ventricular ejection fraction, or left ventricular mass.

The development of heart failure late after myocardial infarction is secondary to progressive ventricular dilatation and a reduction in systolic function, accompanied by a vicious cycle of deleterious activation of the RAS and sympathetic nervous system which promotes further adverse remodelling and depresses cardiac function. In the present study, there was no significant difference in the placebo group between baseline and 12 months in LVESVI ($p=0.07$) or LVEDVI ($p=0.35$), however LVEF did increase significantly by 1.4% ($p=0.014$). A range of definitions of adverse left ventricular remodelling have been suggested; recently, a 12% change in non-indexed LVESV or LVEDV measured using cardiac MRI has been proposed as a cut off indicating adverse (≥12% increase in volumes) or reverse remodelling (≤12% decrease in volumes) in patients following ST-elevation myocardial infarction.[242] Using these cut-off values, of the 90 patients with paired MRI data in the present study, 4 had "adverse remodelling" (3 in the sacubitril/valsartan group and 1 in the valsartan group; $p=0.62$) and 14 had "reverse remodelling" (8 in the sacubitril/valsartan group and 6 in the valsartan group; $p=0.77$). In the placebo arm of the SOLVD-Prevention trial of asymptomatic patients with left ventricular systolic dysfunction, progressive ventricular dilation was observed over 24 months of follow-up. This was attenuated by treatment with the ACE-inhibitor enalapril, and in enalapril treated patients there was little change in left ventricular volumes as measured by radionuclide ventriculograms after 12 months of follow-up but a reduction in volumes was observed at 24 months.[243] It is also notable that the degree of progressive ventricular dilation seen in SOLVD-Prevention (i.e., asymptomatic patients) was less than that seen in patients with symptomatic heart failure in the SOLVD-Treatment trial.[244] In the REVERT trial, 94% of patients were taking an ACE inhibitor or ARB at baseline and in the

placebo arm there were no significant differences seen in the change in LVESVI, LVEDVI or LVEF between baseline in 12 months; in the metoprolol treated patients there were significant reductions in LVESVI and LVEDVI along with an improvement in LVEF.[241] Therefore, the small number of patients with signs of adverse remodelling over 12 months of follow-up in the present study may simply reflect the excellent background neurohumoral antagonist therapy which they were taking along with the relatively short follow-up time.

Randomised data describing the remodelling effect of neprilysin inhibition in patients with left ventricular systolic dysfunction with or without heart failure are limited. As described in Chapter 1, pre-clinical models of myocardial infarction have suggested a beneficial effect on left ventricular volumes and function along with an attenuation of fibrosis, a key process in the progression of adverse remodelling.[142,146] A range of observational studies have reported a reverse remodelling effect of sacubitril/valsartan (summarised in Table 1-2) however the ability of these studies to make conclusions about treatment effect are limited by their observational, non-randomised design. The largest of these, the Prospective Study of Biomarkers, Symptom Improvement, and Ventricular Remodeling During Sacubitril/Valsartan Therapy for Heart Failure (PROVE-HF) reported large improvements in serially echocardiographic measured LVEF and reductions in left ventricular volumes which correlated with the degree of reduction in NT-proBNP in patients with symptomatic HFrEF and a clinical indication to commence sacubitril/valsartan, however these observational data do not support conclusions regarding treatment effect but are hypothesis generating.[153]

In the EVALUATE-HF randomised-controlled trial enrolled patients with HFrEF, the majority of whom were NYHA≥II, sacubitril/valsartan, as compared with enalapril, significantly reduced the secondary echocardiography endpoints of LVESVI by 1.6 (95% CI -3.1, -0.03) mL/m^2, LVEDVI by 2.0 (95% CI -3.7, -0.3) mL/m^2 and LAVI by 2.8 (95% CI -40, -1.6) mL/m^2, with no difference in LVEF after 12 weeks of treatment (0.6% [-0.4, 1.7]).[148] Baseline left ventricular volumes in this trial were lower than those in EVALAUTE-HF (mean enalapril group LVESVI 54.1 mL/m^2 and LVEDVI 79.1 mL/m^2), left ventricular ejection fraction was also lower (33%) and median NT-proBNP higher (595 pg/mL). It

should be noted however that it has been reported previously that echocardiography may underestimate ventricular volumes when compared with results based on cardiac MRI imaging.[245,246] The results of EVALUATE-HF should be considered in context of two important points; firstly the potential remodelling benefit of neprilysin inhibition was assessed in addition to treatment with RAS inhibitors and beta-blockers in the majority of patients, and secondly, the remodelling indices were assessed after only 12 weeks of treatment as it was felt unethical to continue randomised treatment for any longer given the established clinical benefits of sacubitril valsartan as demonstrated in PARADIGM-HF.[138] It may be that a longer period is required to demonstrate a significant additive beneficial remodelling effect.

A second trial, the Pharmacological Reduction of Functional, Ischemic Mitral Regurgitation (PRIME) trial, compared 12 months of randomised treatment with sacubitril/valsartan or valsartan in patients with significant functional mitral regurgitation and LVEF between 25% and <50% and reported a significant reduction in LVEDVI of 7.0 mL/m^2 with no effect on LVESVI or LVEF.[147] Taken together, the data from these trials are suggestive of a small additional beneficial effect of the addition of neprilysin inhibition on reverse remodelling in patients with symptomatic HFrEF.

The key difference in this study to those described above, was that all patients had symptomless left ventricular systolic dysfunction (as well as lower NT-proBNP concentrations). Differences between the remodelling effect of various heart failure therapies have been previously reported in patients with symptomless left ventricular systolic dysfunction and those with symptomatic HFrEF. The ACE inhibitor captopril was the first pharmacological therapy to demonstrate a reduction in mortality and risk of heart failure which was related, in part, to its ability to prevent adverse remodelling in patients with left ventricular systolic dysfunction as a result of an acute myocardial infarction.[46] At around the same time, the ACE inhibitor enalapril was shown in the two randomised placebo-controlled trials of the SOLVD programme to reduce the risk of mortality and hospitalisation for worsening heart failure in patients with established symptomatic HFrEF and reduce the risk of a first hospitalisation for heart failure in patients with asymptomatic left ventricular systolic dysfunction

(LVEF ≤35%).[44,130]As described above, in a small radionuclide ventriculogram sub-study, a greater attenuation of adverse remodelling with ACE-inhibition was seen in patients enrolled in the Treatment study (symptomatic patients) as compared with those in the Prevention arm (asymptomatic patients), however this finding was not replicated in the larger echocardiography sub-study where there was no significant interaction between the study arm and the beneficial remodelling effects of enalapril.[60,243] It is notable that ANP levels were higher in patients in the SOLVD Treatment trial compared with those in the Prevention trial, in which the levels were higher than those of a control group with no history of cardiovascular disease.[247]

Similar to ACE inhibitors, beta-blockers have been shown to improve outcomes in both patients with symptomatic HFrEF and in high-risk patients with left ventricular systolic dysfunction after acute myocardial infarction, and the benefits of this class of drugs are, in part, due to a beneficial effect on left ventricular remodelling. Compared with the results with metoprolol succinate in the MRI substudy of the MERIT-HF trial and in the Randomized Evaluation of Strategies for Left Ventricular Dysfunction Pilot Study (RESOLVD), both of which randomised patients with symptomatic HFrEF (with evidence of elevated natriuretic peptide levels recorded in the RESOLVD study), the significant reduction in LVEDVI and LVEF in the high dose (200 mg once daily) metoprolol succinate group in the REVERT trial was lower in this asymptomatic patient group (who had low natriuretic peptide concentrations at a similar level to those in the present study).[240,241,248] No significant reduction in left ventricular volumes was seen in the low dose metoprolol succinate group (50mg once daily) however there was a significant improvement in LVEF. A similar differential response with a lesser remodelling effect in asymptomatic patients as compared with symptomatic patients has also been seen with ivabradine, a selective inhibitor of the sinus node I_f current.[249,250]

The finding in the present trial of a potential differential treatment effect by the degree of elevation of NT-proBNP level at baseline is perhaps consistent with the greater remodelling effect seen in symptomatic patients with other neurohumoral antagonists when compared with asymptomatic patients as described above. Patients with higher levels of NT-proBNP in this trial appeared

to derive a greater reverse remodelling effect than those with lower NT-proBNP levels. As NT-proBNP is a biomarker of the degree of elevation of left ventricular wall stress (i.e., ongoing stimulus for the process of adverse remodelling), it may be that for the addition of neprilysin inhibition to have any additive favourable remodelling effect to that offered by a RAS inhibitor and beta-blocker, there must be a degree of elevation of left ventricular wall stress as evidence by higher NT-proBNP levels. Furthermore, a drug's effect on left ventricular volumes and remodelling may relate to its effect on altering cardiac load (i.e., reducing preload and afterload). In the present trial I did not observe any reduction in NT-proBNP (suggesting no significant reduction in preload) and systolic blood pressure (i.e., afterload) was lower in the sacubitril/valsartan group although this difference was not statistically significant. Taken together, these results suggest that no significant favourable changes in cardiac loading conditions occurred which may have contributed to any remodelling effect.

4.5.3 Limitations

As with all small mechanistic studies such as this, I was limited by the relatively small sample size. The study sample size, which was based on the power calculation detailed in Chapter 3-11, provided adequate power to detect a mean between-group difference in LVESVI of 6mL/m^2 at 52 weeks, a difference which is thought to represent a minimal clinically meaningful difference and one that is comparable to the treatment effect of ACE-inhibitors and beta-blockers. The standard deviation of change in the present study was smaller than that used in the power calculation, therefore, meaning that there was adequate power to detect a treatment effect of this magnitude. The point estimate and 95% confidence intervals for the effect of treatment did not preclude a smaller treatment difference, but the modest prespecified sample size limited the ability to detect such a difference if it existed.

The present trial deliberately examined the effect of sacubitril/valsartan on late left ventricular remodelling after myocardial infarction, recruiting patients at least 3 months following an acute event. Therefore, I cannot draw any conclusions about the potential effect of neprilysin inhibition on the early and distinctive remodelling in the acute phase of myocardial infarction. It is however of note that the results of the recently presented PARADISE-MI trial did not

report any significant additional benefit on cardiovascular outcomes of the addition of a neprilysin inhibition commenced early (within 7 days) following acute myocardial infarction in an enriched population of patients with left ventricular systolic dysfunction and/or heart failure.[237] This trial did have an echocardiography sub-study, the results of which are yet to be presented at the time of writing.

In the PARADIGM-HF trial, sacubitril/valsartan was superior to the ACE inhibitor enalapril in patients with symptomatic HFrEF. The findings presented in this trial are not directly comparable given the differences in both the patients studied and comparator therapy. It is also possible that a comparison of sacubitril/valsartan with an ACE inhibitor may show different results in terms of remodelling outcomes than those presented. However, VALIANT clearly demonstrated non-inferiority of valsartan compared with captopril in reducing the risk of death or heart failure hospitalisation in patients with myocardial infarction complicated by left ventricular systolic dysfunction, heart failure, or both.[59] Furthermore, an analysis of valsartan versus imputed placebo in VALIANT reported a nearly identical hazard ratio for death from any cause as compared to a pooled estimate of the three seminal ACE-inhibitor post-myocardial infarction trials (SAVE, AIRE and TRACE). In addition, the effect of valsartan on attenuating adverse left ventricular remodelling as measured by echocardiography in the VALIANT cohort was equivalent to that of captopril.[13] Taken together these results confirm that valsartan is as effective as the ACE-inhibitor captopril at reducing the risk of death or heart failure hospitalisation in patient with left ventricular systolic dysfunction, heart failure or both as a result of myocardial infarction. The Optimal Treatment in Myocardial infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL) trial compared the ARB losartan (50mg once daily) to captopril (50mg three times daily) in high-risk patients following myocardial infarction and reported a non-significant trend to a reduction in mortality with captopril compared with losartan.[251] In the HEAAL trial, when compared to the 50mg once daily dose used in OPTIMAAL, a higher dose of losartan (150mg once daily) was shown to reduce the risk of death or heart failure hospitalisation in patients with symptomatic HFrEF.[252] Therefore, the observed non-significant difference in OPTIMAAL is likely explained by the relatively low dose of losartan used as compared to the dose of captopril of

50mg three times daily which was used in both the SAVE trial and VALIANT.[46,59] Taken together, I believe that the evidence presented supports the equivalence of ARB and ACE-inhibitors in high-risk patients following myocardial infarction.

4.5.4 Conclusions

In a population of asymptomatic patients with evidence of adverse left ventricular remodelling and significant ventricular dilatation following a previous myocardial infarction, the addition of a neprilysin inhibitor to standard therapy with a RAS inhibitor and beta blocker did not have any beneficial reverse remodelling effect.

Chapter 5 The effect of neprilysin inhibition on natriuretic peptide levels in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

5.1 Introduction

As described in Chapter 1-3, the natriuretic peptides are a group of endogenous vasoactive peptides released in response to increased cardiac wall stress which aim to counteract the harmful activation of the RAS and sympathetic nervous system through diuretic, natriuretic, sympatholytic, anti-hypertrophy and anti-fibrotic mechanisms of action.[28,76] Three main active forms of natriuretic peptides are in the circulation; atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The formation and active forms of these peptides and their precursor molecules are described in Chapter 1-3.

In both general and at-risk populations (e.g. those with hypertension, coronary artery disease and a previous myocardial infarction), elevated levels of natriuretic peptides are independent predictors of the presence of asymptomatic left ventricular systolic dysfunction and the risk of the development of symptomatic heart failure and mortality.[253-256] The majority of data regarding the prognostic value of natriuretic peptides in patients following myocardial infarction relate to the measurement of these peptides at the time of, or shortly after, acute myocardial infarction when elevated levels are associated with adverse left ventricular remodelling, the development of heart failure and mortality.[82,257-259]

In the Prevention of Events with Angiotensin Converting Enzyme (PEACE) trial in patients with stable coronary artery disease and preserved left ventricular systolic dysfunction, over 50% of whom had had a prior myocardial infarction, elevated BNP and MR-proANP levels were independently associated with a higher risk of cardiovascular mortality and heart failure.[260,261] The EPHESUS trial enrolled patients with heart failure and left ventricular systolic dysfunction, and patients with diabetes with left ventricular systolic dysfunction following acute myocardial infarction; in a post hoc analysis, an increase in BNP at one month

following infarction was an independent predictor of cardiovascular mortality or heart failure hospitalisation.[262] These results suggest that elevated levels of natriuretic peptides measured remote from the time of myocardial infarction may identify patients at risk of future adverse outcomes.

This chapter will describe natriuretic peptide levels measured in patients with asymptomatic left ventricular systolic dysfunction, late after a myocardial infarction, and their relationship with the degree of adverse left ventricular remodelling as measured using cardiac MRI. I will also describe the effect of the addition of a neprilysin inhibitor to standard therapy including a RAS inhibitor and beta-blocker on natriuretic peptide levels and the association with changes in cardiac volume and function. I will also examine cGMP, the major secondary messenger of natriuretic peptide bioactivity, and the effect of treatment with sacubitril/valsartan, compared with valsartan, on its levels in the urine.

5.2 Methods

5.2.1 Study patients and protocol

The patients included in this study were 93 patients who had evidence of persisting left ventricular systolic dysfunction (left ventricular ejection fraction ≤40% measured using echocardiography) without signs or symptoms of heart failure at least 3 months following an acute myocardial infarction. All patients were taking an ACE inhibitor or ARB prior to enrolment and a beta-blocker (unless contraindicated or not tolerated). Eligible patients were randomised 1:1 to sacubitril/valsartan (target dose 97/103mg twice daily) or valsartan (target dose 160mg twice daily) and matching placebo for 52 weeks. The study protocol is detailed in Chapter 3 and baseline characteristics in Table 4-1.

All patients underwent cardiac MRI for assessment of left ventricular and atrial volumes indexed to body surface area pre-randomisation and at 52 weeks as detailed in Chapter 3-6.

Venepuncture was performed pre-randomisation, at 26 weeks, and at 52 weeks as described in Chapter 3-6. Spot urine samples were also collected at the same time points. Mid-regional prohormone of atrial natriuretic peptide mid-regional pro-atrial natriuretic peptide (MR-proANP) (B·R·A·H·M·S KRYPTOR Compact PLUS, Thermo Fisher Diagnostics), BNP (i1000SR, Abbott Laboratories, Abbott Diagnostics) and NT-proBNP (e411, Roche Diagnostics) were measured on clinical immunoassay platforms using the manufacturers' calibrators and quality control materials. α-atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) (aprotinin-treated plasma, α-ANP(1-28), CNP-22 extraction-free enzyme immunoassays (EIAs), Phoenix Pharmaceuticals) were also measured using commercial enzyme-linked immunosorbent assay (ELISA) assays and the manufacturers' quality control materials. Urinary cyclic guanosine monophosphate (cGMP) was measured using a commercially available ELISA (R&D systems, Bio-Techne), and using the manufacturers' quality control materials. All biomarker sample processing and measurements were performed by Philip Stewart, Elaine Butler, Josephine Cooney and Emma Dunning at the Glasgow Biomarker Laboratory, Institute of Cardiovascular and Medical Sciences, University of Glasgow under the supervision of Dr Paul Welsh.

5.2.2 Statistical methods

The distribution of baseline biomarker values was examined by means of histograms and summary statistics. Non-normally distributed biomarkers were log-transformed prior to analysis. Baseline levels are presented as means with standard deviations for normally distributed values, and as medians with interquartile ranges for non-normal distributions. Baseline values are presented in the overall population and by randomised treatment allocation with between-group comparisons made using a two-sample T-test or Wilcoxon rank-sum test for normal and non-normal distributed variables, respectively. Correlation between baseline values of biomarkers and LVESVI, LVEDVI, LVEF and LAVI were calculated by means of a Pearson's correlation coefficient (r) with a linear regression line plotted graphically. The same methods were used to examine the correlation between the 52-week change in biomarker values and change in cardiac MRI parameters.

The treatment effect of sacubitril/valsartan as compared with valsartan on biomarker levels over time was examined by means of a linear regression model adjusted for randomised treatment, baseline value of the outcome and use of diuretics at baseline. The regression model coefficients for the treatment indicator variable are reported as adjusted between-treatment group mean differences or, if required to satisfy modelling assumptions, log transformations were performed, and regression coefficients were back-transformed and are presented as relative differences. In confirmatory analyses, repeated measures analyses were performed adjusting for the main effects of time-point, randomised group and the interaction between time-point and randomised group and for diuretic use at baseline. All analyses were performed on an intention to treat basis as described in Chapter 3-11.

A p-value of <0.05 was considered statistically significant for all analyses. No correction for multiple testing was performed. No imputation for missing data was performed. All analyses were performed by Bethany Stanley (Robertson Centre for Biostatistics) and me using R Studio and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria), and STATA version 16.1 (College Station, TX, USA).

5.3 Results

5.3.1 Baseline levels

The baseline values of natriuretic peptides and urinary cGMP are presented in Table 5-1. Median MR-proANP was 95.0 (IQR 73.0-141) pmol/L and mean ANP 1.2 (SD 0.3) ng/mL. Using the cut-off value of >120 pmol/L, 30 of 93 (32%) patients had elevated levels of MR-proANP. Median BNP and NT-proBNP were 46.3 (23.8-78.7) pg/mL and 230 (123.9-404.2) pg/mL, respectively. Using the cut-off levels of BNP >100 pg/mL and NTproBNP >400 pg/mL, 19 (20.4%) and 25 (27%) patients had baseline levels above these respective thresholds. Mean CNP was 3.3 (0.9) ng/mL and median urinary cGMP was 472 (250-818) pmol/ml. There were no significant differences between the randomised treatment groups in any of the biomarkers at baseline.

Table 5-1 Baseline levels of natriuretic peptides and urinary cGMP

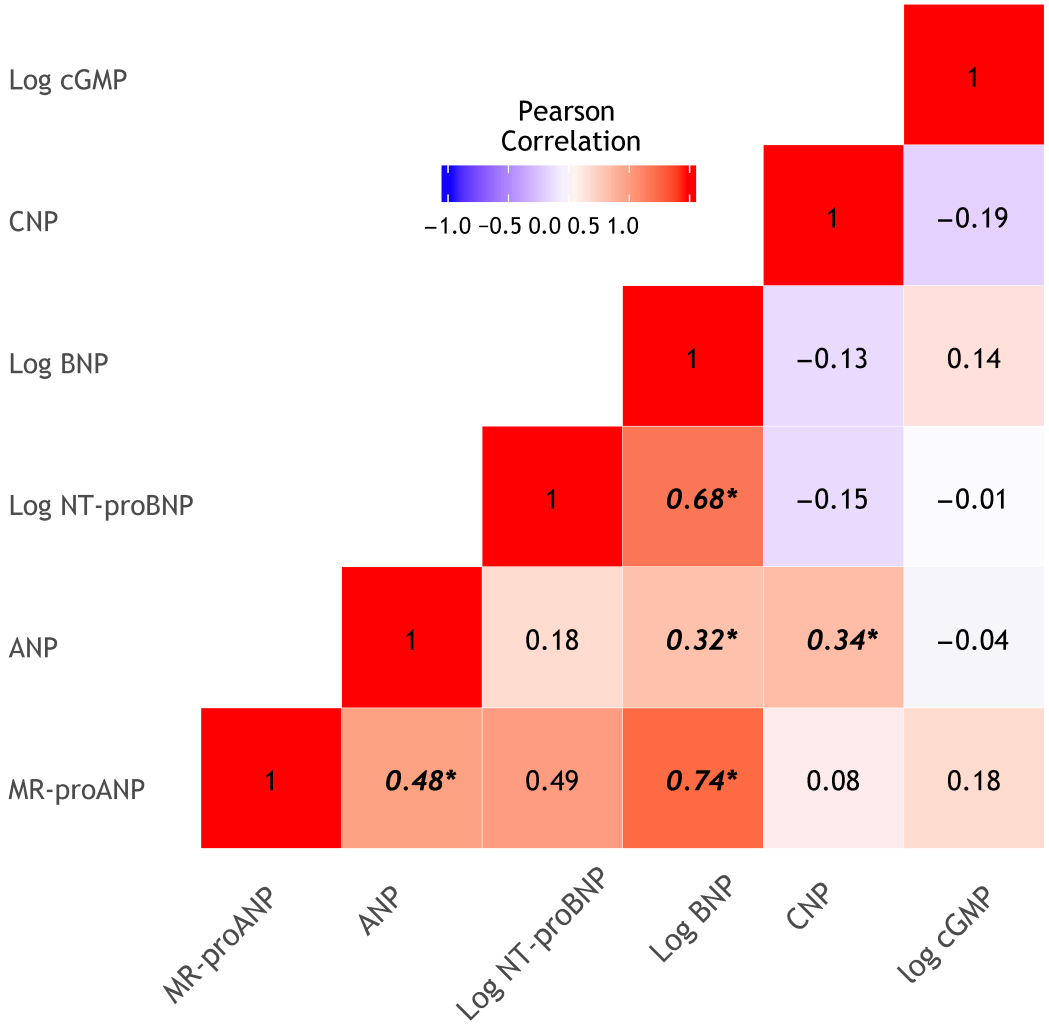
	Overall	Sacubitril/valsartan	Valsartan	P value
	N=93	N=47	N=46	
MR-proANP, pmol/L	95.0 (73.0-141.0)	95.0 (73.0-135.0)	96.0 (66.0-153.0)	0.94
ANP, ng/mL	1.2±0.3	1.1±0.3	1.2±0.3	0.24
NT-proBNP, pg/mL	230 (124-404)	216 (124-404)	242 (124-433)	0.97
BNP, pg/mL	46.3 (23.8-78.7)	36.3 (18.7-70.7)	51.3 (32.1-88.8)	0.14
CNP, ng/mL	3.3±0.9	3.1±0.7	3.4±1.0	0.13
Urinary cGMP, pmol/mL	472 (250-818)	508 (265-841)	378 (204-818)	0.49

Data are presented as means ± standard deviation or median (interquartile range).

5.3.2 Correlation between baseline natriuretic peptide levels and urinary cGMP

The correlations between each natriuretic peptide and urinary cGMP are displayed graphically in Figure 5-1. MR-proANP and ANP were significantly correlated (r=0.48; p<0.001), as were BNP and NT-proBNP (r=0.68; p<0.001). MR-proANP was also significantly correlated with BNP (r=0.74; p<0.001) and NT-proBNP (0.49; p<0.001). There were also significant correlations between ANP and BNP (r=0.32; p=0.002) and CNP (r=0.34; p<0.001). Neither ANP, BNP or CNP were significantly correlated with levels of urinary cGMP at baseline.

Figure 5-1 Pearson correlation coefficients for baseline natriuretic peptides and urinary cGMP



Darker tones indicate stronger correlations.

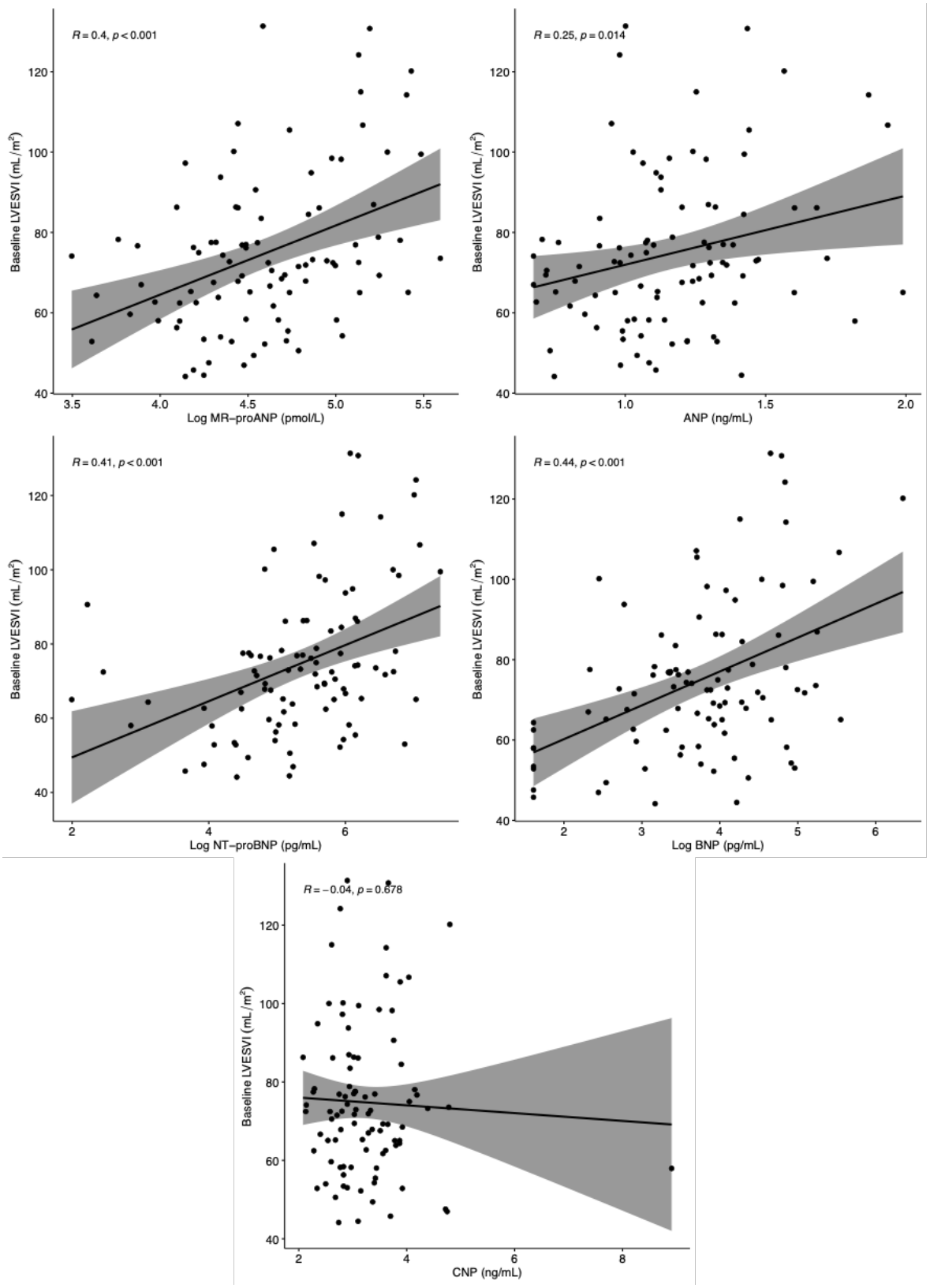
*** = p<0.05**

5.3.3 Correlation between baseline natriuretic peptides and left ventricular and atrial volumes

The relationships between baseline natriuretic peptide levels and baseline cardiac MRI measurements of left ventricular and atrial volumes and left ventricular ejection fraction are shown in Figures 5-2, 5-3, 5-4 and 5-5.

Figure 5-2 shows the relationship between LVESVI and natriuretic peptide levels. In order of magnitude of correlation, BNP ($r=0.44$; $p<0.001$), NT-proBNP ($r=0.41$; $p<0.001$), MR-proANP ($r=0.41$; $p<0.001$), and ANP ($r=0.25$; $p=0.014$) were all significantly correlated with LVESVI. The equivalent results for LVEDVI (Figure 5-3) were BNP ($r=0.44$; $p<0.001$), MR-proANP ($r=0.41$; $p<0.001$), NT-proBNP ($r=0.36$; $p<0.001$) and ANP ($r=0.25$; $p=0.017$). With regards to LVEF (Figure 5-4) NT-proBNP (-0.36; $p<0.001$) BNP (-0.30; $p=0.003$) and MR-proANP ($r=-0.27$; $p=0.01$) displayed significant correlations at baseline but ANP did not ($r=-0.18$; $p=0.081$). Of all the natriuretic peptide correlations, the strongest was seen between LAVI and MR-proANP ($r=0.51$; $p<0.001$) (Figure 5-5). LAVI was also significantly correlated with BNP ($r=0.44$; $p<0.001$), ANP ($r=0.38$; $p<0.001$) and NT-proBNP (0.30; $p=0.003$). There were no significant correlations between CNP and any of the cardiac MRI volumetric measurements.

Figure 5-2 Correlations between baseline natriuretic peptides levels and baseline left ventricular end-systolic volume index



For this figure and all scatter plots presented in this thesis, the solid black line represents a linear regression model with 95% confidence intervals denoted by the shaded grey area.

Figure 5-3 Correlations between baseline natriuretic peptides levels and baseline left ventricular end-diastolic volume index

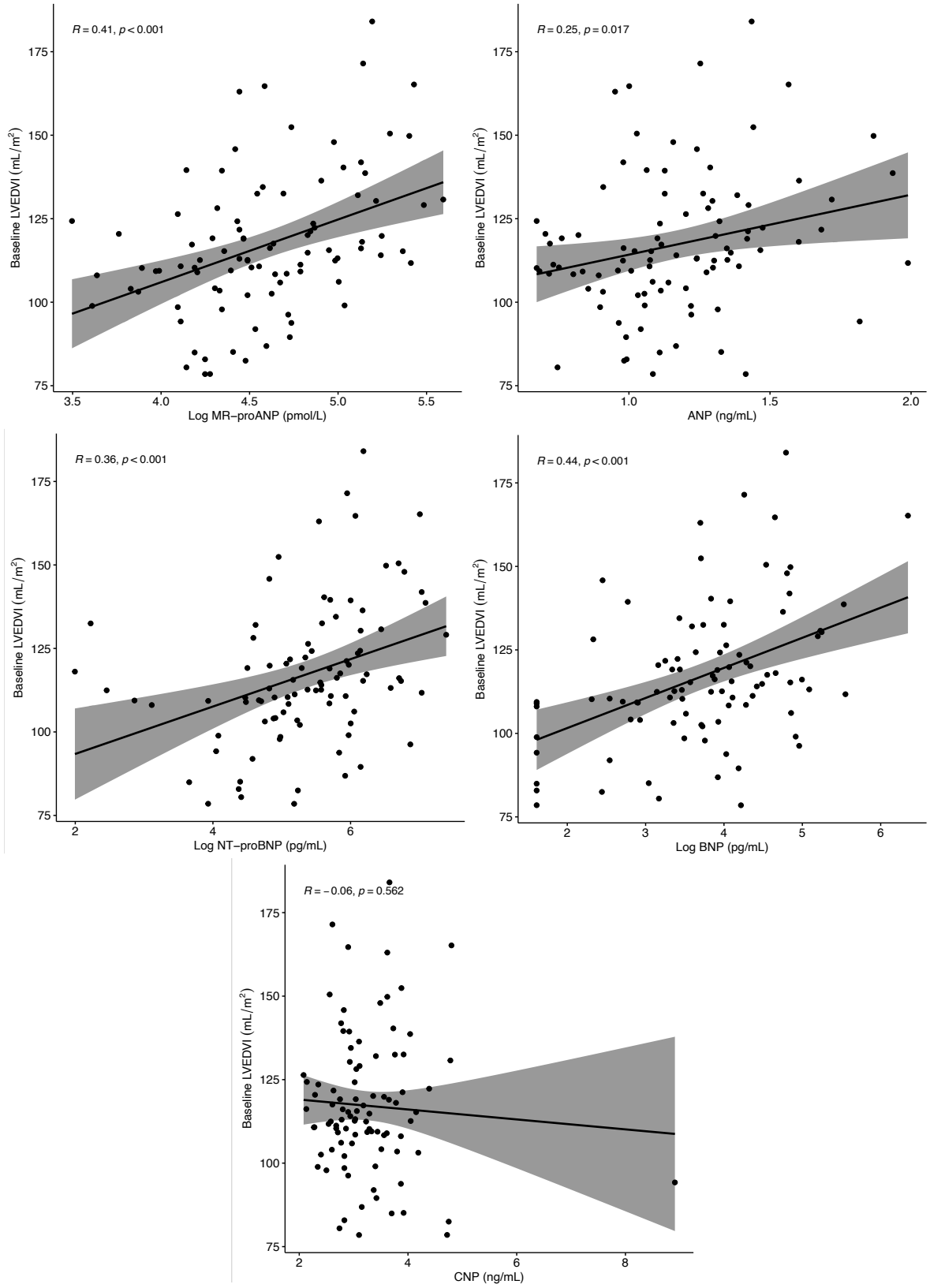
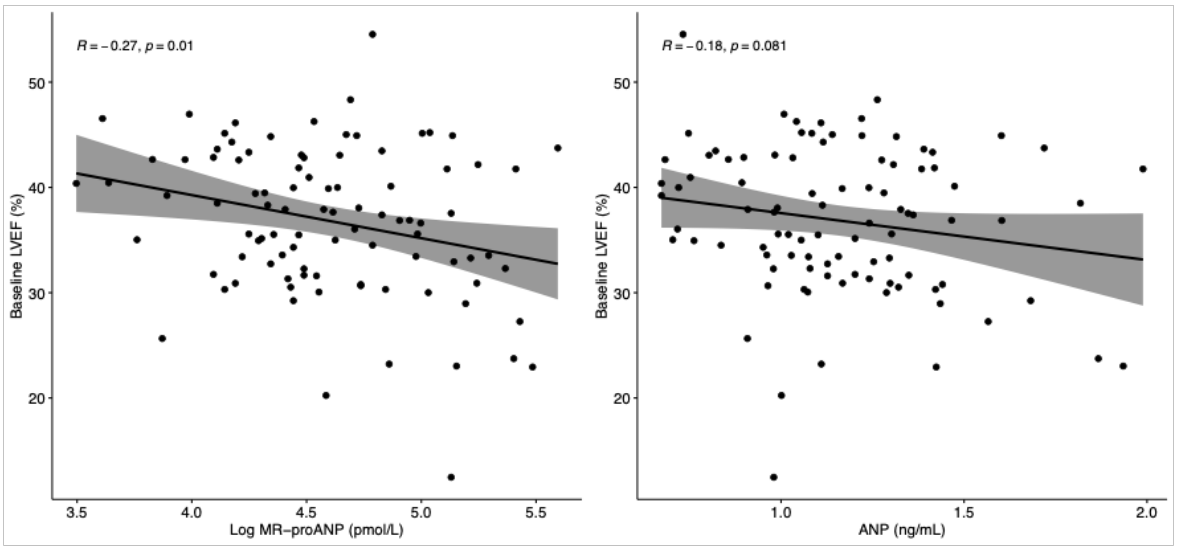
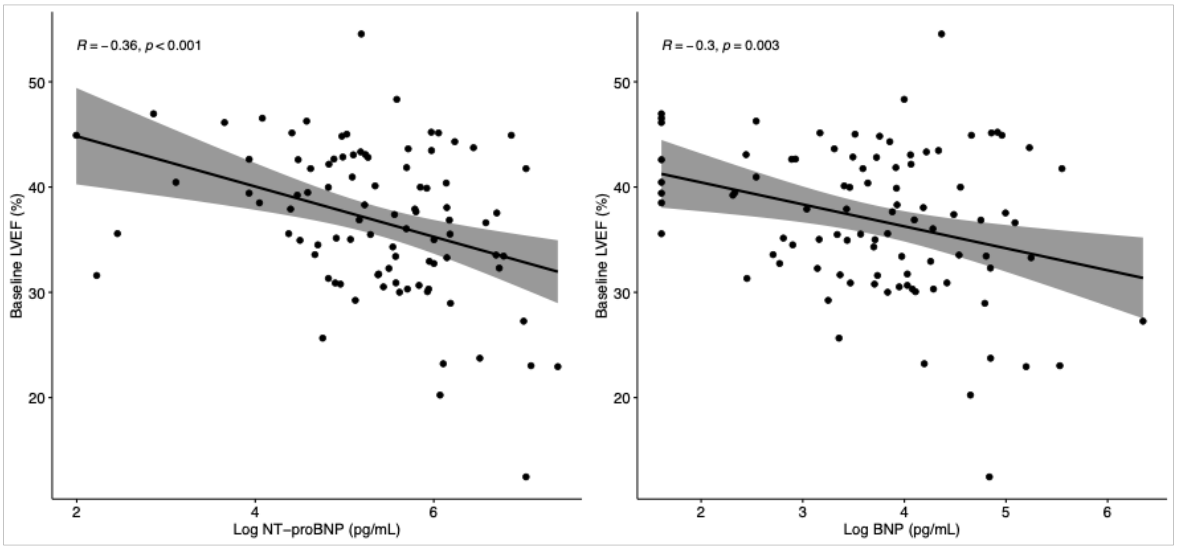


Figure 5-4 Correlations between baseline natriuretic peptides levels and baseline left ventricular ejection fraction





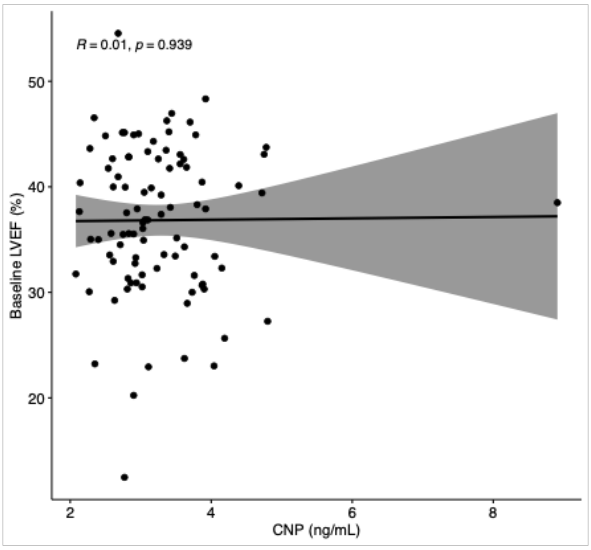
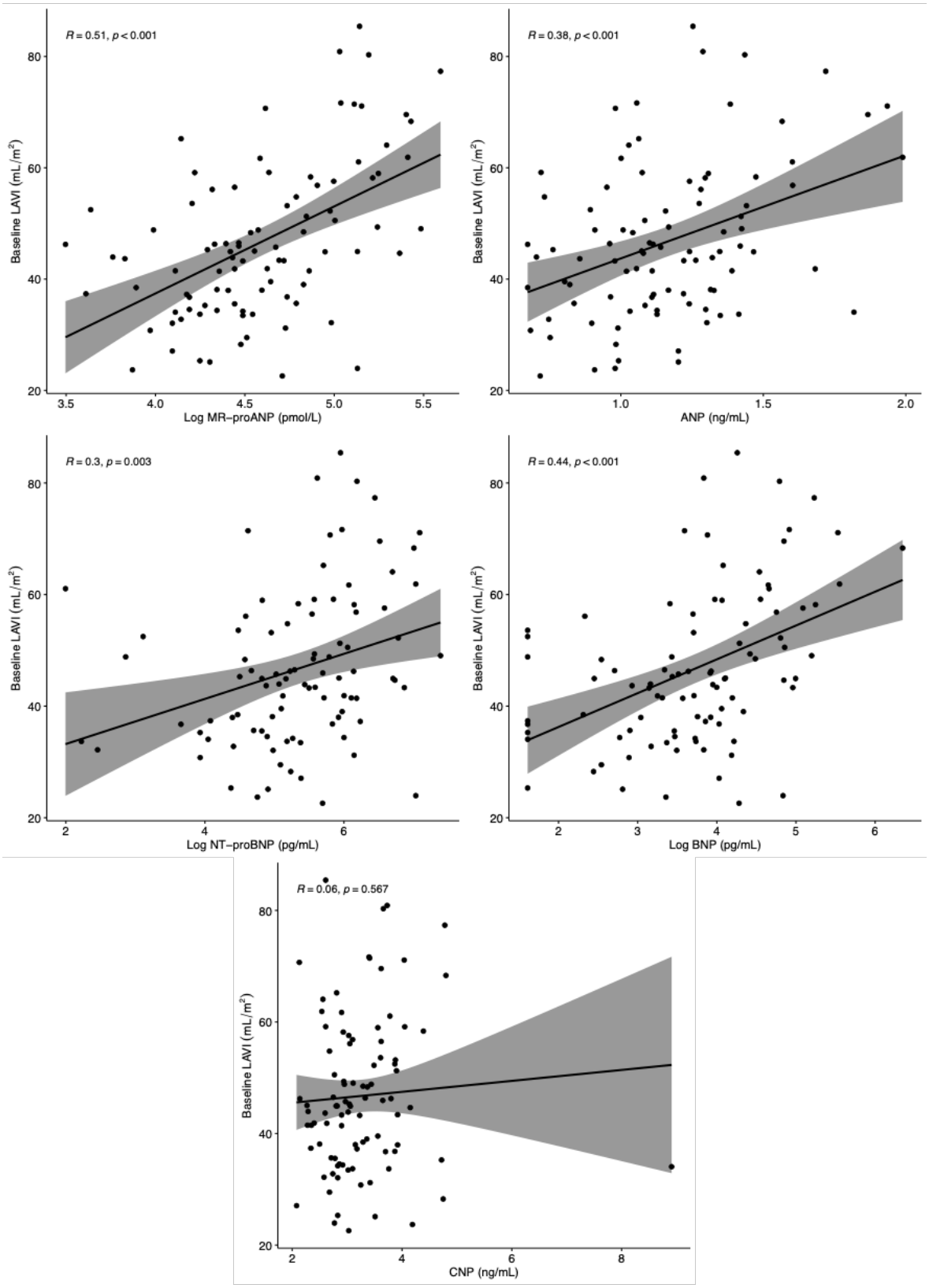


Figure 5-5 Correlations between baseline natriuretic peptides levels and baseline left atrial volume index



5.3.4 Correlation between change in natriuretic peptides and left ventricular and atrial volumes

Data on the change in natriuretic peptide levels and cardiac MRI measurements of left ventricular and atrial volumes between baseline and 52 weeks were available for 90 patients. The correlations between them are displayed in Figures 5-6, 5-7, 5-8, 5-9 and 5-10.

Change in MR-proANP over 52 weeks was significantly correlated with change in LVESVI ($r=0.38$; $p<0.001$), LVEDVI ($r=0.41$; $p<0.001$), and LAVI ($r=0.30$; 0.004) (Figure 5-6). Change in MR-proANP and LVEF were not significantly correlated ($r=-0.08$; $p=0.463$). There were no significant correlations between change in ANP and change in any of the measures of left ventricular remodelling (Figure 5-7) and the same findings were seen when examining the valsartan treated group only in light of the effect of neprilysin inhibition on ANP.

Change in NT-proBNP over 52 weeks was significantly correlated with change in LVESVI ($r=0.38$; $p<0.001$), LVEDVI ($r=0.30$; $p=0.004$), and LVEF ($r=-0.22$; $p=0.039$) (Figure 5-8). Change in NT-proBNP and LAVI were not significantly correlated ($r=-0.16$; $p=0.13$). Results with BNP were similar to those for NT-proBNP but with stronger correlations (Figure 5-9); LVESVI ($r=0.48$; $p<0.001$), LVEDVI ($r=0.43$; $p<0.001$), and LVEF ($r=-0.26$; $p=0.014$) and LAVI ($r=0.20$; $p=0.06$).

Change in CNP was significantly correlated with LVEDVI ($r=-0.27$; $p=0.01$) but not with the change in any other cardiac MRI measurements (Figure 5-10).

Figure 5-6 Correlation between change in MR-proANP and cardiac MRI measurements of left ventricular remodelling at 52 weeks

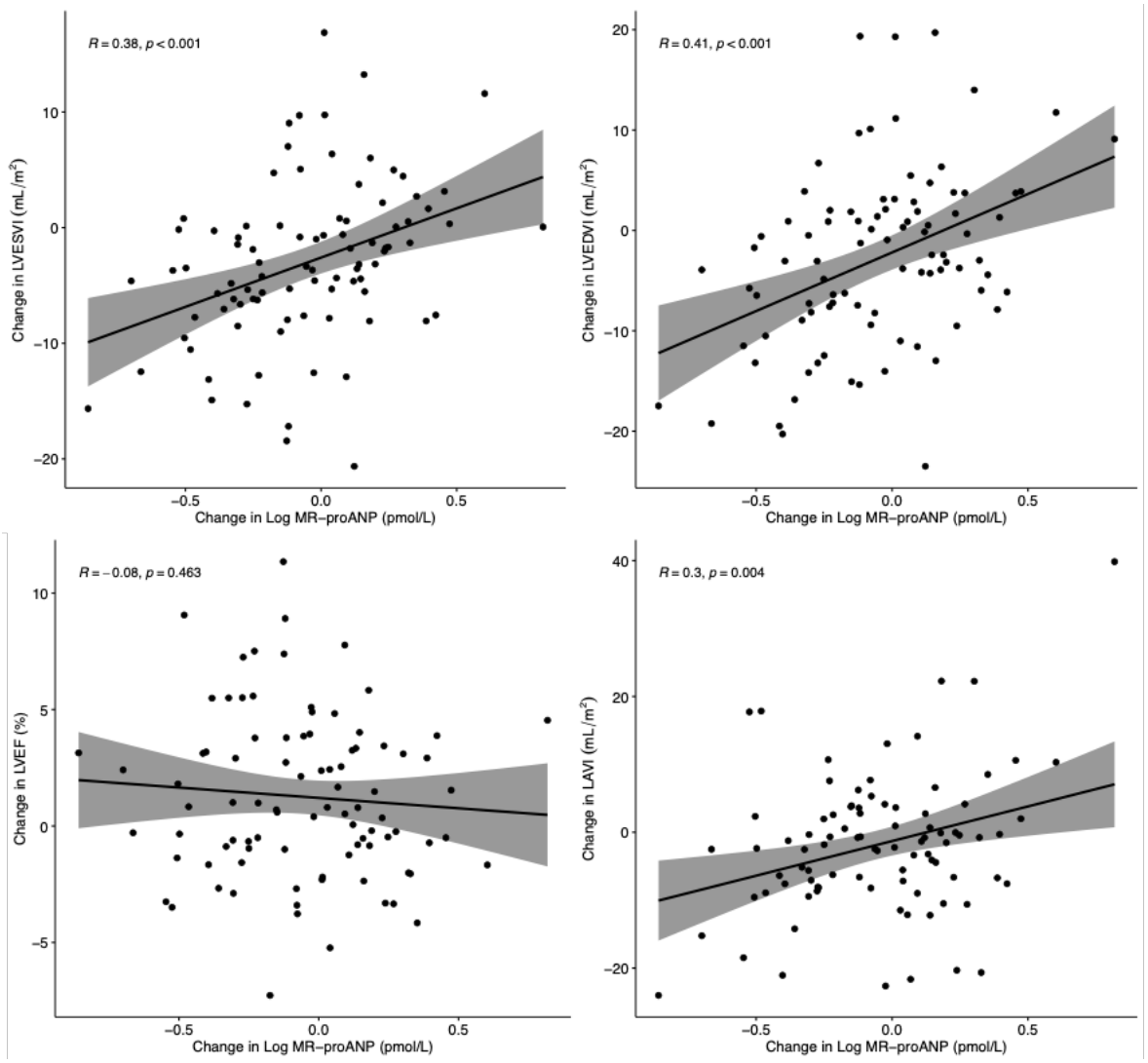


Figure 5-7 Correlation between change in ANP and cardiac MRI measurements of left ventricular remodelling at 52 weeks

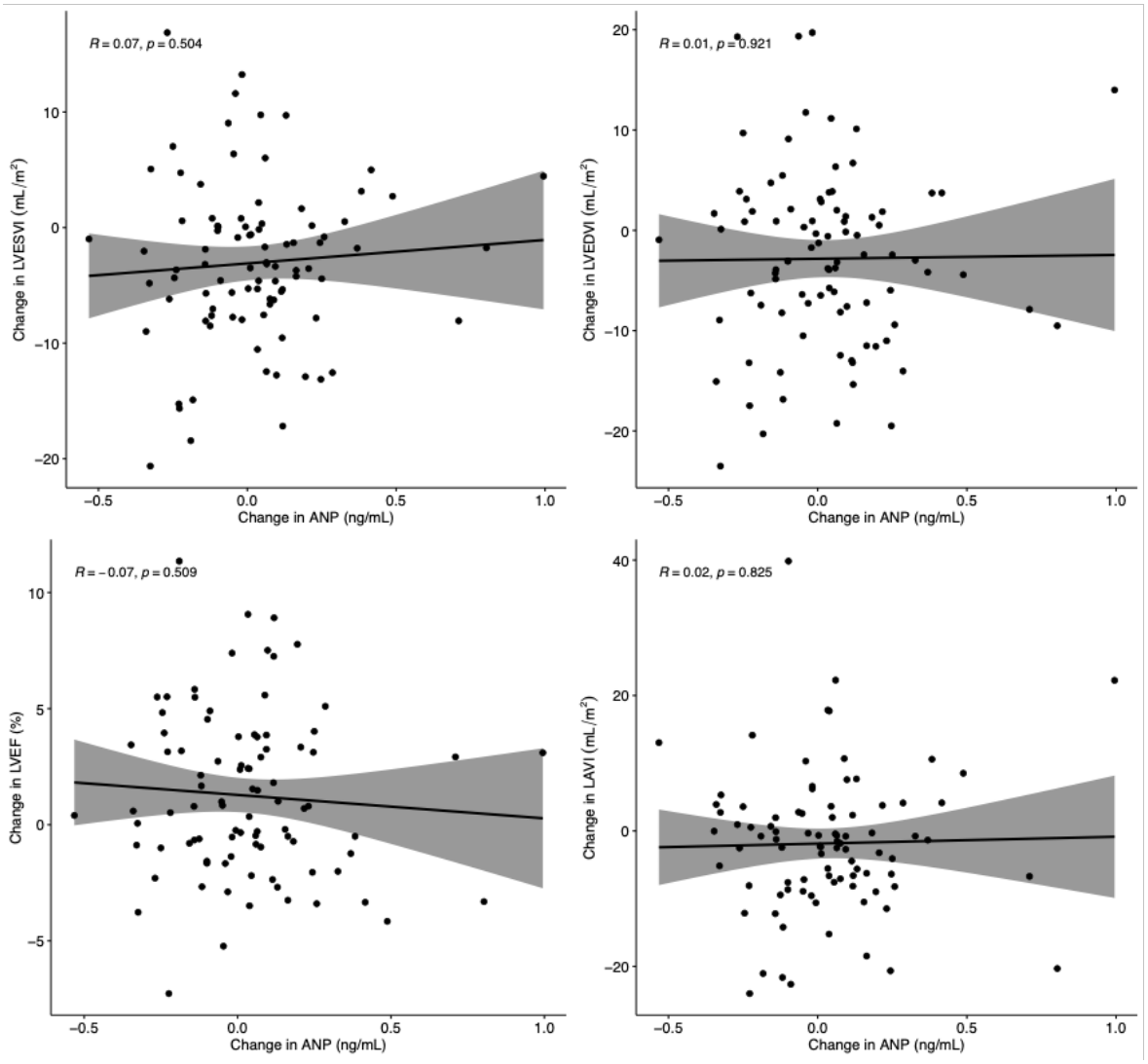
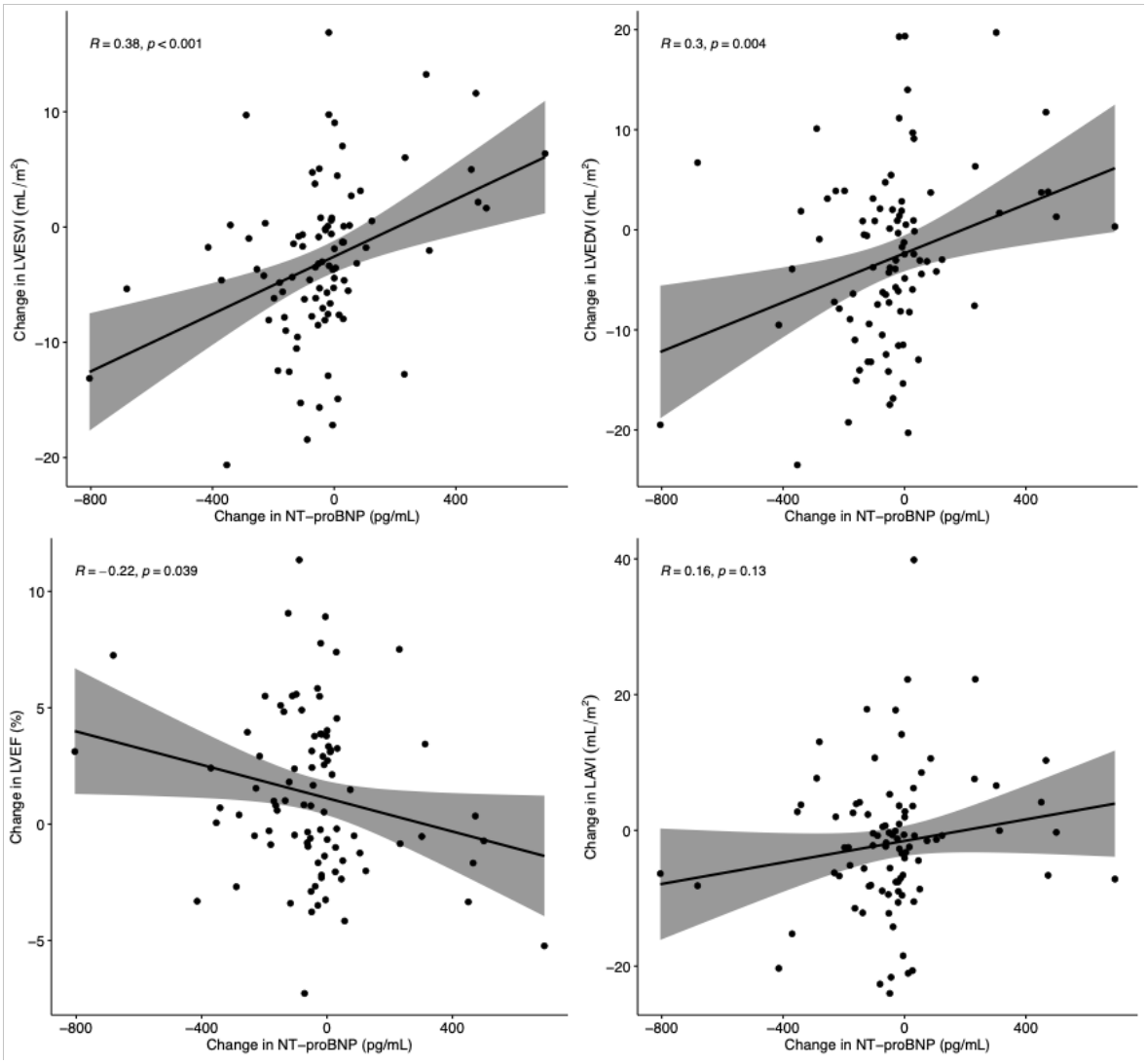


Figure 5-8 Correlation between change in NT-proBNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks



In a sensitivity analysis examining values within the 5-95% centiles of NT-proBNP values, the correlation between change in LVESVI and change in NT-proBNP was broadly similar with Pearson's R=0.29 (p=0.009).

Figure 5-9 Correlation between change in BNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks

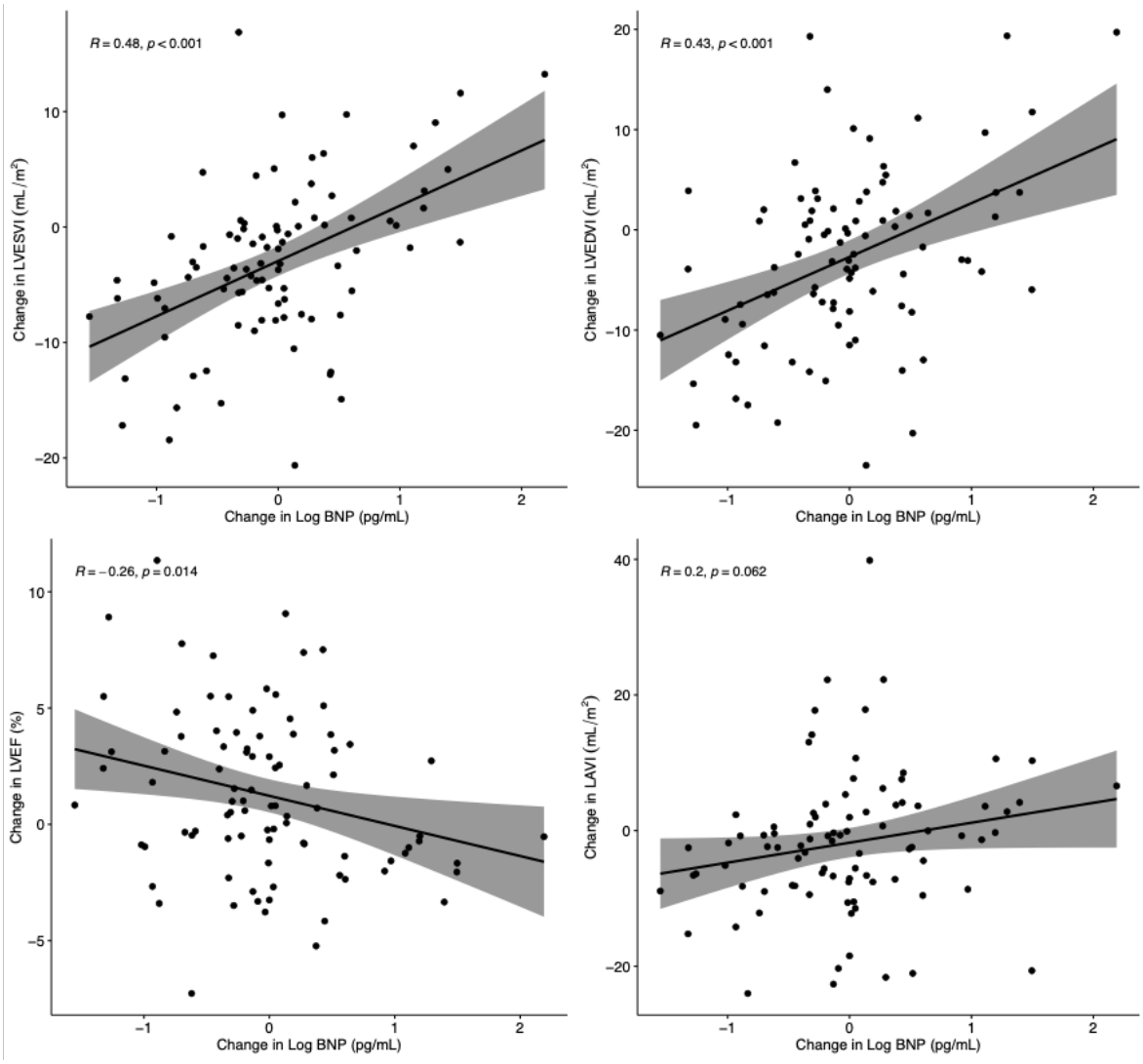
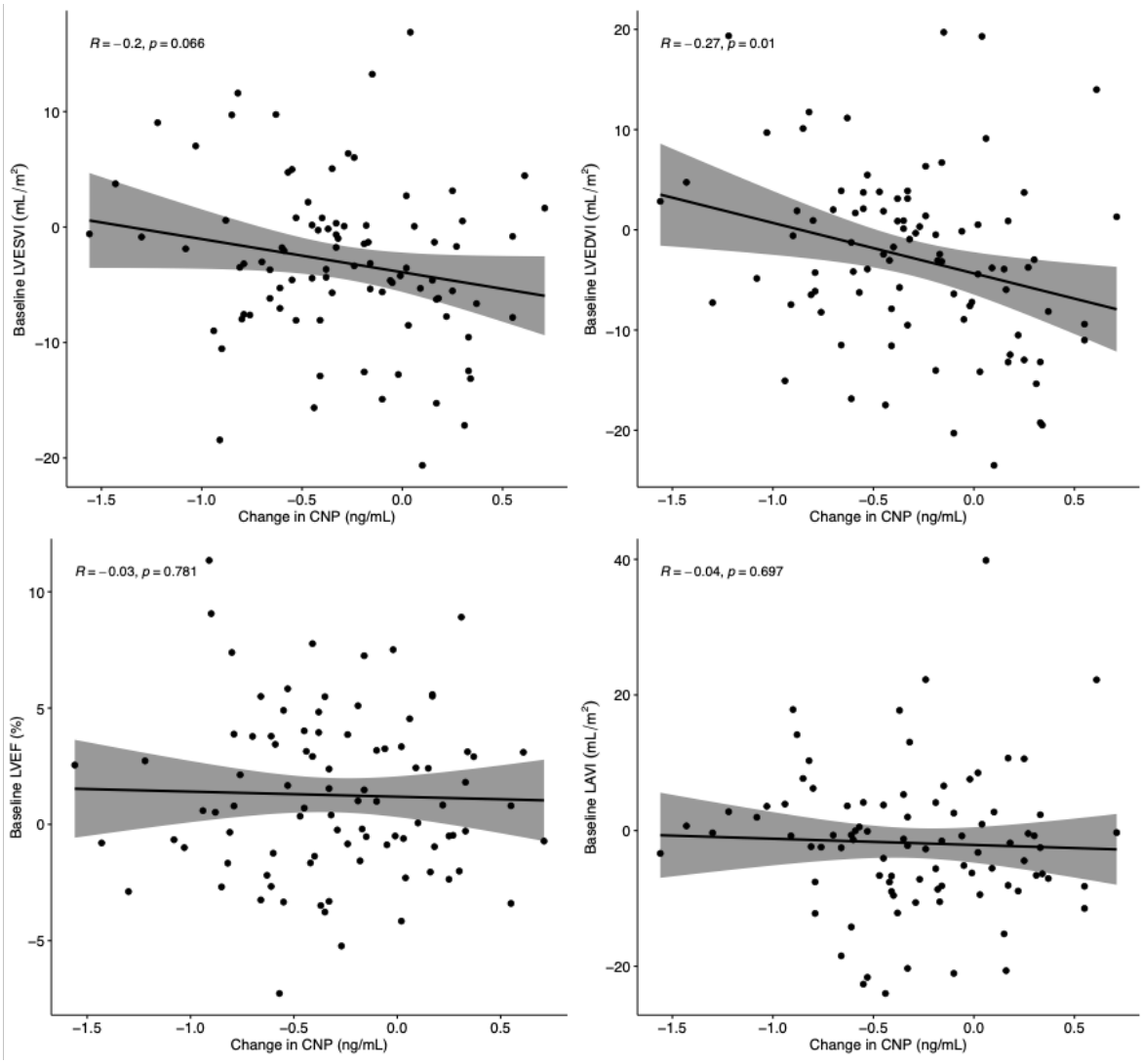


Figure 5-10 Correlation between change in CNP and cardiac MRI measurements of left ventricular remodelling at 52 weeks



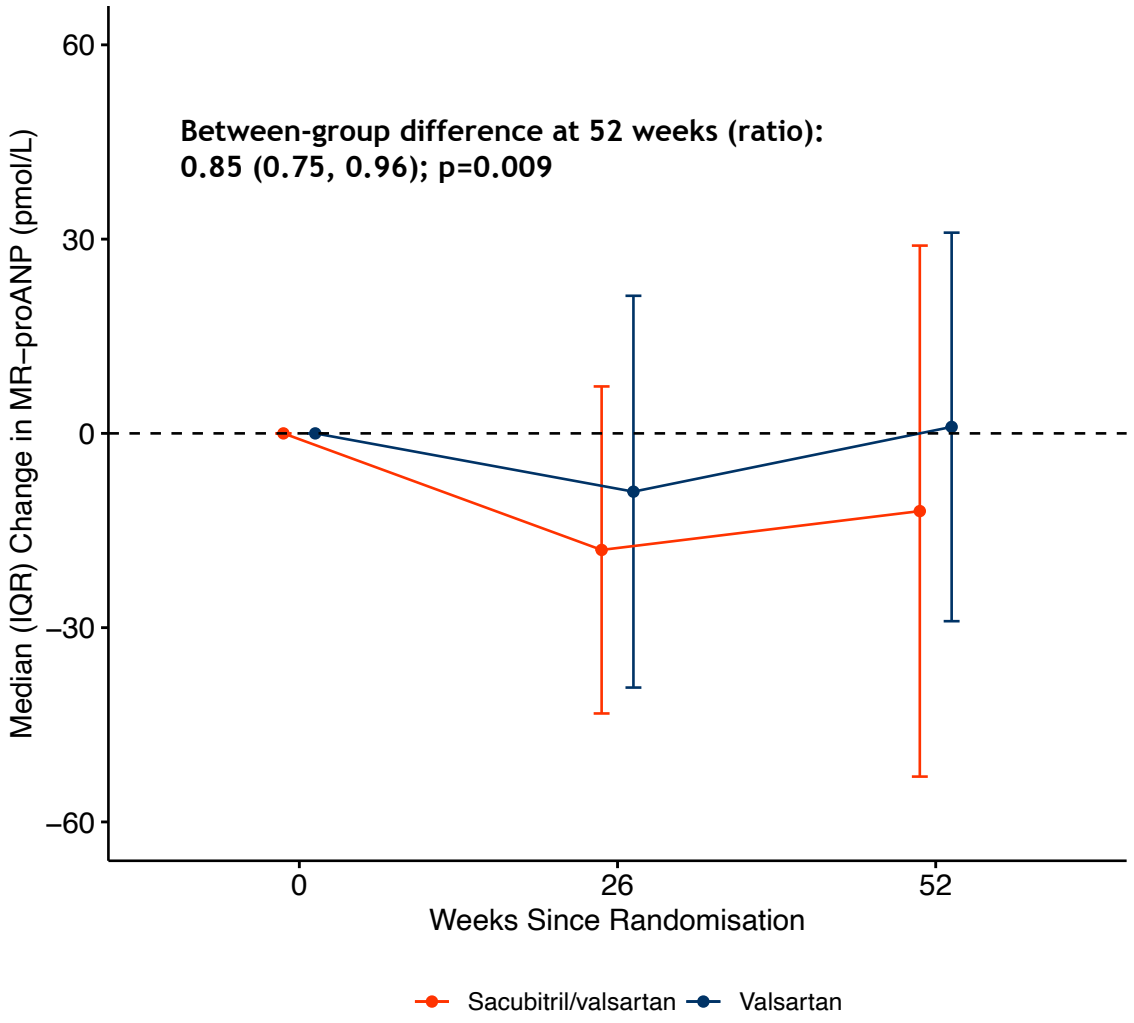
5.3.5 Effect of neprilysin inhibition on natriuretic peptide levels

5.3.5.1 Mid-regional prohormone of atrial natriuretic peptide (MR-proANP)

Median (IQR) MR-proANP at baseline was 96.0 (74.3, 133.8) pmol/L in those randomised to sacubitril/valsartan with follow-up data (n=46) and 96.0 (68.5, 150.0) pmol/L in those randomised to valsartan (n=46). Median change in MR-proANP was -12 (IQR -30.0, 11.0) pmol/L between baseline and 52 weeks in the sacubitril/valsartan group and 1 (-10.0, 20.0) pmol/L in the valsartan group: ratio of adjusted geometric means: 0.85 (95% confidence interval [CI], 0.75, 0.96); p=0.009 (Table 5-2 and Figure 5-11). Similar results were seen in repeated measures modelling with a decrease in MR-proANP at 26 weeks which was non-significant (ratio 0.90 [0.80, 1.01]; p=0.068) and significant at 52 weeks (0.85 [0.75, 0.95]; p=0.006).

Figure 5-11

Change in MR-proANP with sacubitril/valsartan or valsartan from baseline to week 52

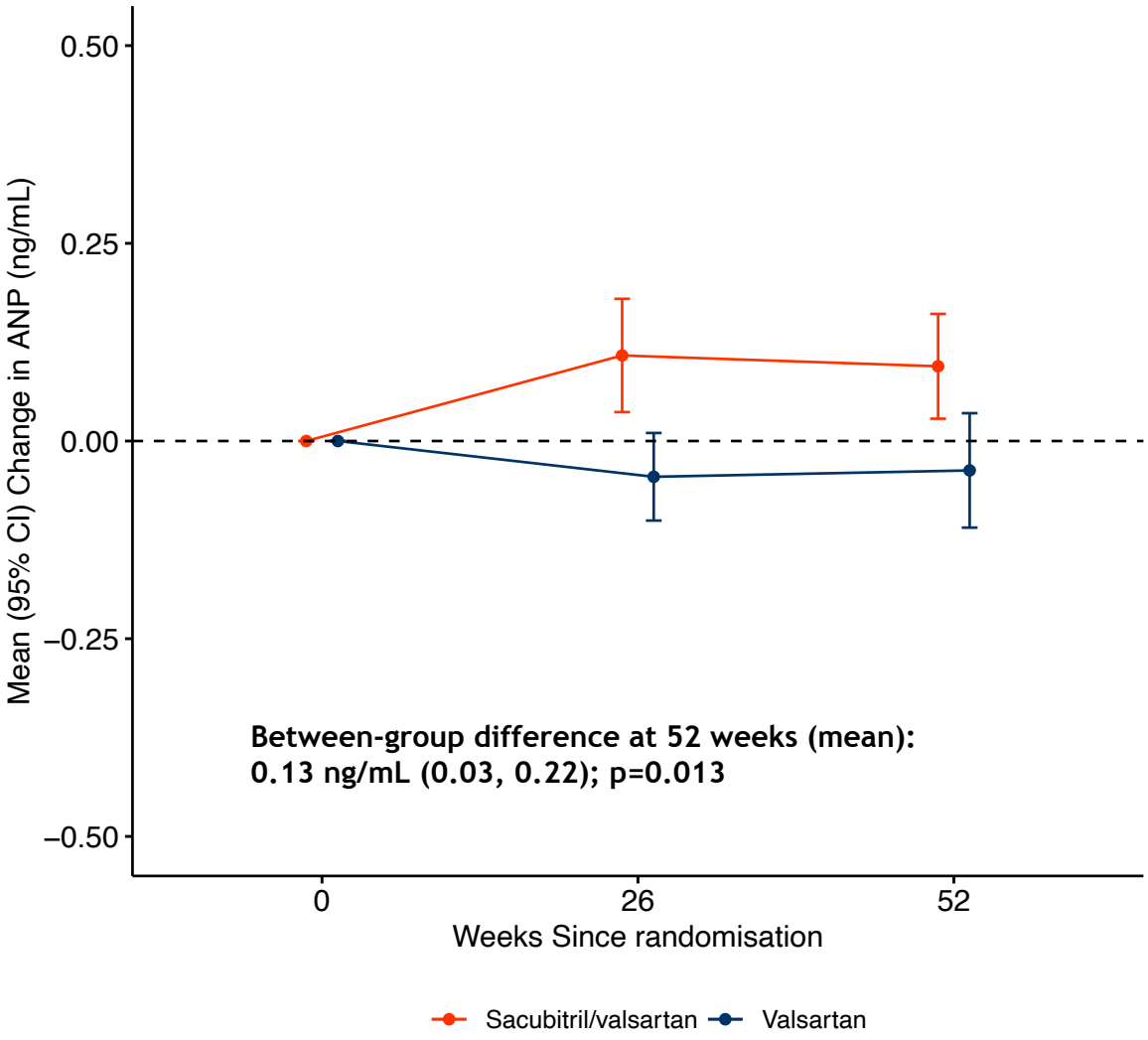


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline MR-proANP and use of diuretics at baseline.

5.3.5.2 Atrial natriuretic peptide (ANP)

Mean (SD) ANP at baseline was 1.12 (0.26) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 1.20 (21.3) ng/mL in those randomised to valsartan (n=46). Mean change in ANP was 0.09 (SD 0.22) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and -0.04 (0.24) ng/mL in the valsartan group: adjusted between-group mean difference 0.13 (95% confidence interval [CI], 0.03,0.22) ng/mL; p=0.013 (Table 5-2 and Figure 5-12). Similar results were seen in repeated measures modelling with significant increases with sacubitril/valsartan compared with valsartan at 26 weeks (p=0.002) and 52 weeks (p=0.008).

Figure 5-12 Change in ANP with sacubitril/valsartan or valsartan from baseline to week 52

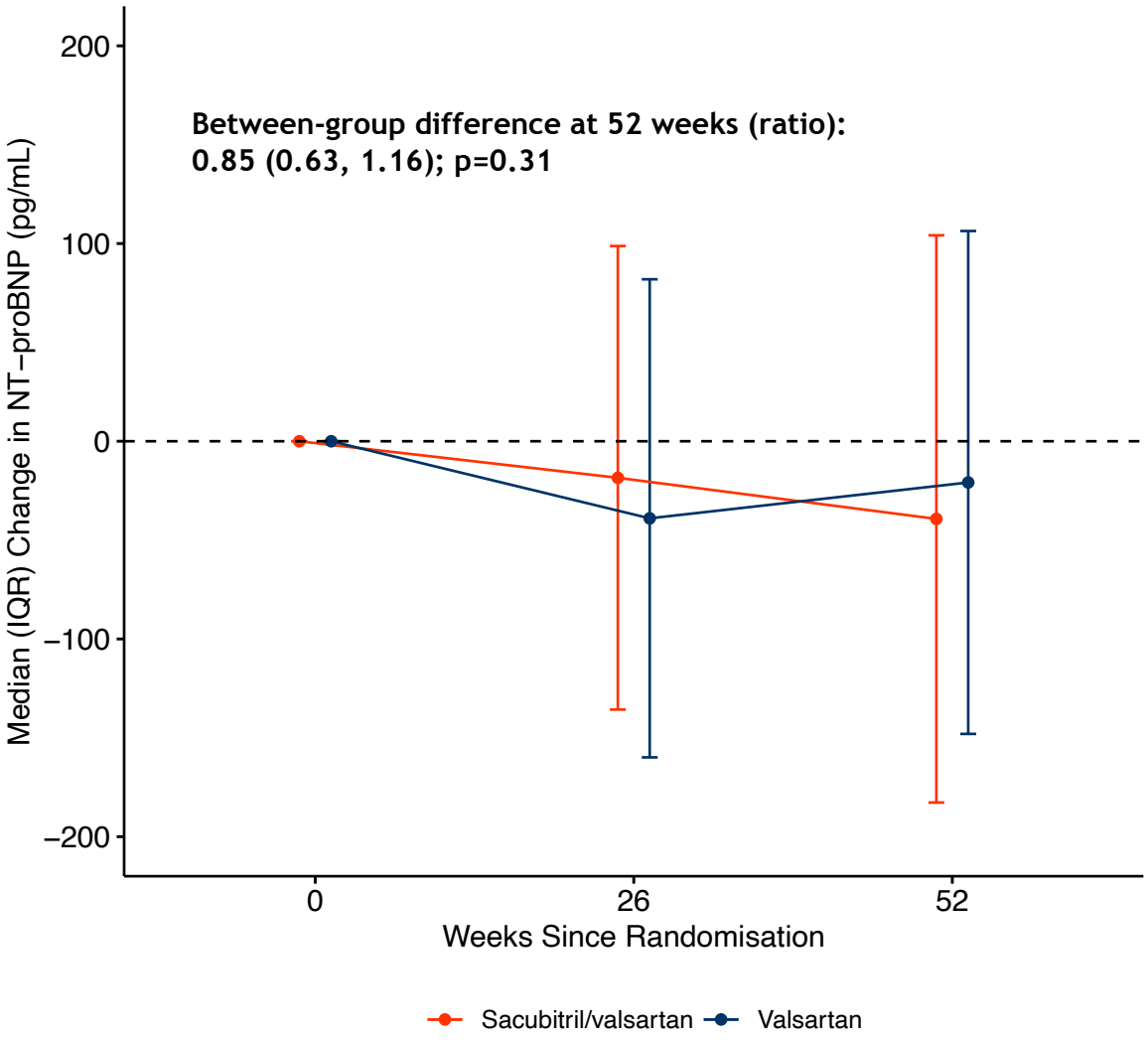


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline ANP, and use of diuretics at baseline.

5.3.5.3 N-terminal prohormone of B-type natriuretic peptide (NT-proBNP)

Median (IQR) NT-proBNP at baseline was 213 (126, 399) pg/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 242 (124, 426) pg/mL in those randomised to valsartan (n=46). Median change in NT-proBNP was -39 (IQR -131, 12) pg/mL between baseline and 52 weeks in the sacubitril/valsartan group and -21 (-104, 23) pg/mL in the valsartan group: ratio of adjusted geometric means: 0.85 (95% confidence interval [CI], 0.63,1.16); p=0.31 (Table 5-2 and Figure 5-13). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 5-13 Change in NT-proBNP with sacubitril/valsartan or valsartan from baseline to week 52

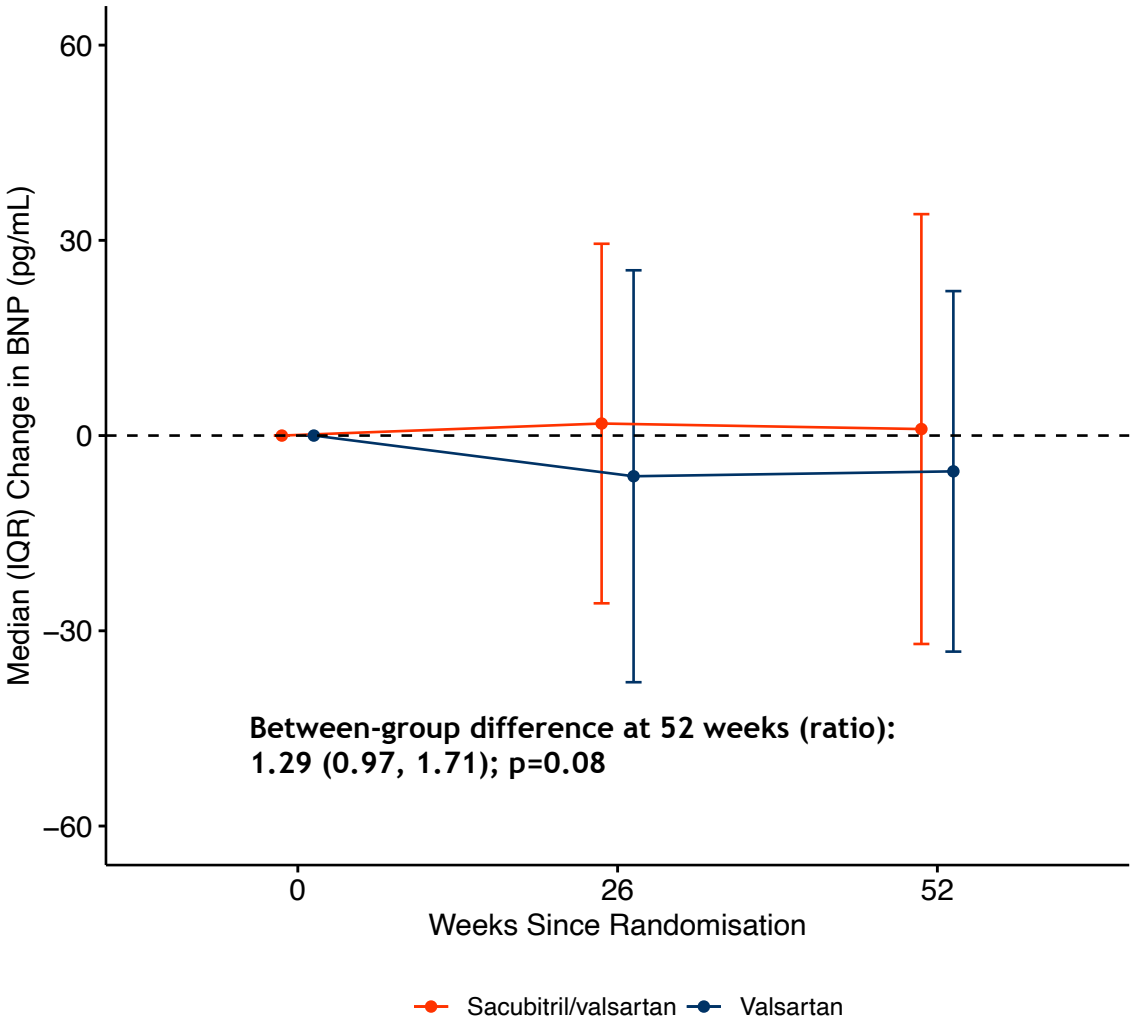


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline NT-proBNP and use of diuretics at baseline.

5.3.5.4 B-type natriuretic peptide (BNP)

Median (IQR) BNP at baseline was 38.5 (19.8, 70.0) pg/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 51.4 (32.5, 86.3) pg/mL in those randomised to valsartan (n=46). Median change in BNP was 1.0 (IQR -9.6, 23.5) pg/mL between baseline and 52 weeks in the sacubitril/valsartan group and -5.5 (-18.9, 8.8) pg/mL in the valsartan group: ratio of adjusted geometric means: 1.29 (95% confidence interval [CI], 0.97,1.71); p=0.08 (Table 5-2 and Figure 5-14). Similar results were seen in repeated measures modelling with increases with sacubitril/valsartan compared with valsartan at 26 (ratio 1.27 [0.97,1.65]) and 52 weeks (1.27 [0.97, 1.66]) which were not statistically significant.

Figure 5-14 Change in BNP with sacubitril/valsartan or valsartan from baseline to week 52

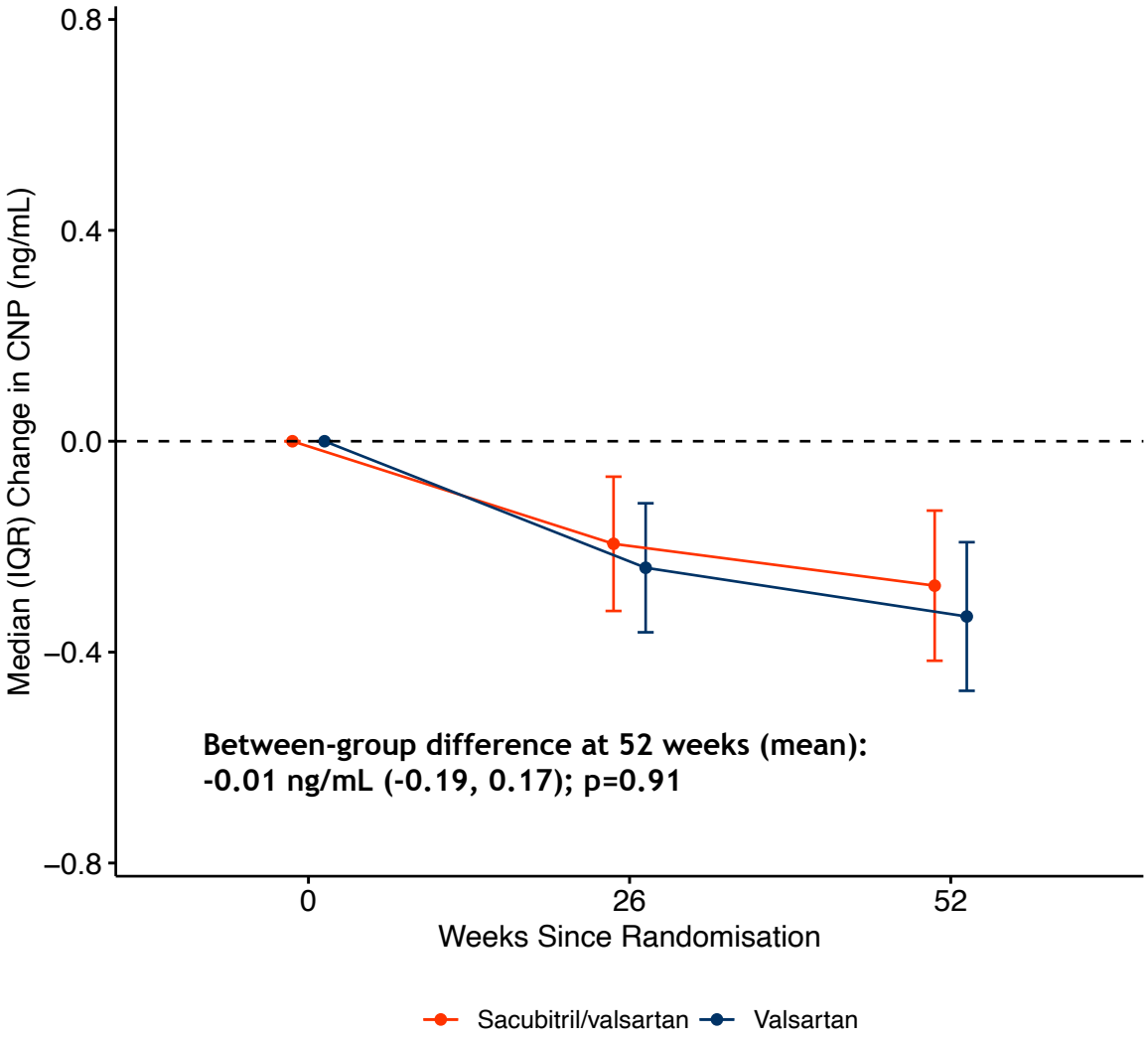


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline BNP and use of diuretics at baseline.

5.3.5.5 C-type natriuretic peptide (CNP)

Mean (SD) CNP at baseline was 3.14 (0.65) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 3.39 (1.02) ng/mL in those randomised to valsartan (n=46). Mean change in CNP was -0.27 (SD 0.48) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and -0.33 (0.47) ng/mL in the valsartan group: adjusted between-group mean difference -0.01 (95% confidence interval [CI], -0.19,0.17) ng/mL; p=0.91 (Table 5-2 and Figure 5-15). Similar results were seen in repeated measures modelling with no significant changes with sacubitril/valsartan compared with valsartan at 26 weeks (p=0.97) and 52 weeks (p=0.91).

Figure 5-15 Change in CNP with sacubitril/valsartan or valsartan from baseline to week 52

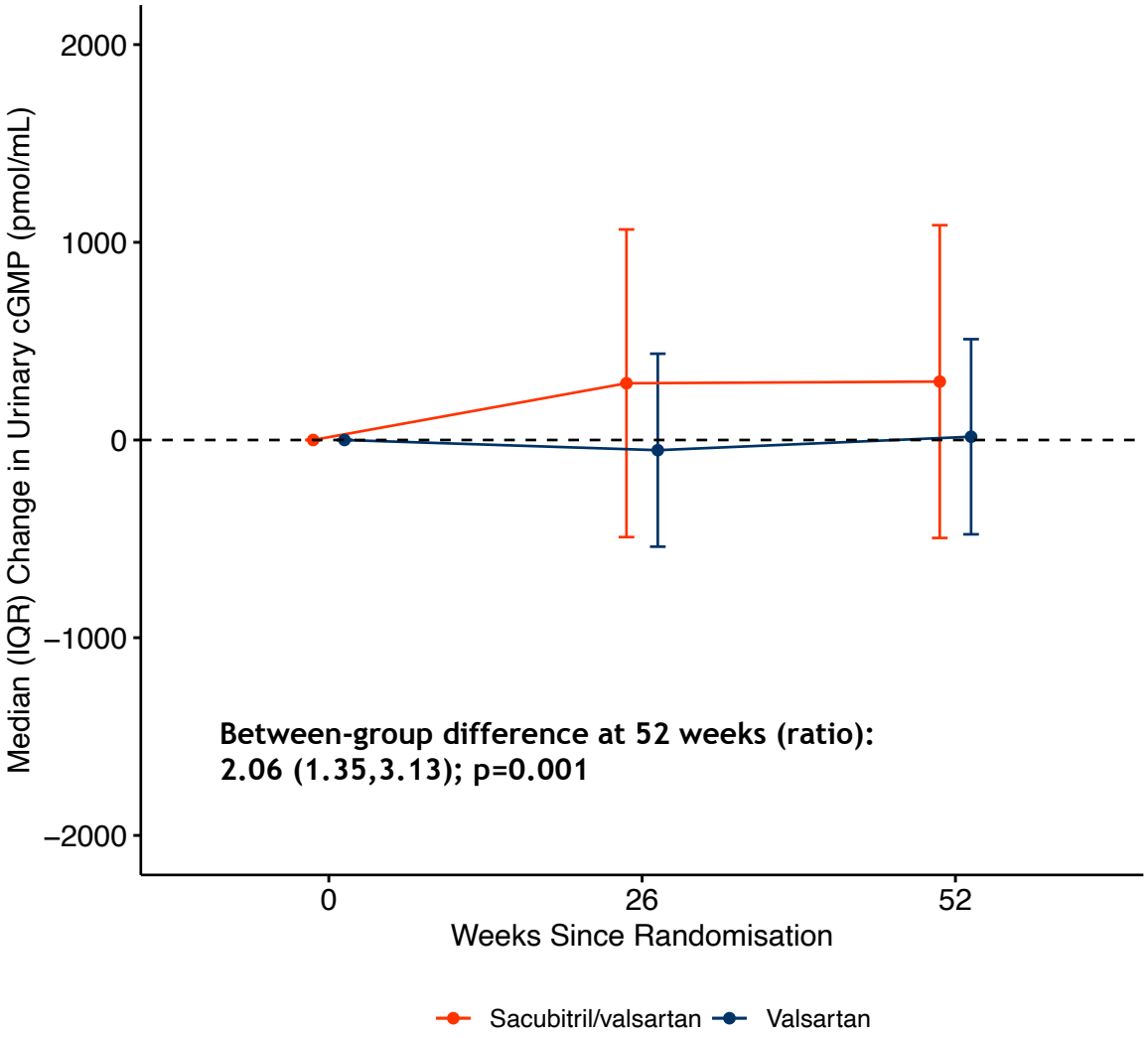


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline CNP and use of diuretics at baseline.

5.3.5.6 Urinary cyclic guanosine monophosphate (cGMP)

Median (IQR) cGMP at baseline was 510 (271, 835) pmol/L in those randomised to sacubitril/valsartan with follow-up data (n=46) and 378 (205, 802) pmol/L in those randomised to valsartan (n=46). Median change in cGMP was 296 (IQR -5, 796) pmol/L between baseline and 52 weeks in the sacubitril/valsartan group and 17 (-241, 252) pmol/L in the valsartan group: ratio of adjusted geometric means: 2.06 (95% confidence interval [CI], 1.35, 3.13); p=0.001 (Table 5-2 and Figure 5-16). Similar results were seen in repeated measures modelling with significant increases in cGMP at 26 weeks (p<0.001) and 52 weeks (p=0.001). When cGMP was indexed to urinary creatinine the ratio of adjusted geometric means was 1.94 (1.54, 2.43); p<0.001.

Figure 5-16 Change in cGMP with sacubitril/valsartan or valsartan from baseline to week 52



Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline cGMP and use of diuretics at baseline.

Table 5-2 Effect of neprilysin inhibition on natriuretic peptide levels

	Sacubitril/valsartan				Valsartan				Between-group difference (95% CI) *	P Value
	n	Baseline	Week 52	Change	n	Baseline	Week 52	Change		
ANP, ng/mL	46	1.12 (0.26)	1.22 (0.34)	0.09 (0.22)	46	1.20 (0.31)	1.16 (0.36)	-0.04 (0.24)	0.13 (0.03, 0.22)	0.013
MR-proANP, pmol/L	46	96.0 (74.3, 133.8)#	82.0 (66.0, 115.8)#	-12.0 (-30.0, 11.0)#	46	96.0 (68.5, 150.0)#	108.0 (66.5, 148.5)#	1.0 (-10.0, 20.0)#	0.85 (0.75, 0.96)	0.009
NT-proBNP, pg/mL	46	213 (126, 399)#	168 (105, 376)#	-39 (-131, 12)#	46	242 (124, 426)#	235 (113, 330)#	-21 (-104, 23)#	0.85 (0.63, 1.16)	0.31
BNP, pg/mL	46	38.5 (19.8, 70.0)#	39.9 (25.4, 92.3)#	1.0 (-9.6, 23.5)#	46	51.4 (32.5, 86.3)#	40.0 (21.7, 95.1)#	-5.5 (-18.9, 8.8)#	1.29 (0.97, 1.71)	0.08
CNP, ng/mL	46	3.14 (0.65)	2.87 (0.52)	-0.27 (0.48)	46	3.39 (1.02)	3.06 (0.93)	-0.33 (0.47)	-0.01 (-0.19, 0.17)	0.91
Urinary cGMP, pmol/mL	46	510 (271, 835)#	847 (454, 1413)#	296 (5, 796)#	46	378 (205, 802)#	420 (189, 808)#	17 (-241, 252)#	2.06 (1.35, 3.13)	0.001

Data presented as mean (SD) unless otherwise stated.

Results reported for those with data available at baseline and 52-weeks.

Median (interquartile range).

*Calculated using a linear model adjusted for randomized treatment, baseline value of the outcome, use of diuretics at baseline.

Between-group differences are reported as ratios of adjusted geometric means for MR-proANP, NT-proBNP, BNP and urinary cGMP from models using log-transformed values. All other outcomes are reported as adjusted mean differences (95% CI).

5.4 Discussion

Elevated levels of natriuretic peptides are a powerful independent predictor of adverse outcomes in patients with HFrEF. In meta-analyses of the-effects of HFrEF treatments, the extent of reduction in lowering natriuretic peptides was significantly correlated with the size of reduction in risk of heart failure hospitalisation but not mortality.[80] The strength of elevated natriuretic peptide levels in identifying of patients at a higher risk of the development of heart failure or subclinical left ventricular systolic dysfunction in both general populations and in high-risk populations is well established.[253-256] Furthermore, elevated levels of natriuretic peptides measured in the early period immediately following acute myocardial infarction identifies patients at elevated risk of adverse left ventricular remodelling and at a higher risk of cardiovascular mortality and heart failure hospitalisation.[82,257-259] Their potential role when measured remote from the time of myocardial infarction in identifying patients at risk of future adverse outcomes and progressive remodelling is less well established. The present study offered an opportunity to examine the relationship between natriuretic peptides levels and the degree of adverse left ventricular remodelling (an independent predictor of the risk of the development of heart failure and mortality) in a well-defined population using cardiac MRI, the gold-standard method of assessing cardiac structure and function.

I observed significant correlations between baseline levels of both ANP and BNP and their precursor molecule fragments MR-proANP and NT-proBNP, which is unsurprising given the equimolar release of these fragments following the enzymatic breakdown of proANP and proBNP by corin and furin.[76] Baseline left atrial and ventricular volumes were significantly correlated (LVESVI $r=0.46$ and LVEDVI $r=0.60$; both $p<0.001$); this relationship between atrial and ventricular distension (and increased wall stress) likely explains the correlations between ANP and BNP, and those of MR-proANP with both BNP and NT-proBNP. Plasma levels of ANP and CNP were also significantly correlated, consistent with previous reports that ANP stimulates release of CNP.[263]

Data on the relationships between natriuretic peptide levels and the degree of adverse remodelling in patients remote from myocardial infarction and those

with asymptomatic left ventricular systolic dysfunction are limited. In the OPTIMAAL trial, at >4 years following acute infarction, elevated NT-proBNP was significantly associated with a greater degree of ventricular dilatation (LVESVI = log NT-proBNP ß coefficient 0.36 [p=0.008] and LVEDVI = log NT-proBNP ß coefficient 0.54 [p=0.0004]).[264] In this cohort, of whom 50% of patients were in NYHA functional class II, greater infarct size was significantly correlated with higher NT-proBNP levels, and this finding was replicated in the present study ($r=0.38$; $p<0.001$). In the context of the correlation between elevated infarct size and degree of ventricular dilation described in Chapter 4, it is likely the relationship between infarct size and NT-proBNP is a surrogate marker for the degree of wall stress on the non-infarct zone myocardium, rather than a direct cause of elevated natriuretic peptide levels, a conclusion which is supported by the observation in OPTIMAAL that in multivariate regression including infarct size, LVEDVI was the only independent predictor of NT-proBNP levels at >4 years following myocardial infarction.[264] With regards to the data related to measurements of other natriuretic peptide levels, in the SOLVD registry, after adjustment for NYHA functional class (37% of patients were NYHA class I), higher levels of ANP were significantly associated with a lower ejection fraction.[84]

The present data demonstrating significant associations between elevated levels of MR-proANP, BNP, NT-proBNP and, to a lesser extent, ANP and a greater degree of left ventricular remodelling are the first, to my knowledge, to describe these relationships in a cohort of exclusively asymptomatic patients with left ventricular systolic dysfunction remote after myocardial infarction (median time from infarction 3.6 years).

The relationship between a greater degree of adverse remodelling late following myocardial infarction and risk of outcomes is well established.[37,38] In addition to the associations demonstrated with metrics of adverse left ventricular remodelling, further support for the measurement of natriuretic peptides is provided by limited data reporting the predictive capability of elevated natriuretic peptide measurements when measured late after the time of acute myocardial infarction. In a sub-study of the SAVE trial which recruited patients with asymptomatic left ventricular systolic dysfunction immediately following myocardial infarction, 471 patients were assessed who remained asymptomatic

at 3-months post infarction (i.e., the same time-point for eligibility for inclusion in the present study).[265] In that study a 1 standard deviation increase in ANP in univariable analysis was associated with a 7% and 10% higher risk of cardiovascular death and severe heart failure, respectively. However, after multivariable adjustment a significant association was only seen with the risk of heart failure. Another finding of note from SAVE was that the ANP levels at 3 months in asymptomatic patients were similar to those seen in the SOLVD treatment arm (i.e., patients with symptomatic HFrEF), suggestive of ongoing increased cardiac filling pressures and wall stress, and ANP only decreased to similar levels to those in the SOLVD prevention arm (i.e., asymptomatic patients) at 1 year following infarct.[247] Unfortunately differences in the assay used in the present study preclude any direct comparisons with these results. In another study of 145 patients in Japan, BNP measured 1 month following acute myocardial infarction was an independent predictor of cardiovascular mortality.[266]

A further novel aspect of the present study was the examination of the change in natriuretic peptides levels and their association with change in ventricular and atrial volumes. When considering the value of any surrogate outcome, it must be demonstrated that a change in the marker is associated with a change in outcome. The present data clearly establish a relationship between a change MR-proANP, NT-proBNP and BNP and a change in ventricular volumes, an established surrogate marker for outcomes adding to similar observations in patients with symptomatic HFrEF.[153,267,268] However it is important to highlight that natriuretic peptide guided treatment has not been demonstrated to improve outcomes in patients with HFrEF.[269]

In summary, these results suggest that measurement of natriuretic peptides may offer easy-to-obtain predictive information on the degree of adverse remodelling in at-risk patients remote from the time of myocardial infarction. The cost and relative ease of measurement relative to echocardiography mean that measurement of natriuretic peptides may be a valuable “gate-keeper” test to identify patients who may have adversely remodelled following myocardial infarction and are therefore at elevated risk of adverse outcomes. I will return to this subject in Chapter 7.

This study also offered novel insights into the potential mechanisms of action behind the established clinical benefits seen with sacubitril/valsartan.[138] A strength of the present work was the use of valsartan as the comparator which allowed a direct examination of the effect of neprilysin inhibition per se on natriuretic peptide levels which are substrates for the enzyme. This differs from previous randomised comparisons which have used an ACE-inhibitor as the comparator, i.e., a comparison of neprilysin and AT1R inhibition versus ACE inhibition alone.[138,148]

Randomised data describing the effect of neprilysin inhibition on natriuretic peptide levels in patients with symptomatic left ventricular systolic dysfunction are provided by the PARADIGM\-HF and PIONEER-HF trials, where sacubitril valsartan, compared with enalapril, significantly reduced NT-proBNP (not a substrate for neprilysin) indicating a reduction in left ventricular wall stress. I did not observe any significant difference in the change in NT-proBNP between baseline and 52 weeks with sacubitril/valsartan as compared with valsartan (i.e., the addition of neprilysin inhibition). This could be considered consistent with the lack of significant effect on left ventricular volumes as the two are significantly correlated both in the present study and in previous reports.[153] It is notable however, that the baseline levels of NT-proBNP in these trials was significantly higher (median 1594 pg/mL and 2536 pg/mL in the enalapril arms of PARADIGM-HF and PIONEER-HF, respectively), which is unsurprising given the inclusion criteria for these trials mandated elevated natriuretic peptide levels and the patients studied differed from the asymptomatic population in the present trial, i.e. symptomatic (NYHA ≥II) HFrEF in PARADIGM-HF and HFrEF patients hospitalised for decompensated heart failure in PIONEER-HF.[138,270] I did however, observe a significant reduction in MR-proANP in patients treated with sacubitril/valsartan suggesting a degree of reduction in cardiac filling pressures and/or negative feedback secondary to the increase in ANP.

As described in Chapter 1-4, neprilysin has a greater affinity for ANP relative to BNP.[108] Consistent with this, in the present study I observed a significant increase in ANP but the small increase in BNP was not statistically significant. Due to the difficulties in measuring ANP in large multi-centre studies because of its short half-life, there are no randomised data examining the effect of

sacubitril/valsartan on ANP. In the observational PROVE-HF study, concentrations of ANP were significantly higher than baseline 14 days after initiation of sacubitril valsartan and the degree of increase in ANP was associated with improvements in ejection fraction and LAVI, a finding that was not replicated in the present study.[271] In another observational study by Nougué and colleagues, in findings similar to those presented here, a significant rise in ANP with sacubitril/valsartan was not correlated with beneficial reverse remodelling changes.[272]

In the PARADIGM-HF trial, BNP was significantly higher at 8-10 weeks following randomisation in the sacubitril/valsartan group as compared with the enalapril group.[273] Again, consistent with the greater affinity that neprilysin has for ANP relative to BNP, in an observational study in 23 patients with HFrEF initiated on sacubitril/valsartan, a greater relative increase in ANP was seen as compared with BNP.[274] Of the three bioactive natriuretic peptides measured, CNP levels showed the least difference between the two treatment groups after 52 weeks of treatment with sacubitril/valsartan or valsartan. This is unsurprising given levels of CNP in patients with heart failure are not significantly elevated compared to controls and therefore is not thought to play a major pathophysiological role.[275]

It appears clear, therefore, from the available data that ANP, rather than BNP or CNP may be the predominant natriuretic peptide mediator underlying the clinical benefits of sacubitril/valsartan, possibly in consort with increased levels of other substrates for neprilysin (Chapter 6). The physiological actions of ANP and BNP are mediated through their activation of the natriuretic peptide receptor A (NPR-A), for which ANP has a greater affinity for, relative to BNP, resulting in increased intracellular levels of the secondary messenger cGMP; an increase in cGMP results in vasodilation, natriuresis as well as anti-hypertrophic and anti-fibrotic effects.[276] The observed increase in urinary cGMP in the present study, therefore, reflects the increase in ANP-mediated activation of NPR-A. Indeed, the urinary cGMP:ANP ratio increased by 89% ($p=0.003$) between baseline and 52 weeks in sacubitril/valsartan treated patients compared with a 66% ($p=0.026$) increase in cGMP:BNP ratio. It is worth considering that this increase in ANP (a substrate for neprilysin) is also in the context of a significant

reduction in MR-proANP (not a substrate for neprilysin) indicating a reduction in the formation of the proANP precursor molecule. Therefore, the rise in ANP represents an increase in bioactive ANP (secondary to a reduction in its breakdown by neprilysin), not an increase in production, as evidenced by a significant increase in the ratio of ANP:MR-proANP of 32%% ($p<0.001$) with sacubitril/valsartan compared with valsartan. An increase in bioactive BNP was also suggested by a 28% increase in the BNP:NT-proBNP ratio ($p=0.02$).

5.4.1 Limitations

The major limitation of the present study was the sample size. A larger sample size may have provided greater statistical power to detect small differences between the treatment groups (e.g., in BNP). A high number of statistical tests were performed with no correction for multiple testing. With regards to the predictive capability of natriuretic peptides to detect left ventricular remodelling, I did not have any patients with ejection fractions of >40% to identify optimal cut-off values to detect patients with persisting left ventricular systolic dysfunction late after myocardial infarction. The degree of change in left ventricular volumes over time was relatively small and this may have limited my ability to detect correlations between the change in natriuretic peptide levels and the degree of cardiac remodelling. Elevated neprilysin levels have been reported to be detectable in patients with HFrEF.[277] I did not measure neprilysin concentrations or activity in the present study but this could provide further insight into the mechanisms behind changes in natriuretic peptide levels.

5.4.2 Conclusion

In this study examining natriuretic peptide levels in patients with asymptomatic left ventricular systolic dysfunction late following a myocardial infarction, I have made several important observations. Firstly, in this patient population elevated levels of ANP and BNP, and their precursor molecule fragments (MR-proANP and NT-proBNP) were significantly correlated with the degree of adverse left ventricular remodelling with higher levels of these peptides indicating greater ventricular dilatation and impairment of systolic function. Secondly, reductions in plasma levels of MR-proANP, NT-proBNP and BNP over 52 weeks were associated with improvement in left ventricular and atrial volumes over the

same period as measured by cardiac MRI. Finally, the addition of neprilysin inhibition to standard therapy in the form of sacubitril/valsartan increased plasma levels of ANP and urinary cGMP, indicating increase ANP-mediated bioactivity, and significantly reduced MR-proANP which is not a substrate for neprilysin and may indicate a reduction in left ventricular filling pressures. No significant differences were observed in concentrations of BNP or CNP (substrates for neprilysin) or NT-proBNP (a marker of elevated left ventricular wall stress and not a substrate for neprilysin).

Chapter 6 The effect of neprilysin inhibition on postulated circulating substrates for neprilysin in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

6.1 Introduction

Neprilysin has numerous substrates in addition to the natriuretic peptides (Chapter 1-4), and it has been suggested that by preventing their breakdown, increased levels of some of these substrates may be one of the mechanisms of action behind the clinical benefits of neprilysin inhibition observed in patients with HFrEF in the PARADIGM-HF trial.[138] Data relating to the effect of sacubitril/valsartan are mainly limited to observational studies examining concentrations of these substrates and these studies are limited in making conclusions regarding treatment effect by the very nature of their observational design.[272] Furthermore, given the overwhelming evidence for clinical benefit in patients with HFrEF, it would not be ethical to randomise these patients to a neprilysin inhibitor. Therefore, the present study population, those with asymptomatic left ventricular systolic dysfunction, are a close pathophysiological group in which to examine the effect of neprilysin inhibition on its substrates in a rigorously performed randomised, active-comparator controlled trial.

This chapter will provide novel data on the effect of neprilysin inhibition on a selection of its postulated circulating neurohumoral and metabolic substrates through the randomised comparison of sacubitril/valsartan compared with valsartan alone.

6.2 Methods

6.2.1 Study patients and protocol

The 93 patients detailed in in the preceding chapters compose the population included in this study. These patients had evidence of persisting left ventricular systolic dysfunction (left ventricular ejection fraction ≤40% measured using echocardiography) with no signs or symptoms of heart failure and were recruited into a trial examining the effect of the addition of neprilysin inhibition to standard therapy at least 3 months following an acute myocardial infarction. All patients were taking an ACE inhibitor or ARB prior to enrolment and a beta-blocker (unless contraindicated or not tolerated). Eligible patients were randomised 1:1 to sacubitril/valsartan (target dose 97/103mg twice daily) or valsartan (target dose 160mg twice daily) and matching placebo for 52 weeks. The study protocol is detailed in Chapter 3 and baseline characteristics in Table 4-1.

Venepuncture was performed pre-randomisation, at 26 weeks, and at 52 weeks as described in Chapter 3. Mid-regional pro-adrenomedullin (MR-proADM) (B·R·A·H·M·S KRYPTOR Compact PLUS, Thermo Fisher Diagnostics) was measured on a clinical immunoassay platform using the manufacturer's calibrators and quality control materials. Endothelin-1 was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D systems, Bio-Techne), and using the manufacturer's quality control materials. GLP-1 (plasma from a BD p800 protease inhibitor vacutainer, using Total GLP-1 assay, Mercodia) and apelin (aprotinin-treated plasma, apelin-36 extraction-free EIAs, Phoenix Pharmaceuticals) were also measured using commercial ELISA assays and the manufacturers' quality control materials.

All biomarker sample processing and measurements were performed by Philip Stewart, Elaine Butler, Josephine Cooney and Emma Dunning at the Glasgow Biomarker Laboratory, Institute of Cardiovascular and Medical Sciences, University of Glasgow under the supervision of Dr Paul Welsh.

6.2.2 Statistical methods

The distribution of baseline biomarker values was examined by means of histograms and summary statistics and non-normally distributed values were log-transformed prior to analysis. Baseline levels are presented as means with standard deviations for normally distributed values, and as medians with interquartile ranges for non-normal distributions. Baseline values are presented in the overall population and by randomised treatment allocation with between-group comparisons made using a two-sample T-test or Wilcoxon rank-sum test for normal and non-normal distributed variables, respectively.

The treatment effect of sacubitril/valsartan as compared with valsartan on biomarker levels over time was examined using a linear regression model which was adjusted for randomised treatment, baseline value of the outcome and use of diuretics at baseline. The regression model coefficients for the treatment indicator variable are reported as between-treatment group adjusted mean differences. Repeated measures analyses were performed as confirmatory analyses and are adjusted for the main effects of time-point, randomised group and the interaction between time-point and randomised group and for diuretic use at baseline. All analyses were performed on an intention to treat basis as described in Chapter 3.

A p-value of <0.05 was considered statistically significant for all analyses. No correction for multiple testing was performed. No imputation for missing data was performed. All analyses were performed by Bethany Stanley (Robertson Centre for Biostatistics) and me using R Studio and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria), and STATA version 16.1 (College Station, TX, USA).

6.3 Results

6.3.1 Baseline values

Baseline values of the substrates for neprilysin detailed overall and by randomised treatment allocation in Table 6-1. There were no significant between-group differences at baseline.

Table 6-1 Baseline levels of circulating neprilysin substrates

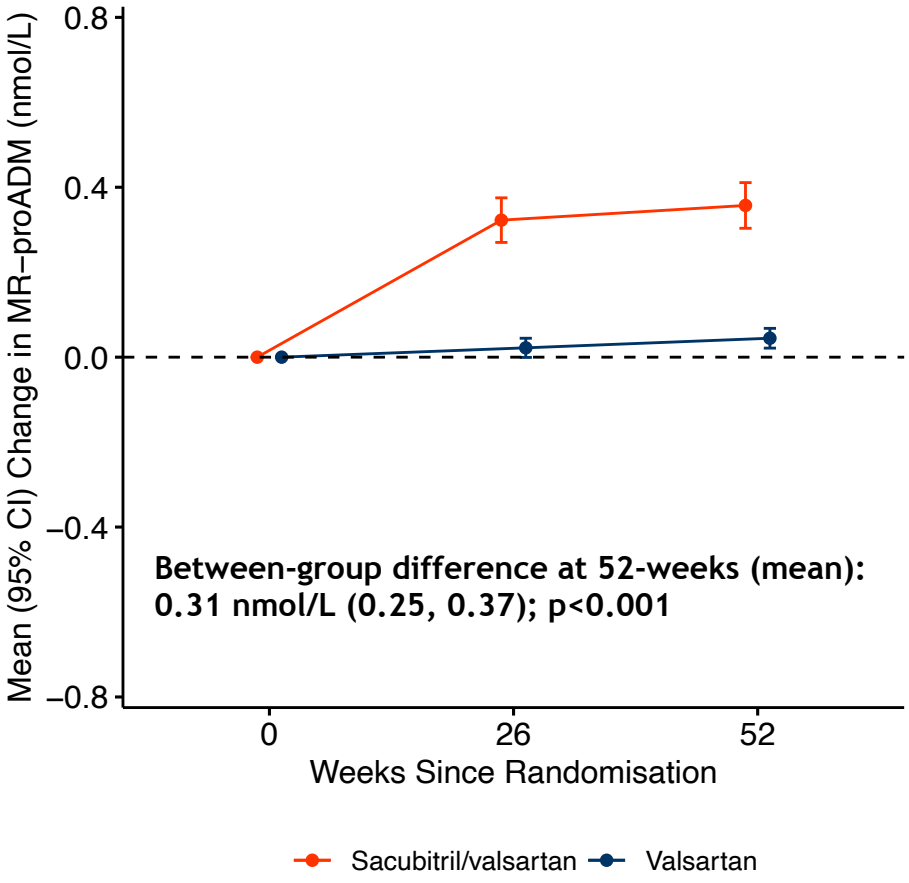
	Sacubitril/valsartan	**Valsartan**	**Total**	**p-value**
	N=47	**N=46**	**N=93**	
MR-proADM (nmol/L)	0.5±0.1	0.5±0.1	0.5±0.1	0.30
GLP-1 (pmol/L)	5.8±3.8	6.1±3.6	6.0±3.7	0.68
Apelin (ng/mL)	1.4±0.5	1.4±0.4	1.4±0.4	0.72
Endothelin-1 (pg/mL)	1.3±0.4	1.3±0.3	1.3±0.3	0.17

Data presented as mean ± standard deviation.

6.3.2MR-proADM

Mean (SD) MR-proADM at baseline was 0.54 (0.11) nmol/L in those randomised to sacubitril/valsartan with follow-up data (n=46) and 0.51 (0.12) nmol/L in those randomised to valsartan (n=46). Mean change in MR-proADM was 0.36 (SD 0.18) nmol/L between baseline and 52 weeks in the sacubitril/valsartan group and 0.04 (0.08) nmol/L in the valsartan group: adjusted between-group mean difference 0.31 (95% confidence interval [CI], 0.25,0.37) nmol/L; p<0.001 (Figure 6-1). Similar results were seen in repeated measures modelling with significant increases with sacubitril/valsartan compared with valsartan at 26 weeks (p<0.001) and 52 weeks (p<0.001)

Figure 6-1 Change in MR-proADM with sacubitril/valsartan or valsartan from baseline to week 52

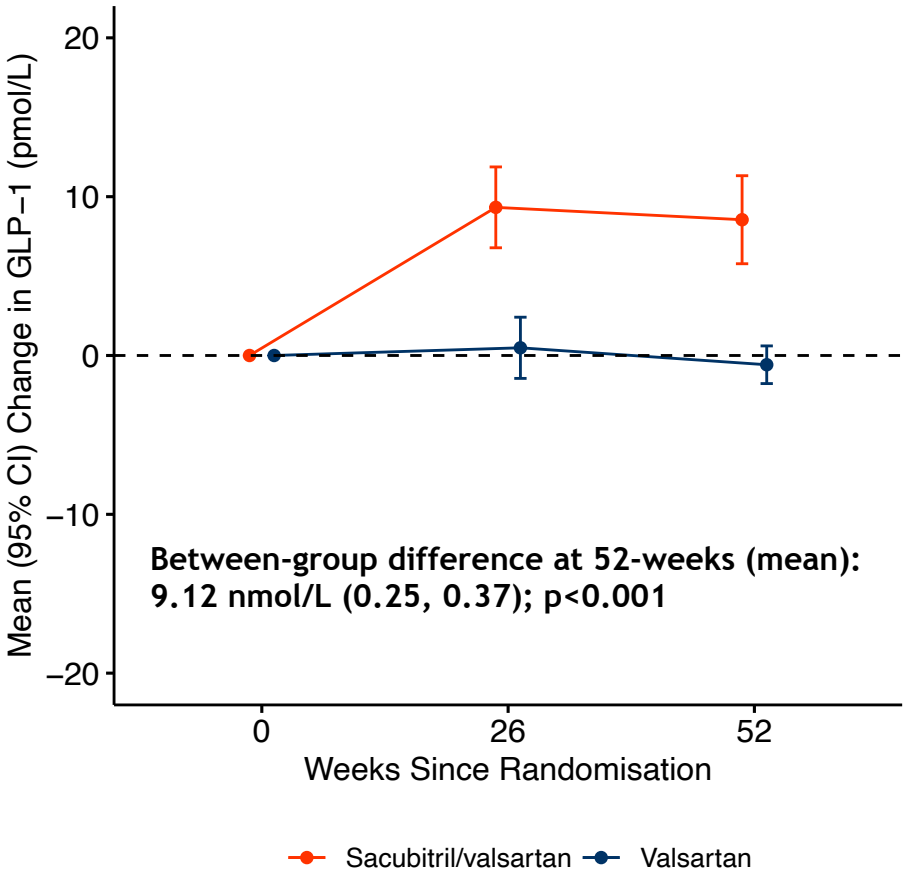


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline MR-proADM, and use of diuretics at baseline.

6.3.3 GLP-1

Mean (SD) GLP-1 at baseline was 5.78 (3.89) pmol/L in those randomised to sacubitril/valsartan with follow-up data (n=46) and 6.12 (3.59) pmol/L in those randomised to valsartan (n=46). Mean change in GLP-1 was 8.55 (SD 9.33) pmol/L between baseline and 52 weeks in the sacubitril/valsartan group and -0.58 (3.99) pmol/L in the valsartan group: adjusted between-group mean difference 9.12 (95% confidence interval [CI], 6.13,12.11) pmol/L; p<0.001 (Figure 6-2). Similar results were seen in repeated measures modelling with significant increases with sacubitril/valsartan compared with valsartan at 26 weeks (p<0.001) and 52 weeks (p<0.001).

Figure 6-2 Change in GLP-1 with sacubitril/valsartan or valsartan from baseline to week 52

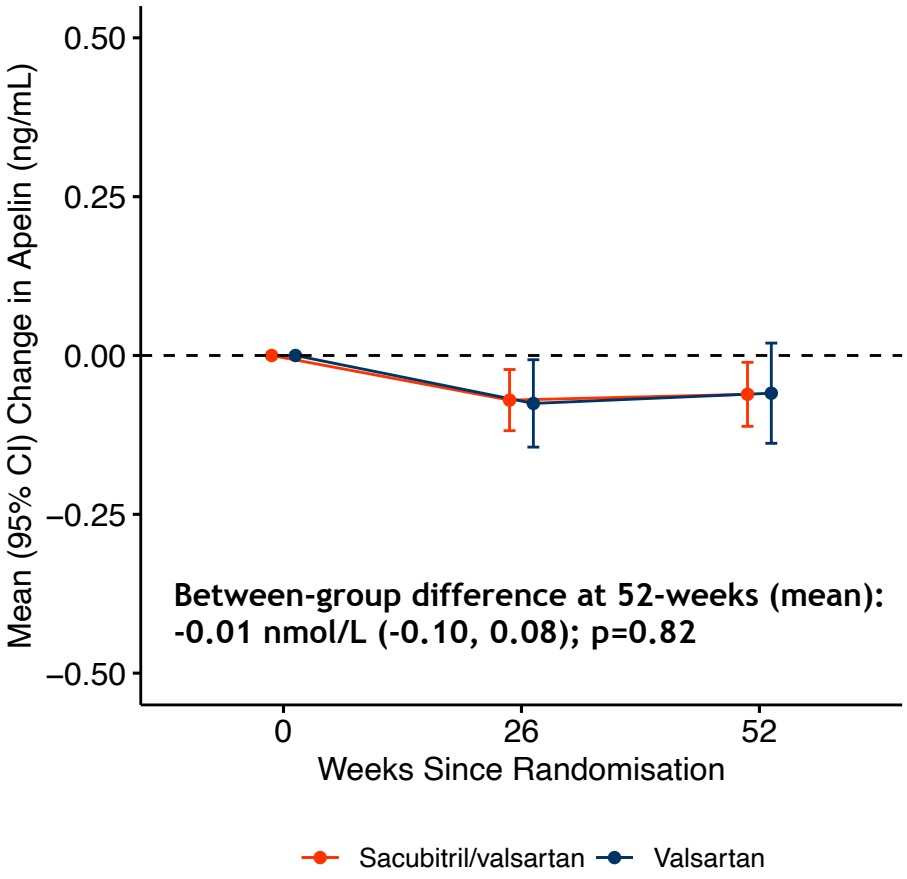


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline GLP-1, and use of diuretics at baseline.

6.3.4 Apelin

Mean (SD) apelin at baseline was 1.40 (0.46) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 1.44 (0.36) ng/mL in those randomised to valsartan (n=46). Mean change in apelin was -0.06 (SD 0.17) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and -0.06 (0.24) ng/mL in the valsartan group: adjusted between-group mean difference -0.01 (95% confidence interval [CI], -0.10, 0.08) ng/mL; p=0.82 (Figure 6-3). Similar results were seen in repeated measures modelling with no significant difference with sacubitril/valsartan compared with valsartan at 26 weeks (p=0.94) and 52 weeks (p=0.81).

Figure 6-3 Change in apelin with sacubitril/valsartan or valsartan from baseline to week 52

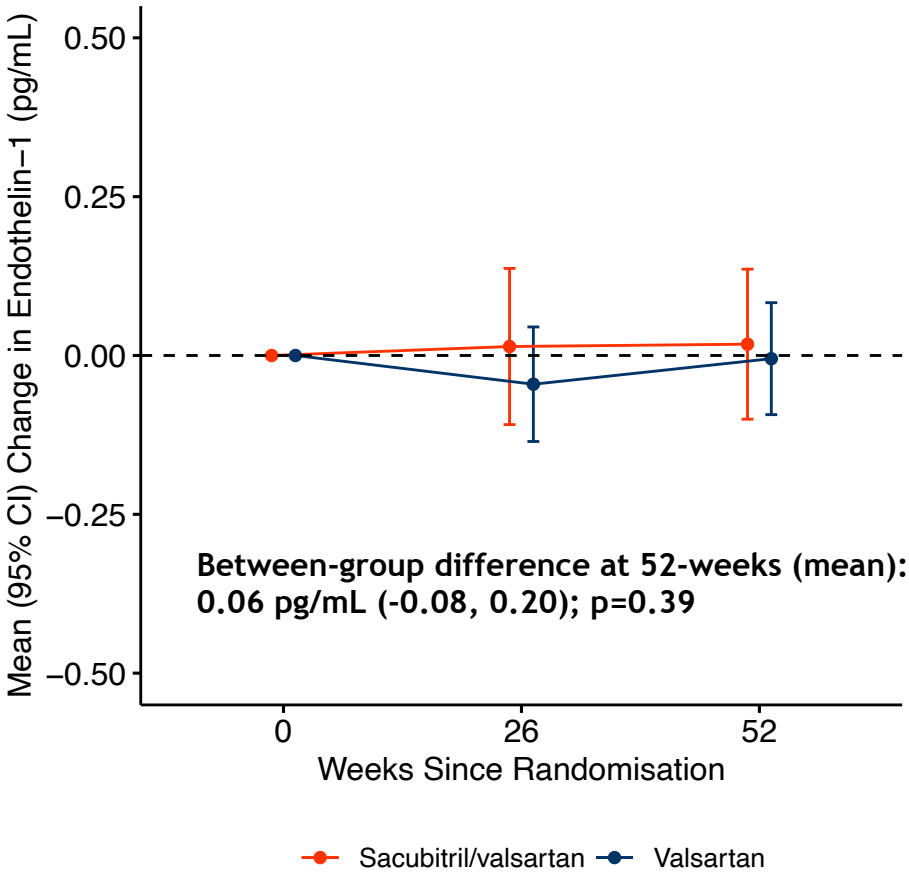


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline apelin, and use of diuretics at baseline.

6.3.5 Endothelin-1

Mean (SD) endothelin-1 at baseline was 1.36 (0.36) pg/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 1.25 (0.32) pg/mL in those randomised to valsartan (n=46). Mean change in endothelin-1 was 0.02 (SD 0.40) pg/mL between baseline and 52 weeks in the sacubitril/valsartan group and -0.01 (0.30) ng/mL in the valsartan group: adjusted between-group mean difference 0.06 (95% confidence interval [CI], -0.08,0.20) pg/mL; p=0.39 (Figure 6-4). Similar results were seen in repeated measures modelling with no significant difference with sacubitril/valsartan compared with valsartan at 26 weeks (p=0.12) and 52 weeks (p=0.31).

Figure 6-4 Change in endothelin-1 with sacubitril/valsartan or valsartan from baseline to week 52



Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline endothelin-1, and use of diuretics at baseline.

6.4 Discussion

Neprilysin is a ubiquitous enzyme with multiple substrates including the natriuretic peptides as described in Chapter 1.4 and Chapter 5.[278] A novel aspect of the study presented in this thesis was the comparison between sacubitril/valsartan and valsartan alone, facilitating a direct examination of the effect of the addition of neprilysin inhibition on parameters of cardiac remodelling as described in Chapter 4, along with its effect on circulating substrates for neprilysin including the natriuretic peptides (Chapter 5) and those described in this Chapter.

Adrenomedullin

Adrenomedullin (ADM) is a circulating vasoactive peptide with a 52-peptide ring structure which is derived from prepro-ADM.[279] PreproADM, a 185 amino acid structure, is processed by carboxypeptidases into proADM which consists of two bioactive peptides, ADM and proadrenomedullin N-terminal 20 peptide (PAMP). ADM which has a 60-fold greater vasodilatory effect than PAMP, is an established substrate for neprilysin.[280] As part of the processing of proADM, a circulating mid-regional fragment is produced, MR-proADM which is what was measured in the present study. Because MR-proADM is not a substrate for neprilysin, the increase in this peptide in the present study, presumably represents an increase in production of preproADM. It has been reported that administration of exogenous ANP results in increased ADM levels, therefore the observed increase in ANP in the present study may explain why I observed an increase in MR-proADM.[281] It should be noted however that, conversely, administration of ADM has been shown to increase ANP. Although the interplay between these two peptides is not clear, both are thought to have beneficial cardiovascular effects.[282] Along with its vasodilatory effect, bioactive ADM has been reported to have natriuretic, diuretic and positive inotropic properties as well as inhibiting RAS activation.[283]

In patients with chronic HFrEF, elevated adrenomedullin levels have been reported, with greater elevations seen with higher NYHA functional limitation class (note levels in NYHA I patients were similar to healthy controls).[284,285] In the present population the median MR-proADM concentration of 0.51 nmol/L was

higher than the assay manufacturer's healthy population median (0.39 nmol/L) but lower than reported in a HFrEF population (0.81 nmol/L). Elevated concentrations of ADM (and its precursor MR-proADM) represent a protective response to increased circulating volume and pressure overload; furthermore, in both an established ovine model of heart failure and in patients with HFrEF administration of exogenous ADM had beneficial renal, haemodynamic and inhibitory neurohumoral effects.[286,287] In preclinical models, administration of a neprilysin inhibitor has been shown to potentiate the vasodilatory effect of adrenomedullin.[288,289] The present data confirm that neprilysin inhibition results in increasing MR-proADM, likely representing an increased production of preproADM, however assay cross-reactivity with bioactive ADM (a substrate for neprilysin) is also possible. I am unable to comment on levels of circulating bioactive ADM but an increase in its precursor molecule and the presence of neprilysin inhibition (inhibiting the breakdown of bioactive ADM), suggest that bio-ADM concentrations should be increased in response to neprilysin inhibition. Indeed, in an observational study of HFrEF patients commenced on sacubitril/valsartan, an increase in bioactive ADM (bioADM) and MR-proADM was seen with an increase in the ratio of bioADM:MR-proADM suggesting reduced neprilysin-related breakdown of bioADM.[290]

Glucagon-like peptide 1 (GLP-1)

GLP-1 is an incretin hormone that is released by intestinal epithelial L-cells in response to food-intake. Its main action through activation of the GLP-1 receptor is to lower blood glucose levels by stimulating pancreatic insulin release in a glucose-dependent manner as well inhibiting glucagon secretion and thereby supressing gluconeogenesis.[291] As well as these glucose-centric actions, GLP-1 has a range of cardio-protective effects, including blood pressure lowering, weight loss and glucose-independent renoprotective effects including natriuresis and inhibition of the RAS. Native GLP-1 (GLP-1[7-36]) has a very short half-life of 2 minutes due to rapid enzymatic degradation by dipeptidyl peptidase-4 (DPP-4). Therefore, pharmacological activation of the GLP-1 receptor can be achieved through inhibition of DPP-IV (increasing endogenous circulating GLP-1[7-36]) or administration of GLP-1 analogues which are resistant to DPP-4 mediated catabolism (GLP-1 receptor agonists). In patients with diabetes, 7 large

randomised, placebo-controlled cardiovascular outcome trials have now been performed with GLP-1 receptor agonists with a meta-analysis reporting a significant reduction in the risk of Major Adverse Cardiovascular Events (MACE), the individual MACE components of cardiovascular death, myocardial infarction, or stroke, as well as a reduction in the risk of heart failure hospitalisation and a composite kidney outcome.[292] It should be noted however that increased GLP-1 bioactivity through use of the DPP-4 inhibitor alogliptin did not reduce the risk of cardiovascular outcomes in patients with type 2 diabetes following myocardial infarction.[293] In patients with HFrEF, GLP-1 receptor agonists have not been shown to have any beneficial effect in terms of reverse remodelling and furthermore, have been shown to increase heart rate, an unwanted adverse effect thought to be secondary to stimulation of sino-atrial node localised GLP-1 receptors.[294,295]

As well as DPP-4 mediated break-down, enzymatic degradation of native GLP-1 occurs by neprilysin at a different site than that of DPP-4.[296] Following degradation by DPP-4, active GLP-1 (GLP-1[7-36]) is metabolised to GLP-1(9-36), the major circulating form of GLP-1. It has been shown that neprilysin can breakdown both GLP-1(7-36) and its metabolite GLP-1(9-36).[297,298] Therefore, neprilysin inhibition could result in increased circulating GLP-1(7-36), which mediates its actions via the native GLP-1 receptor, and also GLP-1(9-36) for which there is increasing evidence of its potential cardioprotective properties independent of activation of the GLP-1 receptor.[299] A detailed review of the possible cardioprotective effects of GLP-1(9-36) (and potentially its smaller peptide fragments) is beyond the scope of the present thesis however in preclinical heart failure models they include improvement in left ventricular function, increased myocardial glucose uptake, improvements in coronary blood flow and a reduction in infarct size in a myocardial infarction model.[299] Indeed, it has been postulated that the failure of DPP-4 inhibitors to show cardiovascular benefits in patients with type 2 diabetes may be, in part, due to their prevention of the breakdown of GLP-1(7-36), and therefore reduced production of the by-product GLP-1(9-36).

Neprilysin inhibition with candoxatril has been demonstrated in a pre-clinical model to result in increased C-terminal GLP-1 (i.e. both GLP-1[7-36] and GLP-1

[9-36]).[296] In patients with type 2 diabetes in the PARADIGM-HF trial, sacubitril/valsartan, as compared with enalapril, was seen to significantly reduce glycated haemoglobin (HbA1c) concentrations as well as reduce the proportion of patients who commenced insulin therapy during trial follow-up suggesting improved glycaemic control.[300] It was suggested that this observation may be due a GLP-1 mediated glucose-lowering effect secondary to neprilysin inhibition. However, in the absence of a DPP-4 inhibitor, the addition of neprilysin inhibition may not increase GLP-1(7-36) significantly due to its rapid degradation by DPP-4. Indeed, in healthy volunteers, sacubitril/valsartan was demonstrated to increase post-prandial concentrations of total GLP-1 compared with placebo, and also to provide an additive effect in terms of increased GLP-1(7-36) when added to the DPP-4 inhibitor sitagliptin, as compared with sitagliptin alone.[301] It may therefore, be that the predominant effect of a neprilysin inhibitor in the absence of a DPP-4 inhibitor is to increase GLP-1(9-36).

I observed a significant increase in GLP-1 at 52 weeks from baseline in patients randomised to sacubitril/valsartan compared with valsartan. The magnitude of increase was large with an almost 3-fold increase from baseline. The GLP-1 assay used in the present study measured total GLP-1 with 93% and 100% specificity for GLP-1 (7-36) and GLP-1(9-36), respectively. Blood samples were taken using specific tubes including a DPP-4 inhibitor which means that I am not able to distinguish which form(s) of GLP-1 was increased by the addition of a neprilysin inhibitor. Samples were not routinely measured fasting due to the variable timing of study visits during the day however, the magnitude of change in GLP-1 with sacubitril/valsartan and degree of statistical significance mean it was unlikely to be a chance finding.

Apelin

Apelin is a circulating vasoactive peptide which is a ligand for the APJ G-protein-coupled receptor which is expressed widely in the body including in the heart, peripheral vasculature and kidneys.[302] Activation of the APJ receptor by apelin results in a reduction in blood pressure secondary to nitric-oxide mediated vasodilatation, increased aquaresis and increased cardiac contractility. In apelin-knockout mice, progressive left ventricular dilatation and systolic dysfunction occur which is fully reversed by the infusion of apelin, suggesting an important

role for apelin in maintaining cardiac function.[303] Apelin expression has been shown to increase in pre-clinical models of myocardial infarction however in a study of patients with left ventricular systolic dysfunction immediately following myocardial infarction, plasma concentrations of apelin were lower than normal controls.[304] In patients with heart failure, apelin concentrations are lower than normal controls and this is thought to be secondary to angiotensin-II antagonism of apelin and APJ expression.[305] Indeed, the angiotensin receptor blocker olmesartan has been shown to increase apelin levels in a rat model of heart failure.[306] Administration of apelin in patients with heart failure has been shown to reduce afterload and increase cardiac output and this has led to an interest in apelin and the APJ receptor as a potential therapeutic target in heart failure.[307]

It was previously demonstrated that angiotensin converting enzyme 2 (ACE-2) is involved in the degradation of circulating apelin. More recently, neprilysin has been suggested to play a role in its breakdown.[308] This study, to my knowledge, is the first to describe the effects of neprilysin inhibition on apelin concentrations in humans. I did not observe any significant change from baseline in apelin levels with sacubitril/valsartan as compared with valsartan alone, i.e., no effect of neprilysin inhibition. The assay used has 100% cross-reactivity with the 3 main circulating forms of apelin (apelin-12, apelin-13 and apelin-36). The mean apelin level at baseline of 1.43 ng/mL in the present study was higher than that seen immediately post myocardial infarction in patients with left ventricular systolic dysfunction but lower than that of controls in the same study.[304] The present results suggest that neprilysin inhibition, in the patient cohort studied, did not have any significant effect on increasing apelin levels. I cannot exclude that in patients with symptomatic HFrEF, who have lower apelin concentrations than those seen in the present study, that neprilysin inhibition may increase apelin levels. It is however worth highlighting that previous work has not demonstrated any correlation between apelin and NT-proBNP concentrations and the same was seen in the present cohort ($r=-0.11$; $p=0.31$).[309] Furthermore, the sample size of the present study may have been insufficient to detect a small between-group difference.

Endothelin-1

Endothelin-1 is a powerful circulating vasoconstrictor peptide, which also causes sodium retention and promotes adverse cardiac remodelling.[310] Elevated levels of endothelin are seen in heart failure and associated with worse outcomes.[311] The reason for examining the effect of sacubitril/valsartan on endothelin-1 concentrations in the present study was the previous observation that sole neprilysin inhibition with candoxatril resulted in elevated levels of endothelin, confirming that it is a substrate for neprilysin.[118] Elevated endothelin concentrations could lead to an increase in afterload which could precipitate a worsening of systolic function in the population studied.

Endothelin concentrations in the present study were similar to those reported in normal controls and lower than those in patients with HFrEF.[311] Reassuringly, endothelin-1 concentrations did not change significantly in either treatment group over 52 weeks of treatment with no significant between-group difference.

6.4.1 Limitations

In addition to the specific limitations of the individual biomarker measurements discussed, the major limitation of the present study was the sample size which reduced the power to be able to detect small between-group differences. A larger study cohort may have detected more significant between-treatment differences. A further limitation is the inability of the present work to make any conclusions regarding the change in biomarker levels and clinical outcomes. Furthermore, the results presented relate to patients without symptomatic HFrEF; different results may have been observed had patients with symptomatic HFrEF had been studied. Finally, the number of statistical tests performed raises the possibility of chance findings.

6.4.2 Conclusion

In this study in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction, the combined angiotensin receptor-neprilysin inhibitor sacubitril/valsartan, compared with the AT1R inhibitor valsartan alone, increased levels of MR-proADM and GLP-1, with no between-treatment group difference in apelin or endothelin-1 concentrations.

Chapter 7 The effect of neprilysin inhibition on cardiac troponin levels in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

7.1 Introduction

The measurement of elevated concentrations of circulating cardiac troponin is an established tool in the diagnosis of acute myocardial infarction.[312] With the advent of high sensitivity cardiac troponin assays, lower concentrations of circulating troponin indicating the presence of sub-clinical chronic myocardial injury are now able to be detected.[313] A growing body of evidence has demonstrated the value of high-sensitivity troponin measurements in predicting future cardiovascular outcomes, even when the value is within the normal reference range (i.e., below the 99th percentile upper reference limit).[314]

At the time of acute myocardial infarction, a higher peak cardiac troponin concentration is associated with larger infarct size and the degree of left ventricular systolic dysfunction, as well as the risk of heart failure during the index admission and following discharge.[315-318] In both general populations and in patients with stable coronary artery disease, a proportion of who had a prior myocardial infarction, elevated levels of troponin are associated with a higher risk of incident heart failure.[314,319-324] Furthermore, in a population of patients free of coronary artery disease who had cardiac magnetic resonance imaging performed, higher baseline troponin was significantly associated with a greater degree of myocardial fibrosis and left ventricular hypertrophy, and the proportion of patients with an LVEF <50%, but not with LVEDV.[325]

The potential value of measuring high-sensitivity troponin in survivors of myocardial infarction remote from the time of infarction, to identify those with adverse left ventricular remodelling at higher risk of future adverse outcomes, has not, to my knowledge, been examined. The present study, therefore, offers a novel opportunity to describe the associations between high-sensitivity cardiac troponin concentrations and the degree of left ventricular remodelling in a cohort of asymptomatic patients with persisting left ventricular systolic dysfunction late after myocardial infarction.

Finally, in patients with symptomatic HFrEF, sacubitril/valsartan, compared with enalapril, has been demonstrated to significantly reduce levels of cardiac troponin indicating a reduction in the degree of myocardial injury.[140,222,326] Furthermore, in patients with heart failure with preserved ejection fraction, sacubitril/valsartan also reduced high-sensitivity troponin T and this reduction was associated with the degree of reduction in NT-proBNP.[327] The main aim of this study was to examine the effect of the addition of neprilysin inhibition to standard therapy with a RAS blocker and beta-blocker on circulating high sensitivity cardiac troponin concentrations in patients with symptomless left ventricular systolic dysfunction late after myocardial infarction.

7.2 Methods

7.2.1 Study population

The patient population described in the preceding chapters were also included in this study. This cohort comprised of 93 patients with asymptomatic left ventricular systolic dysfunction (left ventricular ejection fraction ≤40% measured using echocardiography) who were at least 3 months following an acute myocardial infarction. All patients were taking an ACE inhibitor or ARB and a beta-blocker (unless contraindicated or not tolerated) prior to randomisation. Eligible and consenting patients were randomised 1:1 to sacubitril/valsartan (target dose 97/103mg twice daily) or valsartan (target dose 160mg twice daily) and matching placebo for 52 weeks. The study protocol is detailed in Chapter 3 and baseline characteristics in Table 4-3.

All patients underwent cardiac MRI for assessment of left ventricular and atrial volumes, left ventricular ejection fraction and left ventricular mass pre-randomisation and at 52 weeks. Volumes and mass were indexed to body surface area as detailed in Chapter 3.6.

7.2.2 Measurement of troponin

Venepuncture was performed pre-randomisation, at 26 weeks, and at 52 weeks as described in Chapter 3. High-sensitivity cardiac troponin-I (hs-TnI) was measured using the Architect i1000SR (Abbott Laboratories, Abbott Diagnostics, Abbot Park, IL) clinical immunoassay platform using the manufacturers' calibrators and quality control materials. The lower limit of detection for this assay is 1.2 ng/L. The manufacturer reported 99th centile for the assay is 26.2 ng/L, with sex-specific 99th centiles for women of 15.6 ng/L and 34.2 ng/L in men.

All biomarker sample processing and measurements were performed by Philip Stewart, Elaine Butler, Josephine Cooney and Emma Dunning at the Glasgow Biomarker Laboratory, Institute of Cardiovascular and Medical Sciences, University of Glasgow under the supervision of Dr Paul Welsh.

7.2.3 Statistical methods

The distribution of baseline hs-TnI was examined by means of histograms and summary statistics. As baseline hs-TnI and the change in hs-TnI were non-normally distributed, all values were log-transformed prior to analysis. Baseline levels are presented as medians with interquartile ranges in the overall population and by randomised treatment allocation with between-group comparisons made using a Wilcoxon rank-sum test. Correlations between baseline values of hs-TnI and LVESVI, LVEDVI, LVEF, left atrial volume index LAVI and LVMI were calculated by means of a Pearson's correlation coefficient (r) with a linear regression line plotted graphically. The same methods were used to examine the correlation between the 52-week change in hs-TnI values and change in cardiac MRI parameters.

The treatment effect of sacubitril/valsartan as compared with valsartan on hs-TnI concentrations over time was examined by means of a linear regression model adjusted for randomised treatment, baseline hs-TnI value and use of diuretics at baseline. In order to satisfy modelling assumptions, log transformations were performed, and regression coefficients were back-transformed and are presented as relative differences. In confirmatory analyses, repeated measures analyses were performed adjusting for the main effects of time-point, randomised group and the interaction between time-point and randomised group and for diuretic use at baseline. All analyses were performed on an intention to treat basis as described in Chapter 3.11.

Baseline clinical predictors of baseline LVESVI were examined using a backward-stepwise linear regression model with a p-value set at ≥0.10 for removal of a variable from the model. Variables included were systolic blood pressure, eGFR, age, male sex, time since myocardial infarction, medical history of hypertension or diabetes, revascularisation for myocardial infarction, anterior myocardial infarction, ST-elevation myocardial infarction, current smoking and if the patient was taking a beta-blocker or an MRA. To examine the potential additive predictive ability of measurement of the natriuretic peptides MR-proANP and NT-proBNP along with hs-TnI, these biomarkers were added alone and in combination into models containing the clinical variables identified using the above procedure. Model goodness of fit was assessed by means of the Akaike

information criterion (AIC) with lower values indicating a better fit model and a difference of >2 between two models indicating a significant difference in model fit.

A p-value of <0.05 was considered statistically significant for all analyses. No correction for multiple testing was performed. No imputation for missing data was performed. All analyses were performed by Bethany Stanley (Robertson Centre for Biostatistics) and me using R Studio and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria), and STATA version 16.1 (College Station, TX, USA).

7.3 Results

7.3.1 Baseline troponin values and correlation with degree of cardiac remodelling and natriuretic peptide levels

Plasma concentrations of plasma hs-TnI were detectable above the limit of detection for 90 of 93 (97%) patients at baseline. Median and mean concentrations of hs-TnI were 5.1 (interquartile range 2.8, 7.8) ng/L and 12.5 (standard deviation 41.8), respectively (range 0.6-398.7 ng/L). There was no significant difference between the two treatment groups at baseline (p=0.25). Using sex-specific thresholds (male=34.2 ng/L and female 15.6 ng/L), 8 (8.6%) patients had levels above the 99th centile value for hs-TnI at baseline.

The relationships between baseline hs-TnI levels and baseline cardiac MRI measurements of left ventricular and atrial volumes, left ventricular ejection fraction and left ventricular mass are shown in Figure 7.1. In order of magnitude of correlation, baseline hs-TnI concentration was weakly correlated with LVEDVI (r=0.28, p=0.006), LVESVI (r=0.28, p=0.008), LAVI (r=0.23, p=0.024) and LVMI (r=0.22, 0.034). Baseline hs-TnI was not significantly correlated with LVEF (r=-0.17, p=0.099).

Figure 7.2 displays the association between baseline hs-TnI and concentrations of the natriuretic peptides MR-proANP and NT-proBNP. hs-TnI was significantly correlated with both MR-proANP (r=0.31, [p=0.003]) and NT-proBNP (r=0.26, [p=0.011]).

Figure 7-1 Correlations between baseline troponin I levels and cardiac volumes, function and mass at baseline

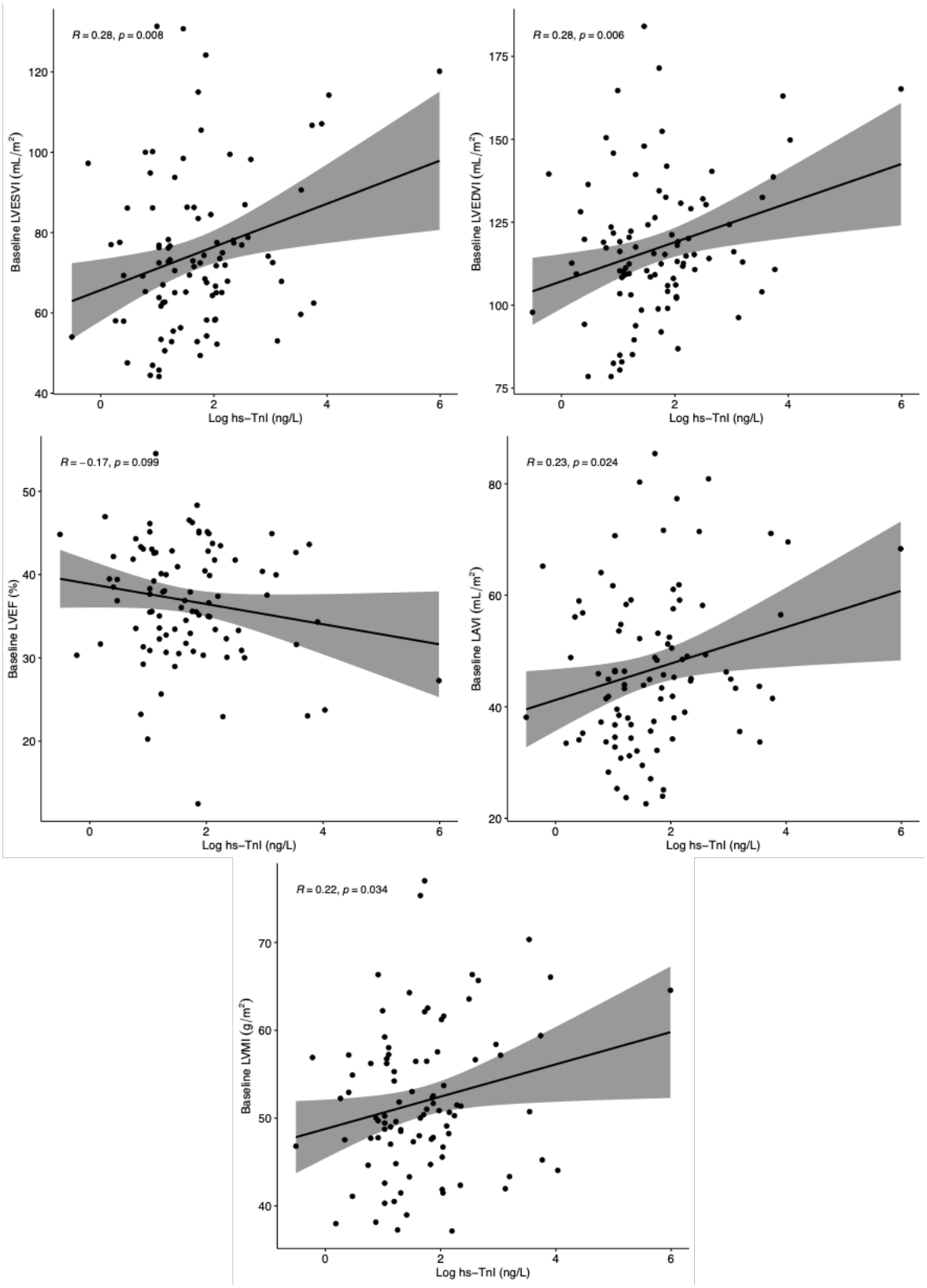
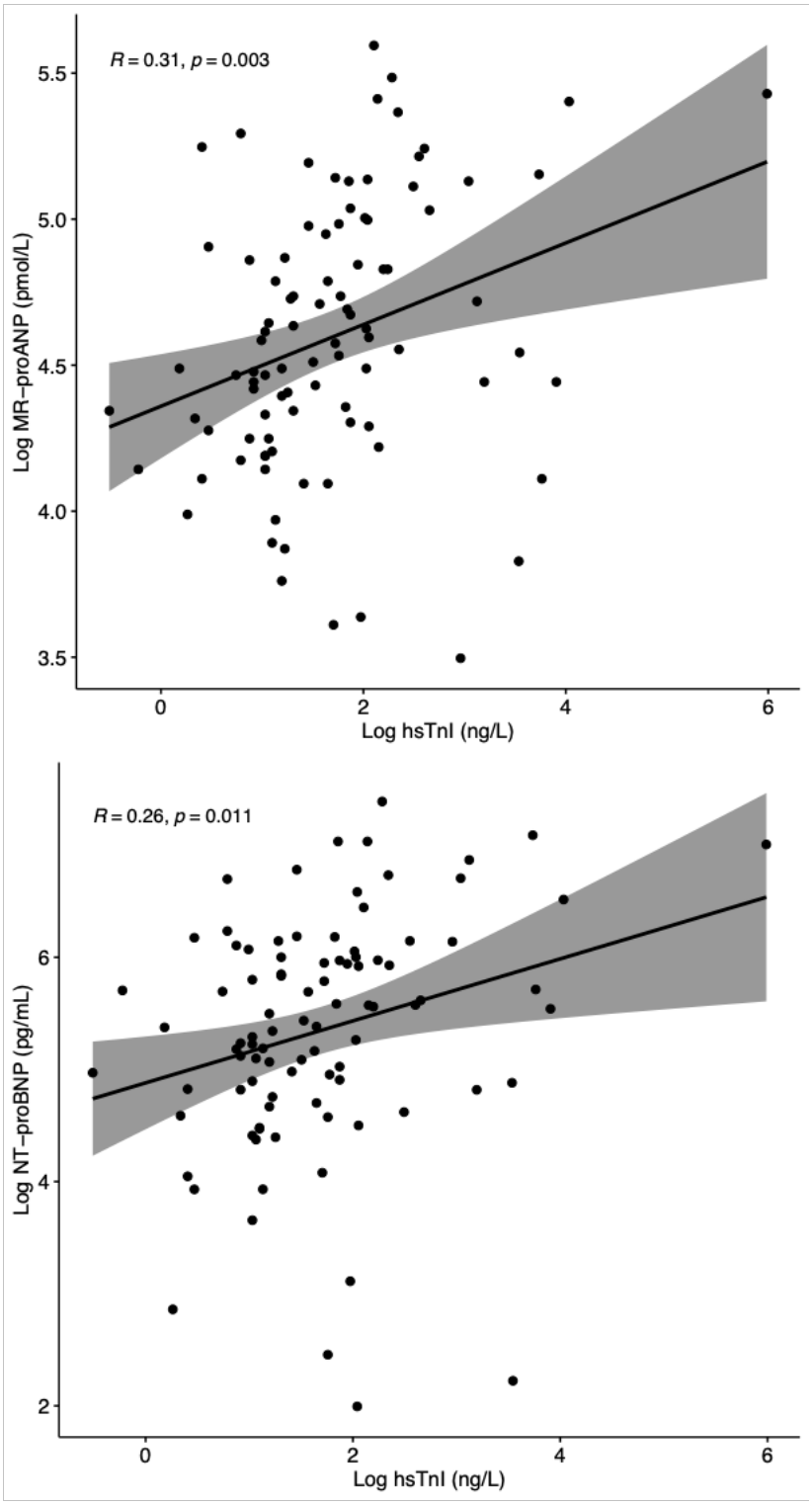


Figure 7-2 Correlation between baseline troponin I and MR-proANP and NT-proBNP concentrations



7.3.2 Baseline troponin and natriuretic peptide levels as predictors of the degree of adverse remodelling

In a backwards-stepwise linear regression model of clinical baseline variables (methods described in Chapter 7.2), eGFR, a history of diabetes, current smoking, and diuretic and mineralocorticoid receptor antagonist use were identified as independent predictors of baseline LVESVI. I tested for the potential of collinearity between diabetes status and MRA use due to the indication for MRA use in patients with diabetes and a low LVEF following acute myocardial infarction (EPHESUS trial), however no significant collinearity was present.

The individual variable beta-coefficients and p-values from regression models including these variables with the addition of baseline MR-proANP and NT-proBNP alone, or in combination along with hs-TnI are displayed in Table 7.1. When added to the model with baseline clinical variables only, both MR-proANP and NT-proBNP alone and in combination were independent predictors of baseline LVESVI. In a model with MR-proANP and hs-TnI, both biomarkers were independent predictors of baseline LVESVI. Similarly, NT-proBNP and hs-TnI were also independent predictors of baseline LVESVI when added to the clinical model. The two models which best predicted baseline LVESVI were a model of clinical variables and both MR-proANP and NT-proBNP (r^2=0.42; AIC 780.8) and the same model with the addition of hs-TnI (r^2=0.44; AIC 779.8). However, in the latter model troponin was not an independent predictor of LVESVI and the delta AIC was <2 between the two models suggesting that the addition of troponin to the model which included both natriuretic peptides did not add significant predictive value. The AIC of a model with the three biomarkers as the only independent variables was 799.0.

Table 7-1 Multivariable predictors of left ventricular end-systolic volume

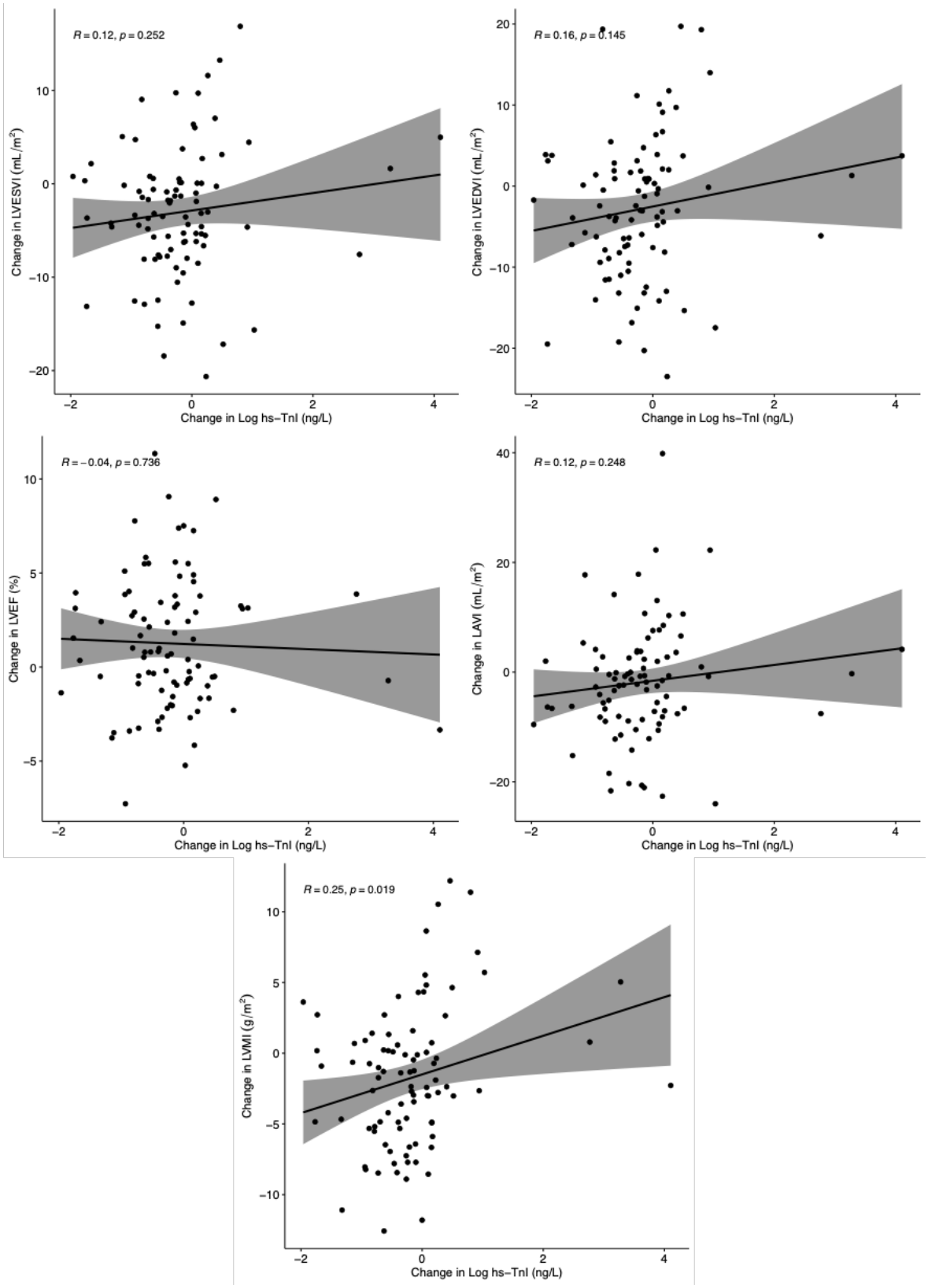
Variable	Basic model	Basic model + MR-proANP	Basic model + MR-proANP + hs-TnI	Basic model + NT-proBNP	Basic model + NT-proBNP + hs-TnI	Basic model + MR-proANP + NT-proBNP	Basic model + MR-proANP + NT-proBNP + hs-TnI
eGFR	ß=-0.3; p=0.045	ß=0.05; p=0.71	ß=0.04; p=0.730	ß=-0.1; p=0.37	ß=-0.08; p=0.49	ß=0.07; p=0.57	ß=0.07; p=0.60
Diabetes	ß=-11.0; p=0.036	ß=-9.3; p=0.047	ß=-10.0; p= 0.031	ß=-11.3; p=0.019	ß=-11.8; p=0.012	ß=-9.9; p=0.03	ß=-10.4; p=0.022
Current	ß=11.6; p=0.038	ß=14.2; p=0.006	ß=12.3; p=0.016	ß=14.4; p=0.006	ß=12.1; p=0.019	ß=15.4; p=0.002	ß=13.7; p=0.007
MRA	ß=9.2; p= 0.17	ß=10.6; p=0.003	ß=10.4; p=0.003	ß=6.6; p=0.067	ß=7.0; p=0.046	ß=8.6; p=0.014	ß=8.6; p=0.013
Diuretic	ß=15.0; p= 0.012	ß=12.5; p=0.019	ß=14.7; p=0.006	ß=14.5; p=0.008	ß=16.7; p=0.002	ß=12.8; p=0.013	ß=14.6; p=0.006
Log MR-proANP	-	ß=20.4; p<0.001	ß=17.3; p<0.001	-	-	ß=15.5; p=0.001	ß=13.5; p=0.006
Log NT-proBNP	-	-	-	ß=7.1; p=<0.001	ß=5.6; p=0.001	ß=4.6; p=0.013	ß=4.2; p=0.025
Log hs-TnI	-	-	ß=3.6; p=0.048	-	ß=4.3; p=0.019	-	ß=3.0; p=0.10
AIC	804.6	785.6	783.3	790.3	786.3	780.8	779.8

7.3.3 Correlation between change in troponin and change in cardiac MRI measures of left ventricular remodelling

Data on the change in hs-TnI concentrations and cardiac MRI measurements of left ventricular and atrial volumes and left ventricular mass between baseline and 52 weeks were available for 90 patients. The correlations between them are displayed in Figure 7.3.

Change in hs-TnI over 52 weeks was not significantly correlated with change in LVESVI (r=0.12; p=0.252), LVEDVI (r=0.16; p=0.145), LVEF (r=-0.04; p=0.736) or LAVI (r=0.12; 0.218). Change in hs-TnI and LVMI over 52 weeks were significantly correlated (r=0.25; p=0.019).

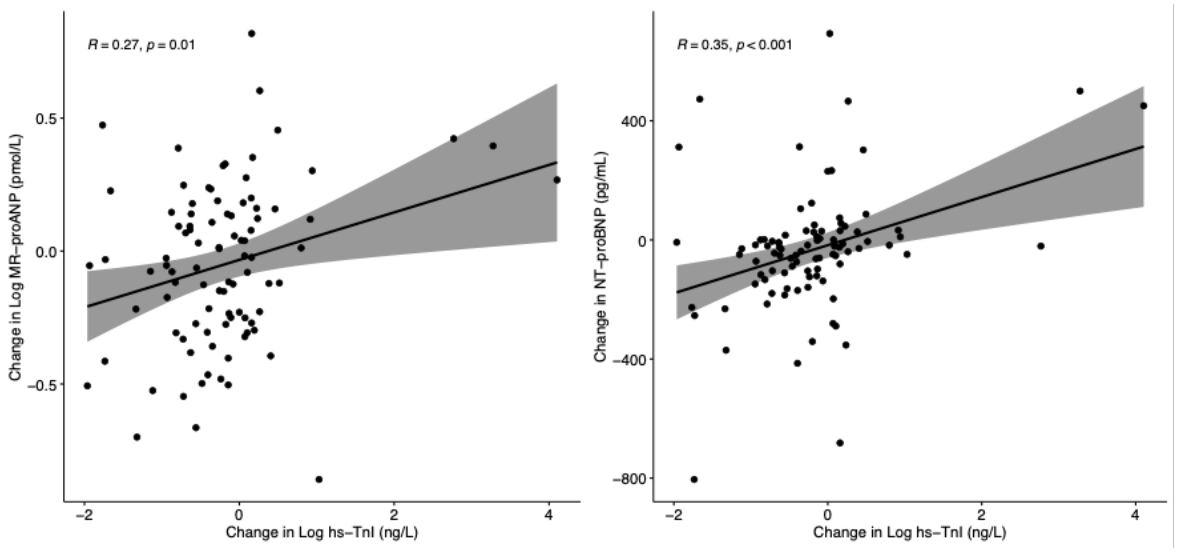
Figure 7-3 Correlations between change in troponin I levels and cardiac volumes, function and mass



7.3.4 Correlation between change in troponin and change in natriuretic peptide levels

The change in hs-TnI between baseline and 52 weeks was significantly correlated with change in MR-proANP (r=0.27;p=0.01) and NT-proBNP (r=0.35;p<0.001) as displayed in Figure 7.4.

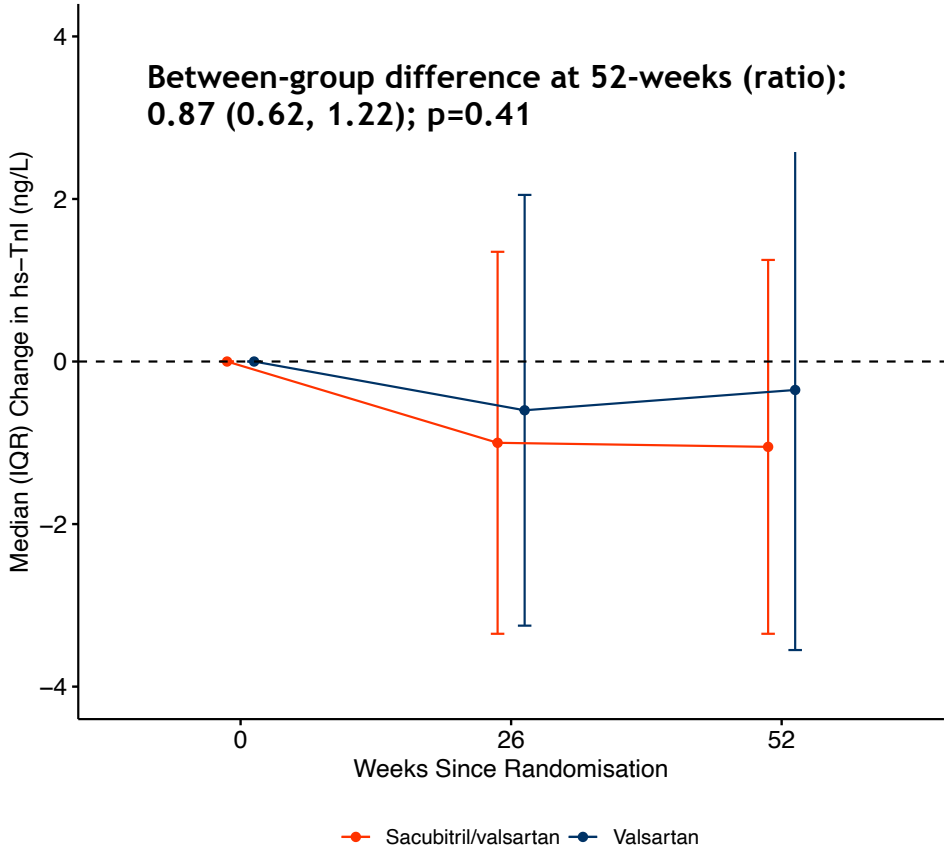
Figure 7-4 Correlation between change in troponin and change in natriuretic peptide levels



7.3.5 Effect of neprilysin inhibition on troponin levels

Median (IQR) hs-TnI at baseline was 3.9 (2.8, 7.4) ng/L in those randomised to sacubitril/valsartan with follow-up data (n=46) and 5.7 (3.0, 8.9) ng/L in those randomised to valsartan (n=46). Median change in hs-TnI was -1.1 (IQR -2.4, -0.1) ng/L between baseline and 52 weeks in the sacubitril/valsartan group and -0.4(-2.4, 0.9) ng/L in the valsartan group: ratio of adjusted geometric means: 0.87 (95% confidence interval [CI], 0.62, 1.22); p=0.41 (Table 7.2 and Figure 7.5). Similar results were seen in repeated measures modelling with a decrease in hs-TnI which was non-significant at 26 weeks (ratio 0.86 [0.65, 1.13]; p=0.28) and at 52 weeks (0.87 [0.66, 1.15]; p=0.34).

Figure 7-5 Change in hs-TnI with sacubitril/valsartan or valsartan from baseline to week 52



Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline hs-TnI and use of diuretics at baseline.

Table 7-2 Change in hs-TnI with sacubitril/valsartan or valsartan from baseline to week 52

	Sacubitril/valsartan				Valsartan				Between-group difference (95% CI) *	P Value
	n	Baseline	Week 52	Change	n	Baseline	Week 52	Change		
hs-TnI, ng/L	46	3.9 (2.8,7.4)[#]	3.1 (2.0, 4.5)[#]	-1.1 (-2.4, -0.1)[#]	46	5.7 (3.0, 8.9)[#]	4.4 (2.7, 7.3)[#]	-0.4 (-2.4, 0.9)[#]	0.87 (0.62, 1.22)	0.41

Results reported for those with data available at baseline and 52-weeks.

Data reported as median (interquartile range)

*Calculated using a linear model adjusted for randomized treatment, baseline hs-TnI, use of diuretics at baseline.

Between-group difference is reported as a ratio of adjusted geometric means (95%CI

7.3.6 Remodelling effect of neprilysin inhibition according to baseline troponin concentration

In the overall trial population median baseline hs-TnI concentration was 5.1 ng/L. Using similar methods to those described in Chapter 4, I examined the effect of the addition of neprilysin inhibition (sacubitril/valsartan versus valsartan alone) in a post hoc subgroup analysis according to baseline hs-TnI concentrations greater than or equal to, or below the median level (5.1 ng/L). These results are summarised in Table 7.3; a greater reverse remodelling effect was seen with sacubitril/valsartan in those with higher troponin levels at baseline with significant interaction p-values for LVESVI (p=0.002), LVEDVI (p=0.036), and LVEF (p=0.03).

Table 7-3 Effect of neprilysin inhibition on left ventricular remodelling according to baseline troponin

	hs-TnI < median	**hs-TnI ≥ median**	**Interaction p value**
LVESVI (ml/m^2)	2.5 (-1.43, 6.5)	-6.4 (-10.4, -2.4)	0.002
LVEDVI (ml/m^2)	0.92 (-4.23, 6.06)	-7.0 (-12.2, -1.7)	0.036
LAVI (ml/m^2)	-0.51 (-6.55, 5.52)	-3.9 (-10.1, 2.32)	0.44
LVEF (%)	-2.1 (-4.1, -0.1)	1.0 (-1.0, 3.1)	0.03
LVMI (g/m^2)	-2.1 (-4.1, -0.1)	1.0 (-1.0, 3.1)	0.20

Median hs-TnI was 5.1 ng/L

Treatment effect calculated using a linear regression model with interaction between treatment group and baseline hs-TnI, adjusted for randomised treatment, baseline value of the outcome, use of diuretics at baseline and time from randomisation to cardiac MRI.

7.4 Discussion

This study which included 93 patients at baseline with asymptomatic left ventricular systolic dysfunction late after myocardial infarction (median time from infarct 3.6 years), the majority of whom had hs-TnI levels within the normal reference range, has several novel findings. Firstly, I observed that in this patient population, higher baseline troponin correlations were correlated with a greater degree of ventricular and atrial dilation and increased left ventricular mass. Furthermore, there were significant (but weak) correlations between baseline markers of increased cardiac wall stress (MR-proANP and NT-proBNP) and troponin concentrations. The inclusion of hs-TnI with either MR-proANP or NT-proBNP, along with clinical variables identified to be predictors of baseline left ventricular dilatation, demonstrated that hs-TnI was an independent predictor of the degree of adverse left ventricular modelling at baseline. Change in troponin from baseline to 52 weeks was significantly correlated with change in MR-proANP, NT-proBNP and left ventricular mass but not with the change in ventricular or atrial volumes. Finally, the addition of a neprilysin inhibitor to standard therapy with a RAS inhibitor and beta-blocker did not significantly reduce hs-TnI over a 52-week treatment period. There was, however, the suggestion of a reverse remodelling effect in those with higher troponin levels at baseline with significant treatment-effect interactions for LVESVI, LVEDVI and LVEF.

Most patients (97%) had detectable hs-TnI levels at baseline, similar to the proportions reported in chronic HFrEF populations.[328] However, the proportion of patients with values greater than the 99th centile upper reference limit (8.6%) in the present study was significantly lower than that reported recently in analyses of HFrEF patients from the EMPEROR-Reduced and DAPA-HF trials, where 68% and 74% of patients, respectively, had a level above the 99th centile of high sensitivity troponin T (hs-TnT).[329,330] This finding, in the context of the lower baseline NT-proBNP concentrations in the present study as compared with those in HFrEF populations, likely reflects a lower degree of chronic myocardial injury due to a lesser degree of left ventricular wall stress.

The relationship between the magnitude of peak cardiac troponin levels at the time of infarction and infarct size, as well as the degree of left ventricular

systolic dysfunction is well established. Higher peak troponin is associated with larger infarcts and a greater degree of systolic dysfunction immediately following acute myocardial infarction and within short-term follow-up, and both of these remodelling metrics are predictors of the risk of development of heart failure.[315,316,318] Troponin measured at this time has also been shown to be predictive of persisting left ventricular systolic dysfunction and the future risk of heart failure hospitalisation. In 168 patients with a first ST-elevation myocardial infarction, peak troponin within 24 hours of infarction was significantly associated with LVEF at 90 days and was an independent predictor of heart failure hospitalisation (12% higher risk per ug/L increase in cardiac troponin-T) within one year.[318]

In the present study, higher troponin concentrations at baseline were correlated with greater ventricular and atrial dilatation and increased left ventricular mass. It should be noted, however, that these correlations, although statistically significant, were weaker than those seen with the natriuretic peptides reported in Chapter 5. There were also positively moderate correlations with hs-TnI and MR-proANP and NT-proBNP; I am not able to differentiate between cause and effect in the relationship of these peptides, but it is most likely bi-directional with increased wall stress leading to chronic myocardial injury and vice-versa. This view is supported by the correlations between change in troponin from baseline to 52 weeks and change in MR-proANP and NT-proBNP. The relationship between troponin concentrations and abnormalities of cardiac structure and function have been reported previously, but predominantly in general population-based studies free of established coronary artery disease or heart failure. In the Dallas Heart study, high-sensitivity cardiac troponin T (hs-TnT) was detectable in 25% of the population of 3546 individuals aged between 30-65.[331] The prevalence of both left ventricular hypertrophy and left ventricular systolic dysfunction was greater with increasing levels of hs-TnT and, after adjustment for traditional risk factors including NT-proBNP, hs-TnT was an independent predictor of all-cause mortality. In a longitudinal study of 4986 participants in the Multi-Ethnic Study of Atherosclerosis (MESA), the highest levels of hs-TnT were associated with a greater prevalence of LVEF <50% and a greater odds of significant left ventricular hypertrophy, however, LVEDV did not differ significantly across troponin concentration categories.[325] Furthermore,

higher baseline hs-TnT concentrations were associated with signs of progressive adverse left ventricular remodelling after 10 years of follow-up as indicated by a >8% increase in LVEDV and a >12% increase in left ventricular mass, including after adjustment for baseline values and established cardiovascular risk factors. In addition, NT-proBNP also increased more during follow-up in patients with higher baseline troponin concentrations, indicating greater ventricular wall stress. In the same cohort, compared with those with undetectable hs-TnT levels, those in the highest hs-TnT quintile at baseline were at a 3-fold higher risk of heart failure hospitalisation over a median of follow-up 12.2 years after adjustment for risk factors including NT-proBNP at baseline.[325] This relationship between higher levels of troponin (including those within the normal reference range) and an elevated risk of cardiovascular outcomes, including heart failure hospitalisation, in general populations and in those with coronary artery disease is well documented in a range of cohort studies.[314,323,332] Similar findings were reported in a sub-study of the Valsartan Heart Failure Trial (Val-HeFT), where a detectable hs-TnT level in patients with HFrEF was an independent predicator of a reduced odds of recovery in LVEF as measured by echocardiography.[333] Conversely, baseline BNP was not an independent predictor of LVEF recovery.

The data above and those presented in this study support the value of high-sensitivity troponin measurement in identifying patients who may have subclinical cardiac dysfunction or adverse remodelling, and the association of elevated troponin concentrations and higher risk of future adverse outcomes, including progressive adverse remodelling and the development of heart failure. Moreover, in the multivariate modelling performed in the population included in the present study, troponin remained an independent predictor of the degree of ventricular dilatation when added to models including the measurement of either NT-proBNP or MR-proANP. Given the relationship between progressive remodelling risk of adverse outcomes, this relationship is perhaps to be expected given that in patients with symptomatic HFrEF (around half of who have an ischaemic aetiology), troponin has been reported to have additive independent predictive value to that of NT-proBNP in predicting the risk of mortality and worsening heart failure events.[329,334]

What about temporal trends in troponin concentrations and the association with remodelling and outcomes? In the present study, change from baseline to 52 weeks in troponin was only weakly correlated with the change in left ventricular mass index but not with change in cardiac volumes or left ventricular ejection fraction. In a study of the large Atherosclerosis Risk in Communities (ARIC) prospective observational cohort in approximately 9000 participants free of coronary heart disease and heart failure at baseline, inclusion of the change in hs-TnT over 6 years improved the discriminatory capacity of models including established risk-factors, NT-proBNP and baseline hs-TnT.[335] Compared to individuals without a detectable hs-TnT concentration, those with detectable hs-TnT were at a 2-fold higher risk of the development of heart failure (and in those with adjudicated events this risk was similar for HFrEF and HFpEF). Of the outcomes examined, a rise in troponin was associated with a higher risk of heart failure relative to coronary heart disease or mortality. Furthermore, in a Canadian cohort study of patients with HFrEF, as compared with patients who had recovery of left ventricular systolic function (i.e., had reverse remodelling) and had no change in troponin over follow-up, patients with evidence of persisting systolic dysfunction had a significant rise in troponin concentrations over follow-up, indicating ongoing myocardial injury.[336] These data suggest that a rise (or fall) in troponin is associated with a higher (or lower) risk of future development of heart failure.

The final section of this chapter relates to the effect of the addition of neprilysin inhibition to standard therapy on troponin concentrations in the present study cohort. In this population of asymptomatic patients with evidence of persisting left ventricular systolic dysfunction late after myocardial infarction, in patients randomised to sacubitril/valsartan, as compared with valsartan, hs-TnI at 52 weeks was 13% lower (95% CI -38%, +22%) than baseline however this difference was not statistically significant (p=0.41). In patients with symptomatic HFrEF, sacubitril/valsartan, compared with enalapril had been shown to significantly reduce troponin indicating a reduction in the degree of myocardial injury.[222] Furthermore, in the PROVE-HF observational study, troponin concentrations were reduced significantly at 30 days from baseline after commencing sacubitril/valsartan with a mean 6.7% reduction at 12 months.[337] Of note, earlier reductions in NT-proBNP were predictive of

subsequent reductions in troponin, and changes of both these biomarkers correlated with improvement in LVEF. Direct comparison of the median hs-TnI level in the present cohort with recent HFrEF cohorts was not possible due to measurement of hs-TnT in three recent trials (PARADIGM-HF, DAPA-HF and EMPEROR-Reduced) and the use of a different hs-TnI assay in another (GALACTIC-HF).[329,330,338,339] However, as detailed above, a lower proportion of patients in the present study had levels above the upper reference limit than in the HFrEF populations described, suggestive of a lower degree of chronic myocardial injury in these asymptomatic patients as compared with symptomatic HFrEF. This finding, along with the lack of significant treatment effect in reducing NT-proBNP in the present cohort indicating a reduction in left ventricular stress, which was observed with sacubitril/valsartan in HFrEF patients, suggests that the relatively smaller sample size may have limited the ability of the present study to detect a smaller absolute difference in troponin between the treatment groups in this asymptomatic population who had evidence of a lower degree of chronic myocardial injury at baseline. The finding of a significant interaction between higher baseline troponin concentrations and a greater reverse remodelling treatment effect in terms of reduction in left ventricular volumes adds to the observed interaction with NT-proBNP reported in Chapter 4 and suggests that the additive benefits of neprilysin inhibition in this patient population may be limited to those patients with evidence of ongoing elevated left ventricular wall stress and chronic myocardial injury.

7.4.1 Limitations

As described above. the relatively small sample size may have limited my ability to detect a small between-group difference in troponin. Minimal changes in left ventricular volumes and NT-proBNP may have limited the present study's ability to detect meaningful correlations between change in troponin and change in left ventricular remodelling parameters. A larger dataset may have identified other significant predictors of left ventricular dilation; for example, most patients in the present study had experienced anterior infarctions and the finding that this was not a predictor of the degree of left ventricular dilatation was somewhat surprising but may simply reflect that it was not a discriminatory factor in the present population. I only measured hs-TnI in the present study; other forms of circulating cardiac troponin, e.g., hs-TnT, when compared with hs-TnI, has been

shown to have differential associations with cardiovascular outcomes. Indeed, in patients with HFrEF, the predictive capability of hs-TnT has been reported to be greater than that of hs-TnI.[340] It is therefore possible, that the relationship between hs-TnT and left ventricular remodelling is different to that described in the present study with hs-TnI.

7.4.2 Conclusions

In patients with symptomless left ventricular systolic dysfunction late after a myocardial infarction, higher circulating levels of hs-TnI were significantly correlated with a greater degree of adverse left ventricular remodelling and were an independent predictor of left ventricular dilation in multivariable modelling, including after adjustment for natriuretic peptide levels. The addition of a neprilysin inhibitor to standard therapy did not significantly reduce hs-TnI after 52 weeks of treatment.

Chapter 8 The effect of neprilysin inhibition on biomarkers of myocardial fibrosis in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction

8.1 Introduction

In patients at risk of the development of heart failure, progressive adverse remodelling can occur due to profibrotic processes promoting replacement of healthy myocardium by collagen and reduced collagen degradation. Higher levels of myocardial fibrosis are associated with increased morbidity and mortality as well as a greater degree of adverse left ventricular remodelling in patients with HFrEF and following myocardial infarction.[264,341–346] Furthermore, the mechanism of benefit of some established HFrEF treatments may be related to their ability to reduce myocardial fibrosis (e.g., mineralocorticoid receptor antagonists) and favourable changes in profibrotic signalling has been reported with sacubitril/valsartan in HFrEF as indicated by the measurement of circulating biomarkers which reflect collagen synthesis, processing and degradation.[347–349]

This chapter will provide novel data on the effect of neprilysin inhibition on a range of biomarkers of fibrotic processes, one of the key mechanisms underlying progressive adverse ventricular remodelling and development of symptomatic heart failure in at-risk patients.

8.2 Methods

8.2.1 Study patients and protocol

The patients included in the present study are the same patient cohort who are described in the preceding chapters; 93 patients who had evidence of persisting left ventricular systolic dysfunction (left ventricular ejection fraction ≤40% measured using echocardiography) with no signs or symptoms of heart failure were recruited into a trial examining the effect of the addition of neprilysin inhibition to standard therapy at least 3 months following an acute myocardial infarction. All patients were taking an ACE inhibitor or ARB prior to enrolment and a beta-blocker (unless contraindicated or not tolerated). Eligible patients were randomised 1:1 to sacubitril/valsartan (target dose 97/103mg twice daily) or valsartan (target dose 160mg twice daily) and matching placebo for 52 weeks. The study protocol is detailed in Chapter 3 and baseline characteristics in Table 4-1.

Venepuncture was performed pre-randomisation, at 26 weeks, and at 52 weeks as described in Chapter 3. Galectin-3 (i1000SR, Abbott Laboratories, Abbott Diagnostics) and growth differentiation factor-15 (GDF-15) (e411, Roche Diagnostics) were measured on clinical immunoassay platforms using the manufacturers calibrators and quality control materials. Tissue inhibitor of metallopeptidase-1 (TIMP-1), matrix metallopeptidase-9 (MMP-9) (using platelet-poor plasma), and soluble suppression of tumorigenicity-2 (sST2) were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D systems, Bio-Techne), and using the manufacturers' quality control materials. Procollagen III N-terminal peptide (PIIINP) (Tecan, IBL International) was also measured using a commercial ELISA assay and the manufacturer's quality control materials.

All biomarker sample processing and measurements were performed by Philip Stewart, Elaine Butler, Josephine Cooney and Emma Dunning at the Glasgow Biomarker Laboratory, Institute of Cardiovascular and Medical Sciences, University of Glasgow under the supervision of Dr Paul Welsh.

Cardiac MRI was performed at pre-randomisation and at 52 weeks as described in Chapter 3-6. T1 mapping images were created using a modified look-locker inversion recovery (MOLLI) sequence in the short axis view of the left ventricle at the base, mid, and apical ventricle. T1 mapping sequences were performed pre- and post-gadolinium administration. For the purposes of measurement of remote zone ECV, regions of interest were drawn in myocardium remote to the area of infarction (defined as myocardium 180 degrees from infarct site) and left ventricular blood pool in the mid ventricular short axis slice. Haematocrit (HCT) was measured at the time of scanning. Extracellular volume (ECV) was calculated as a ratio of corresponding T1 values measured pre- and post-contrast in each of the regions of interest. ECV was calculated using ECV = (1-HCT) × λ, where Lambda (λ)= ΔR1 myocardium /ΔR1 blood, ΔR1= R1 post-contrast - R1 pre-contrast and R1=1/T1.

8.2.2 Statistical methods

The distribution of baseline biomarker values was examined by means of histograms and summary statistics and non-normally distributed values were log-transformed prior to analysis. Baseline levels are presented as means with standard deviations for normally distributed values, and as medians with interquartile ranges for non-normal distributions. Baseline values are presented in the overall population and by randomised treatment allocation with between-group comparisons made using a two-sample T-test or Wilcoxon rank-sum test for normal and non-normal distributed variables, respectively.

The treatment effect of sacubitril/valsartan as compared with valsartan on biomarker levels over time was examined using a linear regression model which was adjusted for randomised treatment, baseline value of the outcome and use of diuretics at baseline. The regression model coefficients for the treatment indicator variable are reported as between-treatment group adjusted mean differences or, if required to satisfy modelling assumptions, log transformations were performed, and regression coefficients were back transformed and are presented as relative differences. Repeated measures analyses were performed as confirmatory analyses and are adjusted for the main effects of time-point, randomised group and the interaction between time-point and randomised group

and for diuretic use at baseline. All analyses were performed on an intention to treat basis as described in Chapter 3.

A p-value of <0.05 was considered statistically significant for all analyses. No correction for multiple testing was performed. No imputation for missing data was performed. All analyses were performed by Bethany Stanley (Robertson Centre for Biostatistics) and me using R Studio and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria), and STATA version 16.1 (College Station, TX, USA).

8.3 Results

8.3.1 Baseline values

Baseline values of the myocardial fibrosis biomarkers are detailed overall and by randomised treatment allocation in Table 8-1. There were no significant between-group differences at baseline. Correlations (expressed as Pearson's r) between baseline circulating biomarker concentrations and baseline ECV fraction as measured by cardiac MRI were as follows: galectin-3=-0.004 (p=0.97), MMP-9 = -0.27 (p=0.031), TIMP-1 = -0.07 (p=0.57) and PIIINP = 0.32 (p=0.004).

Table 8-1 Baseline levels of myocardial fibrosis biomarkers

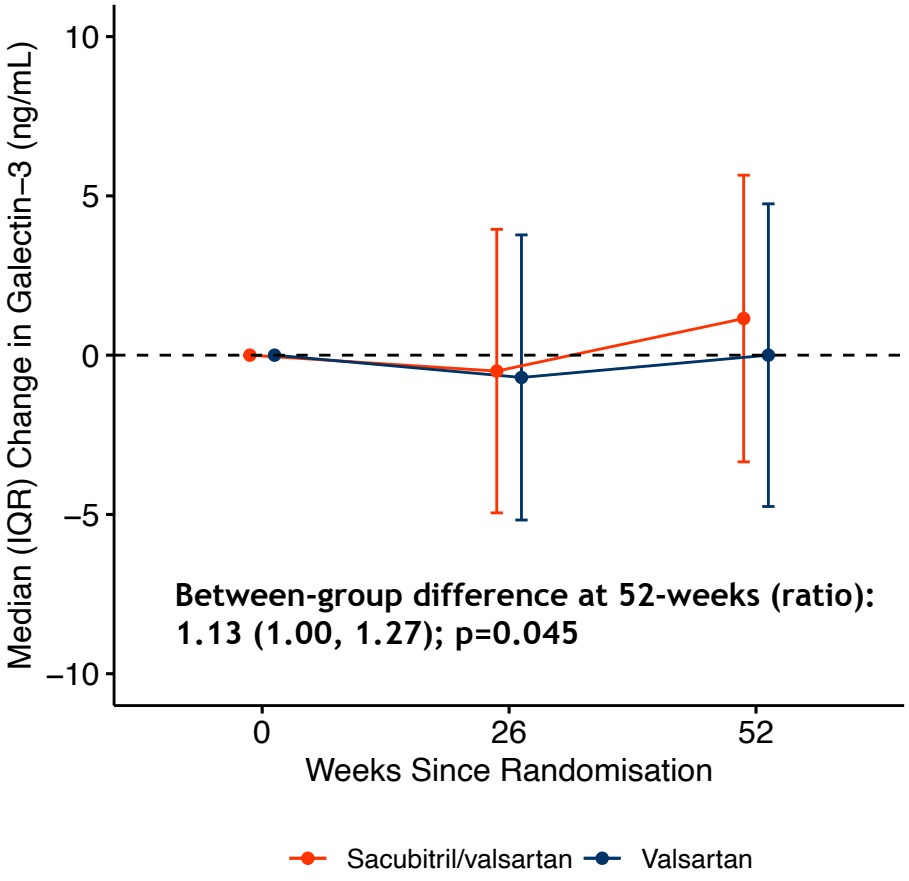
	N=	Sacubitril/valsartan	N=	Valsartan	Total	p-value
Galectin-3 (ng/mL)	46	14.5 (11.1-18.0)	46	12.1 (10.7-15.9)	13.4 (10.9-17.1)	0.13
GDF-15 (pg/mL)	46	1153 (898-1580)	46	1200 (919-1522)	1179 (919-1522)	0.85
sST2 (ng/mL)	46	17.6±6.3	46	16.2±4.8	16.9±5.6	0.24
MMP-9 (ng/mL)	30	38.4 (27.8-47.1)	33	31.1 (26.3-43.8)	35.4 (26.4-47.0)	0.37
TIMP-1 (ng/mL)	46	173.1±35.2	46	171.7±31.2	172.4±33.1	0.84
PIIINP (ng/mL)	46	7.9±3.0	46	7.8±2.5	7.8±2.7	0.86
ECV fraction (%)	37	24.1±2.8	40	23.8±2.5	24.0±2.6	0.54

Data presented as mean ± standard deviation or median (interquartile range)

8.3.2 Galectin-3

Median (IQR) galectin-3 at baseline was 14.5 (11.2, 17.8) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 12.1 (10.7, 15.7) ng/mL in those randomised to valsartan (n=46). Median change in NT-proBNP was 1.2 (IQR -1.2, 3.3) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and 0.0 (-3.0, 1.7) pg/mL in the valsartan group: ratio of adjusted geometric means: 1.13 (95% confidence interval [CI], 1.00, 1.27); p=0.045 (Figure 8-1). In repeated measures modelling there was no significant between-group differences at 26 weeks, but a 14% increase in the sacubitril/valsartan group at 52 weeks (p=0.03).

Figure 8-1 Change in galectin-3 with sacubitril/valsartan or valsartan from baseline to week 52

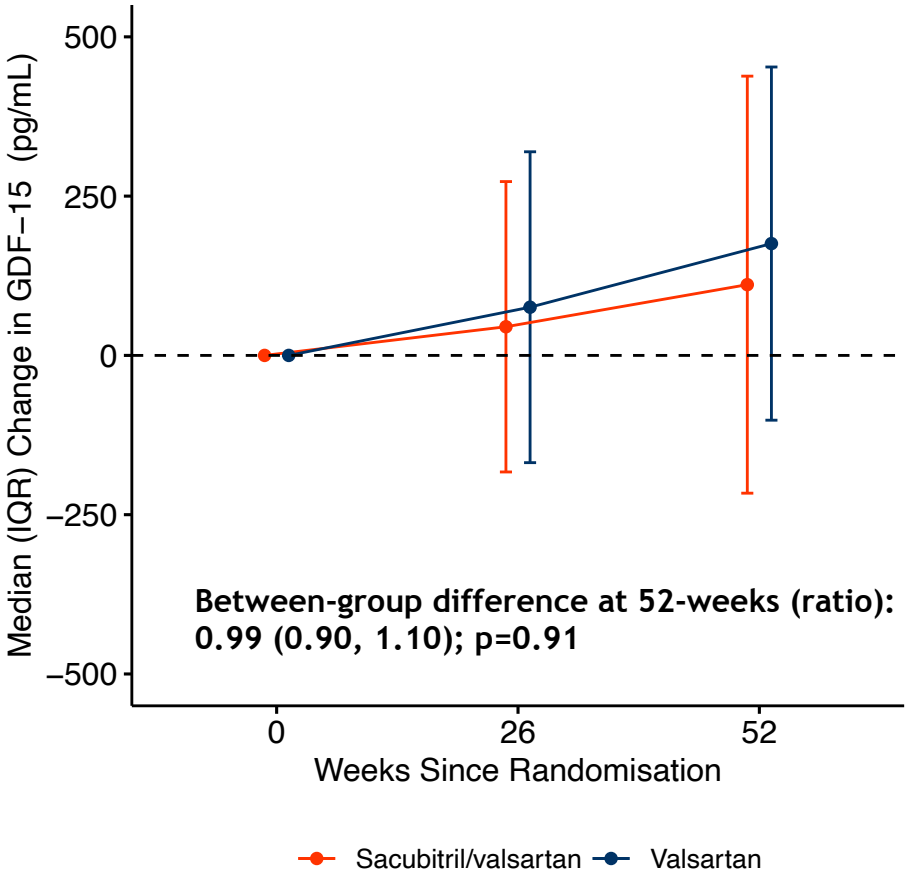


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline galectin-3 and use of diuretics at baseline.

8.3.3 GDF-15

Median (IQR) NT-proBNP at baseline was 1166 (905, 1561) pg/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 1200 (926, 1518) pg/mL in those randomised to valsartan (n=46). Median change in GDF-15 was 111 (IQR -99, 228) pg/mL between baseline and 52 weeks in the sacubitril/valsartan group and 176 (13, 290) pg/mL in the valsartan group: ratio of adjusted geometric means: 0.99 (95% confidence interval [CI], 0.90,1.10); p=0.91 (Figure 8-2). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 8-2 Change in GDF-15 with sacubitril/valsartan or valsartan from baseline to week 52

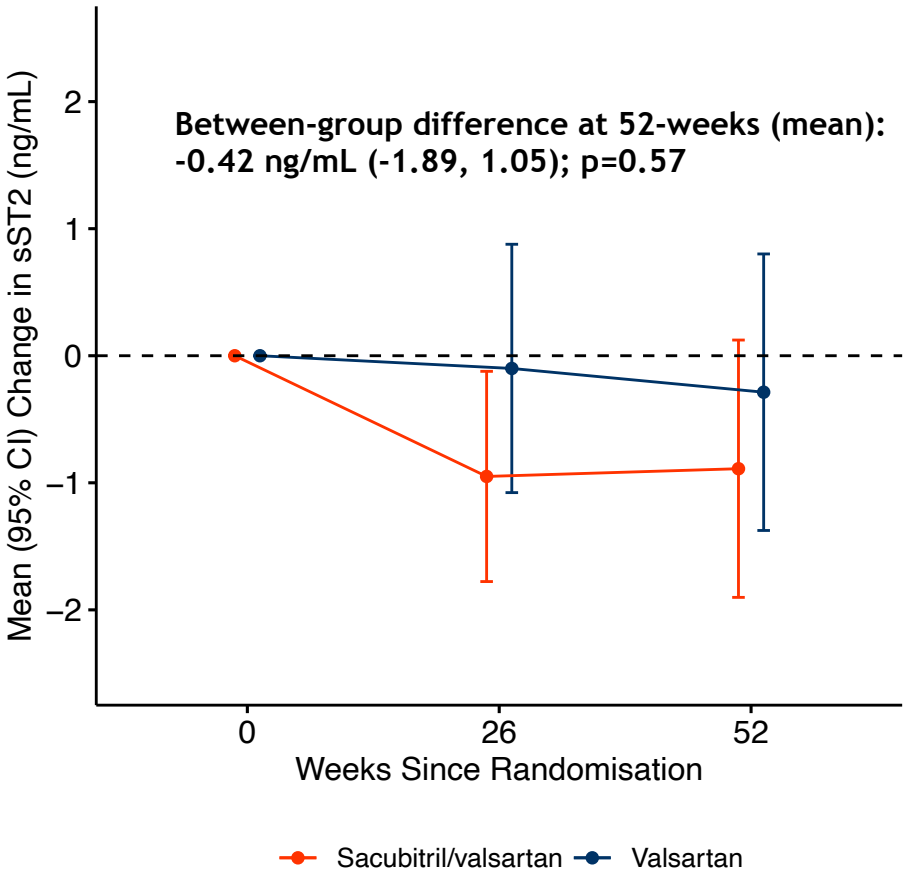


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline GDF-15 and use of diuretics at baseline.

8.3.4sST2

Mean (SD) sST2 at baseline was 17.7 (6.3) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 16.2 (4.8) ng/mL in those randomised to valsartan (n=46). Mean change in sST2 was -0.9 (SD 3.4) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and -0.3 (3.7) ng/mL in the valsartan group: adjusted between-group mean difference -0.42 (95% confidence interval [CI], -1.89, 1.05) ng/mL; p=0.57 (Figure 8-3). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 8-3 Change in sST2 with sacubitril/valsartan or valsartan from baseline to week 52

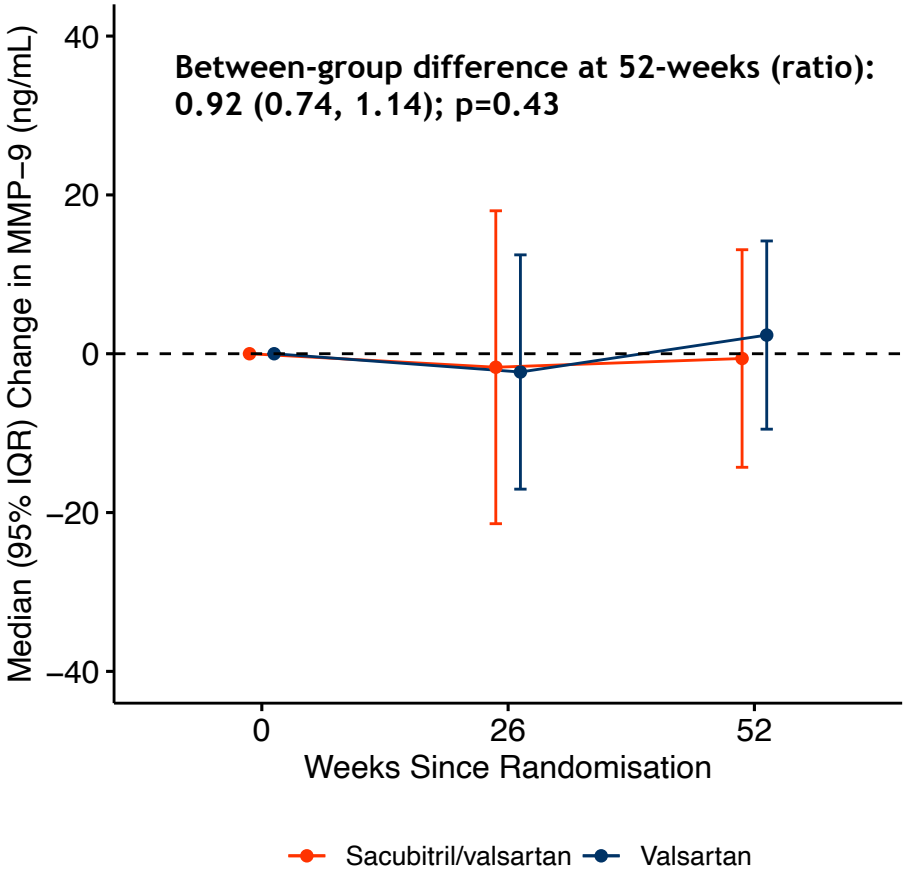


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline sST2, and use of diuretics at baseline.

8.3.5 MMP-9

Median (IQR) MMP-9 at baseline was 41.3 (30.3, 52.8) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=30) and 31.1 (26.3, 43.8) ng/mL in those randomised to valsartan (n=33). Median change in MMP-9 was -0.6 (IQR -8.0, 6.3) pg/mL between baseline and 52 weeks in the sacubitril/valsartan group and 2.1 (-4.5, 7.9) ng/mL in the valsartan group: ratio of adjusted geometric means: 0.92 (95% confidence interval [CI], 0.74,1.14); p=0.43 (Figure 8-4). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 8-4 Change in MMP-9 with sacubitril/valsartan or valsartan from baseline to week 52

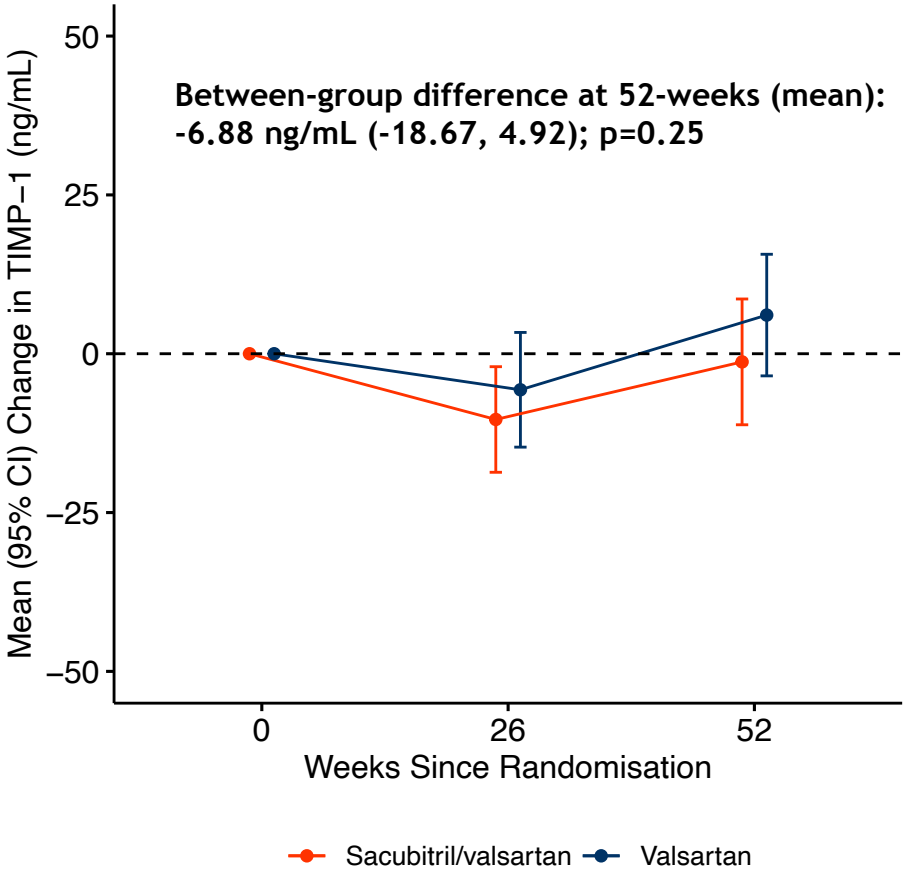


Data presented as median and error bars represent the interquartile range. Between-group difference presented as an adjusted ratio of geometric means calculated using a linear regression model using log-transformed values adjusted for randomised treatment, baseline MMP-9 and use of diuretics at baseline.

8.3.6 TIMP-1

Mean (SD) TIMP-1 at baseline was 172.9 (35.5) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 171.7 (31.2) ng/mL in those randomised to valsartan (n=46). Mean change in TIMP-1 was -1.3 (SD 33.3) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and 6.1 (32.2) ng/mL in the valsartan group: adjusted between-group mean difference -6.88 (95% confidence interval [CI], -18.67, 4.92) ng/mL; p=0.25 (Figure 8-5). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 8-5 Change in TIMP-1 with sacubitril/valsartan or valsartan from baseline to week 52

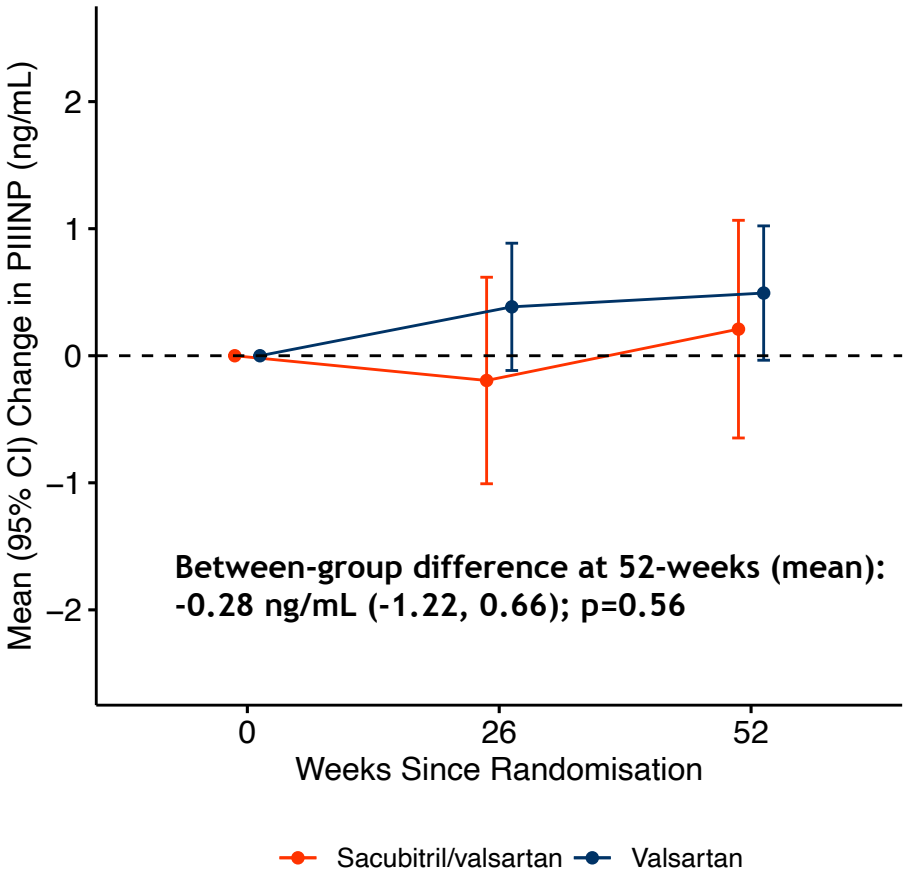


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline TIMP-1, and use of diuretics at baseline.

8.3.7 PIIINP

Mean (SD) PIIINP at baseline was 7.82 (2.96) ng/mL in those randomised to sacubitril/valsartan with follow-up data (n=46) and 7.78 (2.55) ng/mL in those randomised to valsartan (n=46). Mean change in PIIINP was 0.21(SD 2.88) ng/mL between baseline and 52 weeks in the sacubitril/valsartan group and 0.49 (1.78) ng/mL in the valsartan group: adjusted between-group mean difference -0.28 (95% confidence interval [CI], -1.22, 0.66) ng/mL; p=0.56 (Figure 8-6). Similar results were seen in repeated measures modelling with no significant between-group differences at either time point.

Figure 8-6 Change in PIIINP with sacubitril/valsartan or valsartan from baseline to week 52

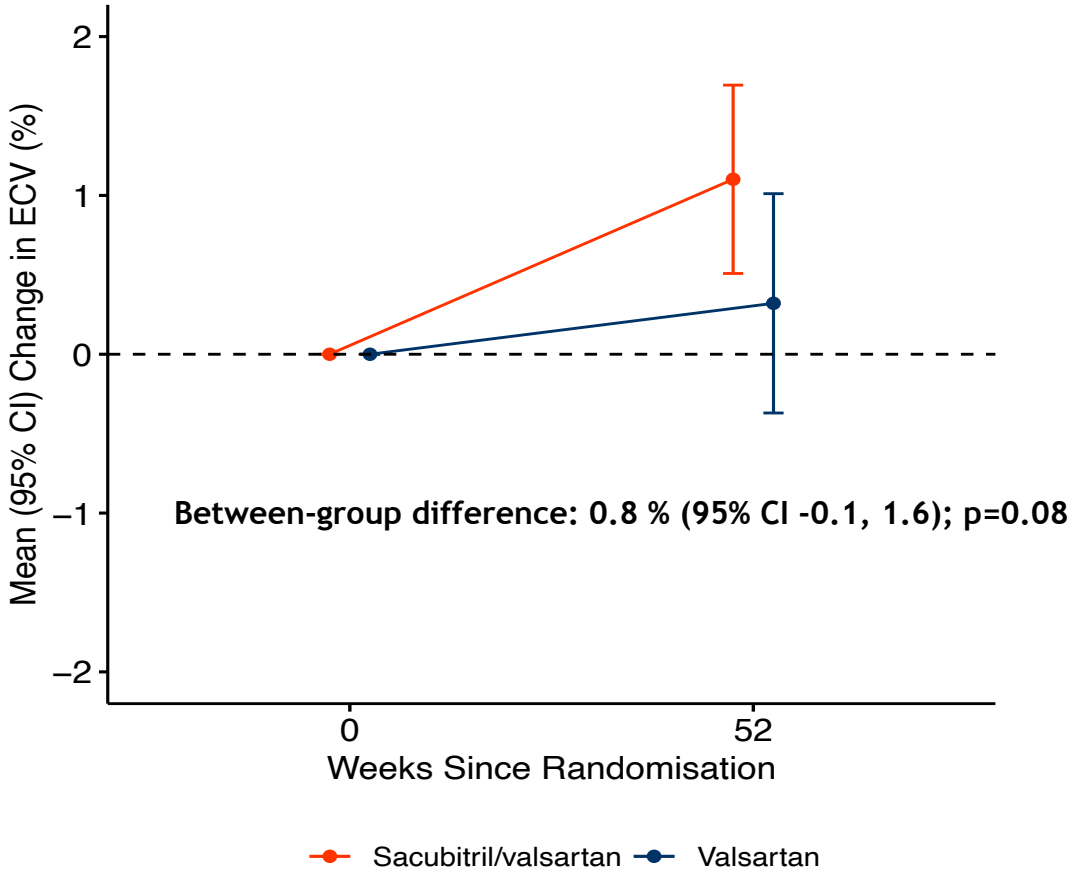


Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline PIIINP, and use of diuretics at baseline.

8.3.8 Effect of neprilysin inhibition on left ventricular remote zone extracellular volume fraction

Mean (SD) remote zone ECV fraction at baseline was 24.1 (2.8) % in those randomised to sacubitril/valsartan with follow-up MRI data (n=37) and 23.8 (2.5) % in those randomised to valsartan (n=40). Remote zone ECV increased by 1.0 (SD 1.7) % between baseline and 52 weeks in the sacubitril/valsartan group and by 0.3 (SD 2.1) % in the valsartan group: adjusted between-group difference 0.8 (95% CI, –0.1, 1.6) %; p=0.08 (Figure 8-7). In a *post hoc* subgroup analysis, the adjusted mean between-treatment group difference in ECV from baseline was 0.2 (95% CI -2.1, 2.4) % in those with NT-proBNP ≥230 pg/mL at baseline, and -1.9 (95% CI -4.2, 0.4) % in those with baseline NT-proBNP <230 pg/mL (Interaction p=0.21).

Figure 8-7 Change in remote zone extracellular volume fraction from baseline to week 52



Data presented as mean and error bars represent 95% CIs. Between-group difference presented as an adjusted mean difference calculated using a linear regression model adjusted for randomised treatment, baseline ECV, use of diuretics at baseline, and time from randomisation to cardiac magnetic resonance imaging.

8.4 Discussion

As previously noted, a novel aspect of the study presented in this thesis was the comparison between sacubitril/valsartan and valsartan alone, facilitating a direct examination of the effect of the addition of neprilysin inhibition on parameters of cardiac remodelling as described in Chapter 4, along with its effect on circulating substrates for neprilysin including the natriuretic peptides (Chapters 5 and 6). Alongside measurement of these substrates for neprilysin, I also measured established circulating and MRI biomarkers of myocardial fibrosis in order to examine the effect of neprilysin inhibition on them, due to the importance of progressive fibrosis in the development of adverse left ventricular remodelling and heart failure.

Key pathophysiological mechanisms driving progressive adverse left ventricular remodelling and the development of heart failure in survivors of myocardial infarction are inflammation and myocardial fibrosis as described in Chapter 1. It is therefore of interest to examine the effect of any new treatment on biomarkers of these profibrotic processes and in the context of any remodelling effect.

Key to the process of myocardial fibrosis is collagen metabolism and several of the biomarkers measured in the present study play a role in the synthesis, processing and degradation of myocardial extracellular matrix collagen and fibroblast activity.[350] Along with aldosterone (which was not measured in this study), soluble ST2 (sST2), galectin-3 (Gal-3), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1),and matrix metalloproteinase 9 (MMP-9) all play a role in the synthesis and degradation of collagen by myocardial fibroblasts. The TIMPs regulate the activity of the MMPs which play a key role in extracellular matrix degradation with elevated TIMP-1 and MMP-9 concentrations associated with a greater degree of adverse remodelling in survivors of myocardial infarction.[264,346] As part of collagen synthesis, the N-terminal fragment of the procollagen type III, is released into the circulation and can be measured as a biomarker for newly synthesised collagen and is one of the few markers described to be associated with histologically proven myocardial fibrosis.[350] Finally, growth differentiation factor-15 (GDF-15) belongs to the transforming growth factor-β family of proteins and levels of GDF-15 increase secondary to

inflammation and elevated levels in heart failure have been reported to be associated with worse outcomes.[351]

How did levels of these fibrosis biomarkers compare to previous populations? In PARADIGM-HF, a trial that enrolled patients with symptomatic HFrEF with elevated natriuretic peptide levels, the median levels of sST2, galectin-3, TIMP-1 and PIIINP were higher, and those of MMP-9 lower, than the published median referent control values.[352] When compared to the PARADIGM-HF median values, the present cohort had lower sST2 (16 vs. 32 ng/mL), galectin-3 (13 vs. 17 ng/mL), MMP-9 (35 vs 64 ng/mL) and higher TIMP-1 (167 vs. 125 ng/mL) and PIIINP (7.4 vs. 4.6 ng/mL) levels. Compared with controls, sST2 and galectin-3 were similar, MMP-9 lower, and TIMP-1 and PIIINP higher. Direct comparisons between these results may be limited to the small sample size in this study and the use of different assays. However, in this context, these results suggest that the present cohort had greater collagen synthesis and less degradation than both the HFrEF and control cohorts (higher TIMP-I and PIIINP, and lower MMP-9 concentrations). sST2, a biomarker that has independent predictive value of the risk of adverse outcomes in HFrEF in addition to troponin and NT-proBNP, was no different than that of the control reference population and only one of the 93 patients had a value >35 ng/mL which is the current prognostic threshold used, above which is associated with worse outcomes in HFrEF.[353] GDF-15 levels in the current cohort (1179 pg/mL) were also lower than those in the PARADIGM-HF trial (1690 pg/ml) but higher than published controls (762 pg/mL).[351]

In the current study, I did not find any significant between-group differences in any of the fibrosis biomarkers with the exception of galectin-3. In PARADIGM-HF, as compared with enalapril, sacubitril/valsartan significantly reduced sST2, TIMP-1, MMP-9 and had no effect on galectin-3, PIIINP and GDF-15. Of note, when analysed in the same way as the PARADIGM-HF trial (ratio of geometric means), the estimate of relative change in TIMP-1 and MMP-9 in PARADIGM-HF was similar than that seen in this trial but the treatment effect estimates in the current trial did not meet statistical significance, probably reflecting a limitation of the smaller sample size in the present study. The lack of difference in ST2 between the treatment groups is likely indicative of the low levels at baseline and does not preclude a lowering effect in HFrEF patients as observed

in PARADIGM-HF. Finally, it is possible that the reduction in sST2 (and those of other profibrotic biomarkers) in PARADIGM-HF was not a *direct* effect of neprilysin inhibition but was secondary to haemodynamic improvements and a reduction in left ventricular wall stress as evidenced by a reduction in NT-proBNP (which was not seen in the present study). In addition to these indirect factors, a direct effect of neprilysin inhibition on reducing pro-fibrotic signalling is to reduce circulating aldosterone levels as was seen in the PARADIGM-HF trial.[348] The finding of the present study of an increase in galectin-3 with the addition of neprilysin inhibition is somewhat surprising. Galectin-3 acts to increase myofibroblast proliferation and fibrogenesis and an increase in galectin-3 is associated with worse cardiovascular outcomes in patients with HFrEF.[354–356] However, change in galectin-3 has not been shown to correlate with left ventricular remodelling following acute myocardial infarction.[357] It is possible that the result (which is of borderline statistical significance [p=0.045] and does not account for multiple testing) is a spurious finding given that no difference was seen at 6 months and no significant difference was seen between sacubitril/valsartan and enalapril in the much larger PARADIGM-HF trial in which the observed effect was directionally opposite to that seen in the present study. Of note however, was the recent observation that the SGLT2 inhibitor empagliflozin increased galectin-3 in context of a favourable significant reverse remodelling effect in a placebo-controlled trial in patients with symptomatic HFrEF.[358] Higher levels of galectin-3 in patients treated with an MRA (a treatment which reduces myocardial fibrosis) have been reported; potential reasons for this may be a negative feedback reaction to the anti-fibrotic effects of MRA, “aldosterone breakthrough” stimulated secretion of galectin-3 or the reduction in eGFR secondary to MRA (as galectin-3 concentrations are strongly correlated with renal function).[355,357] Aldosterone stimulated galectin-3 secretion in the present cohort seems unlikely as in the PARADIGM-HF trial, sacubitril/valsartan, compared with enalapril, reduced aldosterone concentrations in patients with HFrEF.[348]

In the cohort studied, the addition of a neprilysin inhibitor did not have any significant effect on cardiac MRI measured remote zone ECV fraction. Interestingly, ECV increased to a greater degree in patients randomised to sacubitril/valsartan, but this difference was not statistically significant and may

be a spurious observation given that the present study was not powered to detect a difference in ECV and data were not available for 16 of the 93 randomised patients. Furthermore, the increase in ECV would appear to be discordant with the treatment effect estimates of the effect of neprilysin inhibition on MMP-9, TIMP-1 and PIIINP in the present study and those in PARADIGM-HF which were suggestive of a reduction in pro-fibrotic signalling.

8.4.1 Limitations

The limitations of the analyses presented in this chapter are the same as those discussed previously for other biomarkers analysed. Namely, the sample size may have reduced the power to be able to detect small between-group differences. A larger study cohort may have detected more significant between-treatment differences, and indeed some of the biomarkers (e.g., TIMP-1 and MMP-9) were directionally similar to those seen in the much larger PARADIGM-HF cohort. As discussed, the number of statistical tests performed raises the possibility of chance findings and no correction for multiple testing was performed.

8.4.2 Conclusion

In this study in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction, the addition of neprilysin inhibition did not significantly reduce any circulating biomarkers of profibrotic processes or cardiac MRI measured remote zone ECV fraction. Galectin-3 levels significantly increased in patients randomised to sacubitril/valsartan compared with valsartan alone.

Chapter 9 Discussion

9.1 Summary of findings

The development of left ventricular systolic dysfunction following myocardial infarction increases the subsequent risk of the development of symptomatic HFrEF and mortality. Despite substantial advances in the last 3 decades in reducing the risk of heart failure following myocardial infarction, new therapeutic strategies aiming to minimise the development of left ventricular systolic dysfunction at the time of infarction and those which prevent progressive worsening of systolic dysfunction in the months and years following infarction are needed to prevent this common complication.

In Chapter 2 I have detailed the results of a nationwide epidemiological study examining the trends in the occurrence of a first hospitalisation for heart failure in survivors of a myocardial infarction in Scotland between 1991 and 2015. This study included over 175 000 patients with 1.5 million patient-years of follow-up. This study had several important findings. Firstly, over the period examined there was a significant reduction in the incidence of myocardial infarction with annual decline of 2.3% (95% CI 1.3-3.2%; $p<0.001$). The risk of mortality following discharge from myocardial infarction substantially decreased; at 1-year the adjusted risk of death fell by 46% (95% CI 40-52) between 1991 and 2015, with a significant 37% and 36% reduction in the risk of death at 5- and 10-years, respectively. Over a median follow-up time of 6.7 years, 12.2% of patients were admitted to hospital with a primary cause of heart failure following discharge from a myocardial infarction. The incidence of the development of heart failure fell over the period examined; the crude case incidence per 1000 patient-years of heart failure at 1-year following discharge from myocardial infarction decreased from 55.3 in 1991 to 31.3 in 2015 with similar trends for heart failure occurring within 5- and 10-years. Age at time of admission of heart failure within 1-year rose from 70.8 years in 1991 to 76.2 years in 2015. The occurrence of hospitalisation for heart failure was associated with a 3.5-fold increase in the adjusted risk of death compared to a person who did not develop heart failure. However, the risk of death following a heart failure hospitalisation fell by 30% between 1991 and 2015. The data from the study detailed in Chapter 2 highlight that in a contemporary population the development of heart failure in the

months and years following myocardial infarction remains a common occurrence and therefore preventing the development of this complication remains an important focus for research.

In the following Chapters, I have described the methods and results of a prospective, randomised, double-blind, active-comparator trial powered to investigate the effects of the addition of neprilysin inhibition to RAS inhibition on left ventricular volumes in patients with symptomless left ventricular systolic dysfunction late after myocardial infarction. The main findings of this study were that in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction, the addition of a neprilysin inhibitor to RAS inhibition with sacubitril/valsartan, compared with RAS inhibition alone with valsartan, did not have a significant favourable remodelling effect on left atrial or ventricular volumes and did not significantly reduce biomarkers of left ventricular wall stress (NT-proBNP) or myocardial injury (hsTn-I).

Progressive pathological left ventricular remodelling is the common link between myocardial infarction, the development of HFrEF, and worsening of established HFrEF. In the context of the clinical benefits observed with sacubitril/valsartan in patients with HFrEF in the PARADIGM-HF and the established correlation between the effect of HFrEF therapies on left ventricular volumes and function and clinical outcomes, it was not unreasonable to hypothesise that the benefits in HFrEF were due, in part, to a reverse remodelling effect and that this benefit, if present, would extend to patients with asymptomatic left ventricular systolic dysfunction following myocardial infarction (a common pathological precursor of symptomatic HFrEF). My finding that the addition of neprilysin inhibition to standard treatment with a RAS inhibitor and beta-blocker did not have any additive reverse remodelling effect in the population studied in the present trial does not exclude a reverse remodelling effect in patients with HFrEF and the results have to be considered in the context of the population studied (and the differences with patients with HFrEF).

The EVALUATE-HF trial reported a beneficial reverse remodelling effect with sacubitril/valsartan as compared with enalapril in patients with HFrEF, the majority of whom were symptomatic.[148] The inclusion of patients in my study who were exclusively asymptomatic of their left ventricular systolic dysfunction,

as reflected in relatively low NT-proBNP concentrations, is a key distinction between this cohort and that studied in EVALUATE-HF and in 3 recently published trials in patients with symptomatic HFrEF reporting a beneficial remodelling effect with the sodium-glucose co-transporter 2 (SGLT2) inhibitor empagliflozin.[148,358–360] In EVALUATE-HF, a significant correlation between the degree of change in LVESVI and the change in NT-proBNP from baseline was reported, a finding that was replicated in the present trial.[148] This, along with the observed treatment-effect interaction with baseline NT-proBNP level (Chapter 4), raises the possibility that a beneficial reverse remodelling effect with a neprilysin inhibitor may only occur when there is evidence of increased left ventricular wall stress, i.e., elevated natriuretic peptide concentrations. Indeed, the degree of elevation of natriuretic peptide levels in patients with HFrEF correlates with the level of neurohumoral activation, progressive adverse remodelling, symptoms and prognosis.[247,265] Furthermore, if any remodelling effect of neprilysin inhibition is secondary to haemodynamic improvements as a result of increased vasodilation and augmented diuresis (thereby reducing preload and afterload), then it follows that this effect may be attenuated in patients without evidence of increased left ventricular end-diastolic pressure (i.e. elevated natriuretic peptides). Therefore, it is possible that a beneficial remodelling effect with the addition of neprilysin inhibition might have been demonstrated if patients had been enrolled based on elevated natriuretic peptide concentrations, although this is a hypothetical proposal based on a small and *post hoc* sub-group analysis and needs to be tested prospectively.

In EVALUATE-HF, the magnitude of reduction in LVESVI (1.6 mL/m^2) and LVEDVI (2.0 mL/m^2) was less than that seen with an ACE inhibitor and beta-blockers in patients with HFrEF however this may represent the relatively short 12-week follow-up.[60,240] The results in the present study do not exclude a remodelling effect of the magnitude with sacubitril/valsartan seen in EVALUATE-HF, however, my trial was not powered to detect such a difference if it exists. As described in Chapter 4, a differential reverse remodelling effect has previously been reported with other HFrEF pharmacotherapies, with less effect in asymptomatic patients, as compared to symptomatic patients with beta-blockers, ivabradine and possibly ACE-inhibitors.[240,241,243,244,249,250] Given the established clinical benefits with sacubitril/valsartan in patients with

symptomatic HFrEF, I did not think it was ethically justifiable to conduct a 52-week long remodelling study in these patients thereby limiting my ability to assess the remodelling effect of neprilysin inhibition in symptomatic HFrEF patients.

The population studied in the present study were remote (at least 3 months) from the time of index infarction with a median time from infarction of 3.6 years. This aspect of the study design was to avoid the inclusion of patients in whom left ventricular systolic dysfunction was transient following infarction (i.e., patients who would be less likely to demonstrate progressive adverse remodelling). This characteristic of the study cohort means that I cannot exclude a beneficial remodelling effect of the addition of neprilysin inhibition to standard therapy if added immediately following the time of acute infarction. Indeed, it could be argued that the sympatholytic, RAS inhibitory and anti-fibrotic effects of the augmentation of natriuretic peptides and other substrates for neprilysin may be more beneficial in the milieu of acute infarction where there is significant neurohumoral activation and prior to the development of myocardial fibrosis and scar. Indeed, the remodelling effect of the ACE inhibitor captopril in the SAVE trial was evident only in the first year of treatment and was not seen to continue during the second year of follow-up.[37] However, perhaps consistent with the results seen in the present study, the recently published PARADISE-MI trial reported no benefit in reducing the incidence of the primary composite endpoint of cardiovascular death of sacubitril/valsartan, compared with ACE-inhibitor ramipril, when added to standard therapy in a contemporary, high-risk population in the immediate period following acute myocardial infarction.[237] Given the established correlation between the clinical benefits of RAS inhibitors and beta-blockers in high-risk patients following myocardial infarction and their remodelling effect, it is not unreasonable to hypothesise that the lack of observed clinical benefit in PARADISE-MI with the addition of a neprilysin inhibitor suggests that it would be unlikely that there is any substantial additive beneficial remodelling effect in the population studied in that trial. The results of an echocardiographic sub-study from PARADISE-MI have not yet been published at the time of writing this thesis and will provide further evidence regarding this.

In Chapters 5, 6 and 8, I have described the effect of neprilysin inhibition on concentrations of its postulated circulating substrates and biomarkers of myocardial fibrosis. These results provide novel insights into the potential mechanisms of action underlying the clinical benefits observed with sacubitril/valsartan in HFrEF. As described in Chapter 1, neprilysin has a greater affinity for ANP than BNP and CNP, therefore the observation that neprilysin inhibition significantly increased concentrations of ANP but not BNP or CNP is consistent with this (Chapter 5). Increased natriuretic peptide bioactivity was evidenced by the two-fold increase in urinary cGMP:creatinine ratio with sacubitril/valsartan as compared with valsartan; this, along with the reduction in MR-proANP, indicating a reduction in proANP production as it is not a substrate for neprilysin, suggests that increased cGMP activation may be a key mechanism of action underlying the clinical benefits of neprilysin inhibition. I also observed a significant increase in MR-proADM (not a substrate for neprilysin) which is the precursor molecule of bioactive adrenomedullin which is a substrate for neprilysin. A bi-directional relationship between natriuretic peptides and adrenomedullin production has been previously reported, with the exogenous administration of each peptide resulting in increased levels of the other.[282,361] It is possible, therefore, that the observed increase in MR-proADM was secondary to an increase in ANP but I am unable to confirm this hypothesis. Nevertheless, an increase in MR-proADM, and therefore bioactive adrenomedullin with its positive inotropic, antifibrotic and natriuretic effects, is thought to be favourable. Consistent with a previous report that sacubitril/valsartan improved glycaemic control in patients with diabetes in PARADIGM-HF, I observed an increase in the incretin hormone GLP-1 with sacubitril/valsartan as compared with valsartan alone.[300] Along with this anti-hyperglycaemic effect, GLP-1 in its various peptide forms as detailed in Chapter 6, is thought to have beneficial cardiovascular effects including improved cardiac glucose utilization, natriuresis, myocardial function and vasodilation.[299] Apelin, previously identified as a possible substrate for neprilysin, did not differ significantly between the treatments and reassuringly, I did not observe any increase in endothelin-1 with the addition of neprilysin inhibitor to a RAS inhibitor. Finally, I did not observe any significant changes in biomarkers of pro-fibrotic processes in the cohort enrolled in the present study (Chapter 8). The estimates of relative change in TIMP-1 and MMP-9 were similar to those reported in PARADIGM-HF but the results

were non-significant which may reflect the limited ability of the relatively small sample size to detect small between-group differences. Furthermore, when considered in the context of no reduction in NT-proBNP or troponin, the results in the present study are supportive of the hypothesis that the reductions in markers of fibrosis previously reported with sacubitril/valsartan are reflective of a reduction in left ventricular wall stress and injury secondary to haemodynamic changes and left ventricular wall stress (indicated by a reduction in NT-proBNP) rather than a direct effect of neprilysin inhibition itself.

9.2 Strengths

The key strength of this study was the use of valsartan as the comparator agent which allowed me to describe the effect of neprilysin inhibition *per se* on left ventricular remodelling and circulating substrates for neprilysin. This study was powered to detect a 6 mL/m^2 between-group difference in LVESVI, a difference that has previously been shown to represent a minimally important clinical difference. The observed standard deviation of change was less than that used in the power calculation meaning that the trial was adequately powered to detect a difference of this magnitude; as noted previously, I cannot exclude that a smaller treatment effect was present and this would require larger studies to confirm if such a difference exists. Finally, the collection of biomarker samples was near complete for the cohort and the number of patients who underwent follow-up MRI imaging (90 of 92 survivors) was very high with no patients lost to follow-up. Furthermore, this was achieved in the context of the challenges of the COVID-19 pandemic which began during follow-up.

9.3 Limitations

Based on the results presented in this thesis, I am unable to make conclusions regarding the remodelling effect of neprilysin inhibition in patients with symptomatic HFrEF in whom there is a strong guideline recommendation to use of sacubitril/valsartan and in whom I did not feel it was ethically appropriate to conduct a 52-week remodelling study. I only recruited patients with left ventricular systolic dysfunction as a result of a previous myocardial infarction; the remodelling effect of neprilysin inhibition may be less in patients with ischaemic cardiomyopathy compared to those with non-ischemic causes as is

seen with cardiac resynchronization therapy.[362] Previous studies, including PARADIGM-HF, used an ACE-inhibitor as the comparator agent; the results of a comparison between sacubitril/valsartan and an ACE inhibitor may show different results than those presented in this thesis. However, it is notable that in VALIANT, valsartan was found to be equivalent to the ACE inhibitor captopril in reducing the risk of mortality and attenuating adverse left ventricular remodelling in high-risk patients after myocardial infarction.[13,59] The relatively small sample size may have limited my ability to detect small between-group differences in biomarkers. All biomarker analyses are hypothesis-generating, and I am unable to make any conclusion about change in biomarkers and substrates for neprilysin and their relationship with clinical outcomes.

9.4 Future areas of research

The results of the PARADISE-MI trial which reported no additive benefit of the addition of neprilysin inhibition to standard therapy in high-risk survivors of myocardial infarction means that it is unlikely that sacubitril/valsartan will receive a guideline indication in patients with heart failure and/or left ventricular systolic dysfunction complicating acute myocardial infarction.[237] Therefore, its current role in clinical practice remains in survivors remote from myocardial infarction who go onto develop symptomatic HFrEF along with other patients with HFrEF of any cause (i.e., become “PARADIGM-HF like” patients). The identification of patients who are at a higher risk of going on to develop symptomatic HFrEF following myocardial infarction remains challenging; a potential further area of future research could be the use of biomarker measurements performed remote from the time of infarction (e.g., at 3 months) to identify this at-risk population. The finding in Chapter 7 that elevated natriuretic peptide levels (NT-proBNP or MR-proANP) and hs-TnI were independent predictors of the degree of ventricular dilatation at baseline, a powerful predictor of the future development of HFrEF, and the observed treatment-effect interaction with NT-proBNP levels at baseline suggests that measurement of these biomarkers may help identify patients who may stand to benefit from escalation of their medical therapy, e.g. the addition of a neprilysin inhibitor.

As described in Chapter 2, despite contemporary pharmacological and interventional therapies, the development of heart failure in survivors of myocardial infarction remains a frequent occurrence and is associated with an increased risk of mortality. Therefore, new therapeutic strategies which reduce the risk of developing heart failure remain a focus for research to improve outcomes in this patient population. Recently, inhibitors of the sodium-glucose cotransporter 2 (SGLT2) have been demonstrated to reduce the risk of cardiovascular death and worsening heart failure in patients with HFrEF, as well as in patients with heart failure with a mildly reduced or preserved ejection fraction.[363–365] In patients with HFrEF these clinical benefits may be, in part, due to a reverse remodelling effect of SGLT2 inhibitors as reported in three small studies.[358,360,366] Ongoing large, randomised placebo-controlled trials are examining the effect of the addition of an SGLT2 inhibitor to standard therapy in high-risk patients immediately following MI; the DAPA-MI trial with dapagliflozin (NCT04564742) and EMPACT-MI with empagliflozin (NCT04509674). To examine the potential beneficial remodelling of an SGLT2 inhibitor when added to standard therapy in this setting, I, along with colleagues in the Institute of Cardiovascular and Medical Sciences (ICAMS) at the University of Glasgow, have designed a prospective, multi-centre, randomised, placebo-controlled trial examining the effect of the SGLT2 inhibitor empagliflozin on cardiac remodelling in patients with left ventricular systolic dysfunction started shortly after the time of acute myocardial infarction (EMpagliflozin to PREvent worSening of left ventricular volumes and Systolic function after Myocardial Infarction – EMPRESS-MI; NCT05020704). Enrolment into this trial is due to begin in early 2022 and the results are expected to be presented in late 2023.

9.5 Conclusions

In patients with symptomless left ventricular systolic dysfunction because of prior myocardial infarction, the addition of a neprilysin inhibitor to standard therapy including a RAS inhibitor and beta-blocker did not provide any additional beneficial reverse left ventricular remodelling effect. As compared with RAS inhibition alone, the addition of a neprilysin inhibitor did not significantly reduce NT-proBNP, a marker of elevated left ventricular wall stress, or hs-TnI, a marker of myocardial injury.

Appendix 1: Patient Information Sheet

EudraCT 2017-003460-13

Patient Information Sheet

The effects of sacubitril/valsartan compared to valsartan on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction after myocardial infarction: a randomised, double-blinded, active-comparator, cardiac-MR based trial

We would like to invite you to take part in a research trial. Before you decide, it is important that you understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the trial if you wish, and seek clarification if there is anything that is not clear or if you would like further information.

Thank you for taking the time to read this information.

Who is conducting the research?
The research is being carried out by Professor John McMurray, Professor Mark Petrie, Dr. Ross Campbell and Dr. Kieran Docherty from the Department of Cardiology at the Queen Elizabeth University Hospital and the University of Glasgow.

The trial is funded by the British Heart Foundation, and the drug company Novartis who produce Sacubitril/Valsartan. The trial is sponsored by NHS Greater Glasgow and Clyde and the University of Glasgow.

What is the purpose of the trial?
Hearts attacks are a common health problem in the United Kingdom (UK). In the last 30 years, the risk of dying from heart attacks has been reduced through the use of medications and interventions such as angioplasty (using balloons and stents to open up blocked or narrowed heart arteries).

Despite these advances, a proportion of people who survive heart attacks sustain damage to their heart muscle. This means that the heart does not pump blood around the body as efficiently as it should, and can, over time, lead to symptoms such as breathlessness, fluid retention and reduced exercise capacity. This is known as heart failure. The development of heart failure also increases the risk of death in the years following a heart attack. After a heart attack, doctors will usually recommend that people start an ACE (Angiotensin Converting Enzyme) inhibitor or ARB (Angiotensin Receptor Blocker). These two groups of medicines have been shown to help prevent the onset of heart failure and are the current standard treatment for people after a heart attack. Examples of an ACE inhibitor and ARB include the medicines ramipril, enalapril and valsartan.

We wish to test a new medication called sacubitril/valsartan in patients with heart muscle damage after a heart attack. We want to find out whether it is better than a standard treatment at reducing the decline in heart muscle function that occurs in some patients after a heart attack (as this is thought to help reduce the risk of developing heart failure).

In this trial we will compare sacubitril/valsartan with the standard treatment valsartan. Valsartan works by blocking a hormonal system thought to be harmful in patients with heart muscle damage; the new treatment (sacubitril/valsartan) also blocks this system but at the same time boosts another hormonal system that may protect the heart. The new medication is approved for use in the UK for people who have chronic heart failure. Studies have shown that sacubitril/valsartan was better than an ACE inhibitor at reducing the risk of death and hospitalisation for heart failure in patients with chronic heart failure. Sacubitril/valsartan is not approved for treatment following a heart attack in patients who have no symptoms of heart failure, the medical condition that you have.

The aim of this trial is to find out whether sacubitril/valsartan is better than valsartan at slowing the decline in heart muscle contraction and increase in heart chamber size that may occur following a heart attack. If this is shown, our findings may lead to a larger trial to see whether sacubitril/valsartan or a similar drug can reduce the risk of developing heart failure after a heart attack (and improve survival).

Why have I been asked to take part?
You have been invited to take part in this trial as you have had a heart attack in the past which, based on the ultrasound scan of your heart (echocardiogram) you have had, has caused damage to the heart muscle without causing you to have symptoms of heart failure. You are in the group of patients that we think could benefit from sacubitril/valsartan.

Do I have to take part?
No. You are free to decide whether or not you wish to take part. We will describe the trial and go through this information sheet, which we will then give to you. You can take this away and discuss it with other people (e.g. friends, family, GP) before making a decision.

If you decide to take part, you will be asked to sign a consent form to show you have agreed to take part. You are free to withdraw from the trial at any time, without giving reason. This would not affect the standard of care you currently receive or your future treatment.

What does taking part involve?
If you agree to take part in the trial, you will be asked to consent to an ultrasound scan of the heart (echocardiogram) and possibly repeat routine blood tests to assess if you would be suitable for the trial. This will be done at the Queen Elizabeth University Hospital or Glasgow Royal Infirmary. If this confirms you are eligible and agreeable, then we will ask you to consent to the remainder of the trial as outlined below. If these tests indicate some reason you should not take part in the trial then this will be discussed with you in relation to your standard treatment, and you may wish to be rescreened at a later date. If not then we thank you for your interest and consideration of the trial.

We will then perform some additional tests on the same day or on a return visit to the Queen Elizabeth University Hospital. These will include:

- A sample of blood, around 50mls, will be taken from a vein in the arm. This will be stored and used at the end of the study to measure different blood components which are related to the action of the medication and its effect on the heart muscle. With your consent, any samples remaining will be stored for analysis of any future relevant tests as they become available and for use in future ethically approved research studies if appropriate. Where possible, we will ask you to fast overnight for these samples to be taken in the morning. You should take your usual medications, including the study drug as usual. We will not ask you to fast if you are diabetic or unable to do so.
- A sample of urine. This will be used to measure salt and hormone levels in the urine.

- We will perform a cardiac magnetic resonance imaging (MRI) scan. This is the best method we have of looking at the heart in detail and allows us to make very accurate measurements. Unlike X-rays and CT-scans, the MRI scan does not involve any radiation. The scan uses magnets to help take pictures; if, therefore you have any metal implants such as pacemakers, prosthetic joints or plates or aneurysm clips you may not be able to take part. We will discuss this in detail prior to any MRI scan. The scan will take around one hour in total, and you will be asked to lie flat on your back for the duration of the scan with instructions to hold your breath at specific intervals through the scan. We will use a dye (Gadolinium) which is injected into a small plastic tube (cannula/Venflon) in a vein in your arm. This highlights areas of the heart which have been damaged. An information sheet about MRI scanning will be provided to you prior to your first study visit.

After these tests have been performed we will randomly allocate you to either the test medicine (sacubitril/valsartan) or the current standard treatment (valsartan). You will have a 50:50 chance (1 in 2) of being allocated to either drug. This is a "double blind trial", which means that neither you nor your doctor will know what treatment you have been allocated. You will be allocated to one of the following treatment arms:

- **Test arm:** sacubitril/valsartan and a placebo matched to valsartan
- **Standard care arm:** valsartan and placebo matched to sacubitril/valsartan

A placebo is tablet with no medicine in it. The placebo in this study is used to help mask what treatment arm you have been allocated to. Everyone in this study will be allocated to a tablet that contains an active medicine, either sacubitril/valsartan or valsartan. Use of “blinding” is common practice in research studies like this, and helps ensure that the results are unbiased. In an emergency, your study doctor can find out which medication you are taking.

If you have been taking an ACE inhibitor such as ramipril before entering the study you will need to wait at least 36 hours before you start the study medicine. This is because sacubitril/valsartan can cause angioedema (swelling of the face, lips, tongue and or throat, difficulties in breathing) if taken at the same time as an ACE inhibitor. The study doctor will provide you with detailed information on how and when to start taking the study medicine.

You will be required to take the study medicines twice a day; in the morning at approximately 8am and in the evening at approximately 8pm. You will need to take one tablet from two separate bottles at each dose but your study doctor will provide instructions on how to do this correctly. You should swallow the tablets whole with a glass of water. You can take the study medicines with or without food. If you forget to take a dose then skip the missed dose and take the next one at the scheduled time. Do not take a double dose to make up for a missed dose. If you have missed several doses please contact trial team for further advice.

You will remain on the treatment you are allocated along-with the standard medications given to patients after a heart attack for the full study duration of 12 months. Both of the study drugs are tablets which you take twice a day (once in the morning and once at night). There are three dose levels in this study. It is expected that most people will start on dose level 2 (sacubitril/valsartan 49mg/51mg twice daily or valsartan 80mg twice daily). After four weeks treatment, the dose will be escalated to dose level 3 (sacubitril/valsartan 97mg/103 mg twice daily or valsartan 160mg twice daily) for the rest of the study. The goal is to keep you on the highest dose level possible but if you are not able to tolerate a dose or if your study doctor feels it is necessary the dose of study medicine can be reduced to dose level 1 or stopped (permanently or temporarily). If your doctor feels it is necessary, they may also ask to you adjust other non-study medicines. Sometimes the study doctor may need to contact you by telephone about your study medicines. It is important

that you always take the study medicines as directed by the study doctor and attend for each study visit. You should also bring back any empty or used medicine bottles at each study visit. You should also let the study doctor know if you start any new medicines, vitamins or mineral supplements including those that you purchase yourself.

Both of the drugs which we are using can occasionally cause disturbances in the salt levels in the blood and the kidney function. We will keep a close eye on these blood tests with tests taken at regular intervals. We will check your bloods at 1,2, 4 (at which point we will increase the dose of the study drug) and 5 weeks following discharge and then at 3 monthly intervals. These checks will involve a 30-minute visit to hospital and travel expenses and/or free taxis are available if required.

If you stop the study medicine at any point you must wait 36 hours before you start an ACE inhibitor because of the risk of angioedema but the study doctor will provide detailed information about this at the time.

We will provide you with a ***study alert card*** at your first visit. You should carry this with you at all times and show it to any doctor or other healthcare professional (e.g. nurse, pharmacist) who treats you.

Halfway through your involvement in the study (26 weeks) we will ask you to visit the hospital to repeat some of the tests done when you joined the trial over the course of half a day. We will ask you to fast from midnight and will repeat the blood tests when you attend the hospital. We will perform a full medical examination including measurement of your blood pressure. We will also provide you with a plastic bottle to collect a urine sample.

This visit will be repeated at 52 weeks and we will repeat the cardiac MRI scan you had at the beginning of the trial. At this point your involvement in the trial will end. Travel expenses and/or free taxis are available if required for both of these visits. We will also ask to complete a short questionnaire on how you are feeling compared to when you started in the trial.

Some patients, following a heart attack develop symptoms of breathlessness and reduced exercise capacity. This is known as heart failure. If this occurs to you during the period of involvement in the trial, then we will offer you standard treatment with sacubitril/valsartan. Your involvement in the trial will end at this point. If you have been in the trial for longer than 6 months, we will ask if you would be willing to undergo a second MRI scan earlier than planned.

Your General Practitioner (GP) will be informed of your involvement in the trial and will be given contact details for the investigators should they have any questions regarding your treatment and involvement.

What happens at the end of the study?

At the end of twelve months (i.e. the end of your involvement in the study), all participants will stop the study medicine. You will return to normal medical care and your doctor will discuss the best treatment options for you. Standard treatment is an ACE inhibitor or an ARB (including valsartan) and this may be the same treatment that you were on before you began the study. Sacubitril/valsartan will only be available at the end of the study if you develop symptoms of heart failure. If you have been allocated to sacubitril/valsartan you will switch to the ACE inhibitor or ARB tablet as per local practice guidelines and continue on the other tablets which are routine after a heart attack.

Summary of your involvement

- *During your screening visit:* echocardiogram (ultrasound scan), and a blood test if required to assess your suitability for the trial.
- *Week 0* - Recruitment, blood tests, urine sample, clinical examination, cardiac MRI scan, commence study drug.
- *Week 1 (± 3 days):* Blood test and blood pressure check
- *Week 2 (± 3 days):* Blood test and blood pressure check
- *Week 4 (± 3 days):* Blood test, clinical examination and increase dose of study drug
- *Week 5 (± 3 days):* Blood test and blood pressure check
- *Week 14 (± 7 days):* Blood test and blood pressure check
- *Week 26 (± 7 days):* Blood test, urine sample and clinical examination
- *Week 39 (± 7 days):* Blood test and blood pressure check
- *Week 52 (± 7 days):* Blood test, urine sample, questionnaire, clinical examination and cardiac MRI scan

What happens to the information?
Your identity will be completely confidential and known only to the researcher and other NHS staff. This will remain confidential and will be stored in a locked filing cabinet and in a secure NHS database, so that we can contact you to arrange study visits. Your questionnaires and test results will be stored in a secure database at the University of Glasgow within the Robertson Centre for Biostatistics, a specialist centre for managing and analysing data from clinical trials. Your identity will not be passed to the University of Glasgow, or to anyone else out with the NHS. Your data will be handled in accordance with the Data Protection Act 2018, which means that we will keep it safely and will not reveal it to other people, without your permission.

No participants will be named or identified in any way in any public report and it will not be possible to identify any particular individual from the study results. You will be given an option to receive the final study results or a summary of these results.

Since it is important that we make the most of medical research data, we may in future share data from the study with other researchers, both in the UK and in other countries. No personal data will be shared.

The University of Glasgow and NHS Greater Glasgow and Clyde are the sponsor for this study based in the United Kingdom. We will be using information from you and/or your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The University of Glasgow and NHS Greater Glasgow and Clyde will keep identifiable information about you for 10 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out more about how we use your information at http://www.nhsggc.org.uk/patients-and-visitors/faqs/data-protection-privacy/ and/or by contacting one of the study investigators.

What are the possible benefits of taking part?
You may not benefit directly by taking part in the study. It is hoped that by taking part in this research, you will be providing valuable information regarding the best treatment for patients like

yourself who have suffered a heart attack and sustained heart muscle damage. The aim is to gain more information on how best to prevent patients developing heart failure following heart attacks and how best to treat patients in the future.

What are the potential disadvantages and risks of taking part?
Treatment with any medicine can cause side effects. The known side-effects of sacubitril/valsartan and valsartan are listed below. There may be other unforeseen risks or side effects, which are not currently known.

Some symptoms need immediate medical attention:
You may experience symptoms of angioedema (a specific allergic reaction), such as:

- swollen face, lips, tongue or throat
- difficulty in breathing or swallowing
- hives, itching

If you get any of these symptoms, then go to the nearest Accident and Emergency department and show them the study alert card.

The known side-effects of sacubitril/valsartan are listed below. There may be other unforeseen risks currently not known.

Frequency	***Side-effect***
Very common (may affect more than 1 in 10 people)	• low blood pressure (dizziness, light-headedness) • high level of potassium in the blood (shown in a blood test) • decreased kidney function (renal impairment)
Common (may affect up to 1 in 10 people)	• cough • dizziness • diarrhoea • low level of red blood cells (shown in a blood test) • tiredness • (acute) renal failure (severe kidney disorder) • low level of potassium in the blood (shown in a blood test) • headache • fainting • weakness • feeling sick (nausea) • low blood pressure (dizziness, light-headedness) when switching from sitting or lying to standing position • gastritis (stomach pain, nausea) • spinning sensation • low level of sugar in the blood (shown in a blood test)
Uncommon (may affect up to 1 in 100 people)	• allergic reaction with rash and itching • dizziness when switching from sitting to standing position

Valsartan was studied extensively in clinical trials before it was approved for marketing. The most commonly reported side effects of valsartan included: fatigue, muscle aches, vertigo, abdominal pain, cough, worsening kidney function, hypersensitivity (rash, itching), increased liver function tests, decreased red blood cell and sodium levels, low blood pressure, and increases in blood potassium levels. Angioedema (swelling of the face, extremities, eyes, lips, tongue, and difficulty in breathing) has also been reported with valsartan.

If you feel dizzy or light-headed you should not drive or use any tools or machinery.

Having an MRI scan may cause some people to feel claustrophobic. The dye agent injection used (gadolinium) has rarely caused side-effects (injection site discomfort, rash, itch, nausea and vomiting, dizziness, headaches, numbness (paraesthesia) and low blood pressure). Infrequently, the injection may leak out from the vein to the tissues under the skin (extravasation). Allergic reactions to gadolinium dye agents are uncommon but can be serious. We avoid gadolinium in severe kidney failure, due to the risk of nephrogenic systemic fibrosis.

Your study doctor will discuss possible side-effects in more detail with you. Please speak to the study doctor if you suffer any side effects. Contact information for the study team is given at the end of this sheet.

If you are a woman who could become pregnant:
It is not known if sacubitril/valsartan is safe to the unborn child or if it passes into human breast milk and there is limited information available. Valsartan is also not recommended in pregnancy. Therefore, if you are pregnant or breast-feeding, think you may be pregnant, planning to have a baby, or are not willing to use reliable contraception, you will not be able to take part in this study. The study doctor will discuss contraception with you but examples of reliable contraception include:

- Total abstinence when this is in line with your preferred and usual lifestyle
- Female sterilisation or where your partner has already been sterilised
- Use of oral, injected or implanted methods of contraception
- Use of an intrauterine device. These are sometimes called a ‘coil’.

If appropriate, we will advise you about contraception before you decide whether to take part in the study. A pregnancy test will be performed in women who could become pregnant before starting treatment, and at study visits 1 (week 0), 4 (week 4), 6 (week 14), 7 (week 26), 8 (Week 39), and 9 (week 52). If you think you might be pregnant or become pregnant during the study, you should tell the study doctor immediately. You will stop taking the study medicine and will no longer participate in the study, although the study doctor will ask to follow-up on the progress of your pregnancy and health of your baby.

If you are a male whose partner could become pregnant:
To be eligible for this study you will already be taking an ACEI or ARB. These are similar to the study medicines and with the information currently available we do not think that men need to avoid fathering a child whilst taking the study medicines. However, the study doctor will discuss this in more depth with you. If you think your partner may have become pregnant during the study please tell the study doctor immediately. With your partner’s consent the study doctors would like to collect information about your partner’s pregnancy and the health of your partner and their baby.

What will happen to the results of the research study?
The results of this research study will contribute to the understanding and treatment of patients with heart muscle damage after heart attacks. The results will be communicated through national and international meetings and through publications in cardiology journals. Reports or publications

resulting from the study will not contain any personal details. On study completion, the research team will provide a lay summary of the results to all participants where appropriate.

What if something goes wrong?
We do not anticipate that anything will go wrong. In the event that something does go wrong, there are no special compensation arrangements. If you are harmed due to someone's negligence, you may have grounds for legal action for compensation against the sponsor (Greater Glasgow and Clyde Health Board/University of Glasgow), but you may have to pay your legal costs. The normal National Health Service complaints mechanisms are available if you wish to complain or have any concerns (Tel: 0141 201 4500, Email:complaints@ggc.scot.nhs.uk)**I**

If you have a complaint about any aspect of the study?
If you are unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance and the normal NHS complaint mechanisms are also available to you (details above).

Who has reviewed the study?
The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from the University of Glasgow and NHS Greater Glasgow and Clyde, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected. The trial has been designed by the RECOVER-LV trial management group.

If you have any further questions?
The researchers will do their best to answer your questions (office hours are Monday-Friday 9am-5pm). You are encouraged to ask questions at any time during the study. We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study and wish to speak to someone **not** closely linked to the study, please contact **Dr John Payne (0141 951 5652 or email jpayne3@nhs.net)**

If you have any further questions about the study, please contact Dr Kieran Docherty on 07946751750 or email kieran.docherty@glasgow.ac.uk.

THANK YOU FOR TAKING THE TIME TO READ THIS INFORMATION AND FOR CONSIDERING TAKING PART

This trial is funded by a Project Grant from the British Heart Foundation (PG/17/23/32850)

Appendix 2: Consent Forms

IRAS ID: 196627 EudraCT: 2017-003460-13 Study Number: GN16CA007

University of Glasgow

NHS Greater Glasgow and Clyde

Screening Consent Form

Screening Number for this trial:

Title of Project: The effects of sacubitril/valsartan compared to valsartan on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction after myocardial infarction: a randomised, double-blinded, active-comparator, cardiac-MR based trial

Investigators: Prof. John J.V. McMurray, Professor of Cardiology, BHF GCRC
Prof. Mark C. Petrie, Professor of Cardiology, BHF GCRC
Dr. Ross T. Campbell, Clinical Lecturer, BHF GCRC
Dr. Kieran F. Docherty, Clinical Research Fellow, BHF GCRC

Please initial the BOX

I confirm that I have read and understand the information sheet dated 06/05/2019 (version 3.0) for the above study and have had the opportunity to ask questions. ☐

I agree to have echocardiography performed for the purposes of study screening and assessment of eligibility. ☐

I agree to have blood tests checked to assess eligibility for the above study. ☐

----------	----------	----------
Name of Participant	Date	Signature

----------	----------	----------
Name of Researcher	Date	Signature

Original to the researcher, 1 copy to the patient, 1 copy for the patients' notes

IRAS ID: 196627 EudraCT: 2017-003460-13 Study Number: GN16CA007

University of Glasgow

NHS Greater Glasgow and Clyde

Consent Form

Participant Identification Number for this trial:

Title of Project: The effects of sacubitril/valsartan compared to valsartan on left ventricular remodelling in patients with asymptomatic left ventricular systolic dysfunction after myocardial infarction: a randomised, double-blinded, active-comparator, cardiac-MR based trial

Investigators: Prof. John J.V. McMurray, Professor of Cardiology, BHF GCRC
Prof. Mark C. Petrie, Professor of Cardiology, BHF GCRC
Dr. Ross T. Campbell, Clinical Lecturer, BHF GCRC
Dr. Kieran F. Docherty, Clinical Research Fellow, BHF GCRC

Please initial the BOX

I confirm that I have read and understand the information sheet dated 06/05/2019 (version 3.0) for the above study and have had the opportunity to ask questions. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

I give permission for my GP to be made aware of my decision to participate in this study. ☐

I agree to give permission to store samples of my blood and urine for 10 years so that any new tests, relating to heart function and heart failure, can be evaluated as part of this study and future ethically approved studies, aimed at understanding mechanisms of drug action. ☐

I understand that the research team may look at sections of my health record (hospital and general practitioner), where it is relevant to my taking part in the research. I give my permission for the research team to contact my general practitioner, and to have access to my records. Only government regulatory authorities, the research doctor and nurse or representatives of the sponsor (NHS Greater Glasgow and Clyde and Glasgow University) will have access to my medical notes. ☐

I understand that anonymised data from the study may be shared with other researchers both in the UK and elsewhere in the world for further analysis. ☐

If the research produces incidental findings of clinical significance, I will be informed of the results and offered appropriate follow-up and treatment. ☐

I agree to take part in the above study ☐

----------	----------	----------
Name of Participant	Date	Signature
----------	----------	----------
Name of Researcher	Date	Signature

Appendix 3: Cardiac MRI imaging acquisition parameters

	Cine (SSFP)	Tagging	T1 map	T2 map	Cardiac perfusion	LGE
Orientation	VLA, HLA, LVOT, SAX stack	VLA, HLA, LVOT, SAX basal/mid/apex	SAX basal/mid/apex	SAX basal/mid/apex	HLA, SAX basal/mid/apex	VLA, HLA, LVOT, SAX stack
Sequence	SSFP	cine_fl2d9_grid	MOLLI	BEAT_MAP_tfl2d 1_58	Dynamic_tfl_sr_e PAT_std_5.5mins _measures	DE_high_res_tfl2 8_psir_seg
TR, ms	LA 41.r	32.040	280.560	207.39	167.000	900.000
TE, ms	1.51	2.540	1.120	1.32	0.980	1.520
Flip angle, °	50	10	35	12	10	60
Field of view, mm*mm	340x287	255.00x340.00	306.56x360.00	360x288	360.00x360.00	262.50x350.00
Matrix	256x173	168x224	218x256	192x116	192x192	192x256
Slice thickness (mm)	7mm	6.000	8.000	8mm	8.000	8.000
Slice gap (mm)	3mm	n/a	n/a	n/a	n/a	n/a
Voxel size (mm^3)	Acquired 1.66x1.33x7mm recon	Acq- 1.9/1.52/6 Recon - 1.52/1.52/6	Acq = 2.13/1.41/8 Recon - 1.41/1.41/8	Acquired - 2.49x1.88x8 Recon 1.87x1.88x8	Acq - 2.54/1.88/8 Recon - 1.88/1.88/8	Acq - 2.86/1.37/8 Recon – 1.37\1.37/8
Number of slices	1 VLA 1 HLA 1 LVOT	VLA,HLA,LVOT, SAX basal/mid/apex	SAX basal/mid/apex	SAX basal/mid/apex	HLA, SAX basal/mid/apex	1 VLA 1 HLA 1 LVOT

	SAX approx 11 (to cover LV)					SAX approx 11 (to cover LV)
Acceleration	Grappa 3	Grappa - 2	Grappa - 2	Grappa 2	Grappa - 2	Grappa 2
Acquisition time (min:sec)	Heart rate dependant ~ 7s/slice	03:00	05:00	Heart rate dependant ~ 7s/slice	15:00	10:00
Bandwidth (Hz/px)	977	446	1085	1184	1184	1221
ECG gating	Retrospective	Prospective	Triggered	Triggered	Triggered	Triggered

Abbreviations: DCE, dynamic contrast-enhanced; ECG, electrocardiogram; FOV, field of view; GRAPPA, GeneRalized Autocalibrating Partial Parallel Acquisition; HLA, horizontal long axis; LGE, late gadolinium enhancement; LV, left ventricle; LVOT, left ventricular outflow tract; MOLLI, modified Look-Locker inversion-recovery; N/A, not applicable; SAX, short axis; SR-TurboFLASH, saturation recovery Turbo Fast Low-Angle Shot; SSFP, steady-state free precession; TE, echo time; TI, inversion time; TR, repetition time; VLA, vertical long axis.

Appendix 4: Patient Global Assessment of Change Questionnaire

RECOVER-LV – Patient Global Assessment

Subject Number: _____ Subject initials: _____ Date: _____

You are participating in a study to determine whether sacubitril/valsartan has a beneficial effect on measurements of heart function compared to the current gold-standard treatment valsartan in patients with heart muscle dysfunction after a previous heart attack. We would like to know if you feel any difference in your general wellbeing since the start of the study on _____

Please indicate your answer to the following question using a tick (√):

How do you feel compared to when you started in the study?

1: ☐ Markedly improved

2: ☐ Moderately improved

3: ☐ Slightly improved

4: ☐ Unchanged

5: ☐ Slightly worsened

6: ☐ Moderately worsened

7: ☐ Markedly worsened

Please take your time to think about your answer and make sure you have only placed one tick. When you are finished, please return this sheet to your doctor or study nurse.

References

1. Heart & Circulatory Disease Statistics 2021 | BHF. https://www.bhf.org.uk/what-we-do/our-research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2021 (June 15, 2021)
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, Djousse L, Elkind MS v, Ferguson JF, Fornage M, Jordan LC, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 2019.**139**:e56–e528.
3. Leading causes of death, UK - Office for National Statistics. https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/causesofdeath/articles/leadingcausesofdeathuk/2001to2018 (June 15, 2021)
4. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, Abbasi-Kangevari M, Abbastabar H, Abd-Allah F, Abdelalim A, Abdollahi M, Abdollahpour I, Abolhassani H, Aboyans V, Abrams EM, Abreu LG, Abrigo MRM, Abu-Raddad LJ, Abushouk AI, Acebedo A, Ackerman IN, Adabi M, Adamu AA, Adebayo OM, Adekanmbi V, Adelson JD, Adetokunboh OO, Adham D, Afshari M, Afshin A, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* 2020.**396**:1204–1222.
5. Sacks NC, Ash AS, Ghosh K, Rosen AK, Wong JB, Rosen AB. Trends in acute myocardial infarction hospitalizations: Are we seeing the whole picture? *American Heart Journal* 2015.**170**.
6. Fox KAA, Steg PG, Eagle KA, Goodman SG, Anderson FA, Granger CB, Flather MD, Budaj A, Quill A, Gore JM, for the GRACE Investigators. Decline in Rates of Death and Heart Failure in Acute Coronary Syndromes, 1999-2006. *JAMA* 2007.**297**:1892.

7. Capewell S. Trends in case-fatality in 117718 patients admitted with acute myocardial infarction in Scotland. *European Heart Journal* 2000.**21**:1833–1840.
8. Gerber Y, Weston SA, Jiang R, Roger VL. The Changing Epidemiology of Myocardial Infarction in Olmsted County, Minnesota, 1995-2012. *The American Journal of Medicine* 2015.**128**:144–151.
9. Yeh RW, Sidney S, Chandra M, Sorel M, Selby J v, Go AS. Population Trends in the Incidence and Outcomes of Acute Myocardial Infarction. *New England Journal of Medicine* 2010.**362**:2155–2165.
10. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Relations between implementation of new treatments and improved outcomes in patients with non-ST-elevation myocardial infarction during the last 20 years: Experiences from SWEDEHEART registry 1995 to 2014. *European Heart Journal* 2018.**39**:3766–3776.
11. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Improved outcomes in patients with ST-elevation myocardial infarction during the last 20 years are related to implementation of evidence-based treatments: Experiences from the SWEDEHEART registry 1995-2014. *European Heart Journal* 2017.**38**:3056–3065.
12. McManus DD, Gore J, Yarzebski J, Spencer F, Lessard D, Goldberg RJ. Recent Trends in the Incidence, Treatment, and Outcomes of Patients with STEMI and NSTEMI. *The American Journal of Medicine* 2011.**124**:40–47.
13. Solomon SD, Skali H, Anavekar NS, Bourgoun M, Barvik S, Ghali JK, Warnica JW, Khrakovskaya M, Arnold JMO, Schwartz Y, Velazquez EJ, Califf RM, McMurray J v, Pfeffer MA. Changes in ventricular size and function in patients treated with valsartan, captopril, or both after myocardial infarction. *Circulation* 2005.**111**:3411–3419.
14. Sutton MSJ, Pfeffer MA, Moye L, Plappert T, Rouleau JL, Lamas G, Rouleau J, Parker JO, Arnold MO, Sussex B, Braunwald E. Cardiovascular death and left ventricular remodeling two years after myocardial infarction: Baseline predictors and impact of long-term use of captopril - Information from the survival and ventricular enlargement (SAVE) trial. *Circulation* 1997.**96**:3294–3299.

15. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *Journal of the American College of Cardiology* 2000.**35**:569–582.
16. Konstam MA, Kramer DG, Patel AR, Maron MS, Udelson JE. Left Ventricular Remodeling in Heart Failure. *JACC: Cardiovascular Imaging* 2011.**4**:98–108.
17. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 1990.**81**:1161–1172.
18. Conrad N, Judge A, Tran J, Mohseni H, Hedgecott D, Crespillo AP, Allison M, Hemingway H, Cleland JG, McMurray JJ v, Rahimi K. Temporal trends and patterns in heart failure incidence: A population-based study of 4 million individuals. *The Lancet* 2017.**391**:572–580.
19. National Institute for Health and Care Excellence. Chronic heart failure in adults: diagnosis and management NICE guideline [NG106]. 2018. https://www.nice.org.uk/guidance/ng106 (August 24, 2021)
20. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *Journal of the American College of Cardiology* 2000.**35**:569–582.
21. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. *The Lancet* 2006.**367**:356–367.
22. Sutton MGStJ, Sharpe N. Left Ventricular Remodeling After Myocardial Infarction. *Circulation* 2000.**101**:2981–2988.
23. Rienks M, Papageorgiou A-P, Frangogiannis NG, Heymans S. Myocardial Extracellular Matrix. *Circulation Research* 2014.**114**:872–888.
24. Tennant R, Wiggers CJ. The Effect of Coronary Occlusion on Myocardial Contraction. *American Journal of Physiology* 1935.**112**:351–361.
25. Cleutjens JP, Kandala JC, Guarda E, Guntaka R v, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. *Journal of Molecular and Cellular Cardiology* 1995.**27**:1281–1292.
26. Olivetti G, Capasso JM, Sonnenblick EH, Anversa P. Side-to-side slippage of myocytes participates in ventricular wall remodeling acutely after myocardial infarction in rats. *Circulation Research* 1990.**67**:23–34.

27. Banfi C, Cavalca V, Veglia F, Brioschi M, Barcella S, Mussoni L, Boccotti L, Tremoli E, Biglioli P, Agostoni P. Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase-2 in human heart failure. *European Heart Journal* 2005.**26**:481–488.
28. Daniels LB, Maisel AS. Natriuretic Peptides. *Journal of the American College of Cardiology* 2007.**50**:2357–2368.
29. Braunwald E, Rutherford JD. Reversible ischemic left ventricular dysfunction: Evidence for the "hibernating myocardium." *Journal of the American College of Cardiology* 1986.**8**:1467–1470.
30. Solomon SD, Glynn RJ, Greaves S, Ajani U, Rouleau J-L, Menapace F, Arnold JMO, Hennekens C, Pfeffer MA. Recovery of Ventricular Function after Myocardial Infarction in the Reperfusion Era: The Healing and Early Afterload Reducing Therapy Study. *Annals of Internal Medicine* 2001.**134**:451.
31. Stillwell GK. The Law of Laplace. Some clinical applications. *Mayo Clinic proceedings* 1973.**48**:863–869.
32. Hirohata S, Kusachi S, Murakami M, Murakami T, Sano I, Watanabe T, Komatsubara I, Kondo J, Tsuji T. Time dependent alterations of serum matrix metalloproteinase-1 and metalloproteinase-1 tissue inhibitor after successful reperfusion of acute myocardial infarction. *Heart* 1997.**78**:278–284.
33. Bujak M, Frangogiannis N. The role of TGF-β signaling in myocardial infarction and cardiac remodeling. *Cardiovascular Research* 2007.**74**:184–195.
34. Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration. *Cell and Tissue Research 2016 365:3* 2016.**365**:563–581.
35. Garg P, Broadbent DA, Swoboda PP, Foley JRJ, Fent GJ, Musa TA, Ripley DP, Erhayiem B, Dobson LE, McDiarmid AK, Haaf P, Kidambi A, Crandon S, Chew PG, Geest RJ van der, Greenwood JP, Plein S. Extra-cellular expansion in the normal, non-infarcted myocardium is associated with worsening of regional myocardial function after acute myocardial infarction. *Journal of Cardiovascular Magnetic Resonance 2017 19:1* 2017.**19**:1–13.

36. White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* 1987.**76**:44–51.
37. St John Sutton M, Pfeffer MA, Moye L, Plappert T, Rouleau JL, Lamas G, Rouleau J, Parker JO, Arnold MO, Sussex B, Braunwald E. Cardiovascular death and left ventricular remodeling two years after myocardial infarction: baseline predictors and impact of long-term use of captopril: information from the Survival and Ventricular Enlargement (SAVE) trial. *Circulation* 1997.**96**:3294–3299.
38. St John Sutton M, Pfeffer MA, Plappert T, Rouleau JL, Moyé LA, Dagenais GR, Lamas GA, Klein M, Sussex B, Goldman S. Quantitative two-dimensional echocardiographic measurements are major predictors of adverse cardiovascular events after acute myocardial infarction. The protective effects of captopril. *Circulation* 1994.**89**:68–75.
39. Bijl P van der, Abou R, Goedemans L, Gersh BJ, Holmes DR, Ajmone Marsan N, Delgado V, Bax JJ. Left Ventricular Post-Infarct Remodeling. *JACC: Heart Failure* 2020.**8**:131–140.
40. Bolognese L, Neskovic AN, Parodi G, Cerisano G, Buonamici P, Santoro GM, Antoniucci D. Left ventricular remodeling after primary coronary angioplasty: patterns of left ventricular dilation and long-term prognostic implications. *Circulation* 2002.**106**:2351–2357.
41. Bulluck H, Carberry J, Carrick D, McEntegart M, Petrie MC, Eteiba H, Hood S, Watkins S, Lindsay M, Mahrous A, Ford I, Oldroyd KG, Berry C. Redefining Adverse and Reverse Left Ventricular Remodeling by Cardiovascular Magnetic Resonance Following ST-Segment–Elevation Myocardial Infarction and Their Implications on Long-Term Prognosis. *Circulation: Cardiovascular Imaging* 2020.**13**:e009937.
42. Brooks GC, Lee BK, Rao R, Lin F, Morin DP, Zweibel SL, Buxton AE, Pletcher MJ, Vittinghoff E, Olgin JE. Predicting Persistent Left Ventricular Dysfunction Following Myocardial Infarction: PREDiction of ICd Treatment Study (PREDICTS). *Journal of the American College of Cardiology* 2016.**67**:1186.
43. Echouffo-Tcheugui JB, Erqou S, Butler J, Yancy CW, Fonarow GC. Assessing the Risk of Progression From Asymptomatic Left Ventricular Dysfunction to

Overt Heart Failure: A Systematic Overview and Meta-Analysis. *JACC: Heart Failure* 2016.**4**:237–248.

44. SOLVD Investigators, Yusuf S, Pitt B, Davis CE, Hood WB, Cohn JN. Effect of Enalapril on Mortality and the Development of Heart Failure in Asymptomatic Patients with Reduced Left Ventricular Ejection Fractions. *New England Journal of Medicine* 1992.**327**:685–691.
45. Pfeffer MA, Lamas GA, Vaughan DE, Parisi AF, Braunwald E. Effect of Captopril on Progressive Ventricular Dilatation after Anterior Myocardial Infarction. *New England Journal of Medicine* 1988.**319**:80–86.
46. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, Klein M, Lamas GA, Packer M, Rouleau J, Rouleau JL, Rutherford J, Wertheimer JH, Hawkins CM. Effect of Captopril on Mortality and Morbidity in Patients with Left Ventricular Dysfunction after Myocardial Infarction. *New England Journal of Medicine* 1992.**327**:669–677.
47. Pfeffer MA, Pfeffer JM, Steinberg C, Finn P. Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. *Circulation* 1985.**72**:406–412.
48. Maroko PR, Braunwald E. Modification of myocardial infarction size after coronary occlusion. *Annals of Internal Medicine* 1973.**79**:720–733.
49. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circulation Research* 1979.**44**:503–512.
50. Pfeffer MA, Pfeffer JM. Ventricular enlargement and reduced survival after myocardial infarction. *Circulation* 1987.**75**:IV93-7.
51. Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI). *Lancet* 1986.**1**:397–402.
52. Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2.ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *Journal of the American College of Cardiology* 1988.**12**:3A-13A.
53. Morrison LJ, Verbeek PR, McDonald AC, Sawadsky B v, Cook DJ. Mortality and prehospital thrombolysis for acute myocardial infarction: A meta-analysis. *JAMA* 2000.**283**:2686–2692.

54. Sheehan FH, Braunwald E, Canner P, Dodge HT, Gore J, Natta P van, Passamani ER, Williams DO, Zaret B. The effect of intravenous thrombolytic therapy on left ventricular function: a report on tissue-type plasminogen activator and streptokinase from the Thrombolysis in Myocardial Infarction (TIMI Phase I) trial. *Circulation* 1987.**75**:817–829.
55. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. *Lancet* 2003.**361**:13–20.
56. O'Neill W, Timmis GC, Bourdillon PD, Lai P, Ganghadarhan V, Walton J, Ramos R, Laufer N, Gordon S, Schork MA, Pitt B. A Prospective Randomized Clinical Trial of Intracoronary Streptokinase versus Coronary Angioplasty for Acute Myocardial Infarction. *New England Journal of Medicine* 1986.**314**:812–818.
57. The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. *The Lancet* 1993.**342**:821–828.
58. Køber L, Torp-Pedersen C, Carlsen JE, Bagger H, Eliasen P, Lyngborg K, Videbæk J, Cole DS, Auclert L, Pauly NC, Aliot E, Persson S, Camm AJ. A Clinical Trial of the Angiotensin-Converting–Enzyme Inhibitor Trandolapril in Patients with Left Ventricular Dysfunction after Myocardial Infarction. *New England Journal of Medicine* 1995.**333**:1670–1676.
59. Pfeffer MA, McMurray JJV, Velazquez EJ, Rouleau J-L, Køber L, Maggioni AP, Solomon SD, Swedberg K, Werf F van de, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM. Valsartan, Captopril, or Both in Myocardial Infarction Complicated by Heart Failure, Left Ventricular Dysfunction, or Both. *New England Journal of Medicine* 2003.**349**:1893–1906.
60. Greenberg B, Quinones MA, Koilpillai C, Limacher M, Shindler D, Benedict C, Shelton B. Effects of long-term enalapril therapy on cardiac structure and function in patients with left ventricular dysfunction. Results of the SOLVD echocardiography substudy. *Circulation* 1995.**91**:2573–2581.
61. Solomon SD, Hee Shin S, Shah A, Skali H, Desai A, Kober L, Maggioni AP, Rouleau JL, Kelly RY, Hester A, McMurray JJV, Pfeffer MA. Effect of the direct renin inhibitor aliskiren on left ventricular remodelling following

myocardial infarction with systolic dysfunction. *European Heart Journal* 2011.**32**:1227–1234.

62. Dargie HJ. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. *The Lancet* 2001.**357**:1385–1390.
63. Doughty RN, Whalley GA, Walsh HA, Gamble GD, López-Sendón J, Sharpe N, CAPRICORN Echo Substudy Investigators. Effects of carvedilol on left ventricular remodeling after acute myocardial infarction: the CAPRICORN Echo Substudy. *Circulation* 2004.**109**:201–206.
64. Colucci WS, Kolias TJ, Adams KF, Armstrong WF, Ghali JK, Gottlieb SS, Greenberg B, Klibaner MI, Kukin ML, Sugg JE. Metoprolol Reverses Left Ventricular Remodeling in Patients With Asymptomatic Systolic Dysfunction. *Circulation* 2007.**116**:49–56.
65. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a Selective Aldosterone Blocker, in Patients with Left Ventricular Dysfunction after Myocardial Infarction. *New England Journal of Medicine* 2003.**348**:1309–1321.
66. Weir RAP, Mark PB, Petrie CJ, Clements S, Steedman T, Ford I, Ng LL, Squire IB, Wagner GS, McMurray JJ v, Dargie HJ. Left ventricular remodeling after acute myocardial infarction: does eplerenone have an effect? *American heart journal* 2009.**157**:1088–1096.
67. Udelson JE, Feldman AM, Greenberg B, Pitt B, Mukherjee R, Solomon HA, Konstam MA. Randomized, Double-Blind, Multicenter, Placebo-Controlled Study Evaluating the Effect of Aldosterone Antagonism With Eplerenone on Ventricular Remodeling in Patients With Mild-to-Moderate Heart Failure and Left Ventricular Systolic Dysfunction. *Circulation: Heart Failure* 2010.**3**:347–353.
68. Boccanelli A, Mureddu GF, Cacciatore G, Clemenza F, Lenarda A di, Gavazzi A, Porcu M, Latini R, Lucci D, Maggioni A pietro, Masson S, Vanasia M, Simone G de. Anti-remodelling effect of canrenone in patients with mild chronic heart failure (AREA IN-CHF study): final results. *European Journal of Heart Failure* 2009.**11**:68–76.
69. Bold AJ de, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sciences* 1981.**28**:89–94.

70. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. *Nature* 1988.**332**:78–81.
71. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proceedings of the National Academy of Sciences of the United States of America* 2000.**97**:8525–8529.
72. Forssmann W-G, Richter R, Meyer M. The endocrine heart and natriuretic peptides: histochemistry, cell biology, and functional aspects of the renal urodilatin system. *Histochemistry and Cell Biology* 1998.**110**:335–357.
73. Semenov AG, Tamm NN, Seferian KR, Postnikov AB, Karpova NS, Serebryanaya D v, Koshkina E v, Krasnoselsky MI, Katrukha AG. Processing of Pro–B-Type Natriuretic Peptide: Furin and Corin as Candidate Convertases2. *Clinical Chemistry* 2010.**56**:1166–1176.
74. Potter LR. Natriuretic peptide metabolism, clearance and degradation. *FEBS Journal* 2011.**278**:1808–1817.
75. Sudoh T, Minamino N, Kangawa K, Matsuo H. C-Type natriuretic peptide (CNP): A new member of natriuretic peptide family identified in porcine brain. *Biochemical and Biophysical Research Communications* 1990.**168**:863–870.
76. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, Bold MK de, Bold AJ de. Cardiac natriuretic peptides. *Nature Reviews Cardiology* 2020.**17**:698–717.
77. Lang CC, Struthers AD. Interactions between atrial natriuretic factor and the autonomic nervous system. *Clinical Autonomic Research* 1991.**1**:329–336.
78. Potter LR, Yoder AR, Flora DR, Antos LK, Dickey DM. Natriuretic Peptides: Their Structures, Receptors, Physiologic Functions and Therapeutic Applications. *Handbook of experimental pharmacology* 2009. p. 341–366.
79. Nishikimi T, Maeda N, Matsuoka H. The role of natriuretic peptides in cardioprotection. *Cardiovascular Research* 2006.**69**:318–328.
80. Vaduganathan M, Claggett B, Packer M, McMurray JJ v, Rouleau JL, Zile MR, Swedberg K, Solomon SD. Natriuretic Peptides as Biomarkers of Treatment Response in Clinical Trials of Heart Failure. *JACC: Heart Failure* 2018.**6**:564–569.
81. Lemos JA de, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, Hall C, Cannon CP, Braunwald E. The Prognostic Value of B-Type

Natriuretic Peptide in Patients with Acute Coronary Syndromes. *New England Journal of Medicine* 2001.**345**:1014–1021.

82. Omland T, Aakvaag A, Bonarjee VVS, Caidahl K, Lie RT, Nilsen DWT, Sundsfjord JA, Dickstein K. Plasma Brain Natriuretic Peptide as an Indicator of Left Ventricular Systolic Function and Long-term Survival after Acute Myocardial Infarction: Comparison with Plasma Atrial Natriuretic Peptide and N-Terminal Proatrial Natriuretic Peptide. *Circulation* 1996.**93**:1963–1969.
83. Omland T. N-Terminal Pro-B-Type Natriuretic Peptide and Long-Term Mortality in Acute Coronary Syndromes. *Circulation* 2002.**106**:2913–2918.
84. Benedict CR, Johnstone DE, Weiner DH, Bourassa MG, Bittner V, Kay R, Kirlin P, Greenberg B, Kohn RM, Nicklas JM, McIntyre K, Quinones MA, Yusuf S, SOLVD investigators. Relation of neurohumoral activation to clinical variables and degree of ventricular dysfunction: A report from the registry of studies of left ventricular dysfunction. *Journal of the American College of Cardiology* 1994.**23**:1410–1420.
85. O'Connor CM, Starling RC, Hernandez AF, Armstrong PW, Dickstein K, Hasselblad V, Heizer GM, Komajda M, Massie BM, McMurray JJV, Nieminen MS, Reist CJ, Rouleau JL, Swedberg K, Adams KF, Anker SD, Atar D, Battler A, Botero R, Bohidar NR, Butler J, Clausell N, Corbalán R, Costanzo MR, Dahlstrom U, Deckelbaum LI, Diaz R, Dunlap ME, Ezekowitz JA, Feldman D, et al. Effect of Nesiritide in Patients with Acute Decompensated Heart Failure. *New England Journal of Medicine* 2011.**365**:32–43.
86. Packer M, O'Connor C, McMurray JJ v, Wittes J, Abraham WT, Anker SD, Dickstein K, Filippatos G, Holcomb R, Krum H, Maggioni AP, Mebazaa A, Peacock WF, Petrie MC, Ponikowski P, Ruschitzka F, Veldhuisen DJ van, Kowarski LS, Schactman M, Holzmeister J. Effect of Ularitide on Cardiovascular Mortality in Acute Heart Failure. *New England Journal of Medicine* 2017.**376**:1956–1964.
87. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, Seguchi O, Myoishi M, Minamino T, Ohara T, Nagai Y, Nanto S, Watanabe K, Fukuzawa S, Hirayama A, Nakamura N, Kimura K, Fujii K, Ishihara M, Saito Y, Tomoike H, Kitamura S. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 2007.**370**:1483–1493.

88. George SG, Kenny AJ. Studies on the enzymology of purified preparations of brush border from rabbit kidney. *Biochemical Journal* 1973.**134**:43–57.
89. Roques BP, Noble F, Daugé V, Fournié-Zaluski MC, Beaumont A. Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. *Pharmacological reviews* 1993.**45**:87–146.
90. Li C, Chen G, Gerard NP, Gerard C, Bozic CR, Hersh LB. Comparison of the structure and expression of the human and rat neprilysin (endopeptidase 24.11)-encoding genes. *Gene* 1995.**164**:363–366.
91. Oefner C, D'Arcy A, Hennig M, Winkler FK, Dale GE. Structure of human neutral endopeptidase (neprilysin) complexed with phosphoramidon. *Journal of Molecular Biology* 2000.**296**:341–349.
92. Oefner C, Roques BP, Fournie-Zaluski MC, Dale GE. Structural analysis of neprilysin with various specific and potent inhibitors. *Acta Crystallographica Section D: Biological Crystallography* 2004.**60**:392–396.
93. Pankow K, Schwiebs A, Becker M, Siems WE, Krause G, Walther T. Structural Substrate Conditions Required for Neutral Endopeptidase-Mediated Natriuretic Peptide Degradation. *Journal of Molecular Biology* 2009.**393**:496–503.
94. Kerr MA, Kenny AJ. The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. *Biochemical Journal* 1974.**137**:477–488.
95. Kerr MA, Kenny AJ. The molecular weight and properties of a neutral metallo-endopeptidase from rabbit kidney brush border. *The Biochemical journal* 1974.**137**:489–495.
96. Roques BP, Noble F, Daugé V, Fournié-Zaluski MC, Beaumont A. Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. *Pharmacological reviews* 1993.**45**:87–146.
97. Spillantini MG, Sicuteri F, Salmon S, Malfroy B. Characterization of endopeptidase 3.4.24.11 ("enkephalinase") activity in human plasma and cerebrospinal fluid. *Biochemical Pharmacology* 1990.**39**:1353–1356.
98. Yandle T, Richards M, Smith M, Charles C, Livesey J, Espiner E. Assay of endopeptidase-24.11 activity in plasma applied to in vivo studies of endopeptidase inhibitors. *Clinical Chemistry* 1992.**38**:1785–1791.
99. Johnson AR, Coalson JJ, Ashton J, Larumbide M, Erdös EG. Neutral endopeptidase in serum samples from patients with adult respiratory

distress syndrome. Comparison with angiotensin-converting enzyme. *American Review of Respiratory Disease* 1985.**132**:1262–1267.

100. Ronco P, Pollard H, Galceran M, Delauche M, Schwartz JC, Verroust P. Distribution of enkephalinase (membrane metalloendopeptidase, E.C. 3.4.24.11) in rat organs. Detection using a monoclonal antibody. *Laboratory Investigation* 1988.**58**:210–217.
101. Mapp PI, Walsh DA, Kidd BL, Cruwys SC, Polak JM, Blake DR. Localization of the enzyme neutral endopeptidase to the human synovium. *Journal of Rheumatology* 1992.**19**:1838–1844.
102. Gee NS, Bowes MA, Buck P, Kenny AJ. An immunoradiometric assay for endopeptidase-24.11 shows it to be a widely distributed enzyme in pig tissues. *Biochemical Journal* 1985.**228**:119–126.
103. Bowes MA, Kenny AJ. Endopeptidase-24.11 in pig lymph nodes. Purification and immunocytochemical localization in reticular cells. *Biochemical Journal* 1986.**236**:801–810.
104. Charles CJ, Espiner EA, Nicholls MG, Richards AM, Yandle TG, Protter A, Kosoglou T. Clearance receptors and endopeptidase 24.11: Equal role in natriuretic peptide metabolism in conscious sheep. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 1996.**271**.
105. Hashimoto Y, Nakao K, Hama N, Imura H, Mori S, Yamaguchi M, Yasuhara M, Hori R. Clearance Mechanisms of Atrial and Brain Natriuretic Peptides in Rats. *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists* 1994.**11**:60–64.
106. Stephenson SL, Kenny a J. The hydrolysis of alpha-human atrial natriuretic peptide by pig kidney microvillar membranes is initiated by endopeptidase-24.11. *The Biochemical journal* 1987.**243**:183–187.
107. Kenny AJ, Bourne A, Ingram J. Hydrolysis of human and pig brain natriuretic peptides, urodilatin, C-type natriuretic peptide and some C-receptor ligands by endopeptidase-24.11. *Biochemical Journal* 1993.**291**:83–88.
108. Lainchbury JG, Nicholls MG, Espiner EA, Ikram H, Yandle TG, Richards AM. Regional plasma levels of cardiac peptides and their response to acute neutral endopeptidase inhibition in man. *Clinical science (Lond)* 1998.**95**:547–555.

109. Roques BP, Fournié-zaluski MC, Soroca E, Lecomte JM, Malfroy B, Llorens C, Schwartz JC. The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. *Nature* 1980.**288**:286–288.
110. Sybertz EJ, Chiu PJ, Vemulapalli S, Pitts B, Foster CJ, Watkins RW, Barnett A, Haslanger MF. SCH 39370, a neutral metalloendopeptidase inhibitor, potentiates biological responses to atrial natriuretic factor and lowers blood pressure in desoxycorticosterone acetate-sodium hypertensive rats. *The Journal of pharmacology and experimental therapeutics* 1989.**250**:624–631.
111. Shepperson NB, Barclay PL, Bennett JA, Samuels GM. Inhibition of neutral endopeptidase (EC 3.4.24.11) leads to an atrial natriuretic factor-mediated natriuretic, diuretic and antihypertensive response in rodents. *Clinical science (London, England : 1979)* 1991.**80**:265–269.
112. Kukkonen P, Vuolteenaho O, Ruskoaho H. Basal and volume expansion-stimulated plasma atrial natriuretic peptide concentrations and hemodynamics in conscious rats: Effects of sch 39.370, an endopeptidase inhibitor, and c-anf-(4-23), a clearance receptor ligand. *Endocrinology* 1992.**130**:755–765.
113. Sala C, Monopoli A, Casati C, Ardeleani G, Ongini E, Zanchetti A, Morganti A. Hemodynamic and humoral effects of chronic treatment with the neutral endopeptidase inhibitor sch 42495 in spontaneously hypertensive rats. *Journal of Cardiovascular Pharmacology* 1994.**23**:703–708.
114. Charles CJ, Espiner EA, Richards AM, Sybertz EJ. Endopeptidase inhibition in angiotensin-induced hypertension: Effect of SCH 39370 in sheep. *Hypertension* 1995.**26**:89–94.
115. Rademaker MT, Charles CJ, Espiner EA, Nicholls MG, Richards AM, Kosoglou T. Neutral endopeptidase inhibition: Augmented atrial and brain natriuretic peptide, haemodynamic and natriuretic responses in ovine heart failure. *Clinical Science* 1996.**91**:283–291.
116. Northridge DB, Newby DE, Rooney E, Norrie J, Dargie HJ. Comparison of the short-term effects of candoxatril, an orally active neutral endopeptidase inhibitor, and frusemide in the treatment of patients with chronic heart failure. *American heart journal* 1999.**138**:1149–1157.
117. Bevan EG, Connell JM, Doyle J, Carmichael HA, Davies DL, Lorimer AR, McInnes GT. Candoxatril, a neutral endopeptidase inhibitor: efficacy and

tolerability in essential hypertension. *Journal of hypertension* 1992.**10**:607–613.

118. McDowell G, Coutie W, Shaw C, Buchanan KD, Struthers AD, Nicholls DP. The effect of the neutral endopeptidase inhibitor drug, candoxatril, on circulating levels of two of the most potent vasoactive peptides. British journal of clinical pharmacology. 1997 Mar.
119. Ferro CJ, Spratt JC, Haynes WG, Webb DJ. Inhibition of Neutral Endopeptidase Causes Vasoconstriction of Human Resistance Vessels In Vivo. *Circulation* 1998.**97**:2323–2330.
120. Dalzell JR, Seed A, Berry C, Whelan CJ, Petrie MC, Padmanabhan N, Clarke A, Biggerstaff F, Hillier C, McMurray JJ v. Effects of neutral endopeptidase (Neprilysin) inhibition on the response to other vasoactive peptides in small human resistance arteries: Studies with thiorphan and omapatrilat. *Cardiovascular Therapeutics* 2014.**32**:13–18.
121. Seymour AA, Swerdel JN, Abboa-Offei B. Antihypertensive activity during inhibition of neutral endopeptidase and angiotensin converting enzyme. *Journal of cardiovascular pharmacology* 1991.**17**:456–465.
122. Robl JA, Sun CQ, Stevenson J, Ryono DE, Simpkins LM, Cimarusti MP, Dejneka T, Slusarchyk WA, Chao S, Stratton L, Misra RN, Bednarz MS, Asaad MM, Cheung HS, Abboa-Offei BE, Smith PL, Mathers PD, Fox M, Schaeffer TR, Seymour AA, Trippodo NC. Dual metalloprotease inhibitors: Mercaptoacetyl-based fused heterocyclic dipeptide mimetics as inhibitors of angiotensin-converting enzyme and neutral endopeptidase. *Journal of Medicinal Chemistry* 1997.**40**:1570–1577.
123. Trippodo NC, Robl JA, Asaad MM, Fox M, Panchal BC, Schaeffer TR. Effects of omapatrilat in low, normal, and high renin experimental hypertension. *American Journal of Hypertension* 1998.**11**:363–372.
124. Mitchell GF, Izzo JL, Lacourcière Y, Ouellet JP, Neutel J, Qian C, Kerwin LJ, Block AJ, Pfeffer MA. Omapatrilat reduces pulse pressure and proximal aortic stiffness in patients with systolic hypertension: Results of the conduit hemodynamics of omapatrilat international research study. *Circulation* 2002.**105**:2955–2961.
125. Campese VM, Lasseter KC, Ferrario CM, Smith WB, Ruddy MC, Grim CE, Smith RD, Vargas R, Habashy MF, Vesterqvist O, Delaney CL, Liao WC.

Omapatrilat versus lisinopril: Efficacy and neurohormonal profile in salt-sensitive hypertensive patients. *Hypertension* 2001.**38**:1342–1348.

126. Trippodo NC, Robl JA, Asaad MM, Fox M, Panchal BC, Schaeffer TR. Effects of omapatrilat in low, normal, and high renin experimental hypertension. *American Journal of Hypertension* 1998.**11**:363–372.
127. Liao W, Vesterqvist O, Delaney C, Jemal M, Ferreira I, Ford N, Swanson B, Uderman H. Pharmacokinetics and pharmacodynamics of the vasopeptidase inhibitor, omapatrilat in healthy subjects. *British Journal of Clinical Pharmacology* 2003.**56**.
128. Rouleau JL, Pfeffer M a, Stewart DJ, Isaac D, Sestier F, Kerut EK, Porter CB, Proulx G, Qian C, Block a J. Comparison of vasopeptidase inhibitor, omapatrilat, and lisinopril on exercise tolerance and morbidity in patients with heart failure: IMPRESS randomised trial. *Lancet* 2000.**356**:615–620.
129. Packer M, Califf RM, Konstam M a., Krum H, McMurray JJ, Rouleau J-LL, Swedberg K. Comparison of omapatrilat and enalapril in patients with chronic heart failure: The omapatrilat versus enalapril randomized trial of utility in reducing events (OVERTURE). *Circulation* 2002.**106**:920–926.
130. The SOLVD Investigators. Effect of Enalapril on Survival in Patients with Reduced Left Ventricular Ejection Fractions and Congestive Heart Failure. *New England Journal of Medicine* 1991.**325**:293–302.
131. Kostis JB, Packer M, Black HR, Schmieder R, Henry D, Levy E. Omapatrilat and enalapril in patients with hypertension: The Omapatrilat Cardiovascular Treatment vs. Enalapril (OCTAVE) trial. *American Journal of Hypertension* 2004.**17**:103–111.
132. Fryer RM, Segreti J, Banfor PN, Widomski DL, Backes BJ, Lin CW, Ballaron SJ, Cox BF, Trevillyan JM, Reinhart GA, Geldern TW. Effect of bradykinin metabolism inhibitors on evoked hypotension in rats: rank efficacy of enzymes associated with bradykinin-mediated angioedema. *British Journal of Pharmacology* 2009.**153**:947–955.
133. Feng L, Karpinski PH, Sutton P, Liu Y, Hook DF, Hu B, Blacklock TJ, Fanwick PE, Prashad M, Godtfredsen S, Ziltener C. LCZ696: A dual-acting sodium supramolecular complex. *Tetrahedron Letters* 2012.**53**:275–276.
134. Gu J, Noe A, Chandra P, Al-Fayoumi S, Ligueros-Saylan M, Sarangapani R, Maahs S, Ksander G, Rigel DF, Jeng AY, Lin TH, Zheng W, Dole WP. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting

angiotensin receptor-neprilysin inhibitor (ARNi). *J Clin Pharmacol* 2010.**50**:401–414.

135. Hegde LG, Yu C, Renner T, Thibodeaux H, Armstrong SR, Park T, Cheruvu M, Olsufka R, Sandvik ER, Lane CE, Budman J, Hill CM, Klein U, Hegde SS. Concomitant angiotensin AT1 receptor antagonism and neprilysin inhibition produces omapatrilat-like antihypertensive effects without promoting tracheal plasma extravasation in the rat. *Journal of cardiovascular pharmacology* 2011.**57**:495–504.
136. Ruilope LM, Dukat A, Böhm M, Lacourcière Y, Gong J, Lefkowitz MP. Blood-pressure reduction with LCZ696, a novel dual-acting inhibitor of the angiotensin II receptor and neprilysin: a randomised, double-blind, placebo-controlled, active comparator study. *The Lancet* 2010.**375**:1255–1266.
137. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau J, Shi VC, Solomon SD, Swedberg K, Zile MR. Dual angiotensin receptor and neprilysin inhibition as an alternative to angiotensin-converting enzyme inhibition in patients with chronic systolic heart failure: Rationale for and design of the Prospective comparison of ARNI with ACEI to Determine Impact. *European Journal of Heart Failure* 2013.**15**:1062–1073.
138. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR. Angiotensin–Neprilysin Inhibition versus Enalapril in Heart Failure. *New England Journal of Medicine* 2014.**371**:993–1004.
139. Docherty KF, Vaduganathan M, Solomon SD, McMurray JJV. Sacubitril/Valsartan: Neprilysin Inhibition 5 Years After PARADIGM-HF. *JACC Heart failure* 2020.**8**:800–810.
140. Velazquez EJ, Morrow DA, DeVore AD, Duffy CI, Ambrosy AP, McCague K, Rocha R, Braunwald E. Angiotensin–Neprilysin Inhibition in Acute Decompensated Heart Failure. *New England Journal of Medicine* 2019.**380**:539–548.
141. Lueder TG von, Wang BH, Kompa AR, Huang L, Webb R, Jordaan P, Atar D, Krum H. Angiotensin receptor neprilysin inhibitor LCZ696 attenuates cardiac remodeling and dysfunction after myocardial infarction by

reducing cardiac fibrosis and hypertrophy. *Circulation: Heart Failure* 2015.**8**:71–78.

142. Kompa AR, Lu J, Weller TJ, Kelly DJ, Krum H, Lueder TG von, Wang BH. Angiotensin receptor neprilysin inhibition provides superior cardioprotection compared to angiotensin converting enzyme inhibition after experimental myocardial infarction. *International Journal of Cardiology* 2018.**258**:192–198.
143. Suematsu Y, Miura SI, Goto M, Matsuo Y, Arimura T, Kuwano T, Imaizumi S, Iwata A, Yahiro E, Saku K. LCZ696, an angiotensin receptor-neprilysin inhibitor, improves cardiac function with the attenuation of fibrosis in heart failure with reduced ejection fraction in streptozotocin-induced diabetic mice. *European Journal of Heart Failure* 2016.**18**:386–393.
144. Boutagy NE, Feher A, Pfau D, Liu Z, Guerrera NM, Freeburg LA, Womack SJ, Hoenes AC, Zeiss C, Young LH, Spinale FG, Sinusas AJ. Dual Angiotensin Receptor-Neprilysin Inhibition With Sacubitril/Valsartan Attenuates Systolic Dysfunction in Experimental Doxorubicin-Induced Cardiotoxicity. *JACC CardioOncology* 2020.**2**:774–787.
145. Maslov MY, Foianini S, Mayer D, Orlov M v., Lovich MA. Synergy between sacubitril and valsartan leads to hemodynamic, antifibrotic, and exercise tolerance benefits in rats with preexisting heart failure. *American Journal of Physiology - Heart and Circulatory Physiology* 2019.**316**:H289–H297.
146. Pfau D, Thorn SL, Zhang J, Mikush N, Renaud JM, Klein R, deKemp RA, Wu X, Hu X, Sinusas AJ, Young LH, Tirziu D. Angiotensin Receptor Neprilysin Inhibitor Attenuates Myocardial Remodeling and Improves Infarct Perfusion in Experimental Heart Failure. *Scientific Reports* 2019.**9**.
147. Kang DH, Park SJ, Shin SH, Hong GR, Lee S, Kim MS, Yun SC, Song JM, Park SW, Kim JJ. Angiotensin Receptor Neprilysin Inhibitor for Functional Mitral Regurgitation. *Circulation* 2019.**139**:1354–1365.
148. Desai AS, Solomon SD, Shah AM, Claggett BL, Fang JC, Izzo J, McCague K, Abbas CA, Rocha R, Mitchell GF, EVALUATE-HF Investigators. Effect of Sacubitril-Valsartan vs Enalapril on Aortic Stiffness in Patients With Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial. *JAMA* 2019.**322**:1–10.
149. Schmieder RE, Wagner F, Mayr M, Delles C, Ott C, Keicher C, Hrabak-Paar M, Heye T, Aichner S, Khder Y, Yates D, Albrecht D, Langenickel T,

Freyhardt P, Janka R, Bremerich J. The effect of sacubitril/valsartan compared to olmesartan on cardiovascular remodelling in subjects with essential hypertension: the results of a randomized, double-blind, active-controlled study. *European Heart Journal* 2017.

150. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, Shi V, Bransford T, Takeuchi M, Gong J, Lefkowitz M, Packer M, McMurray JJV. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: a phase 2 double-blind randomised controlled trial. *Lancet* 2012.**380**:1387–1395.
151. Martens P, Beliën H, Dupont M, Vandervoort P, Mullens W. The reverse remodeling response to sacubitril/valsartan therapy in heart failure with reduced ejection fraction. *Cardiovascular Therapeutics* 2018.**36**:e12435.
152. Romano G, Vitale G, Ajello L, Agnese V, Bellavia D, Caccamo G, Corrado E, Gesaro G di, Falletta C, Franca E la, Minà C, Storniolo SA, Sarullo FM, Clemenza F. The Effects of Sacubitril/Valsartan on Clinical, Biochemical and Echocardiographic Parameters in Patients with Heart Failure with Reduced Ejection Fraction: The "Hemodynamic Recovery." *Journal of Clinical Medicine* 2019.**8**:2165.
153. Januzzi JL, Prescott MF, Butler J, Felker GM, Maisel AS, McCague K, Camacho A, Piña IL, Rocha RA, Shah AM, Williamson KM, Solomon SD, PROVE-HF Investigators. Association of Change in N-Terminal Pro-B-Type Natriuretic Peptide Following Initiation of Sacubitril-Valsartan Treatment With Cardiac Structure and Function in Patients With Heart Failure With Reduced Ejection Fraction. *JAMA* 2019.**322**:1–11.
154. Liu L-W, Wu P-C, Chiu M-Y, Tu P-F, Fang C-C. Sacubitril/Valsartan Improves Left Ventricular Ejection Fraction and Reverses Cardiac Remodeling in Taiwanese Patients with Heart Failure and Reduced Ejection Fraction. *Acta Cardiologica Sinica* 2020.**36**:125.
155. Castrichini M, Manca P, Nuzzi V, Barbati G, Luca A de, Korcova R, Stolfo D, Lenarda A di, Merlo M, Sinagra G. Sacubitril/Valsartan Induces Global Cardiac Reverse Remodeling in Long-Lasting Heart Failure with Reduced Ejection Fraction: Standard and Advanced Echocardiographic Evidences. *Journal of Clinical Medicine* 2020.**9**:906.
156. Landolfo M, Piani F, Esposti DD, Cosentino E, Bacchelli S, Dormi A, Borghi C. Effects of sacubitril valsartan on clinical and echocardiographic

parameters of outpatients with heart failure and reduced ejection fraction. *International Journal of Cardiology Heart & Vasculature* 2020.**31**.

157. Paolini C, Mugnai G, Dalla Valle C, Volpiana A, Ferraglia A, Frigo AC, Bilato C. Effects and clinical implications of sacubitril/valsartan on left ventricular reverse remodeling in patients affected by chronic heart failure: A 24-month follow-up. *IJC Heart & Vasculature* 2021.**35**:100821.
158. Guerra F, Ammendola E, Ziacchi M, Aspromonte V, Pellegrino PL, Giorno G del, Dell'Era G, Pimpini L, Santoro F, Floris R, Stronati G, Nigro G, Paolisso P, Guido A, Maglia G, Brunetti ND, Carbone A, Gravellone M, Antonicelli R, Cannone M, Accogli M, Russo A dello, Palmisano P. Effect of SAcubitril/Valsartan on left vEntricular ejection fraction and on the potential indication for Implantable Cardioverter Defibrillator in primary prevention: the SAVE-ICD study. *European Journal of Clinical Pharmacology 2021* 2021.:1–8.
159. Rezq A, Saad M, Nozahi M el. Comparison of the Efficacy and Safety of Sacubitril/Valsartan versus Ramipril in Patients With ST-Segment Elevation Myocardial Infarction. *The American Journal of Cardiology* 2021.**143**:7–13.
160. Nichols M, Townsend N, Scarborough P, Rayner M. Trends in age-specific coronary heart disease mortality in the European Union over three decades: 1980-2009. *European Heart Journal* 2013.**34**:3017–3027.
161. Velagaleti RS, Pencina MJ, Murabito JM, Wang TJ, Parikh NI, D'Agostino RB, Levy D, Kannel WB, Vasan RS. Long-term trends in the incidence of heart failure after myocardial infarction. *Circulation* 2008.**118**:2057–2062.
162. Ezekowitz JA, Kaul P, Bakal JA, Armstrong PW, Welsh RC, McAlister FA. Declining In-Hospital Mortality and Increasing Heart Failure Incidence in Elderly Patients With First Myocardial Infarction. *Journal of the American College of Cardiology* 2009.**53**:13–20.
163. Yeh RW, Sidney S, Chandra M, Sorel M, Selby J V., Go AS. Population Trends in the Incidence and Outcomes of Acute Myocardial Infarction. *New England Journal of Medicine* 2010.**362**:2155–2165.
164. McManus DD, Gore J, Yarzebski J, Spencer F, Lessard D, Goldberg RJ. Recent trends in the incidence, treatment, and outcomes of patients with STEMI and NSTEMI. *American Journal of Medicine* 2011.**124**:40–47.
165. Tisminetzky M, Coukos JA, McManus DD, Darling CE, Joffe S, Gore J, Lessard D, Goldberg RJ. Decade-long trends in the magnitude, treatment,

and outcomes of patients aged 30 to 54 years hospitalized with ST-segment elevation and non-ST-segment elevation myocardial infarction. *American Journal of Cardiology* 2014.

166. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Relations between implementation of new treatments and improved outcomes in patients with non-ST-elevation myocardial infarction during the last 20 years: Experiences from SWEDEHEART registry 1995 to 2014. *European Heart Journal* 2018.**39**:3766–3776.
167. Kendrick S, Clarke J. The Scottish Record Linkage System. *Health bulletin* 1993.**51**:72–79.
168. The West of Scotland Coronary Prevention Study Group. Computerised record linkage: compared with traditional patient follow-up methods in clinical trials and illustrated in a prospective epidemiological study. *Journal of clinical epidemiology* 1995.**48**:1441–1452.
169. The Scottish Government. Scottish Index of Multiple Deprivation. https://www.gov.scot/Topics/Statistics/SIMD (September 17, 2018)
170. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. *Statistics in Medicine* 2000.**19**:335–351.
171. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association* 1999.**94**:496.
172. NHS National Services Scotland. ISD Scotland Coding guidelines- Number 26 October 2010. http://www.isdscotland.org/Products-and-Services/Terminology-Services/Clinical-Coding-Guidelines/Docs/coding-guidelines-october10_26_amended.pdf (September 20, 2018)
173. Lewis EF, Velazquez EJ, Solomon SD, Hellkamp AS, McMurray JJV V, Mathias J, Rouleau JL, Maggioni AP, Swedberg K, Kober L, White H, Dalby AJ, Francis GS, Zannad F, Califf RM, Pfeffer MA. Predictors of the first heart failure hospitalization in patients who are stable survivors of myocardial infarction complicated by pulmonary congestion and/or left ventricular dysfunction: A VALIANT study. *European Heart Journal* 2008.**29**:748–756.

174. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Improved outcomes in patients with ST-elevation myocardial infarction during the last 20 years are related to implementation of evidence-based treatments: Experiences from the SWEDEHEART registry 1995-2014. *European Heart Journal* 2017.**38**:3056–3065.
175. Gerber Y, Weston SA, Enriquez-Sarano M, Berardi C, Chamberlain AM, Manemann SM, Jiang R, Dunlay SM, Roger VL. Mortality Associated with Heart Failure after Myocardial Infarction: A Contemporary Community Perspective. *Circulation: Heart Failure* 2016.**9**:1–10.
176. Shafazand M, Rosengren A, Lappas G, Swedberg K, Schaufelberger M. Decreasing trends in the incidence of heart failure after acute myocardial infarction from 1993-2004: a study of 175,216 patients with a first acute myocardial infarction in Sweden. *European journal of heart failure* 2011.**13**:135–141.
177. Desta L, Jernberg T, Spaak J, Hofman-Bang C, Persson H. Risk and predictors of readmission for heart failure following a myocardial infarction between 2004 and 2013: A Swedish nationwide observational study. *International Journal of Cardiology* 2017.**248**:221–226.
178. Hung J, Teng TK, Finn J, Knuiman M, Briffa T, Stewart S, Sanfilippo FM, Ridout S, Hobbs M. Trends From 1996 to 2007 in Incidence and Mortality Outcomes of Heart Failure After Acute Myocardial Infarction: A Population-Based Study of 20 812 Patients With First Acute Myocardial Infarction in Western Australia. *Journal of the American Heart Association* 2013.**2**.
179. Gjesing A, Gislason GH, Køber L, Gustav Smith J, Christensen SB, Gustafsson F, Olsen AMS, Torp-Pedersen C, Andersson C. Nationwide trends in development of heart failure and mortality after first-time myocardial infarction 1997–2010: A Danish cohort study. *European Journal of Internal Medicine* 2014.**25**:731–738.
180. Gho JMIH, Schmidt AF, Pasea L, Koudstaal S, Pujades-Rodriguez M, Denaxas S, Shah AD, Patel RS, Gale CP, Hoes AW, Cleland JG, Hemingway H, Asselbergs FW. An electronic health records cohort study on heart failure following myocardial infarction in England: Incidence and predictors. *BMJ Open* 2018.**8**:1–10.

181. Chen J, Hsieh AF-C, Dharmarajan K, Masoudi FA, Krumholz HM. National Trends in Heart Failure Hospitalization After Acute Myocardial Infarction for Medicare Beneficiaries. *Circulation* 2013.**128**:2577–2584.
182. Wellings J, Kostis JB, Sargsyan D, Cabrera J, Kostis WJ, Myocardial Infarction Data Acquisition System (MIDAS 31) Study Group. Risk Factors and Trends in Incidence of Heart Failure Following Acute Myocardial Infarction. *The American Journal of Cardiology* 2018.**122**:1–5.
183. Sulo G, Igland J, Nygård O, Vollset SE, Ebbing M, Cerqueira C, Egeland GM, Jørgensen T, Tell GS. Trends in the risk of early and late-onset heart failure as an adverse outcome of acute myocardial infarction: A Cardiovascular Disease in Norway project. *European Journal of Preventive Cardiology* 2017.**24**:971–980.
184. Desta L, Jernberg T, Löfman I, Hofman-Bang C, Hagerman I, Spaak J, Persson H. Incidence, Temporal Trends, and Prognostic Impact of Heart Failure Complicating Acute Myocardial Infarction. *JACC: Heart Failure* 2015.**3**:234–242.
185. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005.**366**:1267–1278.
186. Kastrati A, Dibra A, Spaulding C, Laarman GJ, Menichelli M, Valgimigli M, Lorenzo E Di, Kaiser C, Tierala I, Mehilli J, Seyfarth M, Varenne O, Dirksen MT, Percoco G, Varricchio A, Pittl U, Syvänne M, Suttorp MJ, Violini R, Schömig A. Meta-analysis of randomized trials on drug-eluting stents vs. bare-metal stents in patients with acute myocardial infarction. *European Heart Journal* 2007.**28**:2706–2713.
187. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *The New England journal of medicine* 2009.**361**:1045–1057.
188. Roger VL, Weston SA, Gerber Y, Killian JM, Dunlay SM, Jaffe AS, Bell MR, Kors J, Yawn BP, Jacobsen SJ. Trends in incidence, severity, and outcome of hospitalized myocardial infarction. *Circulation* 2010.**121**:863–869.

189. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Relations between implementation of new treatments and improved outcomes in patients with non-ST-elevation myocardial infarction during the last 20 years: experiences from SWEDEHEART registry 1995 to 2014. *European Heart Journal* 2018.**39**:3766–3776.
190. Kontos MC, Fritz LM, Anderson FP, Tatum JL, Ornato JP, Jesse RL. Impact of the troponin standard on the prevalence of acute myocardial infarction. *American Heart Journal* 2003.**146**:446–452.
191. Arruda-Olson AM, Pellikka PA, Bursi F, Jaffe AS, Santrach PJ, Kors JA, Killian JM, Weston SA, Roger VL. Left ventricular function and heart failure in myocardial infarction: Impact of the new definition in the community. *American Heart Journal* 2008.**156**:810–815.
192. Thygesen K, Alpert JS, White HD, on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction: *European Heart Journal* 2007.**28**:2525–2538.
193. Rush CJ, Campbell RT, Jhund PS, Connolly EC, Preiss D, Gardner RS, Petrie MC, McMurray JJ V. Falling Cardiovascular Mortality in Heart Failure With Reduced Ejection Fraction and Implications for Clinical Trials. *JACC Heart failure* 2015.**3**:603–614.
194. Shen L, Jhund PS, Petrie MC, Claggett BL, Barlera S, Cleland JGF, Dargie HJ, Granger CB, Kjekshus J, Køber L, Latini R, Maggioni AP, Packer M, Pitt B, Solomon SD, Swedberg K, Tavazzi L, Wikstrand J, Zannad F, Zile MR, McMurray JJV. Declining Risk of Sudden Death in Heart Failure. *New England Journal of Medicine* 2017.**377**:41–51.
195. Forman DE, Chen AY, Wiviott SD, Wang TY, Magid DJ, Alexander KP. Comparison of Outcomes in Patients Aged <75, 75 to 84, and ≥85 Years With ST-Elevation Myocardial Infarction (from the ACTION Registry-GWTG). *The American Journal of Cardiology* 2010.**106**:1382–1388.
196. Macchia A, Romero M, D'Ettorre A, Mariani J, Tognoni G. Temporal trends of the gaps in post-myocardial infarction secondary prevention strategies of co-morbid and elderly populations vs. younger counterparts: an analysis of three successive cohorts between 2003 and 2008. *European Heart Journal* 2012.**33**:515–522.

197. Gustafsson I, Hvelplund A, Hansen KW, Galatius S, Madsen M, Jensen JS, Tilsted H-H, Terkelsen CJ, Jensen LO, Jørgensen E, Madsen JK, Abildstrøm SZ. Underuse of an invasive strategy for patients with diabetes with acute coronary syndrome: a nationwide study. *Open Heart* 2015.**2**:e000165.
198. Anselmino M, Malmberg K, Öhrvik J, Rydén L, Euro Heart Survey Investigators. Evidence-based medication and revascularization: powerful tools in the management of patients with diabetes and coronary artery disease: a report from the Euro Heart Survey on diabetes and the heart. *European Journal of Cardiovascular Prevention & Rehabilitation* 2008.**15**:216–223.
199. Bagai A, Lu D, Lucas J, Goyal A, Herzog CA, Wang TY, Goodman SG, Roe MT. Temporal trends in utilization of cardiac therapies and outcomes for myocardial infarction by degree of chronic kidney disease: A report from the NCDR Chest Pain-MI registry. *Journal of the American Heart Association* 2018.**7**.
200. Kelly DJ, Gershlick T, Witzenbichler B, Guagliumi G, Fahy M, Dangas G, Mehran R, Stone GW. Incidence and predictors of heart failure following percutaneous coronary intervention in ST-segment elevation myocardial infarction: The HORIZONS-AMI trial. *American Heart Journal* 2011.
201. Lam CSP, McEntegart M, Claggett B, Liu J, Skali H, Lewis E, Køber L, Rouleau J, Velazquez E, Califf R, McMurray JJ, Pfeffer M, Solomon S. Sex differences in clinical characteristics and outcomes after myocardial infarction: Insights from the Valsartan in Acute Myocardial Infarction Trial (VALIANT). *European Journal of Heart Failure* 2015.
202. Nguyen HL, Saczynski JS, Gore JM, Goldberg RJ. Age and sex differences in duration of prehospital delay in patients with acute myocardial infarction a systematic review. *Circulation: Cardiovascular Quality and Outcomes* 2010.
203. Redfors B, Angerås O, Råmunddal T, Petursson P, Haraldsson I, Dworeck C, Odenstedt J, Ioaness D, Ravn-Fischer A, Wellin P, Sjöland H, Tokgozoglu L, Tygesen H, Frick E, Roupe R, Albertsson P, Omerovic E. Trends in Gender Differences in Cardiac Care and Outcome After Acute Myocardial Infarction in Western Sweden: A Report From the Swedish Web System for Enhancement of Evidence-Based Care in Heart Disease Evaluated According

to Recommended Therapies (SWEDEH. *Journal of the American Heart Association* 2015.**4**.

204. Mehta LS, Beckie TM, DeVon HA, Grines CL, Krumholz HM, Johnson MN, Lindley KJ, Vaccarino V, Wang TY, Watson KE, Wenger NK. Acute Myocardial Infarction in Women : A Scientific Statement from the American Heart Association. *Circulation* 2016.**133**:916–947.
205. Bucholz EM, Strait KM, Dreyer RP, Lindau ST, D'Onofrio G, Geda M, Spatz ES, Beltrame JF, Lichtman JH, Lorenze NP, Bueno H, Krumholz HM. Sex differences in young patients with acute myocardial infarction: A VIRGO study analysis. *European Heart Journal: Acute Cardiovascular Care* 2017.**6**:610–622.
206. Wilkinson C, Bebb O, Dondo TB, Munyombwe T, Casadei B, Clarke S, Schiele F, Timmis A, Hall M, Gale CP. Sex differences in quality indicator attainment for myocardial infarction: a nationwide cohort study. *Heart* 2019.**105**:516–523.
207. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, Bold MK de, Bold AJ de. Cardiac natriuretic peptides. *Nature Reviews Cardiology* 2020.**17**:698–717.
208. Bayes-Genis A, Barallat J, Richards AM. A Test in Context: Neprilysin: Function, Inhibition, and Biomarker. *Journal of the American College of Cardiology* 2016.**68**:639–653.
209. Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, Borger MA, Mario C Di, Dickstein K, Ducrocq G, Fernandez-Aviles F, Gershlick AH, Giannuzzi P, Halvorsen S, Huber K, Juni P, Kastrati A, Knuuti J, Lenzen MJ, Mahaffey KW, Valgimigli M, 't Hof A van, Widimsky P, Zahger D. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *European heart journal* 2012.**33**:2569–2619.
210. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Katus HA, Lindahl B, Morrow DA, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow RO, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand J-P, Menasché P, Ravkilde J, Ohman EM, Antman EM, et al. Third universal definition of myocardial infarction. *Circulation* 2012.**126**:2020–2035.
211. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P,

Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt J-U. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European heart journal cardiovascular Imaging* 2015.**16**:233–270.

212. Hundley WG, Bluemke D, Bogaert JG, Friedrich MG, Higgins CB, Lawson MA, McConnell M v, Raman S v, Rossum AC van, Flamm S, Kramer CM, Nagel E, Neubauer S. Society for Cardiovascular Magnetic Resonance guidelines for reporting cardiovascular magnetic resonance examinations. *Journal of Cardiovascular Magnetic Resonance* 2009.**11**:5.
213. Petersen SE, Aung N, Sanghvi MM, Zemrak F, Fung K, Paiva JM, Francis JM, Khanji MY, Lukaschuk E, Lee AM, Carapella V, Kim YJ, Leeson P, Piechnik SK, Neubauer S. Reference ranges for cardiac structure and function using cardiovascular magnetic resonance (CMR) in Caucasians from the UK Biobank population cohort. *Journal of Cardiovascular Magnetic Resonance* 2017.**19**:18.
214. Heydari B, Abdullah S, Pottala J V., Shah R, Abbasi S, Mandry D, Francis SA, Lumish H, Ghoshhajra BB, Hoffmann U, Appelbaum E, Feng JH, Blankstein R, Steigner M, McConnell JP, Harris W, Antman EM, Jerosch-Herold M, Kwong RY, Mozaffarian D, Rimm EB, Caterina R De, Investigators G-P, Group OS, Chan W, Duffy SJ, White DA, Gao XM, Du XJ, Ellims AH, et al. Effect of Omega-3 Acid Ethyl Esters on Left Ventricular Remodeling After Acute Myocardial InfarctionClinical Perspective. *Circulation* 2016.**134**:1885–1899.
215. Bellenger NG, Davies LC, Francis JM, Coats AJ, Pennell DJ. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance* 2000.**2**:271–278.
216. Grothues F, Smith GC, Moon JCC, Bellenger NG, Collins P, Klein HU, Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *The American Journal of Cardiology* 2002.**90**:29–34.

217. Sharpe N, Murphy J, Smith H, Hannan S. Preventive treatment of asymptomatic left ventricular dysfunction following myocardial infarction. *European Heart Journal* 1990.**11**:147–156.
218. Sharpe N, Smith H, Murphy J, Greaves S, Hart H, Gamble G. Early prevention of left ventricular dysfunction after myocardial infarction with angiotensin-converting-enzyme inhibition. *The Lancet* 1991.**337**:872–876.
219. Kramer DG, Trikalinos TA, Kent DM, Antonopoulos G v, Konstam MA, Udelson JE. Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction: a meta-analytic approach. *Journal of the American College of Cardiology* 2010.**56**:392–406.
220. Ponikowski P Voors A Anker S Bueno H Cleland J et. al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). *European heart journal* 2016.**37**:2129–2200.
221. ACE Inhibitor Myocardial Infarction Collaborative Group: M.G. Franzosi, E. Santoro, G. Zuanetti C, Baigent, R. Collins, M. Flather, J. Kjekshus RL, L.S. Liu, A.P. Maggioni, P. Sleight, K. Swedberg GT, Yusuf S. Clinical Investigation and Reports Indications for ACE Inhibitors in the Early Treatment of Acute Myocardial Infarction. 1998.:2202–2212.
222. Packer M, McMurray JJ v, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Belohlávek J, Böhm M, Boytsov S, Burgess LJ, Cabrera W, Calvo C, Chen CH, Dukat A, Duarte YC, Erglis A, Fu M, Gomez E, Gonzàlez-Medina A, Hagège AA, Huang J, Katova T, et al. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation* 2015.**131**:54–61.
223. Lueder TG von, Wang BH, Kompa AR, Huang L, Webb R, Jordaan P, Atar D, Krum H. Angiotensin Receptor Neprilysin Inhibitor LCZ696 Attenuates Cardiac Remodeling and Dysfunction After Myocardial Infarction by Reducing Cardiac Fibrosis and Hypertrophy. *Circulation Heart failure* 2015.**8**:71–78.

224. Gardner BI, Bingham SE, Allen MR, Blatter DD, Anderson JL. Cardiac magnetic resonance versus transthoracic echocardiography for the assessment of cardiac volumes and regional function after myocardial infarction: an intrasubject comparison using simultaneous intrasubject recordings. *Cardiovascular Ultrasound* 2009.**7**:38.
225. Bellenger NG, Davies LC, Francis JM, Coats AJ, Pennell DJ. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance* 2000.**2**:271–278.
226. Pennell DJ. Cardiovascular magnetic resonance. *Circulation* 2010.**121**:692–705.
227. Migrino RQ, Young JB, Ellis SG, White HD, Lundergan CF, Miller DP, Granger CB, Ross AM, Califf RM, Topol EJ. End-systolic volume index at 90 to 180 minutes into reperfusion therapy for acute myocardial infarction is a strong predictor of early and late mortality. The Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-I Angiograph. *Circulation* 1997.**96**:116–121.
228. Eitel I, Pöss J, Jobs A, Eitel C, Waha S de, Barkhausen J, Desch S, Thiele H. Left ventricular global function index assessed by cardiovascular magnetic resonance for the prediction of cardiovascular events in ST-elevation myocardial infarction. *Journal of Cardiovascular Magnetic Resonance* 2015.**17**:1–9.
229. Ugander M, Oki AJ, Hsu LY, Kellman P, Greiser A, Aletras AH, Sibley CT, Chen MY, Patricia Bandettini W, Arai AE. Extracellular volume imaging by magnetic resonance imaging provides insights into overt and sub-clinical myocardial pathology. *European Heart Journal* 2012.**33**:1268.
230. Wong TC, Piehler K, Meier CG, Testa SM, Klock AM, Aneizi AA, Shakesprere J, Kellman P, Shroff SG, Schwartzman DS, Mulukutla SR, Simon MA, Schelbert EB. Association Between Extracellular Matrix Expansion Quantified by Cardiovascular Magnetic Resonance and Short Term Mortality. *Circulation* 2012.**126**:1206.
231. Boyle AJ, Schultz C, Selvanayagam JB, Moir S, Kovacs R, Dib N, Zlotnick D, Al-Omary M, Sugito S, Selvarajah A, Collins N, McLachlan G. Calcium/Calmodulin-Dependent Protein Kinase II Delta Inhibition and

Ventricular Remodeling After Myocardial Infarction. *JAMA Cardiology* 2021.**6**:762.

232. Heydari B, Abdullah S, Pottala J v, Shah R, Abbasi S, Mandry D, Francis SA, Lumish H, Ghoshhajra BB, Hoffmann U, Appelbaum E, Feng JH, Blankstein R, Steigner M, McConnell JP, Harris W, Antman EM, Jerosch-Herold M, Kwong RY, Mozaffarian D, Rimm EB, Caterina R de, Investigators G-P, Group OS, Chan W, Duffy SJ, White DA, Gao XM, Du XJ, Ellims AH, et al. Effect of Omega-3 Acid Ethyl Esters on Left Ventricular Remodeling After Acute Myocardial InfarctionClinical Perspective. *Circulation* 2016.**134**:1885–1899.
233. Dickstein K, Bebchuk J, Wittes J. The high-risk myocardial infarction database initiative. *Progress in Cardiovascular Diseases* 2012.**54**:362–366.
234. Lewis EF, Moye LA, Rouleau JL, Sacks FM, Arnold JMO, Warnica JW, Flaker GC, Braunwald E, Pfeffer MA. Predictors of Late Development of Heart Failure in Stable Survivors of Myocardial Infarction: The CARE Study. *Journal of the American College of Cardiology* 2003.**42**:1446–1453.
235. Lewis EF, Velazquez EJ, Solomon SD, Hellkamp AS, McMurray JJV v, Mathias J, Rouleau JL, Maggioni AP, Swedberg K, Kober L, White H, Dalby AJ, Francis GS, Zannad F, Califf RM, Pfeffer MA. Predictors of the first heart failure hospitalization in patients who are stable survivors of myocardial infarction complicated by pulmonary congestion and/or left ventricular dysfunction: A VALIANT study. *European Heart Journal* 2008.**29**:748–756.
236. Jering KS, Claggett B, Pfeffer MA, Granger C, Køber L, Lewis EF, Maggioni AP, Mann D, McMurray JJV, Rouleau J, Solomon SD, Steg PG, Meer P, Wernsing M, Carter K, Guo W, Zhou Y, Lefkowitz M, Gong J, Wang Y, Merkely B, Macin SM, Shah U, Nicolau JC, Braunwald E. Prospective ARNI vs. ACE inhibitor trial to DetermIne Superiority in reducing heart failure Events after Myocardial Infarction (PARADISE-MI): design and baseline characteristics. *European Journal of Heart Failure* 2021.**23**:1040–1048.
237. Pfeffer MA, Claggett B, Lewis EF, Granger CB, Køber L, Maggioni AP, Mann DL, McMurray JJV, Rouleau J-L, Solomon SD, Steg PG, Berwanger O, Cikes M, Pasquale CG de, East C, Fernandez A, Jering K, Landmesser U, Mehran R, Merkely B, Vaghaiwalla Mody F, Petrie MC, Petrov I, Schou M, Senni M, Sim D, Meer P van der, Lefkowitz M, Zhou Y, Gong J, et al. Angiotensin

Receptor–Neprilysin Inhibition in Acute Myocardial Infarction. *New England Journal of Medicine* 2021.**385**:1845–1855.

238. Oh PC, Choi IS, Ahn T, Moon J, Park Y, Seo JG, Suh SY, Ahn Y, Jeong MH. Predictors of Recovery of Left Ventricular Systolic Dysfunction after Acute Myocardial Infarction: From the Korean Acute Myocardial Infarction Registry and Korean Myocardial Infarction Registry. *Korean Circulation Journal* 2013.**43**:527.
239. McCartney PJ, Eteiba H, Maznyczka AM, McEntegart M, Greenwood JP, Muir DF, Chowdhary S, Gershlick AH, Appleby C, Cotton JM, Wragg A, Curzen N, Oldroyd KG, Lindsay M, Rocchiccioli JP, Shaukat A, Good R, Watkins S, Robertson K, Malkin C, Martin L, Gillespie L, Ford TJ, Petrie MC, Macfarlane PW, Tait RC, Welsh P, Sattar N, Weir RA, Fox KA, et al. Effect of Low-Dose Intracoronary Alteplase During Primary Percutaneous Coronary Intervention on Microvascular Obstruction in Patients With Acute Myocardial Infarction: A Randomized Clinical Trial. *JAMA* 2019.**321**:56–68.
240. Groenning BA, Nilsson JC, Sondergaard L, Fritz-Hansen T, Larsson HBW, Hildebrandt PR. Antiremodeling effects on the left ventricle during beta-blockade with metoprolol in the treatment of chronic heart failure. *Journal of the American College of Cardiology* 2000.**36**:2072–2080.
241. Colucci WS, Kolias TJ, Adams KF, Armstrong WF, Ghali JK, Gottlieb SS, Greenberg B, Klibaner MI, Kukin ML, Sugg JE, REVERT Study Group. Metoprolol Reverses Left Ventricular Remodeling in Patients With Asymptomatic Systolic Dysfunction: The REversal of VEntricular Remodeling with Toprol-XL (REVERT) Trial. *Circulation* 2007.**116**:49–56.
242. Bulluck H, Go YY, Crimi G, Ludman AJ, Rosmini S, Abdel-Gadir A, Bhuva AN, Treibel TA, Fontana M, Pica S, Raineri C, Sirker A, Herrey AS, Manisty C, Groves A, Moon JC, Hausenloy DJ. Defining left ventricular remodeling following acute ST-segment elevation myocardial infarction using cardiovascular magnetic resonance. *Journal of Cardiovascular Magnetic Resonance* 2017.**19**:26.
243. Konstam MA, Kronenberg MW, Rousseau MF, Udelson JE, Melin J, Stewart D, Dolan N, Edens TR, Ahn S, Kinan D, al. E, Howe DM, Kilcoyne L, Metherall J, Benedict C, Yusuf S, Pouleur H. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left

ventricular dilatation in patients with asymptomatic systolic dysfunction. *Circulation* 1993.**88**:2277–2283.

244. Konstam MA, Rousseau MF, Kronenberg MW, Udelson JE, Melin J, Stewart D, Dolan N, Edens TR, Ahn S, Kinan D. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. SOLVD Investigators. *Circulation* 1992.**86**:431–438.
245. Jenkins C, Bricknell K, Chan J, Hanekom L, Marwick TH. Comparison of Two- and Three-Dimensional Echocardiography With Sequential Magnetic Resonance Imaging for Evaluating Left Ventricular Volume and Ejection Fraction Over Time in Patients With Healed Myocardial Infarction. *The American Journal of Cardiology* 2007.**99**:300–306.
246. Gardner BI, Bingham SE, Allen MR, Blatter DD, Anderson JL. Cardiac magnetic resonance versus transthoracic echocardiography for the assessment of cardiac volumes and regional function after myocardial infarction: an intrasubject comparison using simultaneous intrasubject recordings. *Cardiovascular Ultrasound 2009 7:1* 2009.**7**:1–7.
247. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 1990.**82**:1724–1729.
248. Effects of metoprolol CR in patients with ischemic and dilated cardiomyopathy : the randomized evaluation of strategies for left ventricular dysfunction pilot study. *Circulation* 2000.**101**:378–384.
249. Ceconi C, Freedman SB, Tardif JC, Hildebrandt P, McDonagh T, Gueret P, Parrinello G, Robertson M, Steg PG, Tendera M, Ford I, Fox K, Ferrari R. Effect of heart rate reduction by ivabradine on left ventricular remodeling in the echocardiographic substudy of BEAUTIFUL. *International Journal of Cardiology* 2011.**146**:408–414.
250. Tardif J-C, O'Meara E, Komajda M, Böhm M, Borer JS, Ford I, Tavazzi L, Swedberg K, SHIFT Investigators. Effects of selective heart rate reduction with ivabradine on left ventricular remodelling and function: results from the SHIFT echocardiography substudy. *European heart journal* 2011.**32**:2507–2515.

251. Dickstein K, Kjekshus J. Effects of losartan and captopril on mortality and morbidity in high-risk patients after acute myocardial infarction: the OPTIMAAL randomised trial. *The Lancet* 2002.**360**:752–760.
252. Konstam MA, Neaton JD, Dickstein K, Drexler H, Komajda M, Martinez FA, Riegger GAJ, Curie-paris PM. Effects of high-dose versus low-dose losartan on clinical outcomes in patients with heart failure (HEAAL study): a randomised , double-blind trial. *The Lancet* 2009.**374**:1840–1848.
253. Tsutamoto T. Plasma brain natriuretic peptide level as a biochemical marker of morbidity and mortality in patients with asymptomatic or minimally symptomatic left ventricular dysfunction. Comparison with plasma angiotensin II and endothelin-1. *European Heart Journal* 1999.**20**:1799–1807.
254. Ng LL, Loke IW, Davies JE, Geeranavar S, Khunti K, Stone MA, Chin DT, Squire IB. Community screening for left ventricular systolic dysfunction using plasma and urinary natriuretic peptides. *Journal of the American College of Cardiology* 2005.**45**:1043–1050.
255. Goetze JP, Mogelvang R, Maage L, Scharling H, Schnohr P, Sogaard P, Rehfeld JF, Jensen JS. Plasma pro-B-type natriuretic peptide in the general population: Screening for left ventricular hypertrophy and systolic dysfunction. *European Heart Journal* 2006.**27**:3004–3010.
256. Iacovoni A, Maria R de, Gavazzi A, Grosu A, Fontana A, Guideri S, Gori M, Ferrari P, Filippi A, Rutili MR, Donzelli L, Parolini M, Senni M. Natriuretic peptides and the Framingham Risk score for screening of asymptomatic left ventricular systolic dysfunction in high-risk patients in primary care. The DAVID-BERG study. *International Journal of Cardiology* 2013.**168**:5093–5095.
257. Richards AM, Nicholls MG, Yandle TG, Ikram H, Espiner EA, Turner JG, Buttimore RC, Lainchbury JG, Elliott JM, Frampton C, Crozier IG, Smyth DW. Neuroendocrine prediction of left ventricular function and heart failure after acute myocardial infarction. *Heart* 1999.**81**:114–120.
258. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, Buttimore RC, Lainchbury JG, Elliott JM, Ikram H, Crozier IG, Smyth DW. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: New neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation* 1998.**97**:1921–1929.

259. Nagaya N, Nishikimi T, Goto Y, Miyao Y, Kobayashi Y, Morii I, Daikoku S, Matsumoto T, Miyazaki S, Matsuoka H, Takishita S, Kangawa K, Matsuo H, Nonogi H. Plasma brain natriuretic peptide is a biochemical marker for the prediction of progressive ventricular remodeling after acute myocardial infarction. *American Heart Journal* 1998.**135**:21–28.
260. Omland T, Sabatine MS, Jablonski KA, Rice MM, Hsia J, Wergeland R, Landaas S, Rouleau JL, Domanski MJ, Hall C, Pfeffer MA, Braunwald E. Prognostic Value of B-Type Natriuretic Peptides in Patients With Stable Coronary Artery Disease. *Journal of the American College of Cardiology* 2007.**50**:205–214.
261. Sabatine MS, Morrow DA, Lemos JA de, Omland T, Sloan S, Jarolim P, Solomon SD, Pfeffer MA, Braunwald E. Evaluation of Multiple Biomarkers of Cardiovascular Stress for Risk Prediction and Guiding Medical Therapy in Patients with Stable Coronary Disease. *Circulation* 2012.**125**:233.
262. Olivier A, Girerd N, Michel J, Ketelslegers J, Fay R, Vincent J, Bramlage P, Pitt B, Zannad F, Rossignol P. Combined baseline and one-month changes in big endothelin-1 and brain natriuretic peptide plasma concentrations predict clinical outcomes in patients with left ventricular dysfunction after acute myocardial infarction: Insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study. *International Journal of Cardiology* 2017.**241**:344–350.
263. Nazario B, Hu RM, Pedram A, Prins B, Levin ER. Atrial and brain natriuretic peptides stimulate the production and secretion of C-type natriuretic peptide from bovine aortic endothelial cells. *Journal of Clinical Investigation* 1995.**95**:1151–1157.
264. Orn S, Manhenke C, Squire IB, Ng L, Anand I, Dickstein K. Plasma MMP-2, MMP-9 and N-BNP in long-term survivors following complicated myocardial infarction: relation to cardiac magnetic resonance imaging measures of left ventricular structure and function. *Journal of cardiac failure* 2007.**13**:843–849.
265. Vantrimpont P, Rouleau JL, Ciampi A, Harel F, Champlain J de, Bichet D, Moyé LA, Pfeffer M. Two-year time course and significance of neurohumoral activation in the Survival and Ventricular Enlargement (SAVE) Study. *European heart journal* 1998.**19**:1552–1563.

266. Suzuki S, Yoshimura M, Nakayama M, Mizuno Y, Harada E, Ito T, Nakamura S, Abe K, Yamamuro M, Sakamoto T, Saito Y, Nakao K, Yasue H, Ogawa H. Plasma Level of B-Type Natriuretic Peptide as a Prognostic Marker After Acute Myocardial Infarction. *Circulation* 2004.**110**:1387–1391.
267. Daubert MA, Adams K, Yow E, Barnhart HX, Douglas PS, Rimmer S, Norris C, Cooper L, Leifer E, Desvigne-Nickens P, Anstrom K, Fiuzat M, Ezekowitz J, Mark DB, O'Connor CM, Januzzi J, Felker GM. NT-proBNP Goal Achievement Is Associated With Significant Reverse Remodeling and Improved Clinical Outcomes in HFrEF. *JACC: Heart Failure* 2019.**7**:158–168.
268. Weiner RB, Baggish AL, Chen-Tournoux A, Marshall JE, Gaggin HK, Bhardwaj A, Mohammed AA, Rehman SU, Barajas L, Barajas J, Gregory SA, Moore SA, Semigran MJ, Januzzi JL. Improvement in structural and functional echocardiographic parameters during chronic heart failure therapy guided by natriuretic peptides: mechanistic insights from the ProBNP Outpatient Tailored Chronic Heart Failure (PROTECT) study. *European Journal of Heart Failure* 2013.**15**:342–351.
269. Felker GM, Anstrom KJ, Adams KF, Ezekowitz JA, Fiuzat M, Houston-Miller N, Januzzi JL, Mark DB, Piña IL, Passmore G, Whellan DJ, Yang H, Cooper LS, Leifer ES, Desvigne-Nickens P, O 'connor CM. Effect of Natriuretic Peptide–Guided Therapy on Hospitalization or Cardiovascular Mortality in High-Risk Patients With Heart Failure and Reduced Ejection Fraction A Randomized Clinical Trial. *JAMA* 2017.**27705**:713–720.
270. Velazquez EJ, Morrow DA, DeVore AD, Duffy CI, Ambrosy AP, McCague K, Rocha R, Braunwald E. Angiotensin–Neprilysin Inhibition in Acute Decompensated Heart Failure. *New England Journal of Medicine* 2019.**380**:539–548.
271. Murphy SP, Prescott MF, Camacho A, Iyer SR, Maisel AS, Felker GM, Butler J, Piña IL, Ibrahim NE, Abbas C, Burnett JC, Solomon SD, Januzzi JL. Atrial Natriuretic Peptide and Treatment With Sacubitril/Valsartan in Heart Failure With Reduced Ejection Fraction. *JACC: Heart Failure* 2021.**9**:127–136.
272. Nougué H, Pezel T, Picard F, Sadoune M, Arrigo M, Beauvais F, Launay JM, Cohen-Solal A, Vodovar N, Logeart D. Effects of sacubitril/valsartan on neprilysin targets and the metabolism of natriuretic peptides in chronic

heart failure: a mechanistic clinical study. *European Journal of Heart Failure* 2018.**21**:598–605.

273. Myhre PL, Vaduganathan M, Claggett B, Packer M, Desai AS, Rouleau JL, Zile MR, Swedberg K, Lefkowitz M, Shi V, McMurray JJ v, Solomon SD. B-Type Natriuretic Peptide During Treatment With Sacubitril/Valsartan. *Journal of the American College of Cardiology* 2019.**73**:1264–1272.
274. Ibrahim NE, McCarthy CP, Shrestha S, Gaggin HK, Mukai R, Szymonifka J, Apple FS, Burnett JC, Iyer S, Januzzi JL. Effect of Neprilysin Inhibition on Various Natriuretic Peptide Assays. *Journal of the American College of Cardiology* 2019.**73**:1273–1284.
275. Wei CM, Heublein DM, Perrella MA, Lerman A, Rodeheffer RJ, McGregor CG, Edwards WD, Schaff H v, Burnett JC. Natriuretic peptide system in human heart failure. *Circulation* 1993.**88**:1004–1009.
276. Boerrigter G, Lapp H, Burnett JC. Modulation of cGMP in Heart Failure: A New Therapeutic Paradigm. *Handbook of Experimental Pharmacology* 2009.**191**:485–506.
277. Bayés-Genís A, Barallat J, Galán A, Antonio M de, Domingo M, Zamora E, Urrutia A, Lupón J. Soluble Neprilysin Is Predictive of Cardiovascular Death and Heart Failure Hospitalization in Heart Failure Patients. *Journal of the American College of Cardiology* 2015.**65**:657–665.
278. D'Elia E, Iacovoni A, Vaduganathan M, Lorini FL, Perlini S, Senni M. Neprilysin inhibition in heart failure: mechanisms and substrates beyond modulating natriuretic peptides. *European Journal of Heart Failure* 2017.**19**:710–717.
279. Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2005.**25**:2480–2487.
280. Wilkinson IB, McEniery CM, Bongaerts KH, MacCallum H, Webb DJ, Cockcroft JR. Adrenomedullin (ADM) in the human forearm vascular bed: effect of neutral endopeptidase inhibition and comparison with proadrenomedullin NH2-terminal 20 peptide (PAMP). *British Journal of Clinical Pharmacology* 2001.**52**:159–164.
281. Vesely DL, Blankenship M, Douglass MA, McCormick MT, Rodriguez-Paz G, Schocken DD. Atrial natriuretic peptide increases adrenomedullin in the circulation of healthy humans. *Life Sciences* 1996.**59**:243–254.

282. Petrie MC, McDonald JE, Hillier C, Morton JJ, McMurray JJ. Effects of adrenomedullin on angiotensin II stimulated atrial natriuretic peptide and arginine vasopressin secretion in healthy humans. *British journal of clinical pharmacology* 2001.**52**:165–168.
283. Petrie MC, McClure SJ, Love MP, McMurray JJV. Novel neuropeptides in the pathophysiology of heart failure: Adrenomedullin and endothelin-1. *European Journal of Heart Failure* 1999.**1**:25–29.
284. Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H. Increased plasma levels of adrenomedullin in patients with heart failure. *Journal of the American College of Cardiology* 1995.**26**:1424–1431.
285. Jougasaki M, Wei C-M, McKinley LJ, Burnett JC. Elevation of Circulating and Ventricular Adrenomedullin in Human Congestive Heart Failure. *Circulation* 1995.**92**:286–289.
286. Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, Masuda Y, Miyatake K, Kangawa K. Hemodynamic, Renal, and Hormonal Effects of Adrenomedullin Infusion in Patients With Congestive Heart Failure. *Circulation* 2000.**101**:498–503.
287. Rademaker MT, Charles CJ, Espiner EA, Nicholls MG, Richards AM. Long-Term Adrenomedullin Administration in Experimental Heart Failure. *Hypertension* 2002.**40**:667–672.
288. Lisy O, Jougasaki M, Schirger JA, Chen HH, Barclay PT, Burnett JC. Neutral endopeptidase inhibition potentiates the natriuretic actions of adrenomedullin. *American Journal of Physiology-Renal Physiology* 1998.**275**:F410–F414.
289. Rademaker MT, Charles CJ, Cooper GJS, Coy DH, Espiner EA, Lewis LK, Nicholls MG, Richards AM. Combined endopeptidase inhibition and adrenomedullin in sheep with experimental heart failure. *Hypertension (Dallas, Tex : 1979)* 2002.**39**:93–98.
290. Arfsten H, Goliasch G, Bartko PE, Prausmüller S, Spinka G, Cho A, Novak J, Haslacher H, Strunk G, Struck J, Hülsmann M, Pavo N. Increased concentrations of bioactive adrenomedullin subsequently to angiotensin-receptor/neprilysin-inhibitor treatment in chronic systolic heart failure. *British Journal of Clinical Pharmacology* 2021.**87**:916–924.

291. Holst JJ. The Physiology of Glucagon-like Peptide 1. *Physiological Reviews* 2007.**87**:1409–1439.
292. Sattar N, Lee MMY, Kristensen SL, Branch KRH, Prato S del, Khurmi NS, Lam CSP, Lopes RD, McMurray JJ v, Pratley RE, Rosenstock J, Gerstein HC. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of randomised trials. *The Lancet Diabetes & Endocrinology* 2021.
293. White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F, EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *The New England journal of medicine* 2013.**369**:1327–1335.
294. Jorsal A, Kistorp C, Holmager P, Tougaard RS, Nielsen R, Hänselmann A, Nilsson B, Møller JE, Hjort J, Rasmussen J, Boesgaard TW, Schou M, Videbaek L, Gustafsson I, Flyvbjerg A, Wiggers H, Tarnow L. Effect of liraglutide, a glucagon-like peptide-1 analogue, on left ventricular function in stable chronic heart failure patients with and without diabetes (LIVE)-a multicentre, double-blind, randomised, placebo-controlled trial. *European Journal of Heart Failure* 2017.**19**:69–77.
295. Lepore JJ, Olson E, Demopoulos L, Haws T, Fang Z, Barbour AM, Fossler M, Davila-Roman VG, Russell SD, Gropler RJ. Effects of the Novel Long-Acting GLP-1 Agonist, Albiglutide, on Cardiac Function, Cardiac Metabolism, and Exercise Capacity in Patients With Chronic Heart Failure and Reduced Ejection Fraction. *JACC: Heart Failure* 2016.**4**:559–566.
296. Plamboeck A, Holst JJ, Carr RD, Deacon CF. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* 2005.**48**:1882–1890.
297. Li J, Zheng J, Wang S, Lau HK, Fathi A, Wang Q. Cardiovascular Benefits of Native GLP-1 and its Metabolites: An Indicator for GLP-1-Therapy Strategies. *Frontiers in Physiology* 2017.**0**:15.
298. Windeløv JA, Wewer Albrechtsen NJ, Kuhre RE, Jepsen SL, Hornburg D, Pedersen J, Jensen EP, Galsgaard KD, Winther-Sørensen M, Ørgaard A, Deacon CF, Mann M, Kissow H, Hartmann B, Holst JJ. Why is it so difficult

to measure glucagon-like peptide-1 in a mouse? *Diabetologia* 2017.**60**:2066–2075.

299. Ussher JR, Drucker DJ. Cardiovascular Actions of Incretin-Based Therapies. *Circulation Research* 2014.**114**:1788–1803.
300. Seferovic JP, Claggett B, Seidelmann SB, Seely EW, Packer M, Zile MR, Rouleau JL, Swedberg K, Lefkowitz M, Shi VC, Desai AS, McMurray JJ v, Solomon SD. Effect of sacubitril/valsartan versus enalapril on glycaemic control in patients with heart failure and diabetes: a post-hoc analysis from the PARADIGM-HF trial. *The Lancet Diabetes and Endocrinology* 2017.**5**:333–340.
301. Wewer Albrechtsen NJ, Mark PD, Terzic D, Hansen LH, Andersen UØ, Hartmann B, Carr RD, Gustafsson F, Deacon CF, Holst JJ, Goetze JP, Plomgaard P. Sacubitril/Valsartan Augments Postprandial Plasma Concentrations of Active GLP-1 When Combined With Sitagliptin in Men. *The Journal of Clinical Endocrinology & Metabolism* 2019.**104**:3868–3876.
302. Japp AG, Newby DE. The apelin–APJ system in heart failure: Pathophysiologic relevance and therapeutic potential. *Biochemical Pharmacology* 2008.**75**:1882–1892.
303. Kuba K, Zhang L, Imai Y, Arab S, Chen M, Maekawa Y, Leschnik M, Leibbrandt A, Markovic M, Schwaighofer J, Beetz N, Musialek R, Neely GG, Komnenovic V, Kolm U, Metzler B, Ricci R, Hara H, Meixner A, Nghiem M, Chen X, Dawood F, Wong KM, Sarao R, Cukerman E, Kimura A, Hein L, Thalhammer J, Liu PP, Penninger JM. Impaired Heart Contractility in Apelin Gene–Deficient Mice Associated With Aging and Pressure Overload. *Circulation Research* 2007.**101**.
304. Weir RAP, Chong KS, Dalzell JR, Petrie CJ, Murphy CA, Steedman T, Mark PB, McDonagh TA, Dargie HJ, McMurray JJ v. Plasma apelin concentration is depressed following acute myocardial infarction in man. *European journal of heart failure* 2009.**11**:551–558.
305. Dalzell JR, Rocchiccioli JP, Weir RAP, Jackson CE, Padmanabhan N, Gardner RS, Petrie MC, McMurray JJ v. The Emerging Potential of the Apelin-APJ System in Heart Failure. *Journal of cardiac failure* 2015.**21**:489–498.
306. Fukushima H, Kobayashi N, Takeshima H, Koguchi W, Ishimitsu T. Effects of Olmesartan on Apelin/APJ and Akt/Endothelial Nitric Oxide Synthase

Pathway in Dahl Rats With End-stage Heart Failure. *Journal of Cardiovascular Pharmacology* 2010.**55**:83–88.

307. Japp AG, Cruden NL, Barnes G, Gemeren N van, Mathews J, Adamson J, Johnston NR, Denvir MA, Megson IL, Flapan AD, Newby DE. Acute Cardiovascular Effects of Apelin in Humans. *Circulation* 2010.**121**:1818–1827.
308. McKinnie SMK, Fischer C, Tran KMH, Wang W, Mosquera F, Oudit GY, Vederas JC. The Metalloprotease Neprilysin Degrades and Inactivates Apelin Peptides. *ChemBioChem* 2016.**17**:1495–1498.
309. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *European Journal of Heart Failure* 2006.**8**:355–360.
310. Love M, McMurray J. Endothelin in chronic heart failure: current position and future prospects. *Cardiovascular Research* 1996.**31**:665–674.
311. Tsutamoto T, Hisanaga T, Fukai D, Wada A, Maeda Y, Maeda K, Kinoshita M. Prognostic value of plasma soluble intercellular adhesion molecule-1 and endothelin-1 concentration in patients with chronic congestive heart failure. *The American Journal of Cardiology* 1995.**76**:803–808.
312. Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, Borger MA, Mario C di, Dickstein K, Ducrocq G, Fernandez-Aviles F, Gershlick AH, Giannuzzi P, Halvorsen S, Huber K, Juni P, Kastrati A, Knuuti J, Lenzen MJ, Mahaffey KW, Valgimigli M, 't Hof A van, Widimsky P, Zahger D. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *European heart journal* 2012.**33**:2569–2619.
313. Apple FS, Sandoval Y, Jaffe AS, Ordonez-Llanos J, IFCC Task Force on Clinical Applications of Cardiac Bio-Markers. Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. *Clinical chemistry* 2017.**63**:73–81.
314. Omland T, Lemos JA de, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E, Prevention of Events with Angiotensin Converting Enzyme Inhibition (PEACE) Trial Investigators. A sensitive cardiac troponin T assay in stable coronary artery disease. *The New England journal of medicine* 2009.**361**:2538–2547.

315. Steen H, Futterer S, Merten C, Jünger C, Katus HA, Giannitsis E. Relative role of NT-pro BNP and cardiac troponin T at 96 hours for estimation of infarct size and left ventricular function after acute myocardial infarction. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance* 2007.**9**:749–758.
316. Nguyen TL, Phan JAK, Hee L, Moses DA, Otton J, Terreblanche OD, Xiong J, Premawardhana U, Rajaratnam R, Juergens CP, Dimitri HR, French JK, Richards DAB, Thomas L. High-sensitivity troponin T predicts infarct scar characteristics and adverse left ventricular function by cardiac magnetic resonance imaging early after reperfused acute myocardial infarction. *American heart journal* 2015.**170**:715-725.e2.
317. Stelzle D, Shah AS v, Anand A, Strachan FE, Chapman AR, Denvir MA, Mills NL, McAllister DA. High-sensitivity cardiac troponin I and risk of heart failure in patients with suspected acute coronary syndrome: a cohort study. *European Heart Journal Quality of Care & Clinical Outcomes* 2018.**4**:36.
318. Hassan AKM, Bergheanu SC, Hasan-Ali H, Liem SS, Laarse A van der, Wolterbeek R, Atsma DE, Schalij MJ, Jukema JW. Usefulness of peak troponin-T to predict infarct size and long-term outcome in patients with first acute myocardial infarction after primary percutaneous coronary intervention. *The American journal of cardiology* 2009.**103**:779–784.
319. Daniels LB, Laughlin GA, Clopton P, Maisel AS, Barrett-Connor E. Minimally Elevated Cardiac Troponin T and Elevated N-Terminal Pro-B-Type Natriuretic Peptide Predict Mortality in Older Adults. Results From the Rancho Bernardo Study. *Journal of the American College of Cardiology* 2008.**52**:450–459.
320. McKie PM, AbouEzzeddine OF, Scott CG, Mehta R, Rodeheffer RJ, Redfield MM, Burnett JC, Jaffe AS. High-sensitivity troponin I and amino-terminal pro--B-type natriuretic peptide predict heart failure and mortality in the general population. *Clinical chemistry* 2014.**60**:1225–1233.
321. Saunders JT, Nambi V, Lemos JA de, Chambless LE, Virani SS, Boerwinkle E, Hoogeveen RC, Liu X, Astor BC, Mosley TH, Folsom AR, Heiss G, Coresh J, Ballantyne CM. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the

Atherosclerosis Risk in Communities Study. *Circulation* 2011.**123**:1367–1376.

322. Welsh P, Preiss D, Hayward C, Shah ASV, McAllister D, Briggs A, Boachie C, McConnachie A, Padmanabhan S, Welsh C, Woodward M, Campbell A, Porteous D, Mills NL, Sattar N. Cardiac Troponin T and Troponin I in the General Population. *Circulation* 2019.**139**:2754–2764.
323. Jia X, Sun W, Hoogeveen RC, Nambi V, Matsushita K, Folsom AR, Heiss G, Couper DJ, Solomon SD, Boerwinkle E, Shah A, Selvin E, Lemos JA de, Ballantyne CM. High-Sensitivity Troponin I and Incident Coronary Events, Stroke, Heart Failure Hospitalization, and Mortality in the ARIC Study. *Circulation* 2019.**139**:2642–2653.
324. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasan RS, Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012.**126**:1596–1604.
325. Seliger SL, Hong SN, Christenson RH, Kronmal R, Daniels LB, Lima JAC, Lemos JA de, Bertoni A, deFilippi CR. High-Sensitive Cardiac Troponin T as an Early Biochemical Signature for Clinical and Subclinical Heart Failure. *Circulation* 2017.**135**:1494–1505.
326. Morrow DA, Velazquez EJ, DeVore AD, Prescott MF, Duffy CI, Gurmu Y, McCague K, Rocha R, Braunwald E. Cardiovascular biomarkers in patients with acute decompensated heart failure randomized to sacubitril-valsartan or enalapril in the PIONEER-HF trial. *European Heart Journal* 2019.
327. Jhund PS, Claggett BL, Voors AA, Zile MR, Packer M, Pieske BM, Kraigher-Krainer E, Shah AM, Prescott MF, Shi V, Lefkowitz M, McMurray JJV, Solomon SD. Elevation in High-Sensitivity Troponin T in Heart Failure and Preserved Ejection Fraction and Influence of Treatment With the Angiotensin Receptor Neprilysin Inhibitor LCZ696. *Circulation: Heart Failure* 2014.**7**:953–959.
328. Kociol RD, Pang PS, Gheorghiade M, Fonarow GC, O'Connor CM, Felker GM. Troponin Elevation in Heart Failure: Prevalence, Mechanisms, and Clinical Implications. *Journal of the American College of Cardiology* 2010.**56**:1071–1078.

329. Packer M, Januzzi JL, Ferreira JP, Anker SD, Butler J, Filippatos G, Pocock SJ, Brueckmann M, Jamal W, Cotton D, Iwata T, Zannad F. Concentration-dependent clinical and prognostic importance of high-sensitivity cardiac troponin T in heart failure and a reduced ejection fraction and the influence of empagliflozin: the EMPEROR-Reduced trial. *European Journal of Heart Failure* 2021.
330. Berg DD, Docherty KF, Sattar N, Jarolim P, Welsh P, Jhund PS, Anand IS, Chopra V, Boer RA de, Kosiborod MN, Nicolau JC, O'Meara E, Schou M, Hammarstedt A, Langkilde A-M, Lindholm D, Sjöstrand M, McMurray JJV, Sabatine MS, Morrow DA. Serial Assessment of High-Sensitivity Cardiac Troponin and the Effect of Dapagliflozin in Patients with Heart Failure with Reduced Ejection Fraction: An Analysis of the DAPA-HF Trial. *Circulation* 2021.
331. Lemos JA de, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, Hashim I, Berry JD, Das SR, Morrow DA, McGuire DK. Association of Troponin T Detected With a Highly Sensitive Assay and Cardiac Structure and Mortality Risk in the General Population. *JAMA* 2010.**304**:2503.
332. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, Ford I, Trompet S, Stott DJ, Kearney PM, Mooijaart SP, Kiechl S, Angelantonio E di, Sattar N. High-Sensitivity Cardiac Troponin Concentration and Risk of First-Ever Cardiovascular Outcomes in 154,052 Participants. *Journal of the American College of Cardiology* 2017.**70**:558–568.
333. Florea VG, Rector TS, Anand IS, Cohn JN. Heart Failure With Improved Ejection Fraction: Clinical Characteristics, Correlates of Recovery, and Survival. *Circulation: Heart Failure* 2016.**9**.
334. Pocock SJ, Ferreira JP, Gregson J, Anker SD, Butler J, Filippatos G, Gollop ND, Iwata T, Brueckmann M, Januzzi JL, Voors AA, Zannad F, Packer M. Novel biomarker-driven prognostic models to predict morbidity and mortality in chronic heart failure: the EMPEROR-Reduced trial. *European Heart Journal* 2021.
335. McEvoy JW, Chen Y, Ndumele CE, Solomon SD, Nambi V, Ballantyne CM, Blumenthal RS, Coresh J, Selvin E. Six-Year Change in High-Sensitivity Cardiac Troponin T and Risk of Subsequent Coronary Heart Disease, Heart Failure, and Death. *JAMA Cardiology* 2016.**1**:519–528.

336. Howlett JG, Sharma N, Alemayehu WG, Dyck JRB, Anderson T, Fine N, Becker H, White JA, Paterson DI, Thompson RB, Oudit GY, Haykowsky MJ, Ezekowitz JA. Circulating troponin and further left ventricular ejection fraction improvement in patients with previously recovered left ventricular ejection fraction. *ESC Heart Failure* 2020.**7**:2725–2733.
337. Murphy SP, Prescott MF, Maisel AS, Butler J, Piña IL, Felker GM, Ward JH, Williamson KM, Camacho A, Kandanelly RR, Solomon SD, Januzzi JL. Association Between Angiotensin Receptor–Neprilysin Inhibition, Cardiovascular Biomarkers, and Cardiac Remodeling in Heart Failure With Reduced Ejection Fraction. *Circulation: Heart Failure* 2021.:653–662.
338. Rørth R, Jhund PS, Kristensen SL, Desai AS, Køber L, Rouleau JL, Solomon SD, Swedberg K, Zile MR, Packer M, McMurray JJV. The prognostic value of troponin T and N-terminal pro B-type natriuretic peptide, alone and in combination, in heart failure patients with and without diabetes. *European Journal of Heart Failure* 2019.**21**:40.
339. Teerlink JR, Diaz R, Felker GM, McMurray JJV, Metra M, Solomon SD, Adams KF, Anand I, Arias-Mendoza A, Biering-Sørensen T, Böhm M, Bonderman D, Cleland JGF, Corbalan R, Crespo-Leiro MG, Dahlström U, Echeverria LE, Fang JC, Filippatos G, Fonseca C, Goncalvesova E, Goudev AR, Howlett JG, Lanfear DE, Li J, Lund M, Macdonald P, Mareev V, Momomura S, O'Meara E, et al. Cardiac Myosin Activation with Omecamtiv Mecarbil in Systolic Heart Failure. *New England Journal of Medicine* 2021.**384**:105–116.
340. Antonio M de, Lupón J, Galán A, Vila J, Zamora E, Urrutia A, Díez C, Coll R, Altimir S, Bayes-Genis A. Head-to-head comparison of high-sensitivity troponin T and sensitive-contemporary troponin I regarding heart failure risk stratification. *Clinica chimica acta; international journal of clinical chemistry* 2013.**426**:18–24.
341. Webb CS, Bonnema DD, Ahmed SH, Leonardi AH, McClure CD, Clark LL, Stroud RE, Corn WC, Finklea L, Zile MR, Spinale FG. Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: Relation to left ventricular remodeling. *Circulation* 2006.**114**:1020–1027.
342. Schelbert EB, Piehler KM, Zareba KM, Moon JC, Ugander M, Messroghli DR, Valeti US, Chang C-CH, Shroff SG, Diez J, Miller CA, Schmitt M, Kellman P,

Javed Butler ;, Gheorghiade M, Wong TC. Myocardial Fibrosis Quantified by Extracellular Volume Is Associated With Subsequent Hospitalization for Heart Failure, Death, or Both Across the Spectrum of Ejection Fraction and Heart Failure Stage. *Journal of the American Heart Association* 2015.**4**.

343. Duprez DA, Gross MD, Kizer JR, Ix JH, Hundley WG, Jacobs DR. Predictive Value of Collagen Biomarkers for Heart Failure With and Without Preserved Ejection Fraction: MESA (Multi-Ethnic Study of Atherosclerosis). *Journal of the American Heart Association* 2018.**7**.
344. González A, Schelbert EB, Díez J, Butler J. Myocardial Interstitial Fibrosis in Heart Failure: Biological and Translational Perspectives. *Journal of the American College of Cardiology* 2018.**71**:1696–1706.
345. Squire IB, Evans J, Ng LL, Loftus IM, Thompson MM. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. *Journal of cardiac failure* 2004.**10**:328–333.
346. Kelly D, Khan SQ, Thompson M, Cockerill G, Ng LL, Samani N, Squire IB. Plasma tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9: novel indicators of left ventricular remodelling and prognosis after acute myocardial infarction. *European heart journal* 2008.**29**:2116–2124.
347. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of Excessive Extracellular Matrix Turnover May Contribute to Survival Benefit of Spironolactone Therapy in Patients With Congestive Heart Failure Insights From the Randomized Aldactone Evaluation Study (RALES). *Circulation* 2000.**102**:2700–2706.
348. Zile MR, O'Meara E, Claggett B, Prescott MF, Solomon SD, Swedberg K, Packer M, McMurray JJ v, Shi V, Lefkowitz M, Rouleau J. Effects of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFrEF. *Journal of the American College of Cardiology* 2019.**73**:795–806.
349. Iraqi W, Rossignol P, Angioi M, Fay R, Nuée J, Ketelslegers JM, Vincent J, Pitt B, Zannad F. Extracellular cardiac matrix biomarkers in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure: insights from the Eplerenone Post-Acute Myocardial

Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study. *Circulation* 2009.**119**:2471–2479.

350. López B, González A, Ravassa S, Beaumont J, Moreno MU, San José G, Querejeta R, Díez J. Circulating Biomarkers of Myocardial Fibrosis: The Need for a Reappraisal. *Journal of the American College of Cardiology* 2015.**65**:2449–2456.
351. Bouabdallaoui N, Claggett B, Zile MR, McMurray JJV, O'Meara E, Packer M, Prescott MF, Swedberg K, Solomon SD, Rouleau JL. Growth differentiation factor-15 is not modified by sacubitril/valsartan and is an independent marker of risk in patients with heart failure and reduced ejection fraction: the PARADIGM-HF trial. *European Journal of Heart Failure* 2018.**20**:1701–1709.
352. Zile MR, O'Meara E, Claggett B, Prescott MF, Solomon SD, Swedberg K, Packer M, McMurray JJ v, Shi V, Lefkowitz M, Rouleau J. Effects of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFrEF. *Journal of the American College of Cardiology* 2019.**73**:795–806.
353. O'Meara E, Prescott MF, Rouleau JL, Chiang L-M, Solomon SD, Swedberg K, Packer M, McMurray JJ v, Zile MR. Association between sST2 Levels and Cardiovascular Outcomes and Effect of Sacubitril/Valsartan on sST2 Levels: Results From the PARADIGM-HF Trial. *Journal of Cardiac Failure* 2016.**22**:S29–S30.
354. Lopez-Andrés N, Rossignol P, Iraqi W, Fay R, Nuée J, Ghio S, Cleland JGF, Zannad F, Lacolley P. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. *European journal of heart failure* 2012.**14**:74–81.
355. Velde AR van der, Gullestad L, Ueland T, Aukrust P, Guo Y, Adourian A, Muntendam P, Veldhuisen DJ van, Boer RA de. Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH. *Circulation Heart failure* 2013.**6**:219–226.
356. Anand IS, Rector TS, Kuskowski M, Adourian A, Muntendam P, Cohn JN. Baseline and serial measurements of galectin-3 in patients with heart

failure: Relationship to prognosis and effect of treatment with valsartan in the Val-HeFT. *European Journal of Heart Failure* 2013.**15**:511–518.

357. Weir RAP, Petrie CJ, Murphy CA, Clements S, Steedman T, Miller AM, McInnes IB, Squire IB, Ng LL, Dargie HJ, McMurray JJ v. Galectin-3 and cardiac function in survivors of acute myocardial infarction. *Circulation Heart failure* 2013.**6**:492–498.
358. Lee MMY, Brooksbank KJM, Wetherall K, Mangion K, Roditi G, Campbell RT, Berry C, Chong V, Coyle L, Docherty KF, Dreisbach JG, Labinjoh C, Lang NN, Lennie V, McConnachie A, Murphy CL, Petrie CJ, Petrie JR, Speirits IA, Sourbron S, Welsh P, Woodward R, Radjenovic A, Mark PB, McMurray JJ v, Jhund PS, Petrie MC, Sattar N. Effect of Empagliflozin on Left Ventricular Volumes in Patients With Type 2 Diabetes, or Prediabetes, and Heart Failure With Reduced Ejection Fraction (SUGAR-DM-HF). *Circulation* 2021.**143**:516–525.
359. Jensen J, Omar M, Kistorp C, Poulsen MK, Tuxen C, Gustafsson I, Køber L, Gustafsson F, Faber J, Fosbøl EL, Bruun NE, Brønd JC, Forman JL, Videbæk L, Møller JE, Schou M. Twelve weeks of treatment with empagliflozin in patients with heart failure and reduced ejection fraction: A double-blinded, randomized, and placebo-controlled trial. *American Heart Journal* 2020.**228**:47–56.
360. Santos-Gallego CG, Vargas-Delgado AP, Requena-Ibanez JA, Garcia-Ropero A, Mancini D, Pinney S, Macaluso F, Sartori S, Roque M, Sabatel-Perez F, Rodriguez-Cordero A, Zafar MU, Fergus I, Atallah-Lajam F, Contreras JP, Varley C, Moreno PR, Abascal VM, Lala A, Tamler R, Sanz J, Fuster V, Badimon JJ. Randomized Trial of Empagliflozin in Nondiabetic Patients With Heart Failure and Reduced Ejection Fraction. *Journal of the American College of Cardiology* 2021.**77**:243–255.
361. Lainchbury JG, Nicholls MG, Espiner EA, Yandle TG, Lewis LK, Richards AM. Bioactivity and Interactions of Adrenomedullin and Brain Natriuretic Peptide in Patients With Heart Failure. *Hypertension* 1999.**34**:70–75.
362. Barsheshet A, Goldenberg I, Moss AJ, Eldar M, Huang DT, McNitt S, Klein HU, Hall WJ, Brown MW, Goldberger JJ, Goldstein RE, Schuger C, Zareba W, Daubert JP. Response to preventive cardiac resynchronization therapy in patients with ischaemic and nonischaemic cardiomyopathy in MADIT-CRT. *European Heart Journal* 2011.**32**:1622–1630.

363. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Bělohlávek J, Böhm M, Chiang C-E, Chopra VK, Boer RA de, Desai AS, Diez M, Drozdz J, Dukát A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *New England Journal of Medicine* 2019.**381**:1995–2008.

364. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, Januzzi J, Verma S, Tsutsui H, Brueckmann M, Jamal W, Kimura K, Schnee J, Zeller C, Cotton D, Bocchi E, Böhm M, Choi D-J, Chopra V, Chuquiure E, Giannetti N, Janssens S, Zhang J, Gonzalez Juanatey JR, Kaul S, Brunner-La Rocca H-P, Merkely B, Nicholls SJ, Perrone S, Pina I, et al. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. *N Engl J Med* 2020.**383**:1413–1424.

365. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Brunner–La Rocca H-P, Choi D-J, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone S v., Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, et al. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. *New England Journal of Medicine* 2021.**385**:1451–1461.

366. Omar M, Jensen J, Ali M, Frederiksen PH, Kistorp C, Videbæk L, Poulsen MK, Tuxen CD, Möller S, Gustafsson F, Køber L, Schou M, Møller JE. Associations of Empagliflozin With Left Ventricular Volumes, Mass, and Function in Patients With Heart Failure and Reduced Ejection Fraction. *JAMA Cardiology* 2021.**6**:836.