Pathophysiological Role of Aldosterone in Cardiac Remodelling after Myocardial Infarction

by

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List of abbreviations

ACC/AHA	American College of Cardiology/American Heart Association
ACE	angiotensin converting enzyme
ACS	acute coronary syndrome
ACTH	adrenocorticotrophic hormone
AMI	acute myocardial infarction
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
ARB	angiotensin receptor blocker
ARR	aldosterone:renin ratio
AT_1	angiotensin II type 1 (receptor)
AVP	arginine vasopressin
BNP	brain natriuretic peptide
BSA	body surface area
CABG	coronary artery bypass graft
CAPRICORN	Carvedilol Post-Infarct Survival Control in Left-Ventricular Dysfunction trial
CHAPS	Carvedilol Heart Attack Pilot Study
CHD	coronary heart disease
CHF	chronic heart failure
CLARITY-TIMI-28	Clopidogrel as Adjunctive Reperfusion Therapy–Thrombolysis in Myocardial Infarction-28 study
CMR	cardiac magnetic resonance
CRT	cardiac resynchronisation therapy

CT-1	cardiotrophin-1
CV	coefficient of variation
DNA	deoxyribonucleic acid
DTPA	diethylenetriaminepentaacetate
ECAT	European Concerted Action on Thrombosis and Disabilities study
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
ECM	extracellular matrix
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EPHESUS	Eplerenone Post-acute myocardial infarction Heart failure Efficacy and SUrvival Study
ESC	European Society of Cardiology
ET-1	endothelin-1
GM-CSF	granulocyte-macrophage colony-stimulating factor
GP	general practitioner
HEART	Healing and Early Afterload Reducing Therapy study
HF	heart failure
HLA	horizontal long axis
ICD	implantable cardioverter defibrillator
IL-	interleukin-
IR	immunoreactivity
IRA	infarct-related artery
JGA	juxtaglomerular apparatus

LGE-CMR	late gadolinium-enhanced cardiac magnetic resonance
LIF	leukaemia inhibitory factor
LV	left ventricle
LVEDV(I)	left ventricular end-diastolic volume (index)
LVEF	left ventricular ejection fraction
LVESV(I)	left ventricular end-systolic volume (index)
LVMI	left ventricular mass index
LVOT	left ventricular outflow tract
LVSD	left ventricular systolic dysfunction
MCP-1	monocyte chemoattractant protein-1
MDRD	Modification of Diet in Renal Disease
MIP-1α	Macrophage inflammatory protein-1α
MMP	matrix metalloproteinase
MRA	mineralocorticoid receptor antagonist
MRI	magnetic resonance imaging
MVO	microvascular obstruction
NA	noradrenaline
NSTEMI	non-ST-elevation myocardial infarction
NTproBNP	N-terminal pro-brain natriuretic peptide
PAI-1	plasminogen activator inhibitor-1
PCI	percutaneous coronary intervention
PCR	polymerase chain reaction
PRC	plasma renin concentration
PREAMI	Perindopril and Remodeling in Elderly with Acute Myocardial Infarction study

RAAS	renin-angiotensin-aldosterone system
RALES	Randomised aldactone evaluation study
RANTES	regulated on activation normally T cell-expressed and secreted
RNA	ribonucleic acid
RNVG	radionuclide ventriculography
RVEF	right ventricular ejection fraction
RVI	right ventricular infarction
RVSD	right ventricular systolic dysfunction
RW	(Robin Weir – used to indicate researcher responsible for data collection where stated)
SA	short-axis
SD	standard deviation
SEM	standard error of the mean
SF-1	steroidogenic factor-1
SNP	single nucleotide polymorphism
SPECT	single photon emission computed tomography
sST2	soluble ST2
STEMI	ST-elevation myocardial infarction
TE	echo time (during CMR image acquisition)
TGF-β1	transforming growth factor-β1
THAldo	tetrahydroaldosterone
THDOC	tetrahydrodeoxycorticosterone
THS	tetrahydrodeoxycortisol
TI	time to inversion (during CMR image acquisition)
TIMI	Thrombolysis In Myocardial Infarction

TIMP	tissue inhibitor of matrix metalloproteinase
Tn (TnI or TnT)	troponin (I or T)
TNFα	tumour necrosis factor-α
tPA	tissue plasminogen activator
TR	repetition time (during CMR image acquisition)
true-FISP	true fast imaging with steady-state precession
TS	(Tracey Steedman – used to indicate researcher responsible for data collection where stated)
TTE	trans-thoracic echocardiography
turbo-FLASH	turbo fast low angle-shot
U&E's	urea and electrolytes
VALIANT	VALsartan In Acute myocardial iNfarcTion study
VIF	variance inflation factor
VLA	vertical long-axis
vWF	von Willebrand factor
WIG	Western Infirmary General Hospital, Glasgow
4E-LVH	4E-Left Ventricular Hypertrophy study

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Declaration

The experimental design of the work presented in this thesis was that of the author and his supervisors, Professor John McMurray and Professor Henry Dargie. All experimental work was carried out by the author with the exception of the acquisition of a proportion of the cardiac magnetic resonance scans (performed by Tracey Steedman, Glasgow Cardiac Magnetic Resonance Unit, Western Infirmary, Glasgow), and biomarker measurements (laboratories supervised by Dr. JJ Morton, Professor JMC Connell, Dr. A Gracie, Professor IB MacInnes, Professor GD Lowe [all University of Glasgow], and Dr. Iain Squire [University of Leicester] performed the blinded *post-hoc* measurement of the biomarkers under investigation in this thesis).

I declare that this thesis has been composed by myself and is a record of work performed by myself. It has not previously been submitted for a higher degree.

Robin Weir

April 2009

Summary

Acute myocardial infarction (AMI) remains a common and serious manifestation of coronary artery disease. The development of heart failure (HF) and/or evidence of left ventricular systolic dysfunction (LVSD) following AMI increases both in-hospital and longer-term mortality. A series of structural and functional changes occur within the heart in general and within the left ventricle (LV) in particular following AMI, initially providing a stabilising mechanism to maintain the cardiac output but over time becoming maladaptive and leading to progressive ventricular dilatation, dysfunction, HF and premature death. This process is termed remodelling. It is now understood that a complex series of mechanical, genetic and neurohormonal factors, including the mineralocorticoid hormone aldosterone, are implicated in its pathogenesis. Aldosterone antagonism reduces cardiovascular morbidity and mortality in patients with advanced chronic HF and survivors of large AMI who develop HF and/or are diabetic. These benefits could, at least in part, be due to an anti-remodelling action, although this was uncertain at the time I began my research project.

The work contained in this thesis examines cardiac remodelling, and the effects of the mineralocorticoid receptor antagonist eplerenone, in a cohort of 100 patients admitted with AMI, with depressed LV ejection fraction (LVEF) but without HF or diabetes mellitus, using a gold standard modality for LV functional assessment and infarct imaging: late gadolinium-enhanced cardiac magnetic resonance (LGE-CMR). Patients were treated with (double-blinded) eplerenone or placebo for 24 weeks, and underwent serial LGE-CMR scanning and measurement of haematological, urinary and genetic markers thought to be of pathophysiological importance in post-infarction remodelling.

There was, by chance, a significant imbalance in LV function at baseline between the randomised groups. After pre-specified covariate-adjustment, however, I found a significant effect of eplerenone on LV remodelling. Moreover I found that the use of eplerenone in addition to an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker was well-tolerated. I also found an effect of eplerenone on two matrix metalloproteinases (MMPs) considered key enzymes in extracellular matrix turnover. Specifically, eplerenone decreased MMP-2 and attenuated the drop in MMP-9 seen in the placebo group, changes that are protective against remodelling. These findings suggest a potential anti-remodelling effect of eplerenone in 'asymptomatic' LVSD after AMI, i.e. patients in whom eplerenone is not currently indicated.

Patients with limited functional recovery early after AMI resulting in a persistently reduced LVEF require stringent monitoring and may qualify for an implantable cardioverter defibrillator. The use of predictive biomarkers to identify such patients is gaining popularity. I found that two biomarkers, tissue plasminogen activator (tPA) antigen and tissue inhibitor of metalloproteinase-4 (TIMP-4), measured in a blood sample taken a mean of 3 days after AMI, were independent predictors of adverse remodelling. These findings are novel, provide further pathophysiological insights into the inter-related biological systems that underlie remodelling, and may inform future trials aimed at modulating these pathways in order to attenuate remodelling.

LGE-CMR affords detailed characterisation of myocardium. In keeping with previous studies, infarct volume, endocardial extent and transmurality predicted LV

remodelling. In addition I also found that the presence of microcirculatory dysfunction, defined as persistent microvascular obstruction (MVO) within the infarcted region on baseline LGE-CMR, divided my study population into two distinct groups with opposite remodelling outcomes. Patients with late MVO progressively remodelled, while those without reverse remodelled over 24 weeks. From these results, I propose that late MVO be used as an indicator of adverse ventricular remodelling. This may enhance the risk-stratification of survivors of AMI.

Aldosterone has a number of detrimental effects on the cardiovascular system and is strongly implicated in the pathogenesis of remodelling. I observed direct correlations between aldosterone sampled at baseline and CMR parameters of remodelling. Analysis by treatment group revealed an association between change in aldosterone over time and parameters of remodelling in placebo- but not eplerenone-treated patients, despite higher circulating aldosterone concentrations in the latter group. We propose that the cardiac effects of aldosterone display a temporal variation after AMI, specifically that circulating aldosterone in the first few days after infarction is key in selecting a remodelling pathway but that over the following weeks and months circulating aldosterone is less influential in potentiating the remodelling process. I also found a novel relationship between aldosterone and infarct volume, which merits further investigation as the role of aldosterone in the pathophysiology of remodelling is further described.

My trial design afforded the opportunity to examine the relationships of certain novel biomarkers with LV function and other established biomarkers after AMI. I demonstrated that circulating concentrations of the peptide apelin were reduced over

24 weeks after AMI compared to healthy controls but bore no relationship to LV function. Separately, I showed that concentrations of the soluble interleukin-1 receptor family member ST2 fell significantly over time after AMI and correlated with early and medium-term LV function and infarct volume. I detected a novel relationship between ST2 and aldosterone, which may suggest a pathophysiological role for ST2 in post-infarction remodelling and merits further investigation.

These studies provide further insights into the roles of aldosterone and of selective mineralocorticoid receptor antagonism in cardiac remodelling in a relatively under-studied population: survivors of AMI with 'asymptomatic' LVSD. The primary results may inform clinical trials powered to detect a mortality benefit in this patient group. The data provided on the use of established and recently-discovered biomarkers in the prediction of medium-term LV function after AMI represents a highly topical area for further studies. Finally, pre-discharge CMR was safe in AMI patients, and facilitated the detection of additional findings that positively influenced the management of almost one-quarter of the trial cohort. These findings may lead to greater uptake of this increasingly-available modality, to enhance the early management and risk stratification of survivors of large AMI.

Chapter 1

Introduction

1.1 Background

Improvements in the primary prevention of coronary artery disease, predominantly through more rigorous diagnosis and aggressive control of cardiac risk factors, have led to a decline in premature coronary heart disease (CHD) in most Western countries. Despite this, acute myocardial infarction (AMI) remains an important global clinical and socio-economic problem. In the United Kingdom, based on incidence rates of AMI of 600 per 100 000 in men and 200 per 100 000 in women under the age of 70 years, an estimated 123 000 persons aged 75 or less will suffer an AMI in 2008; this figure would be expected to rise further if elderly patients were included.[1, 2] Data from USA estimate that 770 000 Americans will suffer a first AMI, and a further 430 000 will experience a recurrent AMI, by the end of 2008, during which as many as 175 000 will suffer a silent AMI.[3] The combined expense of direct treatment of, loss of earnings/workforce by, and informal caring for CHD patients is estimated at over £7.9 billion per year to the UK economy.[1]

In Scotland, although the age-standardised mortality rates following AMI have fallen between 1994 and 2004 (from 223 to 140 per 100 000), cardiovascular morbidity and mortality remain high.[1, 4] Moreover, clinical evidence of heart failure or imaging evidence of left ventricular systolic dysfunction (LVSD) following AMI further impair the prognosis.[5-10] In a recent international registry incorporating data from 5573 patients admitted consecutively with AMI to one of 84 centres in 9 countries between 1999 and 2001, collected in conjunction with the VALsartan In Acute myocardial iNfarcTion (VALIANT) trial, the adjusted hazard ratio for death during admission with AMI complicated by heart failure, LVSD or both was 4.12.[10] The

development of heart failure, LVSD or both after AMI is therefore of considerable clinical and prognostic relevance.

Review of epidemiologic and clinical trial data suggest that heart failure will complicate 30-40% of admissions with AMI.[11] The incidence of LVSD after AMI is less well-documented and subject to variability in definitions and modes of assessment, but overall LVSD will occur in 25-60% of AMI, while heart failure will occur in at least 50% of patients with LVSD.[11] There is strong evidence that heart failure [6, 7, 10, 12-15] and LVSD [10, 16-18] early after AMI are independently and additively detrimental to short- and long-term prognosis. LVSD in the absence of heart failure early after AMI ("asymptomatic LVSD") is also a significantly morbid condition, carrying a higher in-hospital mortality than patients displaying signs of heart failure post-AMI in the context of preserved LV systolic function.[10]

What happens over time to patients with LVSD after AMI? In response to the acute deterioration in LV systolic function precipitated by AMI, a series of mechanical, neurohormonal and genetic processes are stimulated, the combined result of which is the promotion of complex changes in the architecture and function of the LV, in an initial compensatory response to maintain cardiac output: this process is post-infarction LV remodelling.[19, 20] If left unchecked, this early adaptive response will result in progressive LV dilatation, geometric alteration and dysfunction, leading inexorably towards the development of heart failure, with its associated morbidity and mortality. Results of several AMI studies have consistently shown a close relationship between LV remodelling and long-term clinical outcomes, which have led to the acceptance of LV remodelling as a major predictor of the clinical course of heart

failure.[21] From evidence derived from several studies performed over the last two decades, it is now apparent that a single measure of LV volumes and LV ejection fraction (LVEF) early after AMI predicts major cardiovascular outcomes, and that serial changes in LV volumes and LVEF correlate closely with progression to heart failure, cardiogenic shock, recurrent AMI and short- and long-term survival.[22-27]

Studies of AMI patients designed to detect a mortality benefit following a specific intervention generally require large patient numbers to allow adequate power. The strong relationship between LV remodelling and major cardiovascular end-points, however, raises the possibility that the results of a relatively small study of remodelling can be used to inform larger clinical trials powered to assess the effects of that intervention on hard clinical end-points.

The pathophysiology of post-infarction LV remodelling is being increasingly described, and provides multiple insights into the natural history of myocardial infarction in addition to providing several targets for strategies to ameliorate LV function, cardiovascular morbidity and survival. This thesis will focus on post-infarction LV remodelling in a cohort of patients with 'asymptomatic' LVSD early after AMI.

1.2 Cardiac remodelling:

Cardiac remodelling has been defined by the International Forum on Cardiac Remodelling as the alterations in genome expression, molecules, cells and interstitium that are manifested clinically as changes in the size, shape and function of the heart after cardiac injury.[21] Physiological remodelling occurs in normal growth from infancy to adulthood, and in pregnancy, in which the remodelling is not only an appropriate physiological response to the increased circulating blood volume, but is also reversible. Pathophysiological remodelling can occur in a variety of environments, including pressure overload (eg. hypertension, aortic stenosis, hypertrophic obstructive cardiomyopathy), volume overload (eg. valvular regurgitation), myopericarditis, idiopathic dilated cardiomyopathy and AMI. In many of these conditions, remodelling is an adaptive phenomenon which allows maintenance of an adequate cardiac output despite abnormal loading conditions, but in post-infarction remodelling the early adaptive response is rapidly superseded by a chronic maladaptive process. Post-infarction remodelling of the LV is the most extensively studied of these processes, and is the focus of this thesis.

1.2.1 Post-infarction remodelling

Acute occlusion of a coronary artery, especially in the absence of a collateral circulation, results in an abrupt cessation of systolic contraction within the region of myocardium subtended by the infarct-related artery.[28] This is caused by a combination of acute myocyte loss and stunned myocardium, ie. viable myocytes which retain the potential to recover contractility but which are rendered severely hypo- or non-contractile due to the acute deterioration in their pericellular environment.[29] Within one hour of coronary occlusion, geometric changes occur due to the abrupt increase in

loading conditions – the infarct zone stretches and the LV begins to dilate.[30] This response is in part adaptive – stretching of myocytes in the non-ischaemic regions of the LV allows partial compensation for the acute reduction in stroke volume and LVEF caused by the infarct via the Frank-Starling mechanism.[24, 31, 32] Ongoing myocyte loss and chronic increases in ventricular wall stress, however, lead to progressive LV dilatation and dysfunction.[19]

Studies examining the timecourse of LV geometric changes following AMI have led to an arbitrary division of post-infarction remodelling into early (<72 hours after coronary occlusion) and late (≥72 hours) phases.[30, 33, 34] Although there is some overlap, the major geometric alterations that occur in early remodelling are largely confined to the infarct zone and consist of stretching, infarct expansion and thinning, while late remodelling involves stretching of the entire LV myocardium, including the peri-infarct zone and remote, non-ischaemic myocardium, with subsequent hypertrophy, distortion of ventricular shape, and deterioration in systolic function.

While acute thrombotic occlusion of the infarct-related artery is the index event, what actually stimulates the remodelling process? As a direct consequence of acute regional myocardial dysfunction, two functionally disparate triggers have been identified – mechanical and neurohormonal.

1.2.2 Mechanical triggers

Regional wall stress has been shown consistently to be a major precipitant of post-infarction LV remodelling.[19, 24, 31, 32, 35] As described above, the initial changes in LV morphology are in part compensatory as they improve cardiac output in the acute

stages of infarction, but as the LV dilates, further demands are made on the surviving non-ischaemic myocardium due to increased wall stress. The law of La Place states that wall stress is directly proportional to the product of intracavitary pressure and radius, and inversely proportional to wall thickness.[36] As the cavity dilates, wall stress therefore increases to maintain intracavitary pressure, and hypertrophy of the non-infarcted myocardium occurs.

Intact myocardium consists of three major components: myocytes, extracellular matrix (ECM) and the capillary microcirculation derived from the coronary arteries. The ECM is an essential structural component of healthy myocardium, and is composed of predominantly type I collagen, with lesser amounts of type III collagen.[37] The ECM acts as a stress-tolerant scaffold which maintains the structural relationship between myocytes, contractile filaments and capillaries. Excessive wall stress, which develops very early after coronary occlusion, can directly and indirectly influence the ECM. Direct stretch as the LV enlarges can cause lengthening and weakening of collagen fibres; indirectly, stretch of surface mechanotransducers known as integrins can result in intracellular signalling triggering the release of growth factors and cytokines which, in turn, activate a family of enzymes responsible for the natural turnover of the ECM – the matrix metalloproteinases (MMPs); these will be described separately.[38]

Various components of the LV remodelling process are therefore stimulated mechanically as a consequence of increased wall stress following AMI. There is, however, an overlap between the mechanical and neurohormonal stimulation of remodelling. Mechanical stretch of myocytes in vitro provoked accumulation of

angiotensin II, a key neurohormonal influence on remodelling, within 30 minutes suggesting that in the dilating heart such stretch might provide a separate means of angiotensin II activation outwith the renin-angiotensin-aldosterone system (RAAS).[39]

1.2.3 Neurohormonal triggers

Plasma levels of several neurohormones rise rapidly early after AMI. The acute reduction in haemodynamic stability caused by regional infarction stimulates the sympathetic nervous system. Plasma noradrenaline (NA) levels rise acutely in an initial compensatory response to maintain a perfusing cardiac output.[40] Continued sympathetic overactivity leads, however, to further increased wall stress; NA excess also stimulates release of inflammatory cytokines, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).[40-42] Hypoperfusion of renal glomeruli promotes feedback renin release and activation of the RAAS; excess NA contributes by stimulating β1-receptors on cells of the juxtaglomerular apparatus (JGA), promoting further renin secretion.[43] Plasma angiotensin II and aldosterone levels rise, both of which have specific roles in remodelling. The major neurohormonal triggers of LV remodelling in the early post-infarction period are therefore the sympathetic nervous system and the RAAS. The respective roles of NA, the hormones of the RAAS, natriuretic peptides and cytokines in post-infarction remodelling will be discussed separately.

1.2.4 Changes within myocytes during LV remodelling

Myocyte loss occurs not only at the time of the acute myocardial injury, but continues thereafter as an ongoing feature of the remodelling process. For many years, necrosis was thought to be the sole method of myocyte death following AMI, but evidence

from a series of animal models has led to new insights into the changes that occur in myocytes after coronary occlusion.[44]

The classical necrosis model of acutely ischaemic myocytes undergoing cell membrane lysis, influx of extracellular fluid, swelling, activation of proteolytic enzymes, and leakage of intracellular contents has been modified by the combined apoptosis-necrosis model.[45, 46] Within the peri-infarct zone and within non-ischaemic myocardium remote from the infarct, there is evidence of apoptosis acutely (within hours) and continuing into the late remodelling phase.[47, 48] Apoptosis is an energy-requiring process triggered by the activation of a family of cysteine proteases known as caspases; leakage of myocyte contents, especially cytochrome C, is thought to stimulate these caspases which then promote apoptosis.[46, 49] The relative contributions of apoptosis and necrosis to myocyte loss are probably species-specific; interesting data from the rat infarction model suggest that apoptosis is the predominant mode of myocyte death within the first 2-4 hours following AMI, with necrosis predominating from 6-24 hours.[50, 51]

Myocyte death is an ongoing process, but non-ischaemic myocytes also undergo structural changes. Myocardial stretch has been shown to promote angiotensin II accumulation within myocytes, but also to induce expression of various hypertrophy-associated genes, including *c-fos, c-jun, c-myc* and *Erg-1*, in addition to a number of growth factors including transforming growth factor β1 (TGF-β1), insulin-like growth factor-1 and endothelin-1 (ET-1).[39, 52, 53] Myocytes are terminally differentiated cells and as such have limited if any potential for further division. Their response to these trophic stimuli is therefore to undergo hypertrophy, which contributes to the

progressive structural changes within the LV and maintains wall stress, further exacerbating the remodelling process.

Changes within myocytes at a molecular level are beyond the scope of this thesis.

1.2.5 Consequences of remodelling on global LV function

Following AMI the combination of mechanical stimulation and neurohormonal activation promotes morphologic changes within the LV in an initial attempt to stabilise cardiac output. Ongoing myocyte loss, hypertrophy of existing myocytes and persistently elevated wall stress continue to drive remodelling, leading to progressive LV impairment, heart failure and death. Replacement fibrosis within the infarct zone occurs early in the remodelling process, and is an appropriate healing response, but fibrosis is also seen in myocardium remote from the infarct, where it is termed reactive fibrosis.[54-58] Such reactive fibrosis is detrimental to cardiac function and contributes to progressive diastolic and systolic dysfunction. Aldosterone has been strongly implicated in its pathogenesis and will be described separately.[59]

Post-infarction remodelling is therefore a complex process, the end result of which is a progressive deterioration in LV function. The RAAS in general and aldosterone in particular have been the subject of several recent studies on LV remodelling, and will provide the focus for this thesis.

1.3 The RAAS and the role of aldosterone in LV remodelling:

1.3.1 The classical RAAS

The classical description of the RAAS depicted a cascade of neurohormonal activation that occurred mainly within the bloodstream, but which was dependent on important tissue-based factors.[60] In this model, the precursor α2-globulin angiotensinogen, synthesised and stored in the liver, is released into the bloodstream where it is converted by kidney-derived plasma renin activity to form angiotensin I, an inactive decapeptide (Figure 1.1).

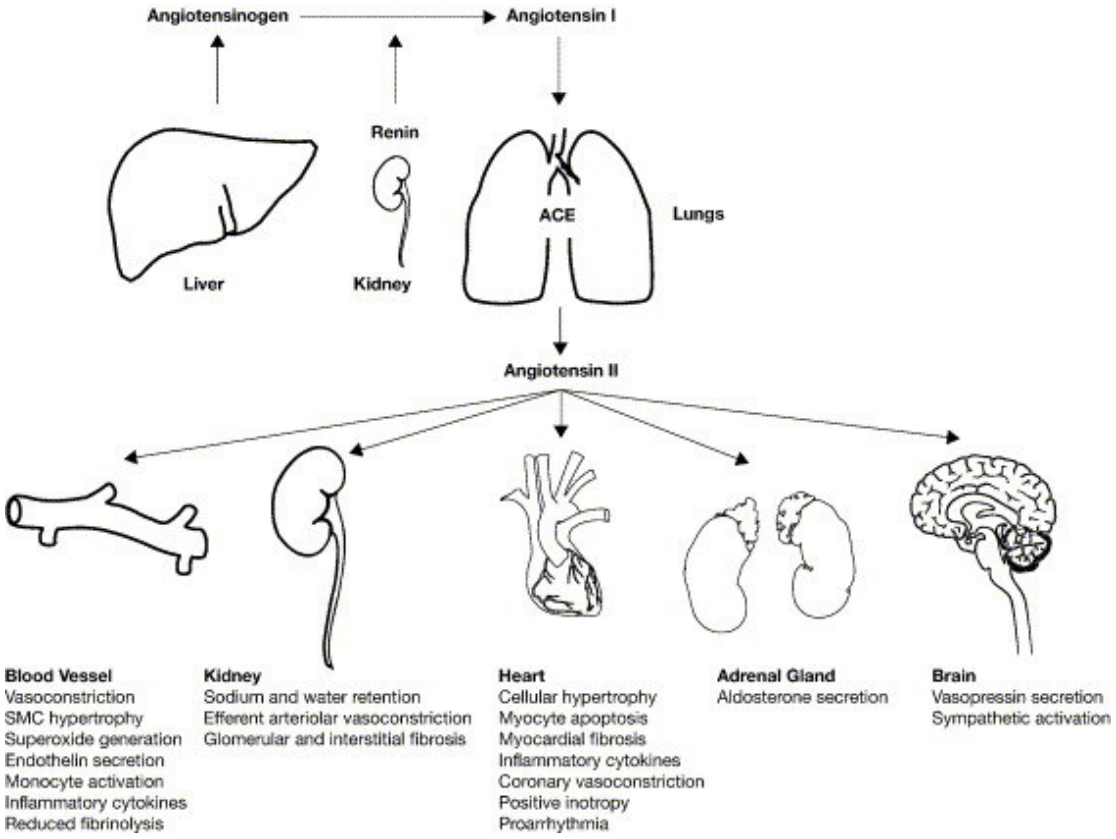


Figure 1.1 The classical renin-angiotensin-aldosterone system, illustrating the pathologic effects of angiotensin II.

Angiotensin-converting enzyme (ACE), a dipeptidyl-carboxypeptidase, then cleaves off an octapeptide histidyl-leucine from the inactive angiotensin I, producing angiotensin II. ACE also inactivates bradykinin. ACE is endothelial-based and found in greatest quantities in the lungs although it is found in endothelium elsewhere. Angiotensin II is a biologically active molecule with a wide range of actions (Figure 1.1). In addition it stimulates aldosterone synthesis within the zona glomerulosa of the adrenal cortex. A counter-regulatory response to RAAS activation is mediated by ACE2, the newly-described homologue of ACE, which hydrolyses angiotensin I to angiotensin-(1-9) and angiotensin II to angiotensin-(1-7).[61] ACE2 appears to have a protective role: ACE2 knockout mice undergo progressive ventricular dilatation and dysfunction, while ACE2 expression is up-regulated in macrophages, smooth muscle cells, cardiomyocytes and endothelial cells within the heart in the setting of AMI, and in chronic heart failure (CHF).[62, 63]

For many years this classical pathway was accepted as the sole means of angiotensin II and subsequently aldosterone production. Observations that drugs that block the RAAS continued to exert beneficial effects on LV function, progression to heart failure and death even after the initial increases in circulating RAAS components following large AMI had returned to baseline, independent of blood pressure-lowering effects, led to theories of non-circulatory RAAS.[40, 64-68] Several body tissues, including the heart, contain or synthesise various components of the RAAS in a variety of animal models, suggesting a role for tissue-based RAAS.[69-72] Growing evidence suggests that cardiac RAAS not only exists but is regulated independently of circulatory RAAS: tissue-based RAAS responds experimentally to myocardial stretch and growth factors rather than the systemic factors related to changes in blood

pressure and circulating volaemia which regulate circulatory RAAS.[73-76] Moreover, non-ACE dependent pathways of angiotensin I conversion to angiotensin II have been identified, the best described of which involves the enzyme chymase, which is released from cardiac and vascular mast cells, mesenchymal and endothelial cells.[77] That a combination of an ACE inhibitor and a chymase inhibitor was necessary to inhibit the vasoconstrictor response to angiotensin I infusions in patients with CHF confirms a dual pathway of angiotensin II synthesis.[77, 78]

The RAAS is therefore more complex than the classical theory suggests. Two of its effector hormones merit particular attention as they have been shown to be of critical importance in the pathogenesis of post-infarction remodelling: angiotensin II and aldosterone.

1.3.2 Angiotensin II

Following large AMI, the combination of increased renin release in response to renal hypoperfusion, sympathetic activation of β1-receptors on JGA cells, mechanical stretching of myocytes, and non-ACE dependent synthesis results in increased local and circulating angiotensin II. The effects of angiotensin II serve to potentiate and exacerbate post-infarction remodelling. Angiotensin II is a potent vasoconstrictor and promotes salt and water retention, both directly and through aldosterone release, further increasing LV wall stress and promoting hypertrophy. It has direct toxic effects on myocytes in addition to stimulating apoptosis, further increasing the demands on the remaining viable myocytes.[79] Cardiac fibroblasts have a high density of angiotensin II type 1 (AT_1) receptors.[80] Activation of these AT_1 receptors stimulates the release of not only enzymes that lyse and degrade collagen but also

cytokines, including tumour necrosis factor-α (TNFα) and interleukin (IL)-1β, which feed back to up-regulate fibroblastic expression of the AT_1 receptor making the cells more sensitive to angiotensin II.[81] In this way, angiotensin II promotes progressive disordering of the ECM, myocyte loss and ongoing pressure and volume loading of the infarcted heart.

Angiotensin II was not measured in the study on which this thesis is based. Aldosterone, the release of which is influenced by angiotensin II levels, provides the focus of this research and is described in detail in the following section.

1.3.3 Aldosterone

***Biosynthesis*:**

Aldosterone is a mineralocorticoid hormone synthesised in the zona glomerulosa of the adrenal cortex. Its main endocrine function is sodium and potassium homeostasis, which it achieves through reabsorption of sodium and excretion of potassium through the distal tubules and collecting ducts of the nephron.[82, 83] The biosynthetic pathway requires the sequential activity of several enzymes; deoxycorticosterone is ultimately converted to aldosterone by a sequence of hydroxylation reactions, all of which are catalysed by aldosterone synthase (Figure 1.2).

Release of aldosterone from the adrenal cortex is stimulated mainly by increased potassium and angiotensin II levels, and to a lesser extent by sodium deficiency, adrenocorticotrophic hormone (ACTH), catecholamines and serotonin.[83]

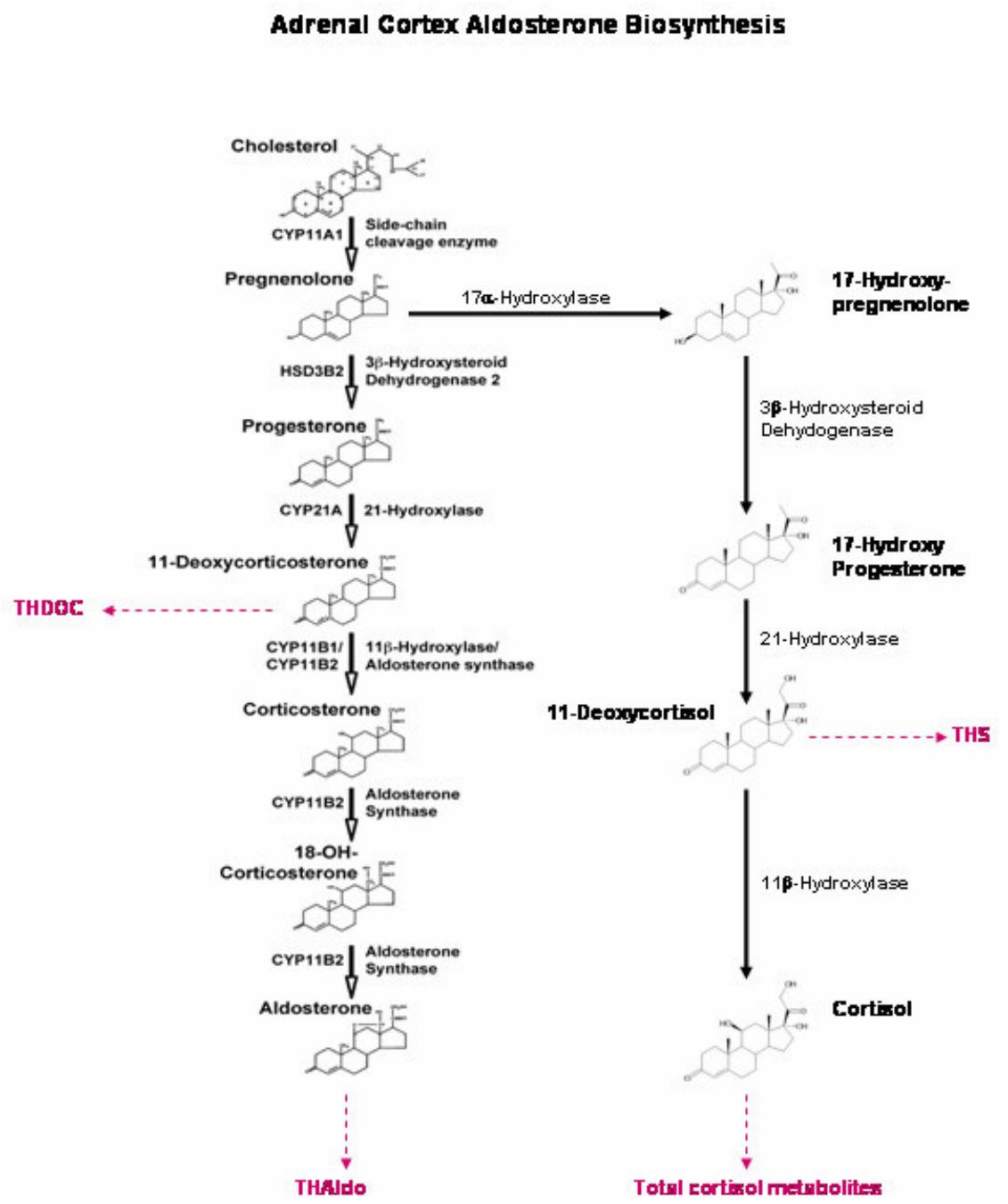


Figure 1.2 Adrenal cortical biosynthesis and degradation of aldosterone and cortisol. Urinary steroid metabolites are shown in purple. THDOC = tetrahydrodeoxycorticosterone, THAldo = tetrahydroaldosterone, THS = tetrahydrodeoxycortisol.

***Cardiovascular effects of aldosterone*:**

Excess circulating aldosterone has numerous adverse effects on the cardiovascular system, including promotion of myocardial interstitial and perivascular fibrosis, stimulation of myocyte apoptosis, mediation of baroreceptor dysfunction, prevention

of myocardial neuronal re-uptake of norepinephrine, increase in sympathetic drive, and potentiation of fluid overload and electrolyte imbalance.[84-89] Many of these deleterious effects on the cardiovascular system in general are pertinent to post-infarction pathophysiology and remodelling.

***Aldosterone production following AMI*:**

The increase in circulating angiotensin II in the early aftermath of AMI described above promotes aldosterone release from the adrenal cortex. Local intracardiac production of aldosterone has also been demonstrated in the rat heart, and has been confirmed in the failing human heart and in hypertensive patients without LVSD, although it is noteworthy that doubt has been cast on the pathophysiological significance of cardiac steroid production by separate research groups.[90-94] Intracardiac aldosterone synthesis is triggered shortly after AMI in the rat heart, allowing local intracardiac aldosterone concentrations far in excess of systemic levels.[92] Additionally, plasma aldosterone extraction across the acutely infarcted human heart has recently been demonstrated, and the transcardiac aldosterone gradient correlates with left ventricular volumes and plasma levels of PIIINP, a marker of matrix collagen turnover, suggesting that aldosterone extraction stimulates remodelling in patients with AMI.[94] Through a variety of mechanisms, therefore, circulating and local aldosterone levels increase significantly early after AMI. Aldosterone is therefore in a prime position to influence remodelling. Elevated aldosterone levels correlate directly with mortality in CHF.[95]

***Effects of aldosterone following AMI*:**

Aldosterone exerts its tissue effects via the mineralocorticoid receptor. These receptors have been identified in the kidney, colon, brain, lung and heart.[96] Within the heart, mineralocorticoid receptors are present on myocytes, fibroblasts and the endothelial cells of large vessels.[97, 98] The high aldosterone state that develops acutely after AMI exerts a wide range of actions through these target organs which contribute to and exacerbate LV remodelling. The mineralocorticoid receptor, however, has a similar affinity for aldosterone and cortisol; for aldosterone to interact with the mineralocorticoid receptor, the receptor has to be 'protected' by the cellular enzyme 11-β hydroxysteroid dehydrogenase.[99] This enzyme is expressed in a variety of organs including the kidney, but the burden of evidence suggests that it is not expressed in the heart, thus potentially exposing the intracardiac mineralocorticoid receptors to the effects of circulating cortisol.[100]

The most dramatic and best-documented effect of aldosterone in the heart is the development of significant fibrosis.[84, 101, 102] Myocardial fibrosis is experimentally induced in the rat by aldosterone in salt-loaded conditions; this effect is attenuated by concomitant administration of the selective mineralocorticoid receptor antagonist (MRA) eplerenone.[86] Interestingly these profibrotic effects of hyperaldosteronism only appear to occur in the presence of a high salt environment.[103] The exact mechanisms by which aldosterone and sodium combine to promote fibrosis are unclear, but the resultant effect appears to be stimulation of mineralocorticoid receptors on cardiac fibroblasts, promoting pathological ECM turnover. Post-infarction fibrosis was traditionally considered to develop as a reparative phenomenon to strengthen the infarcted segment, but it is now clear that in addition to replacement

fibrosis at the site of the scar, reactive fibrosis occurs elsewhere, in myocardium remote from the infarct zone.[37, 104, 105] Such widespread fibrosis contributes to progressive diastolic dysfunction, systolic dysfunction, geometric alteration and arrhythmic potential. The causative relationship between aldosterone and fibrosis has been confirmed by several animal and human studies in which aldosterone antagonism has led to attenuation or even reversal of fibrosis, with concomitant improvement in LV function. [106, 107]

Through mineralocorticoid receptor activation in the kidney, vasculature and endothelium, excess aldosterone following AMI also leads to sodium/water retention (aggravating the loading conditions on the infarcted LV), potassium loss (increasing risk of arrhythmia), perivascular fibrosis and endothelial dysfunction, all of which are deleterious in this crucial post-infarction phase.[86]

1.4 'Upstream' inhibition of the RAAS – the roles of ACE inhibitors and ARBs

Neurohormonal modulation of the RAAS has become a key component in the management of patients with left ventricular systolic dysfunction LVSD, in the context of both CHF and AMI. Following early work confirming an anti-remodelling effect of the ACE inhibitor captopril in a rat AMI model, ACE inhibitors have been shown in landmark trials to improve survival and, when assessed, to attenuate remodelling in both AMI and CHF patients.[23, 66, 68, 108-113] ACE inhibitors are now the cornerstone of medical therapy for these conditions. Fewer clinical data are available on the effects of AT_1-receptor blockers (ARBs) on remodelling. Limited data from CHF populations suggest a beneficial effect of ARBs on remodelling, while a

comparable effect to that of captopril on post-infarction remodelling has recently been demonstrated using the ARB valsartan.[113-117]

***'Aldosterone escape' and the effects of aldosterone blockade*:**

Upstream inhibition of the RAAS with ACE inhibitors or ARBs leads to early reductions in plasma aldosterone, but over time aldosterone levels start to increase. This phenomenon is known as aldosterone escape.[118] It is thought to result from aldosterone secretion consequent to the hyperkalaemic effects of ACE inhibitors/ARBs together with non-ACE dependent angiotensin II/aldosterone synthesis; it is seen in up to 40% of CHF patients, can occur despite combined ACE inhibitor and ARB use, and has been documented as early as 3 days after commencement of an ACE inhibitor following AMI.[115, 118, 119] As aldosterone exerts a multitude of deleterious effects in the post-infarction phase, mineralocorticoid receptor antagonism represents an attractive adjunct to ACE and/or AT_1-receptor inhibition.

It is perhaps unsurprising that clinical trials of mineralocorticoid receptor antagonists have shown beneficial effects on cardiovascular outcomes in patients with hypertension, CHF and AMI. For example, the use of eplerenone in hypertensive patients enrolled into the 4E-LVH Study resulted in comparable reductions in blood pressure and LV mass to those seen with an ACE inhibitor.[120] In the Randomised Aldactone Evaluation Study (RALES), the addition of the aldosterone receptor antagonist spironolactone significantly reduced cardiovascular morbidity and mortality in patients with advanced CHF, most of whom were on an ACE inhibitor.[121] In a sub-study of RALES, spironolactone evoked a reduction in PIIINP compared to

placebo, suggesting an anti-remodelling effect.[122] More recently eplerenone has been shown to provide significant benefit on major cardiovascular outcomes in AMI patients with reduced LVEF and heart failure, or diabetes, again with a high baseline uptake of ACE inhibitors or ARBs in the eplerenone post-acute myocardial infarction heart failure efficacy and survival study (EPHESUS); no imaging sub-study has been reported.[123]

The striking morbidity and mortality benefits reported in RALES and EPHESUS have led to the inclusion of aldosterone antagonists in European and American guidelines for the management of CHF and AMI.[124-128] That mineralocorticoid receptor antagonists should exert an anti-remodelling effect in both of these conditions would appear logical in view of the strong pro-fibrotic effects of aldosterone. Experimental models have confirmed an antifibrotic effect of aldosterone blockade on reactive but not reparative fibrosis, alleviating fears over the safety of aldosterone antagonism following AMI.[106] Despite these data, the evidence for a positive effect of aldosterone blockers on LV remodelling from clinical trials is surprisingly weak. Neither RALES nor EPHESUS, the landmark aldosterone antagonist trials in CHF and AMI respectively, included an imaging sub-study. Evidence from small clinical trials suggests a beneficial effect of aldosterone blockade on LV remodelling: early (day 1) administration of spironolactone to a cohort of 65 patients with reperfused first anterior AMI, treated with ACE inhibitor, led to reductions in LV volumes, improvements in LVEF (measured by invasive contrast ventriculography) and attenuation of PIIINP rise compared to placebo, and more recently reversal of LV remodelling was observed in a small cardiac magnetic resonance (CMR) study (n=51) of patients with mild/moderate CHF and LVEF<40% with the combination of the

ARB candesartan/spironolactone versus candesartan/placebo.[94, 129] Two larger recent CHF trials focusing on serial change in LV function have, however, failed to demonstrate a positive effect on LV remodelling with aldosterone blockade on top of baseline ACE inhibitor/ARB therapy in mild/moderate CHF patients.[130, 131] While theoretically attractive as a method of attenuating pathological remodelling, the role of aldosterone blockade is therefore not yet clear.

1.5 Aldosterone synthase polymorphisms

The final step in the biosynthetic pathway of aldosterone in the zona glomerulosa of the adrenal cortex is catalysed by the enzyme aldosterone synthase (Figure 1.2). Aldosterone synthase is a mixed-function oxidase of the cytochrome P450 family of haem-containing enzymes, and is encoded by CYP11B2, a 9-exon gene located in humans on chromosome 8q22.[132] Two common mutations in CYP11B2 have been identified: a single nucleotide polymorphism (SNP) involving substitution from C to T at position -344, and a mutation in the sequence of intron 2 of the gene, allowing three haplotypes to be described.[133] The -344T/C polymorphism is of particular interest as it lies within the binding site for a transcription factor essential in steroid biosynthesis: steroidogenic factor-1 (SF-1).[134] The excretion of aldosterone is influenced by these mutations, but with significant inter-species and inter-racial variability. In Caucasian populations the -344T allele has been associated with higher excretion rates of aldosterone, while in African American populations the -344C allele has not only been linked to higher aldosterone release but also to adverse prognosis in CHF and a tendency towards adverse ventricular remodelling.[134, 135] Polymorphic variations in CYP11B2 are associated with variations in LV size and mass in healthy populations free of cardiovascular disease, and have been shown to predict

improvement in LVEF in CHF patients being commenced on medical therapy inclusive of ACE inhibitors.[136, 137]

Such variations in the synthesis, release and excretion of aldosterone, a key hormone in LV remodelling, understandably engendered interest in the influence of SNPs in CYP11B2 on post-infarction remodelling. Results from two recent studies have been disappointing, however. A German population-based study revealed no difference in allele frequency between patients who had sustained AMI 5 years previously and the general population; moreover, within the post-MI cohort there was no association between either allele and severity of LVSD, LV mass nor diastolic function.[138] In a separate study analysing the influence of common genetic mutations on the early remodelling process in 266 French patients with incident anterior AMI, no association between any mutation, including -344T/C, and LV remodelling out to 1 year after AMI was found.[139] It is noteworthy, however, that TTE was the imaging modality employed and that the definition of LV remodelling used was change in (Δ) LV end-diastolic volume (LVEDV) over time. LV end-systolic volume (LVESV) and the change therein has consistently been demonstrated to be a more powerful predictor of survival, and the most appropriate means of assessing LV remodelling, following AMI.[22]

Aldosterone therefore plays an important role in post-infarction LV remodelling, but key questions persist regarding the additional benefits of aldosterone blockade on LV remodelling in the context of upstream pharmacological inhibition of the RAAS, and the influence of CYP11B2 and its common mutations in the remodelling process; both of these issues were analysed in this thesis through assessing the effects of the

selective MRA eplerenone on LV remodelling in a cohort of patients with LVSD early after AMI but without clinical or radiological evidence of heart failure..

1.6 The role of revascularisation in post-infarction LV remodelling

Acute coronary occlusion is the index event that triggers the various mechanical, neurohormonal and genetic factors that drive the remodelling process. Patency of the infarct-related artery (IRA) is therefore an attractive and obvious target in the limitation of myocardial damage and prevention of remodelling. The critical importance of a patent IRA (the "open artery hypothesis") has been demonstrated in various observational and clinical studies over the last two decades. Patients with an open IRA, or else an occluded IRA with adequate collateral blood supply, 30 days after index (non-reperfused) AMI sustained significantly less LV dilatation that patients with an occluded IRA and no collateral supply.[140] Comparison of patients treated with or without early thrombolytic revealed early improvements in LV function that were attributable to thrombolytic therapy, but ongoing attenuation of LV remodelling up to 6 weeks that was attributable to patency of the IRA.[141] Studies of both early and late (up to two weeks after AMI) percutaneous reperfusion have confirmed beneficial effects on medium- and long-term LV remodelling in those with a persistently patent IRA.[26, 142, 143]

The importance of microcirculatory dysfunction following reperfusion therapy has been increasingly appreciated in recent years. The role of such microvascular obstruction in LV remodelling is discussed separately (Chapter 1.11.4).

1.7 The role of neurohormonal activation in post-infarction LV remodelling

1.7.1 Natriuretic peptides

A number of natriuretic peptides are released in high quantities early after AMI.[40] Two of these in particular have attracted considerable interest due to their potential in assessing prognosis and monitoring disease progression – BNP and N-terminal pro-BNP (NTproBNP). BNP is a 32 amino acid neurohormone synthesised in ventricular myocardium and released in response to ventricular myocardial stretch and pressure overload.[144] As BNP is released, a 76 amino acid residue – the N-terminal fragment – is also released: NTproBNP. BNP is elevated in symptomatic and asymptomatic LVSD and predicts mortality across all grades of severity of CHF.[145-147] NTproBNP also predicts mortality in CHF.[148]

BNP and NTproBNP have been extensively studied in the setting of AMI. BNP is a powerful predictor of the development of heart failure, recurrent AMI and death in both ST-elevation and non-ST-elevation acute coronary syndromes.[149-151] NTproBNP not only predicts major adverse cardiovascular outcomes after AMI but has been shown to be an even stronger predictor of survival than LVEF.[151, 152]

The relationship between these natriuretic peptides and parameters of LV function after AMI is less consistent. Data from studies employing a variety of imaging modalities reveal associations between BNP and certain LV measurements but not others. In a radionuclear study, BNP correlated with size of perfusion defect but neither LV volumes nor LVEF.[153] Similarly, BNP showed a close relationship with CMR-measured infarct scar, and also with LVEF, in a stable post-AMI cohort but no

consistent relationship with LV volumes.[154] Despite relatively weak correlations between BNP, LVEF and certain LV volumetric measurements in various studies there is a lack of a consistent relationship between BNP and LV parameters in the acute and chronic stages of myocardial infarction.[155, 156] NTproBNP is also subject to these inconsistencies. Despite its prognostic benefit in AMI there is no consistent agreement between NTproBNP levels and LV parameters after AMI; indeed, when NTproBNP was measured in three groups of patients with CHD – stable angina, unstable angina and AMI – the weakest correlation between LVEF and NTproBNP was seen in the AMI group.[157, 158]

These natriuretic peptides are of undoubted prognostic benefit in AMI. Their relationship with LV volumes and function after AMI is less clear; I analysed further this relationship in the cohort of patients studied in this thesis, in order to determine whether eplerenone affects BNP and/or NTproBNP after AMI.

1.7.2 Noradrenaline

NA is a catecholamine synthesised in the adrenal medulla from dopamine, under the influence of dopamine β-hydroxylase. It has a combined role of hormone and neurotransmitter and is an important effector molecule of the sympathetic nervous system. NA may also have an important role in LV remodelling. Direct toxic effects on myocytes in response to NA have been demonstrated in vitro, including β-receptor induced promotion of apoptosis and α-receptor induced myocyte hypertrophy.[159-161] Prolonged infusion of the β-agonist isoproterenol caused a dose-dependent increase in LV volumes and reduced survival in healthy rats.[162] The sympathetic nervous system is activated rapidly in response to acute infarction and NA levels have been shown to

remain elevated up to 90 days after AMI.[40] Not only can NA exert these toxic effects on surviving myocytes, it also influences other pathophysiological processes integral to remodelling. RAAS activation is potentiated through β1-receptor activation on JGA cells, excess circulating NA promotes release of ANP, BNP and inflammatory cytokines, and through its chronotropic and inotropic actions, NA serves to increase loading conditions on the infarcted LV.

NA may have a role in monitoring progression of post-infarction remodelling. I therefore planned to characterise the influence of NA on change in LV function after AMI, and to assess the effects of eplerenone on this marker of sympathetic activation.

1.7.3 Arginine vasopressin

Arginine vasopressin (AVP) is a peptide hormone synthesised in the hypothalamus and released from the posterior pituitary. Its primary effect is to increase renal reabsorption of water. Marked increases in AVP have been documented within 6 hours of infarction, which then decline irrespective of progression to heart failure.[163-165] ACE inhibitors have no effect on AVP levels after AMI, while a specific AVP antagonist in an ovine AMI model failed to influence LV function or cardiac biomarker rise.[166, 167]

The role of AVP following AMI is unclear. I planned to characterise its relationship, if any, to change in LV function after AMI and to assess whether eplerenone has any effect on serial AVP measurement.

1.7.4 Apelin

Apelin is an endogenous ligand for the G-protein coupled APJ receptor which was isolated only 10 years ago.[168] It has been shown to have potent inotropic effects in both normal and failing rat hearts in addition to a diuretic effect (mediated via suppression of AVP activity and release) and, peculiarly, a nitric oxide-dependent vasodilating effect.[168-170] These properties suggest a potential role for apelin in cardiovascular homeostasis. Results of two studies in CHF populations have been conflicting, showing either consistently lower or higher apelin levels than age-matched controls.[171, 172] The natural history of apelin in the AMI setting had not yet been described; this was characterised this for the first time in the work in this thesis.

1.8 The role of matrix metalloproteinases (MMPs) in post-infarction LV remodelling

That changes within the structure of the ECM are integral to LV remodelling has inevitably led to interest in the endogenous regulation of ECM components. This function is performed in animal and human myocardium by the MMPs, a family of zinc-dependent proteases essential for the physiological turnover of the ECM in not only myocardium but also bone growth, reproduction and wound healing. MMPs are synthesised as proenzymes that bind to ECM components but remain inactive; zinc-dependent cleavage activates the MMP and promotes collagen degradation.[173, 174]

Each cell type found within the normal myocardium can, under physiological or pathological conditions, synthesise and release MMPs; cardiac fibroblasts have attracted the greatest attention.[175] MMPs are sub-classified according to substrate specificity and/or structure.

Of particular interest in AMI, and therefore extensively studied in animal models and humans, are the gelatinases – MMP-2 (gelatinase A) and MMP-9 (gelatinase B). Results from various genetic animal models have provided strong insights into the relative contributions of the MMPs to LV remodelling. MMP-2-null mice experience less early LV rupture and less significant late LV remodelling than control wild-type mice after experimental infarction.[176] Similar findings of reduced early rupture and attenuated late remodelling have been demonstrated in MMP-9-null mice following AMI.[177, 178] In mice, gelatinase activity appears to relate to both early myocardial destabilisation and later ventricular remodelling.

A number of studies examining the roles of the MMPs and their endogenous inhibitors - the tissue inhibitors of matrix metalloproteinase (TIMPs) – have been undertaken in humans. From these it is increasingly apparent that there are inter-species differences and significant temporal variations in the activation and function of both MMPs and TIMPs. In the first few days following AMI in humans, MMP-2 levels are consistently elevated and have been shown to correlate inversely with NTproBNP.[179, 180] The relationship between MMP-2 and LV function is variable in humans: a strong inverse correlation between MMP-2 levels and LV volumes in the first few days after AMI has been reported, but several studies have shown no relationship either in the immediate post-infarction or follow-up between MMP-2 and LV parameters.[179-181] The role of MMP-2, seemingly integral to LV remodelling in the mouse model, is as yet unclear in human post-infarction LV remodelling.

In contrast to MMP-2, evidence from human studies suggests a more significant role for MMP-9. The release of MMP-9 after AMI appears to be biphasic, peaking within the first 12 hours then falling to a plateau which remains higher than age-matched controls, and which persists for at least three months.[179, 181] In the early phase, peak MMP-9 is inversely related to baseline LVEF and strongly correlated to the change in LV end-diastolic volume between baseline and 3 months after AMI. Peak MMP-9 also predicts the development of heart failure over the 2 years following the index infarct.[182] The relationship between MMP-9 level and LVEF is lost in the plateau phase, and interestingly at three months follow-up, the plateau MMP-9 level is greater in patients with less severe LVSD.[181] MMP-9 may therefore have a dual role in separate temporal periods following AMI in humans: an early collagen-lysing, pro-remodelling action but perhaps a protective role in the first few months after the acute infarct period, limiting progressive LV impairment. Consistent with this theory, early MMP-9 correlates with baseline NTproBNP but this correlation is lost over time.[157] In a separate small study (n=52) in which LV function was assessed by CMR at least 4 years after AMI, an inverse correlation was again seen between MMP-9 and LVEF (but not LV volumes).[157] Whether MMP-9 exacerbates progressive LV dysfunction, or whether this relationship to LVEF distant from the infarct represents an appropriate adaptive response, remains unclear. That MMP-9 exerts time-dependent actions in the remodelling process is, however, evident from these data.

Interest has focused on the role of TIMPs in remodelling, particularly in the early stages of AMI when MMP activity is at its highest, in terms of the mechanism of remodelling and (potentially) therapeutic potential. Four TIMPs have been identified to date. TIMP-1 levels are increased are increased in patients presenting with acute

coronary syndromes and remain high for at least 6 months thereafter.[180, 181] One study showed a correlation between TIMP-1 and NTproBNP at one week and one month after AMI but the significance of this is unclear. TIMP-1 deficiency does, however, amplify LV dilation after AMI in the mouse.[183] TIMP-2 levels measured in the first few days after AMI are modestly elevated and remain so over the next 6 months at least, while TIMP-4 falls immediately after AMI to levels lower than age-matched controls and remain low out to 6 months.[181] No relationship has been identified so far between TIMP-1,2 or 4 and either baseline or change in LV volumes and LVEF following AMI in humans.

Pharmacologic suppression of MMP activity early after AMI is theoretically attractive as a means of limiting myocardial damage and attenuating remodelling. Animal studies using selective MMP inhibitors have revealed mixed results. Studies involving inhibition of MMP-2 and MMP-9 among others have failed to reproduce the beneficial effects on LV function reported by other groups.[184-186] Broad-spectrum MMP inhibition is not attractive due to not only musculoskeletal side-effects but also theoretical suppression of the appropriate reparative actions of MMPs after large AMI. The largest human trial of an oral MMP inhibitor, with a high affinity for MMP-2,3,8,9,13 and 14, failed to show any benefit on LV remodelling in patients with low LVEF after AMI over 3 months.[187]

Despite the lack of success of MMP inhibition, the roles of individual MMPs in remodelling and the therapeutic potential of more selective MMP inhibition continues to drive research into the respective roles of MMPs and TIMPs in humans. MMP-3 has recently been demonstrated to be of potential interest in post-infarction

remodelling in humans and merits further study.[188] In this study of remodelling in patients with reduced LVEF after AMI that constitutes the focus of this thesis, I analysed further the roles and assessed the response to treatment of MMP-2, MMP-3, MMP-9 and TIMP-1, TIMP-2 and TIMP-4 in the human remodelling process.

1.9 The role of cytokines in post-infarction LV remodelling

Cytokines represent a further category of biologically-active molecules that have been implicated in the pathogenesis of remodelling. These are relatively low molecular weight (8-30kDa) protein molecules that can be synthesised in and secreted by a variety of cell types in different tissues. In this respect they differ from polypeptide neurohormones which tend to be released from specialised cells in specific tissues. Over 30 classes of cytokines have been recognised to date.

Interest in the potential role of cytokines in LV remodelling was stimulated by the discovery of increased levels of the cytokine TNFα in patients with CHF.[189] Cytokines effect a wide range of actions, some of which involve myocytes, fibroblasts and the ECM, hence the generation of significant interest into a potential aetiological role in remodelling. Most research to date in this field has focused on the "pro-inflammatory" cytokines - TNFα, IL-1 and IL-6.

In vitro studies have demonstrated myocyte hypertrophy in response to TNFα or IL-1β, although this may be indirect and mediated via reactive oxygen species.[190-192] Myocardial hypertrophy is also seen in mice over-expressing IL-6.[193] IL-1β and TNFα have also been shown to promote myocyte apoptosis and to exert negative inotropism in vitro and in vivo; interestingly in rats infused with TNFα, this negative

inotropic effect was fully reversible as soon as the TNFα infusion was discontinued.[194-197] Inflammatory cytokines therefore promote changes within myocytes – hypertrophy, apoptosis and functional impairment – all of which are seen in pathological remodelling.

Cytokines also adversely affect cardiac fibroblasts. In vitro, TNFα inhibits fibroblastic collagen gene expression and thus collagen synthesis.[198] IL-1β exerts an antiproliferative effect on cardiac fibroblasts, while two cytokines of the IL-6 family – leukaemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1) – promote fibroblast cell growth.[199] LIF has also been shown to inhibit differentiation of cardiac fibroblasts into myofibroblasts, suggesting an autocrine/paracrine regulatory function in matrix remodelling.[200]

In addition to their effects on myocytes and fibroblasts, cytokines also appear to exert direct and indirect effects on the ECM. Infusion of TNFα not only causes progressive LV dilatation but also degradation of the ECM in rats.[196] Within cultured fibroblasts, TNFα has been shown to upregulate pro-MMP-3, and IL-1β to upregulate a number of MMPs including MMP-2 and MMP-9.[198] Consistent with these in vitro findings, over-expression of TNFα promotes enhanced MMP activity and reduced LVEF acutely, although over time MMP activity falls and TIMP activity rises.[196] Through such variations in the MMP:TIMP balance, cytokines can indirectly modulate ECM turnover.

The pro-inflammatory cytokines therefore affect the major components of the remodelling process: myocytes, cardiac fibroblasts and the ECM. Several other

cytokines have also shown correlation with parameters of remodelling. Monocyte chemoattractant protein-1 (MCP-1) appears to be protective in the short-term, promoting wound healing following experimental AMI in mice, but to potentiate adverse remodelling in the longer-term.[201, 202] Macrophage inflammatory protein-1α (MIP-1α), regulated on activation normally T cell-expressed and secreted (RANTES) and MCP-1 are all elevated in the first week after AMI in humans and remain elevated over the first month in those who develop heart failure; in these high risk patients, weak correlations have been identified between MIP-1α and LV volumes/LVEF.[203] The recently-discovered IL-18 correlates inversely with LVEF after AMI.[204]

There is strong experimental evidence that cytokines play a role in remodelling. Further indirect evidence is provided by the marked reduction in inflammatory cytokines seen in CHF patients following treatment with β-blockers, which are of proven mortality benefit in CHF.[205, 206] Moreover, significant interplay occurs between the cytokines and other systems integral to pathological remodelling. TNFα upregulates cardiac fibroblastic expression of the AT_1 receptor and also promotes angiotensin II release, thereby making the fibroblasts more sensitive to the effects of angiotensin II and promoting RAAS activation.[81] TNFα also appears to upregulate ACE activity, further stimulating the RAAS.[207] NA release due to sympathetic overactivity also activates pro-inflammatory cytokines, while the interaction between cytokines and the MMP:TIMP system has been discussed.[42]

In my study cohort I investigated further the roles of a variety of pro- and anti-inflammatory cytokines on post-infarction LV remodelling. I also examined for the first time the role of a novel biomarker related to cytokine activity in the post-

infarction period – serum soluble ST2 (sST2). ST2 is a member of the IL-1 receptor (IL-1R) family with transmembrane (ST2L) and soluble (sST2) isoforms and may be relevant as a biomarker of prognostic significance in AMI and in both acute and chronic heart failure.[208] ST2L is membrane-bound with 3 extracellular immunoglobulin G (IgG) domains, a single transmembrane domain, and an intracellular domain homologous to Toll-like receptors and other IL-1Rs.[209] sST2 lacks the transmembrane and intracellular domains and is thought to function as a decoy receptor which neutralises IL-33, recently demonstrated to be the ligand for this receptor.[210] IL-33 exerts anti-hypertrophic effects in cultured cardiomyocytes that are antagonised by administration of sST2, and reduces myocardial fibrosis and cardiomyocyte hypertrophy after experimental pressure overload in mice, although interestingly these effects are not seen in mice lacking the ST2 gene.[211, 212] These data suggest a possible cardioprotective role for the IL-33/ST2 signalling pathway. In human studies, serum sST2 is elevated early after AMI and correlates with the myocardial-bound fraction of the enzyme creatine kinase and (inversely with) LVEF.[209] Serum sST2 correlates with NTproBNP in patients admitted with ST-elevation AMI (STEMI) and predicts subsequent 30-day mortality and heart failure.[213, 214] Additionally, serum sST2 is elevated and is of predictive value in acutely dyspnoeic patients with and without decompensated acute and chronic heart failure.[211, 215, 216]

That sST2 predicts adverse cardiovascular outcome, and is related to LVEF after AMI, suggests that it may have a role in post-infarction LV remodelling but this has yet to be evaluated. I therefore examined for the first time the relationships between serum

sST2, parameters of LV function, and a variety of circulating mediators in our trial cohort.

1.10 The role of haemostatic markers in post-infarction LV remodelling:

Myocardial infarction is the product of a multifactorial process culminating in the acute occlusion of the affected coronary artery. Substantial research has been undertaken into the role of the coagulation cascade in the acute stages of infarction and is beyond the scope of this thesis. Of relevance, however, is the evidence of a pro-thrombotic state that not only puts the population at greater risk of developing CHD but also adversely affects prognosis after AMI.[217-220]

Tissue plasminogen activator (tPA) is a serine protease secreted from endothelial cells in response to vascular injury. It has a key role in the coagulation-fibrinolysis cascade, converting inactive plasminogen to the fibrinolytic molecule plasmin, which then degrades fibrin. Plasminogen activator inhibitor-1 (PAI-1) is a potent endogenous inhibitor of tPA (and urokinase) which is released by activated platelets and neutralises tPA. von Willebrand factor (vWF) is an acute phase protein stored in endothelial cells and platelets which is released rapidly at the site of vascular injury and which is crucial for platelet aggregation.

Interest in the potential use of these three markers of haemostasis in risk stratification and prognosis in cardiovascular disease has been stimulated by epidemiological and clinical studies. Both tPA antigen and vWF predict the 2-year occurrence of AMI or sudden cardiac death in patients with angina pectoris of varying severity.[218] In survivors of both non-Q-wave and Q-wave AMI (including STEMI), vWF strongly

predicts the 30-day major adverse cardiovascular event rate (including death and heart failure).[219, 220] In prolonged (10 year) follow-up of 123 survivors of AMI, both vWF and tPA antigen were strong independent predictors of cardiovascular mortality.[221]

That elevated tPA antigen predicts adverse outcome appears counter-intuitive, as it would be expected to promote endogenous fibrinolysis. Free, active tPA is difficult to measure in plasma thus tPA antigen is frequently measured instead; tPA antigen acts as a surrogate marker of the tPA-PAI-1 complex, however, thus elevated tPA antigen indirectly suggests elevated PAI-1.[217, 222] In keeping with this, strong correlation between tPA antigen and PAI-1 was confirmed in the European Concerted Action on Thrombosis and disabilities angina pectoris study group (ECAT).[218] PAI-1 is elevated early after STEMI, has a weak inverse correlation to baseline LVEF, and predicts 30-day occurrence of heart failure and death.[220, 223]

The roles of tPA, PAI-1 and vWF in the post-infarction phase are unclear. They may represent the severity of the ischaemic vascular injury, endothelial dysfunction, success of reperfusion or platelet activation. That they have been shown to predict major adverse events including heart failure after AMI suggests that they may have a role in predicting LV remodelling or indeed its pathophysiology. Plasmin has been shown to activate pro-MMPs thereby promoting ECM degradation.[183] Markedly increased PAI-1 expression in myocytes and perivascular cardiac mast cells has been demonstrated following AMI in mice, contributing to replacement, reactive and perivascular fibrosis, key factors in LV remodelling.[224] Interestingly less fibrosis occurred in PAI-1 knockout mice.

In this thesis I analysed in greater detail the potential predictive and/or pathophysiological roles of tPA antigen (as a surrogate of the tPA-PAI-1 complex) and vWF in post-infarction LV remodelling.

1.11 Non-invasive assessment of ventricular function

European and American guidelines recommend that all patients who have suffered AMI should undergo a formal evaluation of LV function, ideally pre-discharge.[126-128] The exact means of LV assessment is not stipulated in the American College of Cardiology/American Heart Association (ACC/AHA) guidelines, while the European Society of Cardiology (ESC) guidelines recommend that trans-thoracic echocardiography (TTE) should be performed in all patients; other modalities may be used if available.

1.11.1 Comparison of contemporary imaging modalities

Unenhanced 2-dimensional (2-D) TTE is the most widely-used method of assessing LV function following AMI.[11] More reproducible methods are favoured, however, as TTE carries significant inter- and intra-observer variability that limit its applicability; these include radionuclide ventriculography (RNVG), single photon emission computed tomography (SPECT), contrast-enhanced TTE, invasive contrast angiography, and cardiac magnetic resonance imaging (CMR). CMR affords not only the gold-standard means of assessment of LV volumes and ejection fraction, but also allows assessment of myocardial viability, perfusion, regional function and imaging of the thorax and upper abdominal viscera.[225-232] CMR is also superior to first pass RNVG and gated blood-pool SPECT, the traditional modalities of choice, in imaging and quantifying RV function.[226, 233, 234]

Of the various parameters of LV function that can be measured via non-invasive imaging, LVEF is the most relevant in clinical practice. Several important (and expensive) decisions rely on accurate knowledge of LVEF in patients with prior AMI, such as qualification for implantable cardioverter defibrillator (ICD) insertion or even cardiac resynchronisation therapy (CRT). Pharmacological therapy is also influenced by LVEF: ESC guidelines state that in patients with LVEF ≤40% following AMI, long-term ACE inhibitor and β-blocker therapy is indicated as is aldosterone blockade, if either heart failure or diabetes are present (in the absence of significant renal dysfunction or hyperkalaemia).[128] Such management decisions are rendered even more difficult by the lack of interchangeability between LVEF estimates measured by different modalities. This was demonstrated clearly in a study of 52 patients with CHF and LVSD, all of whom underwent TTE, CMR and RNVG within a 4-week period of clinical stability.[235] The CMR and RNVG scans were interpretable in all cases, but the M-mode and 2-D Simpson's biplane TTE images were of sufficient quality for analysis in only 86% and 69% of patients respectively. The LVEF measured by each imaging modality was significantly different from all other techniques except for CMR and 2-D Simpson's biplane TTE, and even between these two quantities the Bland-Altman limits of agreement were unacceptably wide.

The ideal imaging modality should be widely-available, accurate, reproducible and safe for the patient. TTE remains the most widely-available imaging modality although the availability of CMR is increasing. The accuracy and reproducibility of CMR compare favourably to those of TTE, RNVG and invasive contrast angiography.[235-240] Moreover, CMR allows significant sample size reduction

compared to TTE.[236] CMR is non-invasive, uses no radiation, and has been shown to be safe early after AMI, although specific safety questionnaires must be completed prior to consenting to the investigation and entering the designated MRI scanning room.[241] The limitations of CMR scanning are discussed in the Methods section. CMR therefore fulfils several of the criteria for the "ideal" imaging modality.

In summary, CMR is now considered the gold-standard means of measurement of LV mass, volumes and LVEF, particularly in patients with LVSD in whom the geometrical assumptions on which planar imaging techniques necessarily depend, fail to account for the changes that occur in LV morphology. Volumetric analysis is performed on CMR by dividing the LV into a stack of short-axis slices, which removes the need for any geometric assumption. In addition, the excellent spatial and temporal resolution afforded by CMR allows accurate delineation of endocardial and epicardial borders.

1.11.2 CMR in LV remodelling

Left ventricular end-systolic volume (LVESV) is a powerful predictor of survival following AMI, serial change in which has been the focus of a number of clinical studies of LV remodelling in both AMI and CHF populations.[22, 29, 117, 242, 243] All patients in this study will undergo CMR imaging in which LVEF, LV volumes, mass and stroke volume will be measured. LV remodelling is defined as the change in LVESV, indexed to body surface area (BSA), over time.

1.11.3 Late gadolinium enhancement CMR (LGE-CMR)

It has been known for many years that regions of acute and chronic myocardial necrosis, such as infarction, exhibit a higher signal intensity on T1-weighted MRI images following administration of gadolinium-based contrast agents.[244] Such agents shorten the T1 relaxation time, thereby increasing the contrast between tissues depending on their relative content of contrast agent. This has led to numerous studies of myocardial infarction models utilising a variety of pulse sequences to differentiate normal from abnormal myocardium.

LGE-CMR allows assessment of myocardial viability in acute and chronic MI. If the heart is imaged 15-30 minutes after injection of a contrast agent, an increase in the T1 signal intensity will be seen in regions of myocardium with increased extracellular space or abnormal wash-in/washout characteristics (such as infarcted tissue), and will appear bright compared to the normal myocardium. Application of an inversion-recovery pre-pulse serves to enhance the contrast between abnormal (bright) and normal (dark) myocardium.[245]

Following AMI, therefore, the intravenous administration of a gadolinium-based contrast agent allows visualisation of abnormal region(s) of myocardium. Animal infarct models have shown a close correlation between the delayed enhanced region on LGE-CMR images and triphenyltetrazolium chloride-stained slices of the whole heart, signifying infarcted myocardium.[246] In humans with ischaemic LVSD, the transmural extent of the delayed enhanced region on LGE-CMR correlates inversely with improvement in regional contractility following revascularisation.[247] This technique therefore appears to allow in vivo visualisation of abnormal regions of

myocardium consistent with infarction. It should be stressed, however, that delayed enhancement on LGE-CMR is not a specific sign of infarction – it simply indicates that the normal fluid homeostasis within the abnormal segment has been disrupted. Delayed contrast enhancement has been reported in a variety of conditions including myopericarditis, hypertrophic and dilated cardiomyopathies, infiltrative cardiac disorders, cardiac neoplasia and in the transplanted heart.

In our cohort of AMI patients, the use of LGE-CMR should allow visualisation of abnormal regional myocardium consistent with acute infarction. We can then not only record the precise anatomical location of the infarcted area, but also calculate its transmurality and endocardial extent and relate these to LV remodelling.

1.11.4 Microvascular obstruction

Relief of the occlusive obstruction within the IRA is a key component in the acute management of MI and the attenuation of LV remodelling. Despite patency of the IRA, however, abnormal microvascular perfusion is related to worse outcome.[248, 249] Such abnormal perfusion is termed microvascular obstruction (MVO).

CMR facilitates examination of myocardial perfusion. There are two methods:

(i) first-pass perfusion imaging: myocardial enhancement is analysed immediately after contrast injection – this represents "early MVO"

(ii) LGE-CMR: within the hyperenhanced "bright" region as described above, a central hypoenhanced region is frequently seen after large AMI. From the initial reporting of this hypoenhanced core, a series of animal experiments have confirmed a precise anatomical correlation between the

hypoenhanced core and markedly reduced blood flow, in addition to biopsy evidence of necrotic debris.[250-252] This appearance has been termed "late MVO".

The influence of MVO on remodelling after AMI has led to conflicting results. Studies using myocardial contrast echocardiography to determine presence and extent of MVO have consistently shown strong correlations between its presence and adverse remodelling.[253, 254] CMR studies have revealed strong correlations between early MVO and greater LV volumes at baseline, more significant remodelling, and increased risk of major adverse cardiovascular events post-infarct.[255, 256]

Results of a recent study using late MVO after reperfused AMI with confirmed patency of the IRA contradict many of the studies using early MVO. Although the presence and extent of late MVO did correlate with higher cardiac biomarkers, higher LVESV, larger infarct size and lower LVEF at baseline, it had no influence on remodelling over the ensuing 4 months.[257] The major limitation of this study was the small patient number (n=40).

One of the technical issues in the analysis of early MVO using CMR is the need for ultrafast acquisition schemes, as the goal is to acquire multiple slices through the heart (preferably within one heart-beat) to assess the relative perfusion of the myocardium following bolus contrast injection. Current imaging protocols, including those used in our centre, allow only 3-5 slices in total from LV base to apex per heart-beat. Acquisition of delayed contrast-enhanced images, using fast gradient echo sequences with an inversion-recovery pre-pulse, allows much greater spatial resolution and

coverage of the entire LV. This results in reliable quantification of not only infarct extent but also the presence and extent of MVO. Consequently late MVO is now considered to be a more precise estimate of microvascular dysfunction.

The role of MVO in LV remodelling therefore remains somewhat controversial. Using late MVO, I planned to examine this further in the cohort of AMI patients studied in this thesis.

1.12 Aims

On the strength of available evidence, aldosterone plays a major role in post-infarction LV remodelling, and indeed aldosterone blockade is now indicated in addition to ACE inhibitor and β-blocker therapy (both of proven benefit in attenuating adverse remodelling) in patients with reduced LVEF and either heart failure or established diabetes mellitus after AMI. Whether aldosterone blockade exerts further anti-remodelling benefit in the presence of ACE inhibitors and β-blockers remains controversial, and results of several small studies are conflicting.

The hypothesis that this thesis will test is whether inhibition of the effects of aldosterone reduces remodelling after myocardial infarction in a cohort of patients with LVSD early after AMI, who have been treated with standard secondary preventive therapy. This will be accomplished using a randomised, double-blinded study of the effects of the selective MRA eplerenone on LV remodelling over 24 weeks compared to placebo. As patients with LVSD early after AMI who are diabetic or who develop heart failure should receive eplerenone according to current guidelines, such patients will be excluded from this study. This thesis will thus focus on 'asymptomatic' LVSD after AMI.

CMR will be used as the imaging modality in this study, as it represents the current gold-standard method for measuring LV volumes, mass and LVEF.

The roles of the various biomarkers discussed in LV remodelling, and the influence of eplerenone thereon, will also be examined.

The specific aims of this study are as follows:

Effects of Eplerenone

- To assess the effect of eplerenone on the change in (Δ) LV end-systolic volume index (LVESVI) over 24 weeks, compared to placebo – this is the **primary end-point**

- To assess the effect of eplerenone on Δ LV end-diastolic volume index (LVEDVI), Δ LV ejection fraction (LVEF), Δ LV mass index and Δ LV infarct volume index over 24 weeks, compared to placebo.

- To assess the effect of eplerenone on biomarkers related to the pathophysiology of LV remodelling: BNP, NTproBNP, NA, AVP, renin, aldosterone, MMPs, TIMPs, pro- and anti-inflammatory cytokines and markers of haemostasis

Predictors of post-infarction LV remodelling

- To assess further the roles of biomarkers related to the molecular and cellular processes that underlie the macroscopic changes that occur in LV remodelling, using the current gold-standard means of quantifying gross changes in LV structure. These include:
    - BNP and NTproBNP
    - Noradrenaline
    - AVP
    - MMP-2, MMP-3 and MMP-9

- TIMP-1, TIMP-2 and TIMP-4
- Several families of cytokines
- tPA antigen
- vWF

- To analyse further the predictive value of late MVO in LV remodelling, measured using LGE-CMR.

The role of aldosterone in post-infarction LV remodelling

- To analyse the relationships (if any) among:
    - plasma renin and aldosterone concentrations and ceCMR parameters of LV function following AMI across the entire study population, and by treatment group
    - urinary steroid metabolite excretion rates and parameters of LV remodelling
    - plasma aldosterone concentrations and parameters of LV remodelling (if any) of the -344T/C SNP in CYP11B2

Novel markers

- The natural history of the novel peptide apelin following AMI will be characterised for the first time.
- The relationships (if any) between the novel biomarker serum soluble ST2 and parameters of LV remodelling, and between ST2 and a variety of circulating neurohormones of prognostic and/or pathophysiological significance in post-infarction remodelling will be examined for the first time.

Additional findings

- To describe the additional intra- and extra-cardiac abnormalities detected by performing pre-discharge CMR imaging in addition to standard TTE in a cohort of post-AMI patients.

Chapter 2

Materials and Methods

2.1 Introduction

The principal technique used for this thesis was contrast-enhanced cardiac magnetic resonance imaging (LGE-CMR). This was supplemented by transthoracic echocardiography, electrocardiography, and sampling and measurement of a variety of circulating, urinary and genetic markers. In this chapter the background, methods, apparatus and protocols used for these techniques will be outlined.

2.2 Subjects

2.2.1 Inclusion and exclusion criteria

Inclusion criteria:

1. Age ≥18 years
2. AMI within last 1-14 days (definition in section 2.2.3)
3. LVEF <40% on screening 2-D TTE
4. Ability to give written informed consent

Exclusion criteria:

1. Clinical and/or radiological heart failure (ie. Killip score ≥I) [258]
2. Established diabetes mellitus
3. Documented pre-existing LVSD (ie. prior to index AMI)
4. Serum creatinine > 220μmol/l*
5. Serum potassium > 5.0mmol/l*
6. MRI-incompatible (ferrous) prosthesis
7. Claustrophobia causing inability to tolerate CMR
8. Pregnancy

9. Addison's disease
10. Concurrent use of potent inhibitors of CYP3A4: potassium-sparing diuretics, clarithromycin, nefazodone, itraconazole, ketoconazole, ritonavir, nelfinavir, tacrolimus, cyclosporin.**
11. Concurrent use of CYP3A4 inducers: phenytoin, carbamazepine, rifampicin or St. John's Wort

* upper limits of serum creatinine and potassium as used in EPHESUS [123]

** Concurrent use of mild-to-moderate inhibitors of CYP3A4 was allowed, but in their presence the dose of eplerenone remained at 25mg o.d. throughout the trial duration. These include: amiodarone, diltiazem, verapamil, erythromycin, fluconazole and saquinavir.

2.2.2 Recruitment

All consecutive patients admitted directly to the Western Infirmary, Glasgow (WIG) with AMI, or transferred from peripheral hospitals to the WIG for further in-patient management following admission with AMI, were potentially eligible. During the study recruitment phase, all patients without obvious contraindications to enrolment underwent screening TTE, performed by one operator (RW), in order to estimate LVEF (Simpson's biplane rule).[259] Patients with insufficient endocardial definition to allow accurate planimetry were excluded from recruitment. This screening process continued until the pre-specified target population (n=100) had been recruited. The TTE was performed as early as was feasible after admission and the report was made available immediately to the ward team; consistent with EPHESUS, patients with a single measurement of LVEF <40% at any time after the AMI and before enrolment

were eligible (although EPHESUS was less homogeneous in its recruitment, employing any of three different imaging modalities – TTE, RNVG and invasive contrast angiography – to estimate LVEF).[123]

2.2.3 Definition of AMI

AMI has been subject to significant re-definition in the last decade, as new and more sensitive biomarkers become available, in particular troponin T (TnT) and TnI, which are very sensitive markers of myocyte necrosis. The definition of AMI used in this study, in which recruitment commenced in 2005, was that recommended by the British Cardiac Society Working Group in 2004.[260] Classified as "acute coronary syndrome with clinical myocardial infarction", this required either or both of a history of typical ischaemic cardiac chest pain and ECG changes consistent with ischaemia, together with a maximum TnT release of >1.0 ng/ml or TnI release of >0.5 ng/ml. In this study, in which the enrolled patients had necessarily sustained large infarcts, the peak Tn was markedly elevated in all cases. As patients initially admitted to and managed in hospitals other than the WIG were included, many Tn measurements were made in the biochemistry laboratories of these referring hospitals. Different hospital trusts use either TnT or TnI, with different coefficients of variation (CV) which were not recorded at the time, thus the Tn results for the patients enrolled in this study show a mixture of TnT and TnI. Troponin was therefore used as an inclusion criterion only; no further analysis of troponin was performed in the study.

2.2.4 Acute management of patients

The trial did not interfere with the in-patient management in any way. If percutaneous coronary intervention (PCI) was planned, either in the acute infarction phase or the

early post-infarction period, this was given priority over, and performed prior to ceCMR. Patients who had undergone coronary angiography but who were to be referred for coronary artery bypass surgery (CABG) prior to randomisation were excluded. The prescription of standard secondary preventive therapies, including ACE inhibitors and β-blockers, was entirely at the discretion of the admitting consultant. Likewise, the decision to withdraw patients from the trial who developed pulmonary oedema after enrolment (who would therefore have qualified for eplerenone therapy based on the EPHESUS trial and current guidelines) was also at the discretion of the consultant, although it is noteworthy that eplerenone was not being used routinely at that time in Glasgow in these patients.

2.3 Trial outline

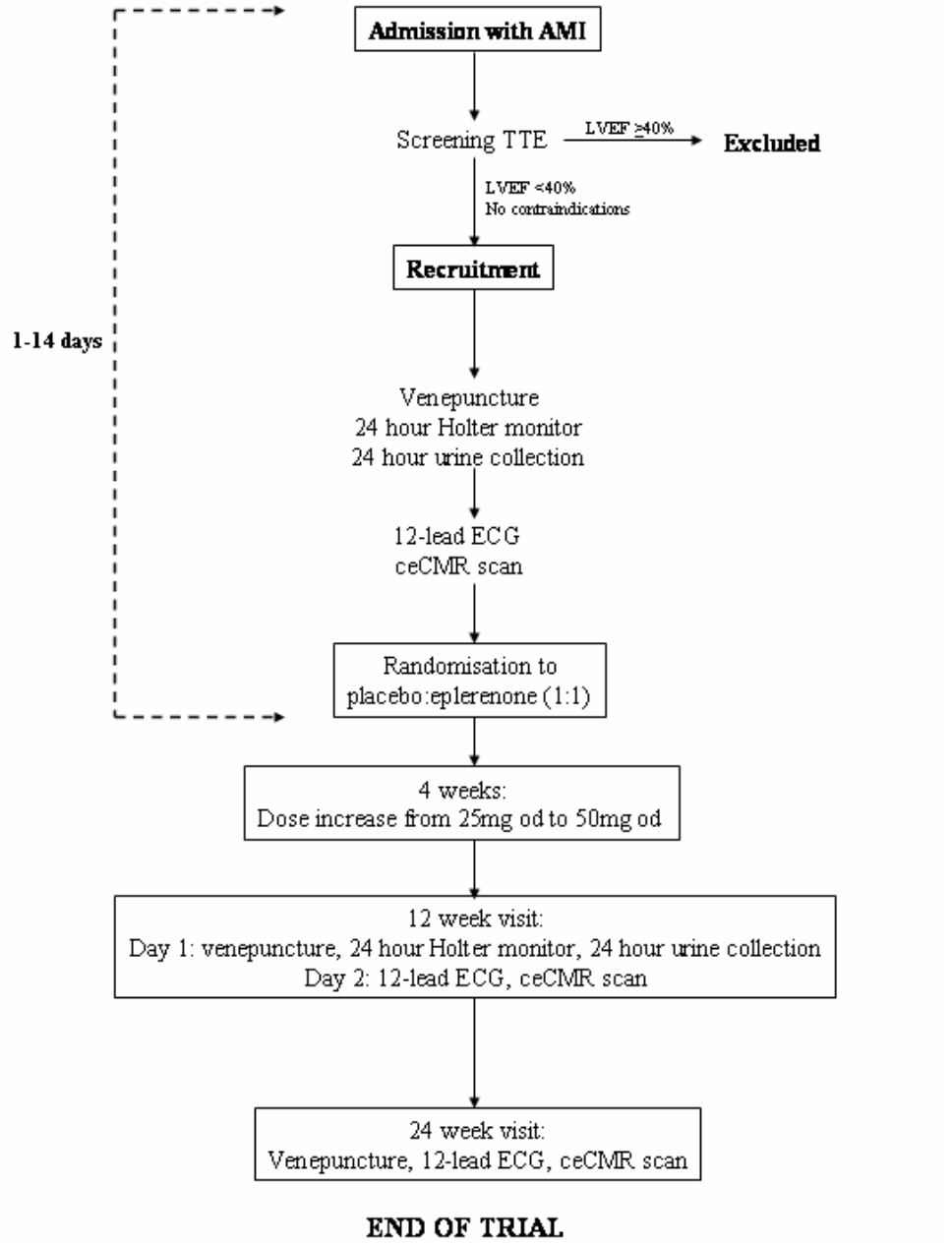


Figure 2.1 Timeline of patient involvement in trial, from acute admission until trial completion.

2.3.1 Randomisation

After obtaining written, informed consent (Appendix I-II), enrolled patients were assigned an arbitrary unique identifier to be used for the duration of the trial. A form

filled out by one investigator (RW) including each patient's name, date of birth and unique identifier, was then sent to the WIG Pharmacy Clinical Trials Unit, where double-blinded randomisation was performed.

Each patient underwent a series of baseline investigations, as indicated in the timeline (Figure 2.1). None of these investigations took place on day 1 according to protocol, as this was deemed a potentially unstable period. Assuming clinical stability, however, as early as possible after the first 24 hours of hospital admission, the following were performed:

(i) full physical examination – including measurement of height, weight and resting haemodynamics

(ii) retrieval and digital scanning of 1st (acute admission) 12-lead ECG

(iii) 24 hour urine collection performed

(iv) 24 hour Holter monitor performed

(v) venepuncture – 50ml blood withdrawn for biomarker analysis

(vi) ceCMR with simultaneous digitally-acquired 12-lead ECG

LGE-CMR was performed as early as possible after day 1. Once the baseline investigations were completed for each patient, study drug was commenced in addition to the secondary preventive medications prescribed by the ward team. Patients were discharged with a 4-week supply of study drug initially; at the 4-week visit, a new 8-week supply was provided, and at the 12-week visit a further 12-week supply was given. On receipt of each new supply of study drug, patients returned the remainder of the previous supply, which was immediately returned to the WIG Pharmacy.

Storage, dispensing, monitoring of study drug uptake (based on counting the number of tablets in all returned bottles) and safe-guarding of the randomisation code were performed entirely by the WIG Pharmacy. The randomisation code was only released after the final analysis of the final LGE-CMR scan had been completed.

2.3.2 12-week visit

This comprised a 2-day visit to the WIG. On day 1 a number of the baseline investigations were repeated:

(i) full physical examination

(ii) 24 hour urine collection performed

(iii) 24 hour Holter monitor performed

(iv) venepuncture – 50ml blood withdrawn for biomarker analysis

Both the 24 hour urine sample and Holter monitor were returned on day 2, on which the second protocol-mandated LGE-CMR was performed, with a simultaneous digital 12-lead ECG.

2.3.3 24-week visit

This was the final visit, and consisted of one day at the WIG. The following were performed:

(i) full physical examination

(ii) venepuncture – 50ml blood withdrawn for biomarker analysis

(iii) digital 12-lead ECG

(iv) LGE-CMR

Unused study drug was then returned, and the patient's involvement in the trial ceased.

2.3.4 Dose of study drug

For ease of prescription, only one strength of tablet was used for the study drug. Each tablet contained either 25mg eplerenone or matched placebo. Consistent with the doses used in EPHESUS, the starting dose of eplerenone (or placebo) was 25mg o.d. After 4 weeks, this dose was increased to 50mg o.d. for the remainder of the trial. Exceptions to this rule included patients with a serum potassium >5.0 mmol/l at 4 weeks (drug either reduced to alternate days or temporarily with-held – Table 2.1) or those who were simultaneously prescribed mild-to-moderate CYP3A4 inhibitors (who remained on 25mg o.d throughout the trial).

Serum Potassium (mmol/l)	Action	Dose adjustment
< 5.0	Increase	25mg o.d. up to 50mg o.d.
5.0 – 5.4	Maintain	No dose adjustment – remain on 25mg o.d. Recheck in 1 week; increase if potassium <5.0 mmol/l
5.5 – 5.9	Decrease	25mg o.d. to 25mg. on alternate days
≥ 6.0	With-hold	N/A

Table 2.1 Dose adjustment of study drug at 4 weeks, according to serum biochemistry.

2.3.5 Monitoring of renal function

Although patients with significant renal impairment were excluded, all patients enrolled into the study had not only sustained a significant amount of myocardial damage, but also had been recently commenced (in the majority of cases) on at least one potentially nephrotoxic drug, to which eplerenone was then added in 50%. Close monitoring of renal function was therefore mandatory. All measurement of serum

urea, creatinine and electrolytes (U&Es) was performed by the WIG routine biochemistry laboratory. Protocol-mandated U&Es were measured at six time-points over the 24-week trial period (see timeline): prior to and 2 days after commencing study drug, then at 4, 5, 12 and 24 weeks. Additional time-points were used if clinically indicated. The major dose change (25mg to 50mg o.d.) was at 4 weeks, hence the U&Es measurement at 5 weeks. For all time-points after 4 weeks, the drug dose was adjusted as in Table 2.2. All U&Es results were available on the same day on which venepuncture was undertaken, and were acted upon immediately if necessary.

Serum Potassium (mmol/l)	**Action**	**Dose adjustment**
< 5.0	Increase Maintain	25mg alternate days to 25mg o.d. If on 50mg o.d. already
5.0 – 5.4	Maintain	No dose adjustment
5.5 – 5.9	Decrease	50mg o.d. to 25mg o.d. 25mg o.d. to 25mg alternate days 25mg alternate days to with-hold
≥ 6.0	With-hold	Recheck within 1 week and re-challenge at 25mg o.d.

Table 2.2 Dose adjustment of study drug at any time-point after 4 weeks, according to serum biochemistry.

2.4 TTE protocol (screening only)

The TTE examination was performed with the patient in the left lateral decubitus position. Images were acquired from standard parasternal and apical views. Ultrasound data were acquired with an Acuson CV70 ultrasound scanner and a 3.5 MHz transducer (Siemens). Standard LV M-mode measurements included LV end-diastolic diameter, LV end-systolic diameter and myocardial wall thickness at end-diastole. LVEF was estimated from the apical 4-chamber and 2-chamber views utilising Simpson's biplane rule.[259] Simpson's biplane analysis was performed by one

observer (RW), and only those with LVEF less than 40% were approached regarding study recruitment. Manual planimetry was used to trace the endocardial contours at end-systole and end-diastole in both the apical 4-chamber and 2-chamber views; by convention the papillary muscles were included within the LV volumes (not LV mass). The study protocol mandated that patients with insufficient endocardial definition to allow accurate planimetry were excluded from recruitment, thus all 100 patients had endocardial definition of sufficient quality to allow accurate volumetric analysis. Contrast was not used.

2.5 CMR protocol

CMR was performed using a 1.5T Siemens Sonata Magnetom with a phased-array chest coil, during breath-hold, and gated to the ECG. Each scan was performed by one of two experienced operators (RW, TS), both of whom are Advanced Life Support-qualified; RW was present throughout all scans thereby providing constant medical cover. Prior to entering the controlled zone, an MRI safety checklist was performed and signed by both patient and qualified MRI personnel (Appendix III). The importance of keeping as still as possible, and maintaining adequate breath-holds, was reinforced verbally prior to commencement of the scan. End-expiration is optimal for consistent breath-holding and was preferred.

2.5.1 Preparation of patient for scan

The following steps were performed in all cases:

- Patient demographic details entered on scanner database
- The power injector (Medrad Spectris, Volkach, Germany) for administration of the contrast agent – gadolinium diethylenetriaminepentaacetic acid (DTPA), GE Healthcare – was prepared. The Medrad injector contains two quick-fit syringes connected by a Y-tubed delivery set. Into syringe A, gadolinium-DTPA was drawn at a dose of 0.1mmol/kg. Syringe B contained 50ml 0.9% NaCl
- Patient placed on table
- Siemens active Brooker ECG electrodes placed on patient's anterior chest wall, and position varied to obtain an optimal R wave
- A 20G IV cannula was placed in a peripheral vein, and connected to the Medrad injector.
- The phased-array chest coil (Siemens CP body array flex) was applied and aligned
- Patient, wearing ear protectors or headphones, enters scanner
- Medrad power injector armed

2.5.2 LV structure and function

All CMR scans commenced with a multi-slice breath-hold localiser. Each of the localisers described in this section used the same protocol:

Protocol: Multislice single-shot breath-hold true fast imaging with steady-state precession (trueFISP) localiser with transverse, sagittal and coronal slices. Settings: field of view = 360mm, field of view phase = 81.3%, slice thickness = 6mm, repetition time (TR) = 3.41ms, echo time (TE) = 1.71ms, flip angle = 60^{o}, averages = 1, phase resolution = 80%, phase oversampling = 0%

From these images, the best axial image depicting the LV and septum was selected (Figure 2.2 A). If no suitable image was produced by the initial localiser, a second axial localiser was performed using the coronal images until the closest match to Figure 2.2 A was obtained. This was used to plan 3 vertical long axis (VLA) parallel localisers along the long axis of the LV from the mid-point of the mitral valve to the apex.

From the resulting VLA scan, 3 horizontal long axis (HLA) localisers were then planned, using the mid-point of the mitral valve and the LV apex to prescribe the orientation (Figure 2.2 B). This resulted in 3 HLA slices (Figure 2.2 C). Using the atrioventricular groove as a landmark, 3 short axis (SA) localiser slices were planned, with the most basal slice positioned in the atria to depict the left ventricular outflow (Figure 2.2 C). From the resulting SA images, 3 long axis views can be prescribed – the 4-chamber, 2-chamber and left ventricular outflow tract (LVOT) views (Figure 2.2 D).

***Cinematographic (cine) imaging*:**

Having thus acquired 3 orthogonal long axis views, cine studies were acquired in each of these 3 orientations, as follows:

Protocol: trueFISP breath-hold cine. Settings: field of view = 360mm, field of view phase = 81.3%, slice thickness = 8mm, TR = 47.4ms, TE = 1.58ms, flip angle = 60^{o}, averages = 1, measurements = 1, phase resolution = 65%, phase oversampling = 20%, segments = 15

These images were used for visual analysis of structure and long axis function.

***Short axis cine stack*:**

Quantitative volumetric assessment of ventricular function requires that the LV be divided into a stack of SA slices from base to apex. Measurements from each slice are then summed to provide overall ventricular mass and volumes, from which LVEF can be calculated. The SA stack was prescribed from the 4-chamber HLA cine already acquired. Using the end-diastolic image from this view, the cursor was positioned in an orientation across the mitral valve plane through the atrioventricular groove (as a marker of the most basal SA slice) as in Figure 2.2 E. The most basal slice that results is depicted in Figure 2.2 F. The slice position was then incremented by 10mm moving towards the apex of the LV and repeated until the LV was completely covered; inter-slice gaps of 2 mm were used. A representation of the final SA cine stack is shown in Figure 2.2 G. The same protocol was used for all SA cine slices:

Protocol: trueFISP breath-hold cine. Settings: field of view = 340mm, field of view phase = 81.3%, slice thickness = 8mm, interslice gap = 2mm, TR = 47.4ms, TE = 1.58ms, flip angle = 60^{o}, averages = 1, measurements = 1, phase resolution = 65%, phase oversampling = 20%, segments = 15

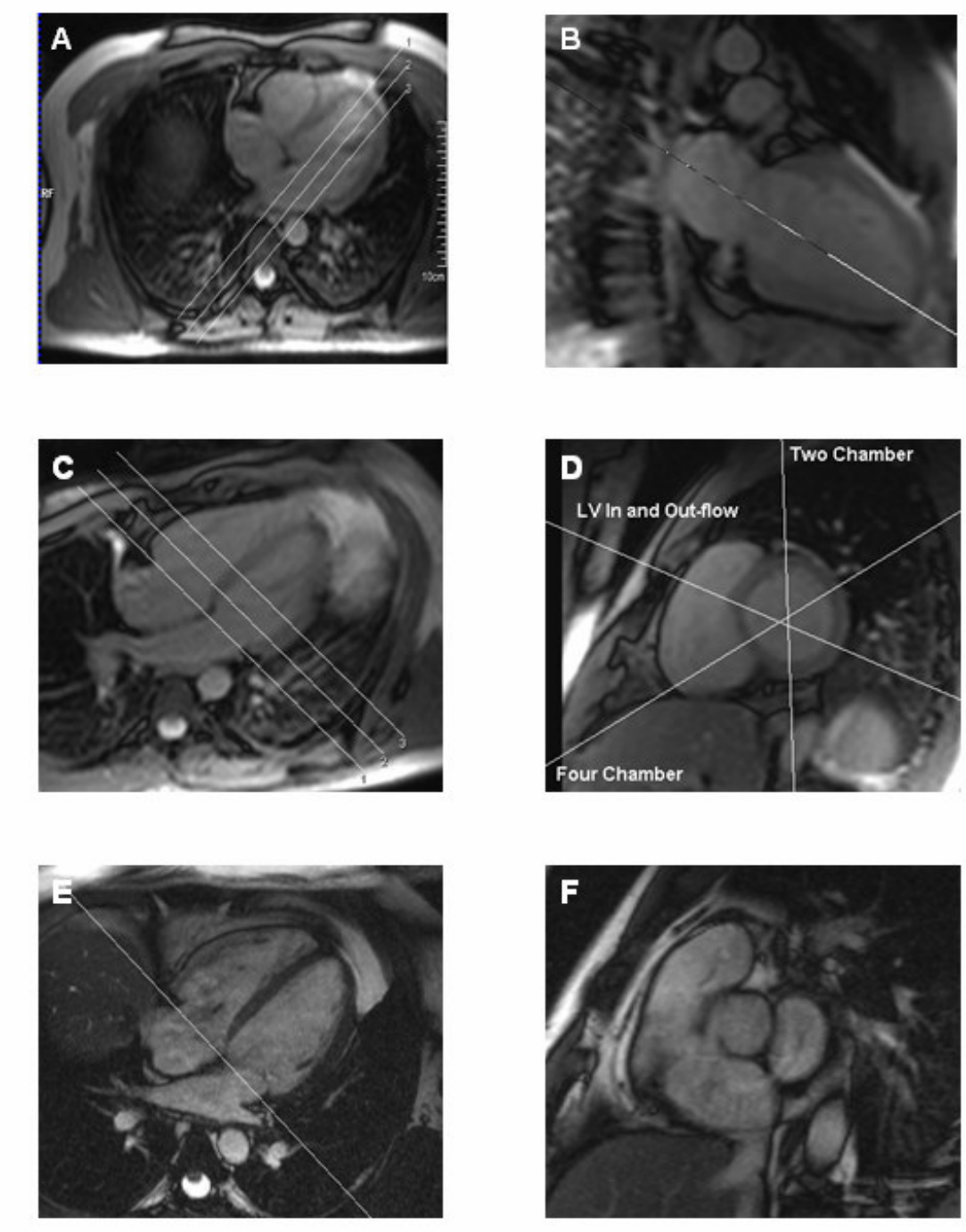
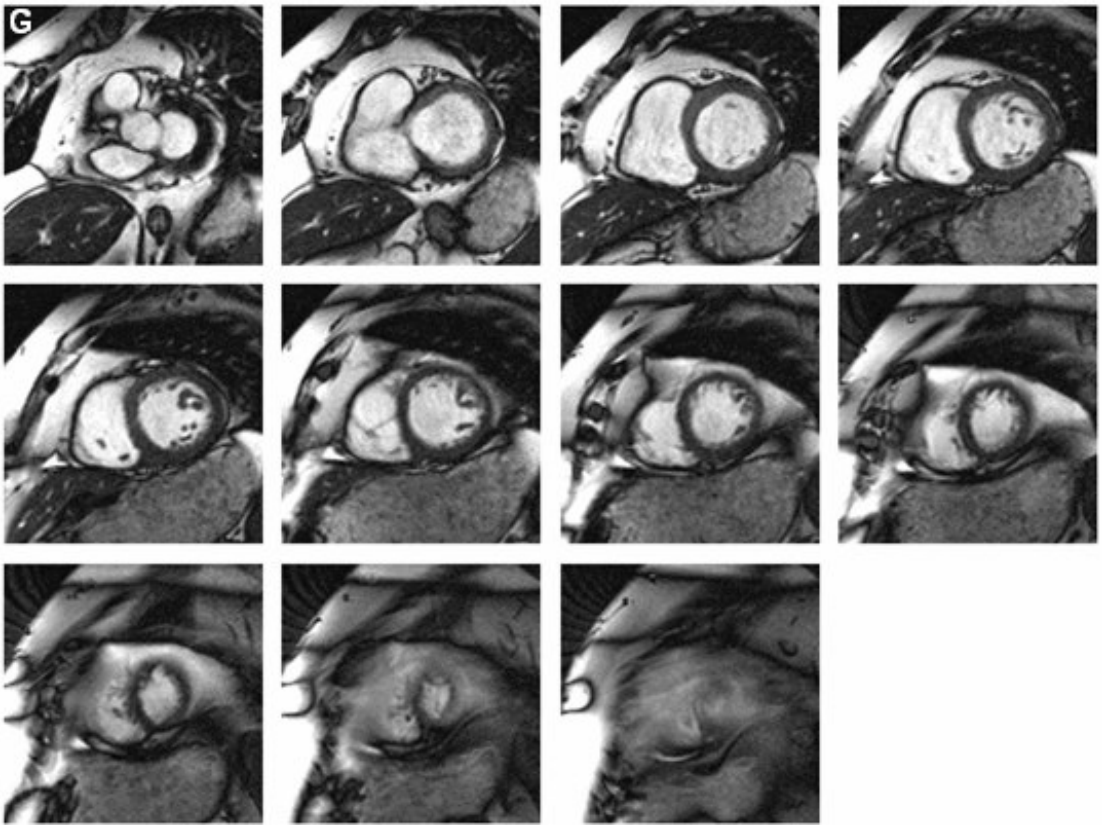


Figure 2.2 Planning of cine CMR image acquisition. From transverse, sagittal and coronal scout images, the best image depicting the LV and septum is selected (A) and utilised to produce a

vertical long-axis (VLA) localiser (B). Prescribing an orientation through the apex and mid-point of the mitral valve (B – orientation line) creates a horizontal long-axis (HLA) localiser (C). Using the atrioventricular grooves as landmarks (C), a perpendicular plane to this HLA localiser is prescribed (D), based on which three orthogonal long-axis planes can be planned (2-chamber, 4-chamber and LV outflow tract views). Cine images are acquired for each of these three long-axis orientations. Finally a short-axis cine stack is planned on the 4-chamber HLA cine image (E). A short-axis image is acquired of the base of the LV (F), from which slice position is advanced at 10mm intervals from base to apex, creating a short-axis cine stack (G).



2.5.3 Contrast-enhanced imaging

Both first-pass perfusion imaging and delayed contrast enhancement imaging were performed on all patients, to detect the presence of early and late MVO respectively (and to measure infarct size and characteristics in the latter). In all cases, the dose of gadolinium-DTPA used was 0.1mmol/kg.

***First-pass perfusion imaging*:**

The visualisation of first-pass myocardial perfusion following bolus contrast injection requires ultra-high speed MR imaging. A balanced single-shot turbo fast low angle-shot (FLASH) sequence with a saturation recovery pre-pulse before each slice was employed. This typically allowed 4 SA slices (copied from the cine SA stack) to be acquired per heart-beat. No breath-hold was required. The weight-adjusted dose of gadolinium-DTPA was delivered into a peripheral vein via the Medrad power injector at a constant rate of 6ml/s, followed by 0.9% NaCl flush. The first-pass protocol was as follows:

Protocol: first-pass single-shot turbo-FLASH sequence with saturation recovery preparation, 4 slices per heartbeat. Non-breath-hold, controlled respiration. Settings: field of view = 340mm, field of view phase = 81.3%, slice thickness = 8mm, TR = 183ms, TE = 0.99ms, flip angle = 8^{o}, averages = 1, measurements = 60, time to inversion (TI) = 100ms

***Imaging of early microvascular obstruction*:**

2 minutes after contrast injection, images were acquired for the determination of early MVO. This required a single-shot steady-state free precession sequence with a non-selective inversion pulse. No breath-hold was required. Typically 3-5 SA slices per heartbeat were acquired, copied from the SA cine stack. A single-shot sequence was acquired at each of four time-points: 2, 3, 4 and 5 minutes after contrast injection. The protocol for early MVO imaging was as follows:

Protocol: ECG trigger, 100 lines field of view = 270 x 360, slice thickness = 8mm, interslice gap = 2mm, flip angle 30^{o}, TE 1.2ms, TR 2.7ms, TI 200-350ms, bandwidth/ pixel = 980 Hz, matrix 256.

***Late gadolinium-enhancement imaging*:**

15 minutes after bolus contrast injection in all scans, LGE-CMR images were acquired. This utilised a contrast-sensitive segmented inversion recovery sequence to acquire a second stack of SA images (positions copied from the SA cine stack). Images were also acquired in three long axis views, with orientations copied from the cine 4-chamber, 2-chamber and LVOT views. Adequate breath-holding was essential for each acquisition. The protocol was as follows:

Protocol: Breath-hold segmented re-phased Turbo-FLASH sequence with non-selective inversion pulse with non-slice selective inversion-recovery.

Constant settings: field of view = 340mm, field of view phase = 81.3%, slice thickness = 8mm, interslice gap = 2mm, TE = 4.3ms, flip angle = 30^{o}, averages = 2, segments = 25, phase resolution = 65%, trigger delay = 0, trigger pulse = 2 (but dependent to an extent on heart-rate: trigger on pulse 1 if bradycardic, or pulse >2 if tachycardic).

Variable settings:

The acquisition window was set greater than RR-interval and TR just under to allow for diastolic imaging. TI was 220ms (for initial scan) and adjusted according to image quality by 10ms steps within the range 200-300ms to optimise image quality. The TI

was often varied between image acquisition, and if the quality of the preceding image was poor it was repeated until an image of adequate quality was obtained. Optimal TI and TR produced a diastolic image with black (nulled) myocardium and bright late enhancement area (Figure 2.3 A). If late MVO was present, it appeared as a dark hypoenhanced core within the bright hyperenhanced area (Figure 2.3 B).

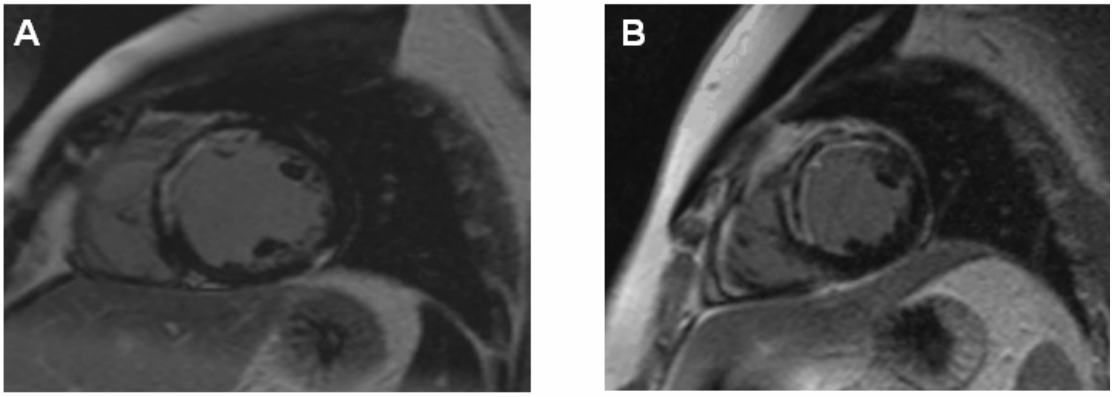


Figure 2.3 Mid-ventricular short-axis slices acquired using a contrast-sensitive segmented inversion recovery sequence 15 mintues after injection of gadolinium-DTPA, from two separate patients admitted with anterior STEMI. (A) Subendocardial region of hyperenhancement affecting the anteroseptal wall of the LV, surrounded by normal (nulled, dark) myocardium; there is no MVO present. (B) Full-thickness infarction of the anteroseptal wall of the LV reveals hyperenhancement throughout wall, from endocardium to epicardium, with a hypoenhanced core – this core represents (late) MVO.

2.6 CMR analysis methodology

2.6.1 LV volumes, mass and ejection fraction

Postprocessing was performed using commercially-available Argus software (Siemens, Erlangen). The number of slices required to cover the LV in end-diastole and end-systole varied from scan to scan dependent on the long axis diameter of the LV. End-systole was chosen as the point where the total LV blood pool was smallest and end-diastole as the point where it was largest. The most basal LV slice at both end-systole and end-diastole was defined as that in which the blood pool was surrounded by 50% or more of ventricular myocardium; papillary muscles were excluded from the LV volumes and included in the LV mass.[261] Manual planimetry, performed on all CMR scans in random order by one observer (RW) blinded to treatment allocation, was used to trace the epicardial and endocardial contours of each SA slice acquired in the cine-stack. Simple addition of the individual slice volumes in this stack of contiguous slices covering the entire LV then allowed calculation of LVESV and LVEDV (ml). LV stroke volume (LVSV) was then calculated as follows: LVSV = LVEDV – LVESV. LVEF was calculated: LVEF = LVSV / LVEDV (%). LV myocardial mass (LVM) was estimated to be the mean of the total difference between the inner and outer circumferences of the LV myocardium in end-diastole and end-systole, multiplied by the myocardial density (taken as 1.05 g/cm^3). All CMR measurements were adjusted for total BSA, creating the following indexed quantities: LVESV index (LVESVI), LVEDV index (LVEDVI), LV mass index (LVMI) and LV infarct volume index.[262]

2.6.2 First-pass perfusion and early microvascular obstruction analysis

Quantitative analysis of the size and extent of both the first-pass defect and early MVO tends to be imprecise as it requires multiple geometric assumptions due to the limited number of SA slices acquired during ultrafast imaging. Such quantitative analysis was not performed in this study. In purely qualitative terms, the presence or absence of a first-pass defect and/or early MVO was recorded for each scan, but no further analysis of these images was performed.

2.6.3 Analysis of delayed hyper-enhanced images

Analysis of the delayed enhancement images was also performed using Argus software (Siemens, Erlangen). The perimeter of the hyper-enhanced region on each SA slice was traced by one observer (RW); Argus software facilitated summation of the volume of enhanced tissue from each slice, producing a measure of infarct volume (ml). The mass of hyper-enhanced tissue could be calculated by multiplication of the volume by the myocardial density factor (1.05g/cm^3).

One major issue relevant to analysis of late hyper-enhanced images pertains to partial volume effect.[263] The thickness of each slice is 8mm, but within this the pattern of late hyper-enhancement is not homogeneous. This irregularity can result in blurring of the infarct border. Towards the periphery of the hyper-enhanced area in some slices, therefore, regions are occasionally seen wherein the brightness level is intermediate between normal (black, nulled) myocardium and bright, hyper-enhanced myocardium. There is a lack of universal consensus on whether (and how) to account for this partial volume effect in the quantitative measurement of late hyper-enhanced tissue. In this study, in keeping with departmental policy and in order to maintain consistency in our

results, we decided to include everything that was hyper-enhanced, taking no account of partial volume effects.

2.6.4 Infarct characteristics

For each patient, infarct volume and mass were calculated on all three LGE-CMR scans. In addition, in the initial (acute) LGE-CMR scan, the anatomical location, transmurality and endocardial extent of the infarct were recorded, as was the presence or absence of late MVO. If present, the region of hypo-enhancement representing late MVO was manually planimetered in identical fashion to the delayed LGE-CMR images.

***Definitions*:**

Infarcted LV myocardium on LGE-CMR was defined as regional delayed hyper-enhancement involving at least the subendocardium (Figure 2.3 A).

Early MVO was defined as the appearance of at least one segment of hypo-enhancement surrounded by hyper-enhancement on images acquired between 2 and 5 minutes post contrast.[250]

Late MVO was defined as late hypo-enhancement within a hyper-enhanced region on the LGE-CMR images which persisted for ≥10minutes after contrast injection (Figure 2.3 B).[264, 265]

The anatomical location of the infarct was based on the AHA standardised 17-segment model (Figure 2.4).[266] Infarct location was categorised as anterior, lateral or

inferior, defined as the location containing the highest percentage of infarcted myocardium.

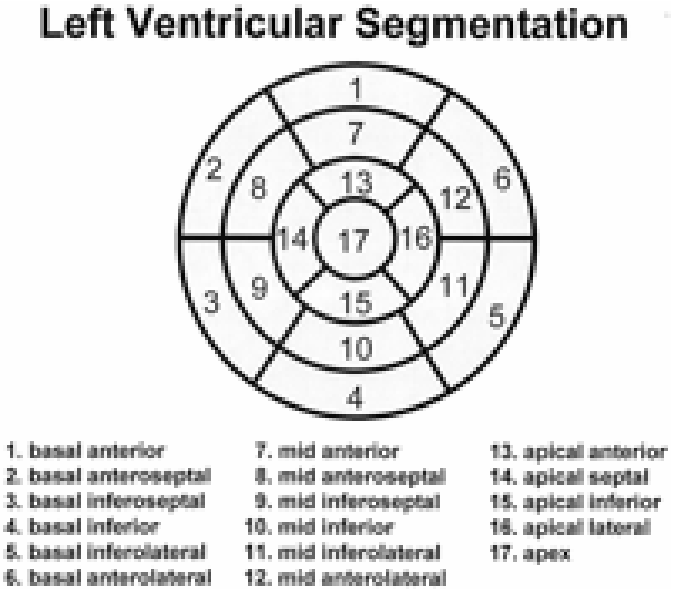


Figure 2.4 17-segment AHA model depicting anatomic segments on short-axis CMR images.[266]

Infarct transmurality was calculated by visually deciding the transmural extent per segment in quarters (1 - 1-25%, 2 – 26-50%, 3 – 51-75%, 4 – 76-100%) and calculating the mean. This produced a transmurality score.[247]

Endocardial extent of the infarct was calculated by measuring the circumferential extent of the infarct at each of the three SA slices used in the AHA segmentation model, and calculating the mean.[266]

LV thrombi were defined as filling defects within the LV cavity, typically adherent to regions of hypokinesis/akinesis, which displayed a constant (low) signal intensity during first-pass imaging, and which on delayed-enhancement imaging were readily distinguishable from scarred myocardium (Chapter 9).

2.7 Coronary angiography

Decisions regarding diagnostic coronary angiography and/or PCI were made by the consultant cardiologist in charge of each patient and were independent of the trial. When undertaken, a qualitative report of the procedure was made available (performed by at least one experienced observer unrelated to the trial). For descriptive purposes, we have defined a significant stenosis as ≥70% luminal narrowing, moderate disease as 50-74%, mild disease as 25-49% and normal as <25% or plaque disease only. Where possible, the culprit vessel was identified and whether or not PCI was undertaken was recorded. Successful culprit vessel PCI was defined as Thrombolysis In Myocardial Infarction (TIMI) 3 flow at the end of the procedure, with no residual stenosis >50% (TIMI Grade Flow defined in Appendix IX).

2.8 Blood sampling

Venepuncture was performed as early as was feasible after enrolment. In total 50ml of venous blood was withdrawn and aliquotted into a variety of collecting tubes for individual biomarker analysis, described below. In an ideal setting, all samples would have been performed in fasting patients at the same time of day (early in the morning) to account for the diurnal variation in certain neurohomones, particularly those of the RAAS. Despite the best efforts of the investigators, the very nature of the patients involved in this trial and the fact that it was based on an acute ward made this difficult. A minority of samples were therefore taken in the afternoons, and frequently the patients were not fasted. The investigators did ensure, however, that all neurohormone samples were taken after 15-20 minutes of supine rest.

2.8.1 Measurement of BNP and NTproBNP

Blood for BNP and NTproBNP analysis was collected in chilled tubes containing potassium ethylenediamine-tetraacetic acid (EDTA) [1mg/ml blood] and aprotinin (50 KIU/ml blood) and centrifuged immediately at 3000 rpm for 15 minutes at ambient temperature. Plasma was extracted and frozen in aliquots at -70^{o}C until batched analysis in a blinded fashion. BNP was measured using a radioimmunoradiometric assay (Shionoria kit, CIS France). The CV for this assay was <5%, with a limit of detection of 1pg/ml. NTproBNP was measured using a chemiluminescent assay kit (Roche Diagnostics) on an Elecsys 2010 autoanalyser (CV <2%, limit of detection of 5pg/ml).

2.8.2 Measurement of noradrenaline and AVP

Blood for NA and AVP was collected in lithium heparin (20 U/ml) tubes and centrifuged immediately at 3000 rpm for 15 minutes at ambient temperature. Plasma was extracted in aliquots and frozen at -70^{o}C until blinded batched analysis. Plasma NA was assayed by high performance liquid chromatography and electrochemical detection; the within and between assay CV were both <10%, and the normal range in healthy volunteers is < 4nmol/l. AVP was measured by radioimmunoassay; the assay sensitivity was 0.5pg/ml. Both were previously-validated in-house assays.[267, 268]

2.8.3 Measurement of plasma renin concentration (PRC) and aldosterone

After 15-20 minutes of supine rest, blood was withdrawn into tubes containing potassium EDTA (1mg/ml blood) for measurement of plasma renin concentration (PRC) and aldosterone. Both samples per patient were centrifuged at 3000 bpm for 15 minutes at ambient temperature. Aliquots of separated plasma were then frozen at -

20°C until batched, blinded analysis. Plasma aldosterone concentration was measured using a solid phase (coated tube) radioimmunoassay kit (Diagnostic Products Corporation UK Ltd). Intra- and inter batch precision was <10% over the sample concentration range.

PRC level was measured by chemiluminescent immunoassay using the Diasorin S.p.A (Italy) method on the Liaison platform. Intra- and inter-batch precision was <10% over the sample concentration range.

2.8.4 Measurement of MMPs and TIMPs

Blood for MMP and TIMP analysis was collected in chilled tubes containing potassium EDTA (1mg/ml blood) and aprotinin (50 KIU/ml blood) and centrifuged immediately at 3000 rpm for 15 minutes at ambient temperature. Plasma was extracted and frozen in aliquots at -20°C in the WIG until later blinded batched analysis. After study completion, the frozen samples from the entire cohort were transferred to the Department of Cardiovascular Sciences, Leicester Royal Infirmary, where measurement of the MMPs and TIMPs was performed by Dr. Iain Squire. Plasma concentrations of MMP-2, -3 and -9 and TIMP-1, -2 and -4 were measured using commercially-available enzyme-linked immunoabsorbent assays (ELISA), Amersham Ltd., Amersham, UK. Each plasma concentration listed in the results section represents the mean of duplicate measurements. The intra- and inter-assay CV was <10% for all six assays.

2.8.5 Measurement of cytokines

Blood for cytokine analysis was collected in plain tubes and allowed to clot at room temperature. Samples were then centrifuged at 3000 rpm for 15 minutes at ambient

temperature, and the supernatant serum aliquotted into Eppendorff tubes, prior to being frozen and stored at -20°C until later blinded batched analysis.

Serum cytokines were analysed in a 20-plex human cytokine assay (Biosource, Invitrogen) for simultaneous quantification of eotaxin, RANTES, IL-1Ra, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p40), IL-15, IP-10, MIG, MIP-1α, MIP-1β, MCP-1 and TNFα. This assay was run according to the manufacturer's procedure and read on the Bio-Plex suspension array system.

2.8.6 Measurement of serum soluble ST2

Blood for sST2 analysis was collected in plain tubes and allowed to clot at room temperature. Samples were then centrifuged at 3000 rpm for 15 minutes at ambient temperature, and the supernatant serum immediately frozen and stored at -70°C until later blinded batched analysis. Serum sST2 was quantified using a human IL-1 R4/ST2 ELISA (R&D Systems), with a lower limit of detection of 32 pg/ml.[269]

2.8.7 Measurement of tPA antigen and vWF

Blood for tPA antigen and vWF was collected into potassium citrate-containing tubes and centrifuged within one hour at 3000 rpm for 15 minutes at ambient temperature. Plasma was extracted and stored at -70°C until batched blinded analysis after completion of the trial. tPA antigen was measured with a commercially available ELISA kit manufactured by Hyphen Biomed (Neuville-sur-Oise, France); the CV for this assay was 9% across the entire range. vWF was measured using an in-house ELISA using antibodies obtained from DAKO plc. (High Wycombe, UK), for which the CV was 7% across the entire range.

2.8.8 Measurement of apelin

Venous blood samples were collected in potassium EDTA-containing tubes (1mg/ml blood). The samples were then spun at 3000 rpm for 15 mins at ambient temperature and plasma extracted and frozen in aliquots at -70^{o}C until analysis. Apelin assays were performed using the Apelin-12 microplate ELISA assay kit (Phoenix Pharmaceuticals) according to the manufacturer's instructions. The antibody used in this apelin assay cross-reacts 100% with Apelin-12, 13 and 36. The assay therefore included each of these peptides if present in plasma.

As we are the first group to characterise plasma apelin following AMI in man, we used a control population for comparison of plasma apelin concentration. This control population consisted of 32 patients with no history of cardiac events, and a normal ECG, TTE and NTproBNP concentration, data kindly donated by Dr. KS Chong (Scottish National Advanced Heart Failure Service, Glasgow Royal Infirmary). Although the control apelin samples were analysed in a separate batch from the study patients, the methodology in sample preparation and analysis was identical, and was performed using the same equipment in the same laboratory.

As this was a first-in-man study, we not only examined the variations in plasma concentrations of apelin that occur after AMI but also the relationship between apelin and LV remodelling, and the inter-relationships between plasma apelin and other peptide neurohormones of interest: NTproBNP, NA and AVP.

2.8.9 Measurement of urinary steroid metabolites

After completion of each 24-hour urinary collection, total volume was noted and a 20ml aliquot withdrawn into a plain universal container which was immediately frozen and stored at -20^{o}C until blinded, batched analysis after completion of the trial. Extraction of urinary steroids was performed using a Sep-pak C18 cartridge (Waters, USA), and 24-hour excretion rates of each steroid were determined by gas chromatography-mass spectrometry (performed by Ms. Mary Ingram, Glasgow Cardiovascular Research Centre), using the method of Shackleton.[270]

2.8.10 Genetic analysis of aldosterone synthase (CYP11B2)

Genomic deoxyribonucleic acid (DNA) was extracted from leucocytes using a variation of the method of Sambrook.[271] Samples of whole blood, withdrawn (into EDTA-containing tubes) at the time of recruitment into the trial and stored at -20^{o}C until batched analysis after trial completion, were gently warmed and 40 ml of cell lysis mix added prior to centrifugation at 1660g for 10 minutes at 4°C. The resulting pellet was re-suspended in nucleic lysis mix, with 10% sodium dodecyl sulphate and proteinase K added, and incubated overnight at 37°C. Following the addition of 1 ml of 6-molar sodium chloride and 5 ml of phenol:chloroform:isoamyl alcohol, the samples were again centrifuged at 1660g for 20 minutes at 4°C. The supernatant was removed and ethanol added. DNA was spooled out with a glass rod, washed in 70% ethanol, allowed to air dry and then suspended in buffer and stored at 4°C. Genomic DNA extraction was kindly performed by Dr. Gordon Inglis.

DNA was quantified spectrophotometrically using a NanoDrop ND1000 (Nanodrop, USA). Polymerase chain reaction (PCR) was then performed, in which a fragment of

the CYP11B2 promoter region was amplified using Thermo-Start DNA Polymerase (ABGene, UK). The quality of the extracted DNA was confirmed by electrophoresis on agarose gels, and PCR product clean-up performed by treating the remaining PCR product with AMPure (Agencourt, USA) according to the manufacturer's published instructions. The resultant purified product was then transferred to a new PCR plate, and DNA sequencing was performed using the BigDye Terminator v3.1 (Applied Biosystems, USA). A second clean-up was performed using CleanSEQ (Agencourt, USA) according to the manufacturer's published instructions.

In the final phase, the DNA sequencing reaction products were analysed using an Applied Biosystems automated DNA sequencer. The sequencing results were analysed using SeqScape Version 2.1.1.

The above experimental work and analysis was performed entirely by Dr. Gordon Inglis, Dr. Eleanor Davies and Matthew Rutherford, within the British Heart Foundation Glasgow Cardiovascular Research Centre, under the overall supervision of Professor John MC Connell. I had no personal role in the laboratory-based genetic sequencing and analysis, but performed all analysis of results relevant to this thesis.

2.9 ECGs and Holter monitors

Digital ECGs scanned or recorded at 4 time-points (acute admission, CMR scan 1, CMR scan 2 and CMR scan 3) were sent to a core lab in Duke University Medical Centre, North Carolina, USA, as were the Holter monitors (performed pre-randomisation and at 3 months in all patients). These data are being used in conjunction with other ECG-CMR studies from Europe and USA to improve the

understanding of the evolution of myocardial infarction on CMR, and have provided a number of sub-studies which have generated several abstracts and should result in future publications, but are not included in this thesis.

2.10 Funding of the trial

The trial was funded by a research grant from Pfizer UK, who additionally provided the WIG Pharmacy Clinical Trials Unit with the study drug and matched placebo.

2.11 Conduct and monitoring of the trial

The trial was sponsored by the North Glasgow University Hospitals NHS Trust, which arranged quality control assessments at regular intervals throughout via independent auditors. Annual safety reports were additionally submitted to the Medicines and Healthcare Regulatory Authority (MHRA).

The general practitioners (GPs) of patients involved in the trial were informed in writing of their inclusion (Appendix IV), and were provided with several telephone numbers and an e-mail address for any queries regarding the trial. Patients were provided with a laminated trial card and advised to keep this with them at all times. Adverse events – both serious and unexpected – are defined in Appendix V. All adverse events were reported to one investigator (RW) either directly by the patients or via the GP. If any adverse event was deemed to be serious and unexpected (by RW in collaboration with either Prof. HJ Dargie or Prof. JJV McMurray) it was reported to the MHRA within 7 working days. All adverse events were recorded in the case report form which was created for each patient and stored in a locked room in the WIG Cardiology Department.

The trial protocol was approved by the local ethics committee, and is registered on www.clinicaltrials.gov ID: NCT00132093. The study complies with the Declaration of Helsinki.

2.12 Pharmacovigilance

This is covered in Appendix VI.

2.13 Statistical methods

The statistical methodology pertaining to each study in this thesis is described separately, within the relevant chapters.

Chapter 3

A study of the effects of eplerenone on left ventricular remodelling after acute myocardial infarction

3.1 Introduction

In response to AMI, through a combination of mechanical triggers and neurohormonal activation, morphologic changes occur within the left ventricle as part of an adaptive response to the acute decrease in cardiac output, but over time this process of remodelling becomes maladaptive, as discussed earlier. Aldosterone has been strongly implicated in the pathogenesis of post-infarction remodelling, in particular the development of both replacement and reactive fibrosis within infarcted and non-infarct zone myocardium respectively.[54-59] Aldosterone antagonism reduces cardiovascular morbidity and mortality in advanced CHF, and in survivors of AMI with resultant LVSD and heart failure (or diabetes mellitus).[121, 123] At the time of writing this thesis, however, the effects of aldosterone antagonsim on post-infarction remodelling were uncertain, with confliciting data arising from a series of (small) clinical trials.[94, 129-131] Variations in the definition of remodelling, and in the reproducibility of the imaging modalities used to assess serial change in LV function have, to an extent, undermined previous trials.

This study was designed to determine the effects of the selective mineralocorticoid receptor antagonist eplerenone on LV remodelling (defined as the change in LVESVI) in survivors of AMI with resultant LVSD but without heart failure (or diabetes mellitus) over 24 weeks, using CMR as the imaging modality. This was the primary end-point. The effects of eplerenone on change in LVEDVI, LVEF, LVMI and, through delayed, contrast-enhanced imaging, change in infarct volume were included as secondary end-points.

3.2 Methods

3.2.1 Recruitment of patients

The study cohort consisted of 100 patients admitted with AMI with no evidence of heart failure nor prior history of diabetes mellitus, all of whom were required to have LVEF <40% on screening echocardiography. Following baseline investigations including LGE-CMR scanning, the patients were randomised in a double-blinded 1:1 fashion to placebo or eplerenone, and followed up over 24 weeks. LGE-CMR scanning was repeated at 12 and 24 weeks. Inclusion/exclusion criteria, methodology of screening, recruitment, randomisation to study drug, and trial protocols have been described in detail in Chapter 2 (2.1 – 2.3).

3.2.2 Echocardiography and LGE-CMR techniques and analysis

Screening echocardiography was performed as described in Chapter 2 (2.4). All recruited patients were studied using the CMR techniques described in detail in Chapter 2 (2.5), and CMR analysis was performed as described in Chapter 2 (2.6).

3.2.3 Statistical methods

The primary outcome of LV remodelling was defined as the between treatment difference in change in LVESV between baseline and 24 weeks. Within the Glasgow Cardiac Magnetic Resonance Unit (based in the Western Infirmary) we have a wealth of (non-BSA-indexed) CMR volumetric data from the follow-up of patients with AMI either for clinical purposes or enrolled in previous research trials, using which I calculated that 45 patients per treatment group would provide around 90% power to detect a treatment difference of 10ml in LVESV (α level 0.05). A total sample size of

100 was therefore chosen to allow a 10% discontinuation rate due to deaths and drop-outs. Only patients with both a baseline and 24-week follow-up scan were analysed and this was done on an intention-to-treat basis. Inter-group comparisons were made using paired sample *t*-tests for continuous variables and Chi-squared test for categorical variables. Paired *t*-tests were used to detect changes in LGE-CMR measurements within each treatment group over the 24 weeks of the study, and differences between these changes were analysed using an unpaired *t* test. A probability value of $p<0.05$ was considered significant.

There was a pre-specified plan to adjust for covariates that were imbalanced at baseline, namely the use of a stepwise selection model to select baseline variables predictive of the primary outcome with a p value <0.10. The variables selected in this manner were then inserted into a linear regression analysis model to adjust the trial end-points for the baseline imbalances.

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA). Professor Ian Ford was consulted prior to commencement of the project and acted as a statistical co-supervisor throughout. In particular, he was instrumental in constructing the statistical strategy employed, including verification of the power calculations, pre-specification of the covariate adjustment methodology and assistance in the *post hoc* analysis of results, in addition to co-authoring the primary results manuscript of the thesis.

3.3 Patient demographics

3.3.1 Screening

Commencing in April 2005, a total of 420 consecutive patients either admitted or transferred to the WIG with AMI were screened to obtain the target study population (n=100). Recruitment was completed in April 2006, and follow-up was finally completed in September 2006.

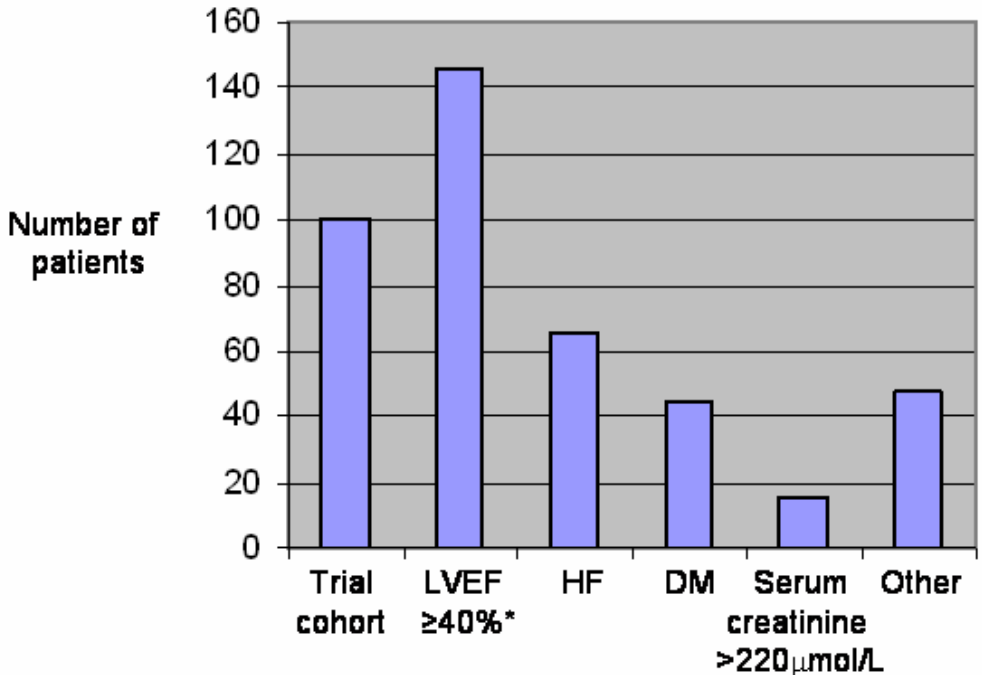


Figure 3.1 Bar chart displaying the outcomes of the 420 patients screened during the trial recruitment phase. * LVEF ≥40% in patients with neither diabetes mellitus nor heart failure. For breakdown of the "Other" column, see text. [Key: HF – heart failure; DM – diabetes mellitus]

44 of the 420 patients screened (10.5%) had established diabetes mellitus and 66 (15.7%) displayed clinical and/or radiological heart failure and were thus excluded; screening TTE was not performed in these patients. Of the remaining 310 patients, 146 (47.1%) had LVEF ≥40%, 17 (5.5%) were excluded on the grounds of dementia, frailty or neuropsychiatric disturbance, 16 (5.2%) had a serum creatinine >220μmol/l,

10 (3.2%) refused, 7 (2.3%) had MRI-incompatible ferrous prostheses, 7 (2.3%) required in-patient CABG, 3 (1.0%) died prior to screening and 4 (1.3%) were excluded due to chronic therapy with the CYP3A4 inducer carbamazepine (n=1), primary biliary cirrhosis (n=1), Addison's disease (n=1) and refractory postural hypotension (n=1). The remaining 100 patients constituted the study cohort. The screening log is depicted graphically in Figure 3.1.

Of relevance, a total of 256 patients underwent screening TTE with satisfactory image quality for analysis; of these 146 (57.0%) had LVEF ≥40% and 110 (43.0%) had LVEF <40%.

3.3.2 Baseline characteristics

For the entire study cohort of 100 patients, the mean age (standard deviation [SD]) was 58.9 (12.1) years and the majority (77%) were male, findings consistent with contemporary AMI studies including EPHESUS.[123] Within the study population 7 patients (7%) had sustained a previous MI, 5 (5%) had undergone prior CABG surgery and 2 (2%) had undergone PCI. An echocardiogram had been performed on each patient with a prior cardiac event or intervention, at some point in time between that previous event and the index AMI that led to inclusion in this study, confirming unimpaired LV systolic function prior to the index AMI, ie. none of the 100 patients enrolled in this study were known to have pre-existing LVSD.

The characteristics of the infarct are described in detail in Chapter 6; 89 patients (89%) sustained STEMI while 11 (11%) suffered non-STEMI (NSTEMI). On admission 12-lead ECG criteria, the site of the AMI was anterior (± lateral) in 55% and inferior (±

posterior) in 45%. Cardiovascular risk factor profile included cigarette smoking in 55 (55%), hypertension in 35 (35%) and dyslipidaemia (defined as serum total cholesterol >5mmol/l and/or on lipid-lowering therapy prior to admission) in 25 (25%). 6 patients (6%) had suffered a previous cerebrovascular event, while in 37 (37%) patients a first-degree relative had been diagnosed with CHD at an age <60 years.

Baseline demographic data according to treatment group (eplerenone or placebo) are shown in Table 3.1.

	Eplerenone (n=50)	**Placebo (n=50)**	**p**
Patient characteristics			
Age (years)	61.0 (12.0)	56.8 (12.0)	0.081
Male	37 (74%)	40 (80%)	0.481
BMI (kg/m^2)	31.2 (4.5)	30.5 (4.0)	0.364
AMI characteristics			
Type: STEMI /	44 (88%)	45 (90%)	
NSTEMI	6 (12%)	5 (10%)	0.752
Site: Anterior (± lateral) /	27 (54%) /	28 (56%) /	
Inferior (± posterior)	23 (46%)	22 (44%)	0.843
Haemodynamics			
Blood pressure (mmHg)			
Systolic	114.5 (16.8)	111.5 (15.8)	0.363
Diastolic	71.2 (13.7)	68.7 (9.8)	0.295
Heart rate (bpm)	66.6 (14.6)	65.1 (14.0)	0.582
Medical history			
Previous AMI	3.0 (6.0%)	4.0 (8.0%)	0.699
Previous CABG	3.0 (6.0%)	2.0 (4.0%)	0.650
Previous PCI	1.0 (2.0%)	1.0 (2.0%)	1.000
Angina	16.0 (32.0%)	23.0 (46.0%)	0.154
Current smoker	23.0 (46.0%)	32.0 (64.0%)	0.072
Hypertension	22.0 (44.0%)	13.0 (26.0%)	0.060
Dyslipidaemia*	16.0 (32.0%)	9.0 (18.0%)	0.108
Diabetes mellitus	0	0	-
Previous stroke/TIA	2.0 (4.0%)	4.0 (8.0%)	0.405
Family history of CHD<60	15.0 (30.0%)	22.0 (44.0%)	0.128

Time intervals relevant to admission			
Time from symptom onset to admission (hrs)	12.9 (36.7)	11.7 (33.6)	0.860
Time from symptom onset to 1st CMR scan (hrs)	90.7 (49.6)	103.6 (65.7)	0.271
Time from AMI to study drug (days)	4.7 (2.2)	5.0 (2.2)	0.584
Treatment			
Thrombolysis	26.0 (52.0%)	28.0 (56.0%)	0.692
Primary PCI	12.0 (24.0%)	15.0 (30.0%)	0.367
Rescue PCI	14.0 (28.0%)	12.0 (24.0%)	0.652
Angiography performed	42.0 (84.0%)	43.0 (86.0%)	0.782
PCI performed	38.0 (76.0%)	36.0 (72.0%)	0.652
Glycoprotein IIbIIIa use	26.0 (52.0%)	15.0 (30.0%)	0.020
ceCMR parameters			
LVESVI (ml/m^2)	38.9 (10.2)	48.7 (17.7)	0.001
LVEDVI (ml/m^2)	79.7 (12.7)	89.0 (21.3)	0.009
LVEF (%)$^{\Delta}$	51.5 (8.2)	46.4 (8.5)	0.003
LVMI (g/m^2)	71.1 (11.6)	77.8 (17.1)	0.024
Infarct volume index (ml/m^2)	31.4 (19.4)	35.0 (22.0)	0.381
Laboratory values			
eGFR†	67.1 (15.5)	73.3 (19.5)	0.084
Creatinine (μmol/l)	101.5 (18.4)	98.7 (24.0)	0.520
Urea (mmol/l)	6.7 (2.5)	6.4 (2.8)	0.583
Serum potassium (mmol/l)	4.1 (0.4)	4.2 (0.3)	0.383
Admission medication			
Aspirin	16.0 (32.0%)	7.0 (14.0%)	0.033
Beta blocker	14.0 (28.0%)	4.0 (8.0%)	0.009
ACE inhibitor or ARB	6.0 (12.0%)	6.0 (12.0%)	1.000
Statin	10.0 (20.0%)	5.0 (10.0%)	0.165
Discharge medication			
Aspirin	47.0 (94.0%)	49.0 (98.0%)	0.312
Clopidogrel	42.0 (84.0%)	40.0 (80.0%)	0.607
β-blocker	48.0 (96.0%)	45.0 (90.0%)	0.244
ACE inhibitor or ARB	47.0 (94.0%)	47.0 (94.0%)	1.000
Statin	50.0 (100%)	48.0 (96.0%)	0.156
Frusemide	9.0 (18.0%)	12.0 (24.0%)	0.466

Table 3.1 Baseline characteristics of study population, by treatment group. Data are presented as mean (SD) for continuous variables and number (%) for categorical variables. Inter-group comparisons were made using paired sample *t*-tests for continuous variables and Chi-squared test for categorical variables. * serum cholesterol >5mmol/l and/or on lipid-lowering therapy prior to admission; † eGFR is estimated glomerular filtration rate, calculated using the Modification of Diet in Renal Disease (MDRD) formula; Δ normal range on CMR 58-68% .

There were, by chance, significant differences between the treatment groups (Table 3.1). Patients were significantly mismatched with respect to baseline LV mass and

function. Patients in the eplerenone group had a significantly lower mean LVESVI (38.9 [10.2] v 48.7 [17.7] ml/m^2, p=0.001), LVEDVI (79.7 [12.7] v 89.0 [21.3] ml/m^2, p=0.009) and LVMI (71.1 [11.6] v 77.8 [17.1] g/m^2, p=0.024), and a significantly higher LVEF (51.5 [8.2] v 46.4 [8.5] %, p=0.003) than those in the placebo group. Infarct volume did not differ significantly between treatment groups.

Although there were no significant inter-group differences in the proportion of patients undergoing PCI, there was significantly greater use of glycoprotein IIb/IIIa inhibitors in the eplerenone-treated patients, in whom there was additionally a significantly greater uptake of aspirin and β-blockers at baseline compared to the placebo group. The patients in both treatment groups had unimpaired baseline renal function in general.

3.3.3 Treatment of AMI

During the time-period over which recruitment took place (April 2005 – April 2006), there was no designated primary PCI service in place for the management of STEMI in the North Glasgow University Hospitals NHS Trust. Policy regarding acute reperfusion in STEMI was to use thrombolytic therapy as the first-line treatment unless there was an obvious contraindication or the patient had presented to hospital more than 12 hours after symptom onset; primary PCI was indicated in these circumstances. In September 2005, the thrombolytic window was shortened to 6 hours after symptom onset, after which primary PCI was indicated. Throughout the recruitment phase, the policy of immediate transfer for 'rescue' PCI should thrombolytic therapy have failed to effect satisfactory reperfusion (defined as peak ST-segment resolution of at least 50% with symptomatic improvement) was adopted.

Across the entire cohort, 89 patients (89%) sustained STEMI. Thrombolytic therapy was used in 54 patients (54%), of whom 26 (26%) required rescue PCI. Primary PCI was the acute reperfusion strategy employed in 27 (27%). 8 patients (8%) were admitted with STEMI but received no acute reperfusion therapy: 3 patients (3%) presented later than 24 hours after onset of symptoms with persisting ST-segment elevation but, in view of resolution of symptoms together with clinical and haemodynamic stability, the decision against primary PCI was made by the duty interventional consultant cardiologist; 1 patient (1%) was transferred from a peripheral hospital for consideration of primary PCI but this was not performed as the ECG ST-segment changes had resolved by the time of transfer and the patient was symptom-free and stable; in 4 patients (4%) the initial ST-segment elevation had been missed by the general physicians under whom the patients were initially admitted thus no acute reperfusion was undertaken.

In-patient diagnostic coronary angiography was performed in 85% of the cohort, with follow-on PCI being undertaken in 74% (Appendix VII). Patients requiring in-patient CABG were excluded. The uptake of standard secondary preventive therapies was exceptionally high: discharge prescription included aspirin in 96%, clopidogrel in 82%, β-blocker in 93%, ACE inhibitor or ARB in 94% and a statin in 98%. Despite the protocol-mandated absence of clinical or radiological heart failure at randomisation, 21 patients (21%) were ultimately discharged on a loop diuretic at the discretion of the ward team.

3.3.4 Patient disposition

The placebo and eplerenone randomisation groups each contained 50 patients. 93 patients completed the entire 24 week follow-up. Of the 7 who failed to complete, 3 patients died (all in the eplerenone group – Appendix VIII), 2 patients had devices inserted which precluded further CMR scanning (VVI pacemaker in an eplerenone group patient and an ICD in a placebo group patient), and 2 patients, both in the placebo group, voluntarily withdrew their consent. One patient, in the placebo group, permanently discontinued study drug due to nausea after 14 days but participated in all other aspects of the trial. Paired analysis was therefore performed on 46/50 in the eplerenone group and 47/50 in the placebo group. The mean time from AMI to screening TTE for all study patients was 33.6 hours, and from AMI to first CMR scan was 97.2 hours; study drug was commenced at a mean of 4.8 days after AMI. There were no between-group differences in any of these parameters. The mean daily doses of eplerenone and placebo achieved in the study patients were 43.7mg and 41.8mg respectively.

3.4 Effects of eplerenone on ceCMR parameters

3.4.1 Effects on LVESVI

Within the placebo group there was very little change in LVESVI over the study period, with a mean LVESVI at baseline of 48.6 ml/m^2 and at 24 weeks of 48.7 ml/m^2 (p=0.97). In the eplerenone group, the mean LVESVI decreased from 38.4 ml/m^2 to 37.4 ml/m^2 (p=0.58) over the 24-week follow-up. Although LVESVI decreased in the eplerenone group and increased slightly in the placebo group (Figure 3.2), this effect was non-significant (eplerenone -1.0 ml/m^2 v placebo +0.1 ml/m^2, p=0.72).

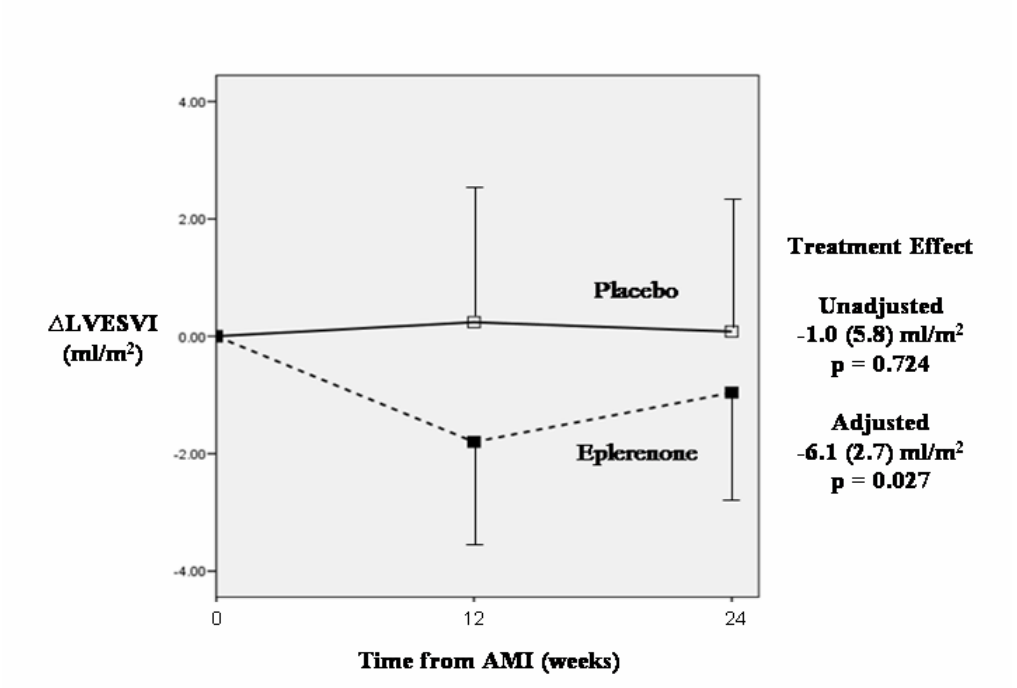


Figure 3.2 Mean (±SEM) changes from baseline in LVESVI (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

The eplerenone group had significantly smaller LV volumes and higher LVEFs at baseline compared to the placebo group. There were also significant imbalances in certain other baseline variables (Table 3.1). Using the covariate adjustment strategy

described in the statistical methods section, and inserting all variables listed in Table 3.1 into the model, fifteen were selected and utilised to adjust the LGE-CMR results for the baseline differences (Table 3.2). Following adjustment, the treatment effect (SD) of eplerenone compared to placebo over 24 weeks on LVESVI was -6.1 (2.7) ml/m^2 (p=0.027).

LVESVI	Age	Thrombolysed
LVEDVI	Hypertension	Glycoprotein IIb/IIIa inhibitor used
LVEF	Dyslipidaemia	eGFR
LVMI	Previous CABG	Aspirin on admission
LV Infarct Volume	Current smoker	β-blocker on admission

Table 3.2 Baseline variables selected for covariate adjustment

3.4.2 Effects on LVEDVI

Mean LVEDVI in the placebo group increased significantly from 88.6 ml/m^2 to 94.9 ml/m^2 (p=0.02), while in eplerenone-treated patients it rose from 79.0 ml/m^2 to 81.2 ml/m^2 (p=0.27) over the 24-week follow-up. There was no significant between-group treatment effect of eplerenone on LVEDVI (eplerenone +2.2 ml/m^2 v placebo +6.2 ml/m^2, p=0.23), but the covariate-adjusted treatment effect was significant (-7.5 [3.4] ml/m^2, p=0.031) – Figure 3.3.

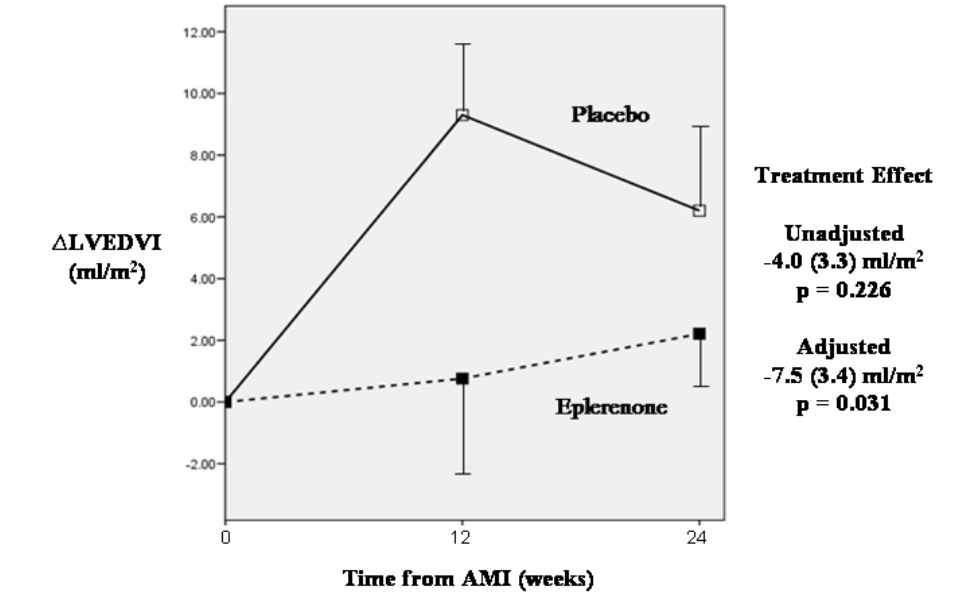


Figure 3.3 Mean (±SEM) changes from baseline in LVEDVI (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

3.4.3 Effects on LVEF

Mean LVEF increased significantly over the course of the study in both treatment groups (Figure 10), from 46.3% to 50.7% (p=0.01) in the placebo group and from 51.7% to 55.3% (p=0.005) in the eplerenone group. There was no significant treatment effect of eplerenone on LVEF (eplerenone +3.6% v placebo +4.4%, p=0.71). Following covariate adjustment the treatment effect remained non-significant (+2.5 [1.8] %, p=0.181).

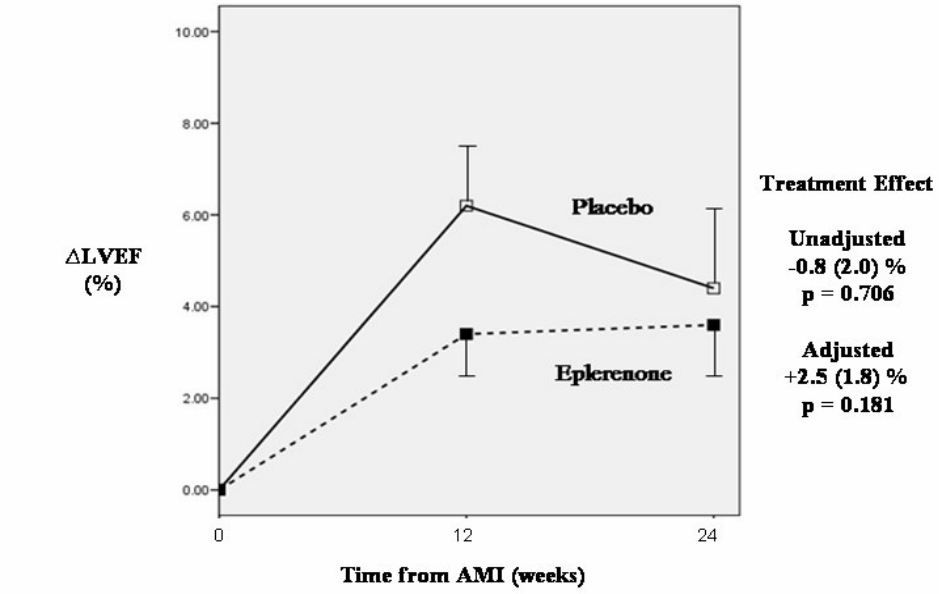


Figure 3.4 Mean (±SEM) changes from baseline in LVEF (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

3.4.4 Effects on LVMI

Mean LVMI decreased significantly within each treatment group over 24 weeks (Figure 3.5). In placebo-treated patients. LVMI fell from 78.1 g/m^2 to 69.7 g/m^2 (p<0.001) and in eplerenone-treated patients, from 70.7 g/m^2 to 64.4 g/m^2 (p<0.001). There was no significant between-group treatment effect (eplerenone -6.2 g/m^2 v placebo -8.4 g/m^2, p=0.31), and this remained non-significant after covariate adjustment (-1.0 [2.2] g/m^2, p=0.660).

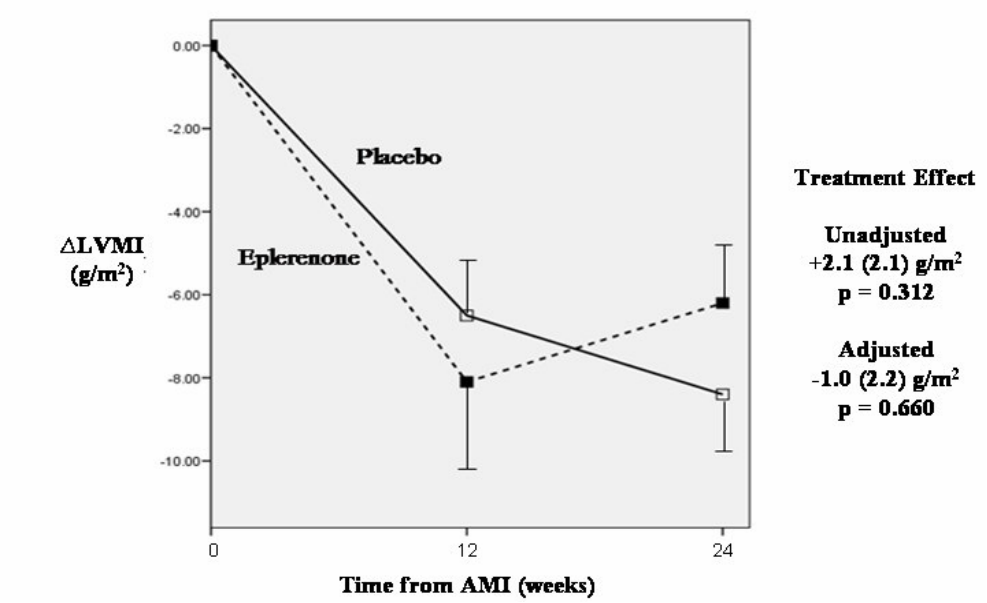


Figure 3.5 Mean (±SEM) changes from baseline in LVMI (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

3.4.5 Effects on LV infarct volume index

Mean LV infarct volume index fell significantly within each treatment group (Figure 12), from 36.4 ml/m^2 to 22.5 ml/m^2 (p<0.001) in the placebo group and from 31.5 ml/m^2 to 19.4 ml/m^2 (p<0.001) in the eplerenone group. There was no significant inter-group difference in Δ LV infarct volume index (eplerenone -12.1 ml/m^2 v placebo -14.0 ml/m^2, p=0.50). After covariate adjustment the treatment effect remained non-significant (-0.9 [1.9] ml/m^2, p=0.626).

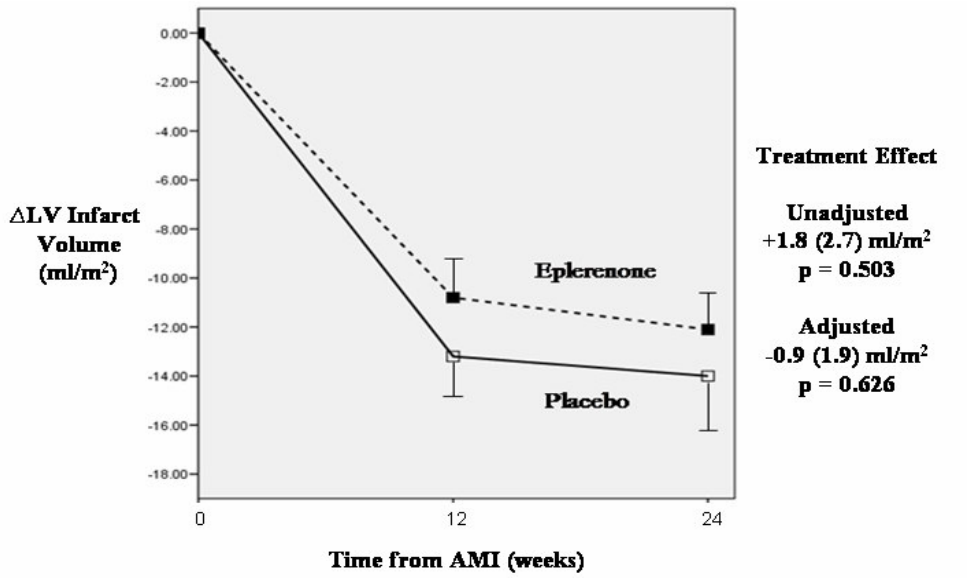


Figure 3.6 Mean (±SEM) changes from baseline in LV infarct volume index (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

3.5 Adverse events, safety and tolerability of eplerenone

Renal function and serum potassium

There were no significant differences in serum creatinine, potassium or eGFR between treatment groups at baseline. None of these changed significantly during follow-up in either treatment group and there were no between-treatment differences in any of these measures (Appendix VIII).

Serious adverse events

Three eplerenone patients died during follow-up: one from ventricular fibrillation on day 7, one from a stroke at 8 weeks and one after a recurrent AMI in a separate coronary arterial territory at 5 months, confirmed at autopsy; no placebo-treated patient died. A small number of patients in each treatment group were re-admitted with biomarker-negative chest pain, recurrent AMI, heart failure or possible arrhythmia (documented in only two patients, both in the placebo group: atrial fibrillation in one, ventricular tachycardia in another) – Appendix VIII.

Other adverse events, safety and tolerability

Three patients in each group developed transient postural hypotension in the first few days after starting study drug which settled in each case. At 24 weeks, mean blood pressure increased by 10.8/1.6mmHg in the eplerenone group and by 10.8/2.6mmHg in the placebo group (between-treatment difference p=0.69). Mean heart rate decreased by 7.4bpm in the eplerenone group and by 6.1bpm in the placebo group (p=0.66). Serum potassium >5.5 but <6.0 mmol/l occurred in two patients, both on eplerenone and both were successfully rechallenged after temporary cessation of study drug. Serum potassium <3.5 mmol/l occurred in two placebo-treated patients. No patient required cessation of study drug due to worsening renal function or developed gynaecomastia. One placebo-treated patient permanently discontinued study drug due to non-specific symptoms.

3.6 Discussion

3.6.1 Study population

In any study designed to examine the serial change in a particular biophysiological variable it is important to examine the substrate on which the research is based. The demography, acute management and chronic treatment of this study population need to be placed in context by comparison with contemporary trials of LV remodelling after AMI in humans. The mean age of patients in this study was just under 60 and the overwhelming majority were male, findings consistent with several contemporary AMI and remodelling studies.[272-278] The pre-specified recruitment strategy excluded patients with known LVSD; in the small number who had suffered a prior MI or who had undergone prior PCI or CABG (total 12%), we stipulated that a LVEF >40% had to have been confirmed on a cardiac imaging study performed at some point in time between the prior cardiac event and the index AMI that led to recruitment in this study. In comparison to the trial populations in recent post-infarction LV remodelling studies, we therefore obtained a 'clean' cohort of patients without known pre-existing LVSD. In contrast to our cohort, the CAPRICORN investigators reported a 25% incidence of prior MI (but no note of any previous LV functional assessment) in the 127 patients enrolled in an echo sub-study examining the effects of carvedilol on LV remodelling after AMI, with similar incidences of prior known CHD in imaging sub-studies of the Carvedilol Heart Attack Pilot Study (CHAPS) and the Healing and Early Afterload Reducing Therapy (HEART) study.[273-276]

89 of the 100 patients in our study cohort suffered STEMI which was acutely reperfused in 81 (91%) via thrombolytic or primary PCI. Overall coronary

angiography was undertaken in 85% and PCI in 74%. In comparison, while the early β blocker studies of LV remodelling employed a high uptake of thrombolytic therapy (97%) prior to commencement of carvedilol, in the CAPRICORN echo sub-study, published in 2004, there was only a 41% rate of thrombolytic and/or PCI, while in the HEART echo sub-study only 72% were thrombolysed prior to assessing the effects of the ACE inhibitor ramipril on LV remodelling after AMI.[273-276]

Historically, remodelling studies examining the effects of ACE inhibitors or β blockers in AMI have necessarily been placebo-controlled thus only half of the patients receive the active drug. Although the uptake of ACE inhibitor therapy was very high at 93% in the CAPRICORN echo sub-study, only 50% received carvedilol.[273] Earlier remodelling studies employed significantly lower prescriptions of known anti-remodelling medications.[272, 274] The discharge prescription of β blockers was 93% and of ACE inhibitors or ARBs 94% within our study cohort. There was also a very high uptake of statins, which have been shown in experimental AMI in rats to attenuate LV remodelling, although there are no such data in humans.[279]

The study population under investigation in this thesis therefore represents a very well-treated cohort with a very high acute revascularisation rate, almost universal uptake of current evidence-based anti-remodelling therapies, and, as each patient had 5 post-discharge trial visits to the Western Infirmary during their 24-week participation in the study, a very closely monitored group of patients. These factors must be kept in mind when attempting to assess the additional benefits on LV remodelling of the study drug.

3.6.2 The effects of eplerenone on LV remodelling

The study was designed to determine the effect of eplerenone on LV remodelling in a cohort of patients with AMI and resultant LVSD, but neither heart failure nor diabetes mellitus, over 24 weeks. Not only were there, by chance, significant baseline imbalances between treatment groups, there was also little evidence of remodelling (defined as ΔLVESVI) in this population of AMI patients treated aggressively with contemporary therapies. Across the entire cohort of 93 patients who completed the 24-week follow-up, mean (SD) LVESVI did not vary significantly over time: 43.6 (15.2) ml/m^2 at baseline vs. 43.1 (20.7) ml/m^2 at 24 weeks, $p = 0.77$. Eplerenone did not significantly affect the pre-specified primary end-point and was only noted to exert an anti-remodelling effect after adjustment for 15 baseline covariates. While these were selected using an appropriate statistical strategy, the results of a 100-patient study corrected for multiple covariates must be treated with caution. That the primary end-point was not met implies that eplerenone did not exert a significant anti-remodelling effect in the srudy population. Nonetheless there was a slight divergence between treatment groups in the primary end-point (ΔLVESVI) occurring despite the superior baseline LV function in the eplerenone group.

Despite the greater predictive accuracy in determining survival after AMI of LVESV over LVEDV (and even LVEF), a number of studies have defined remodelling as serial change in LVEDV rather than LVESV.[22, 130, 131] LVEDVI increased significantly over time in our study cohort as a whole, from 83.9 (18.0) ml/m^2 at baseline to 88.1 (23.0) ml/m^2 at 24 weeks, $p = 0.011$. When analysed by treatment allocation, although LVEDVI increased significantly in the placebo group but not in

the eplerenone group, the between-treatment difference, again, was only significant after covariate adjustment.

An increase in LVESVI after AMI is a powerful predictor of poor clinical outcomes, and attenuation of this change is associated with improved prognosis.[22] The magnitude of the *adjusted* between-treatment differences in LV volumes in this study (6.1 and 7.5 ml/m^2 for LVESVI and LVEDVI respectively) compares favourably with prior remodelling trials despite differences in AMI management and LV assessment. Early studies using captopril <48 hours after Q-wave AMI (72% thrombolysed, baseline LVEF 40.7%) showed significant reductions of 8.0 and 10.4 ml/m^2 in LVESVI and LVEDVI respectively over 3 months compared to placebo.[280] A meta-analysis of three studies from the early 1990s showed small, non-significant changes of 0.5 ml/m^2 in both LVESVI and LVEDVI with ACE inhibitor use over 3 months after thrombolysed AMI, although ACE inhibitors did significantly attenuate remodelling in the non-reperfused sub-group.[281] A further meta-analysis of 8 studies from the 1980s and 1990s revealed small but significant reductions of 3.3 and 4.2 ml in LVESV and LVEDV respectively over 6-12 months in patients with LVEF ≤45% after AMI.[282] More recently, the Perindopril and Remodeling in Elderly with Acute Myocardial Infarction (PREAMI) study showed a significant attenuation of 3.3 ml in LVEDV in 631 elderly patients with LVEF ≥40% treated with perindopril compared to placebo.[283] In a well-treated cohort of patients with a high uptake of anti-remodelling therapies, I have shown comparable, significant effects on covariate-adjusted LV volumes over 6 months using eplerenone. However, while the magnitude and direction of this covariate-adjusted treatment effect is similar to the effect of ACE inhibitors in such prior remodelling studies, the *unadjusted* data do not support a

significant treatment effect of aldosterone antagonism on top of ACE inhibitor/β blocker therapy in this setting.

The use of LGE-CMR in this study afforded *in vivo* visualisation of abnormal regions of myocardium consistent with infarction.[245-247] TTE, the imaging modality employed in the majority of AMI remodelling studies, can only indirectly estimate infarcted myocardium via wall motion scoring and regional wall thickening indices.[275, 276] While infarct volume fell significantly over time in each treatment group, there was no significant between-treatment difference in Δ infarct volume even after covariate-adjustment. This may appear counter-intuitive given the covariate-adjusted effect of eplerenone on serial change in LV volumes. However, early administration of eplerenone has been shown to reduce reactive fibrosis in viable myocardium distant from the infarct site but to have no effect on reparative fibrosis, which is necessary for the structural integrity of the healing scar, in experimental AMI in rats.[106] That eplerenone does not reduce infarct volume more than placebo over time may represent the lack of drug effect on the reparative fibrosis within the infarct site.

Reactive fibrosis in non-infarct zone myocardium should be detectable on delayed LGE-CMR imaging, as this technique simply indicates that the normal fluid homeostasis within the abnormal region has been disrupted (section 1.11.3). Anecdotally, no evidence of non-infarct fibrosis was observed during the 24-week follow-up of our study patients, in whom LGE-CMR images were obtained at all 3 time-points. Reactive fibrosis is a phenomenon frequently seen within the first few months after experimental AMI in rodent models and is attenuated by ACE inhibitors, presumably via reduction in angiotensin II (and thus aldosterone) and possibly via

down-regulation of mineralocorticoid receptor expression in the heart and kidney.[106, 284, 285] In humans, indirect evidence of such fibrosis has been provided by, for example, attenuation of increases in markers of collagen matrix turnover (eg. PIIINP in the RALES study, suggesting an anti-fibrotic effect of spironolactone in patients with advanced CHF).[121] That no macroscopic evidence of such reactive fibrosis in non-infarct sites was seen in our study population may reflect the high uptake of antagonists of the RAAS. Alternatively, the process of non-infarct zone fibrosis may be more chronic in humans and may have been missed by the relatively short 24-week follow-up.

3.6.3 Safety profile of eplerenone in patients presenting with AMI and LVSD

The study was not powered to examine major clinical end-points thus the events listed in Appendix VIII are purely descriptive. In an appropriately-powered study (EPHESUS), eplerenone clearly reduced cardiovascular death and hospitalisation in patients with LVSD and heart failure, or diabetes mellitus, following AMI.[123, 286] That the 3 strokes recorded during follow-up all occurred in eplerenone-treated patients in our study must be treated with caution, however, as a non-significant excess of stroke was seen in the EPHESUS study despite the significant morbidity and mortality reductions.[123]

Eplerenone was well-tolerated biochemically in this study, with no significant change in serum creatinine, eGFR or serum potassium in either treatment group, although it is noteworthy that renal function was normal at baseline across the cohort. In contrast, small but significant increases in creatinine and potassium were seen with the use of spironolactone in RALES (serum creatinine increased by 4-9 μmol/l and potassium by

0.30 mmol/l relative to placebo, p<0.001) and eplerenone in EPHESUS (serum creatinine increased by 3.5 μmol/l and potassium by 0.10 mmol/l relative to placebo, p<0.001).[121, 123] Both studies used the same renal function cut-off for patient inclusion as in this study (serum creatinine ≤220 μmol/l)

In our study cohort, although the absolute number of patients randomised to eplerenone was small (n=50), the use of the drug in conjunction with an ACE inhibitor and β blocker early after AMI in patients with LVSD was safe and well-tolerated.

3.6.4 Limitations

The major limitation of this study is the dependence of the primary results on covariate adjustment, which calls into question the validity of these results. Such adjustment was necessary, however, due to the significant imbalances in LV volumes, mass and function between the treatment groups at baseline. The plan to adjust for any baseline imbalance was pre-specified. We did not pre-specify stratification of randomisation on LGE-CMR outcomes; retrospective analysis of the randomisation process (performed in conjunction with Professor Ian Ford), in chronological order of index event, failed to reveal any aberrant patterns in treatment allocation.

The pre-specified statistical strategy excluded those with an incomplete dataset. Had the worst outcome been assigned to those who died, all 3 of whom were in the eplerenone group, such a strategy may have eliminated an effect of active therapy on remodelling (even after covariate adjustment).

The marked baseline imbalance in LV function is unfortunate, and suggests a potentially important biologic imbalance: placebo-treated patients were perhaps pathophysiologically more likely to remodel to a greater extent than eplerenone-treated patients due to poorer baseline LV function. That there is a divergence in ΔLVESVI between treatment groups in favour of eplerenone despite the superior LV function in patients randomised to eplerenone at baseline, is nonetheless encouraging and does suggest an anti-remodelling effect of eplerenone.

3.6.5 Conclusions

Aggressive management of this cohort of AMI patients with reduced LVEF using contemporary therapies (exclusive of aldosterone antagonists) resulted in very little adverse ventricular remodelling over 24 weeks. Eplerenone had no significant effect on remodelling prior to covariate adjustment. While such statistical adjustment was necessary due to significant baseline imbalances, the potential anti-remodelling effects of eplerenone must be interpreted with caution. Nevertheless, there was a divergence in ΔLVESVI in favour of a beneficial anti-remodelling effect of eplerenone compared to placebo. These results suggest that larger studies powered to detect an improvement in clinical outcomes in a broader patient population than that enrolled in EPHESUS may be worthwhile, ie. patients with 'asymptomatic' LVSD, irrespective of heart failure status or diabetes mellitus.

Chapter 4

A study of the use of biomarkers in the prediction of left ventricular functional recovery after acute myocardial infarction

4.1 Introduction

Patients with limited functional recovery early after AMI resulting in a persistently reduced LVEF constitute a population at high risk of malignant arrhythmias, adverse remodelling, progressive LV dilatation and dysfunction, and premature death as previously described. The use of predictive biomarkers to identify such high-risk patients is gaining popularity as these patients require stringent monitoring, and may qualify for device therapies such as implanted cardioverter defibrillators. Additionally, certain management decisions depend on an adequate knowledge of the LVEF, but as discussed earlier the estimates of LVEF provided by various commonly-used imaging modalities are by no means interchangeable, particularly in patients with LVSD (Chapter 1.11.1). CMR imaging is now recognised as the gold standard means of LV structural and functional assessment; that this technique does not depend on geometric assumptions makes it particularly reproducible in those with LVSD (Chapter 1.11.1).

The patients enrolled in the clinical trial on which this thesis is based constitute an ideal population in which to analyse the relationships of a variety of biomarkers with serial change in LV volumes and function, as each patient had been admitted with AMI, was required to have LVSD, and underwent serial LGE-CMR imaging over 24 weeks.

The aim of this study was to attempt to enhance the risk stratification process of survivors of AMI with resultant LVSD by examining the relationships between a variety of biomarkers measured early after AMI and post-infarction remodelling (measured using serial LGE-CMR), the effects of eplerenone on each biomarker, and whether these biomarkers could be used to predict adverse remodelling.

4.2 Methods

4.2.1 Study patients and trial protocol

The study cohort consisted of 100 patients admitted with AMI, with LVEF <40% on screening TTE but without heart failure or diabetes mellitus. Patients were randomised in a double-blinded 1:1 fashion to placebo or eplerenone, and followed up serially over 24 weeks. The screening, recruitment, randomisation process and trial outline are described in detail in Chapter 2 (2.1 – 2.3), and baseline demographic data of the study cohort are shown in Table 3.1.

ceCMR imaging was performed at baseline (pre-randomisation), 12 and 24 weeks; the methods of imaging and analysis are described in detail in Chapter 2 (2.5 – 2.6). Venepuncture for measurement of circulating biomarkers was also performed at baseline (pre-randomisation) and again at 12 and 24 weeks. Individual methods of sample collection, storage and analysis are described in Chapter 2 (2.8).

4.2.2 Statistical methods

Non-normally distributed biomarkers were log-transformed prior to analysis. The change in each biomarker over time was analysed by treatment group, and the effects of eplerenone on the change in each biomarker assessed. Inter-group comparisons were made using paired sample *t*-tests or Mann-Whitney-U tests as appropriate for continuous variables and Chi-squared test for categorical variables. Paired *t*-tests were used to detect changes in ceCMR measurements and biomarkers within each treatment group over the 24 weeks of the study, and differences between these

changes were analysed using an unpaired *t* test. A probability value of $p<0.05$ was considered significant.

As the randomisation groups were, by chance, significantly imbalanced in terms of baseline LV function (Table 3.1), the treatment effect of eplerenone on each biomarker was assessed before and after the pre-specified covariate-adjustment strategy described in Chapter 3 (3.2.3). In brief, variables predictive ($p < 0.10$) of the primary outcome of the study (i.e. remodelling, defined as change in LVESVI), identified via stepwise selection, were inserted into a linear regression analysis model in order to adjust the treatment effect of eplerenone on each biomarker for the baseline imbalance in LV function.

Biomarkers exhibiting logarithmic-normal distribution were log-transformed prior to parametric correlation and multivariable analysis. Pearson's correlation co-efficients were then computed for baseline biomarker concentrations with changes in three LV parameters – LVESVI, LVEDVI and LVEF – from 0 to 24 weeks. Clinical variables predictive of LV remodelling (ΔLVESVI) were selected by inserting all baseline clinical variables into stepwise linear regression analysis. Multivariable regression models were then constructed using the forward selection of the baseline biomarkers selected as significant ($p < 0.05$) univariable predictors of LVESVI at 24 weeks, and adjusted for these clinical predictors.

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) under the guidance of Professor Ian Ford.

4.3 Biomarker concentrations after AMI, and the effects of treatment with eplerenone

Venepuncture for measurement of cardiac biomarkers was performed at a mean 3.7 days (SD 1.8 days; range 1-12 days) after AMI.

4.3.1 The effects of eplerenone on peptide neurohormones

Baseline values of the neurohormones BNP, NTproBNP, NA and AVP according to treatment group are shown in Table 4.1. Due to the wide range of NTproBNP values, a logarithmic transformation was undertaken.

	Baseline Values		
	Placebo (n=50)	**Eplerenone (n=50)**	**p**
BNP (pg/ml)	238.9 (198.2)	250.7 (167.0)	0.75
NTproBNP (pg/ml)	2471 (2580)	2683 (2768)	0.69
Noradrenaline (nmol/l)	3.35 (1.98)	3.17 (1.67)	0.62
AVP (pg/ml)	0.86 (0.94)	0.87 (0.66)	0.95

Table 4.1 Plasma concentrations of peptide neurohormones at baseline (mean 3.7 days after AMI) according to randomisation group. Data are expressed as mean (SD). Normal reference ranges provided within relevant sub-sections below.

BNP

BNP was elevated at baseline in both treatment groups (Table 4.1; normal range <100 pg/ml). There was no significant inter-group difference in baseline BNP. Within each treatment group, BNP levels fell significantly over the 24-week follow-up (Figure 4.1). Using data from only those patients who completed follow-up, mean BNP fell from 236.5 pg/ml to 139.8 pg/ml (p=0.003) in the placebo group and from 248.6 pg/ml to 100.4 pg/ml (p<0.001) in the eplerenone group. The unadjusted treatment effect of eplerenone on BNP compared to placebo was non-significant (eplerenone -148.1 pg/ml v placebo -96.7 pg/ml, p=0.155), although there was a trend towards a continuing reduction in BNP in the eplerenone group from three to six months (Figure 4.1). Following adjustment for the covariates listed in Table 3.2, the treatment effect of eplerenone remained non-significant: the adjusted treatment effect (SD) on BNP was -35.0 (39.6) pg/ml (p=0.380).

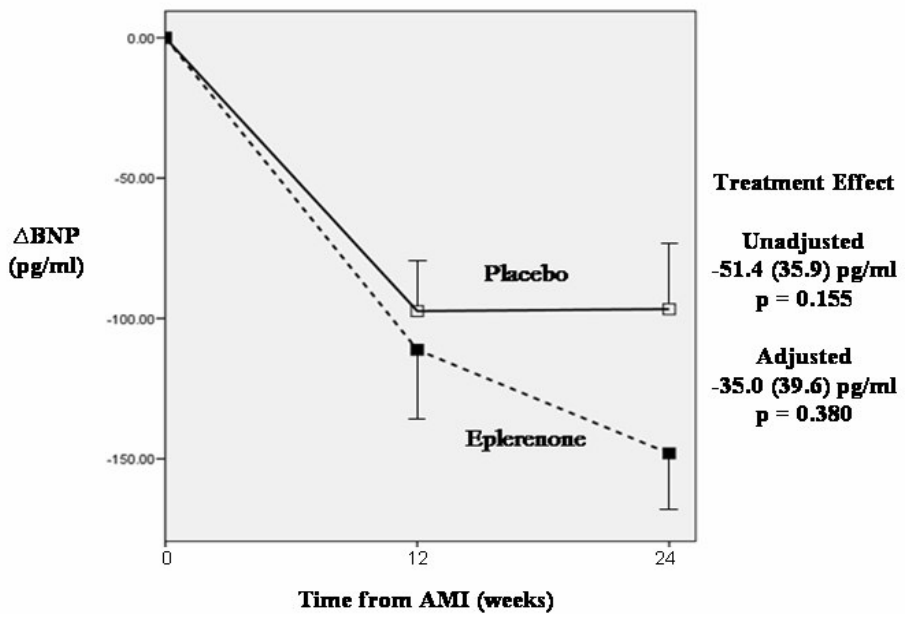


Figure 4.1 Mean (±SEM) changes from baseline in BNP (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

NTproBNP

NTproBNP was elevated in both treatment groups at baseline but with no significant inter-group differences (Table 4.1; normal range <125 pg/ml). Within each treatment group, NTproBNP levels decreased significantly during follow-up. In the placebo group, mean NTproBNP fell from 2526.1 pg/ml to 909.7 pg/ml over 24 weeks ($p<0.001$), while in the eplerenone group, the mean NTproBNP fell from 2649.2 pg/ml to 773.1 pg/ml over the same time period ($p<0.001$). Following logarithmic transformation, mean $\log_{10}$(NTproBNP) decreased from 3.24 to 2.55 units ($p<0.001$) in the placebo group and from 3.28 to 2.59 units ($p<0.001$) in the eplerenone group over the 24-week follow-up (Figure 4.2).

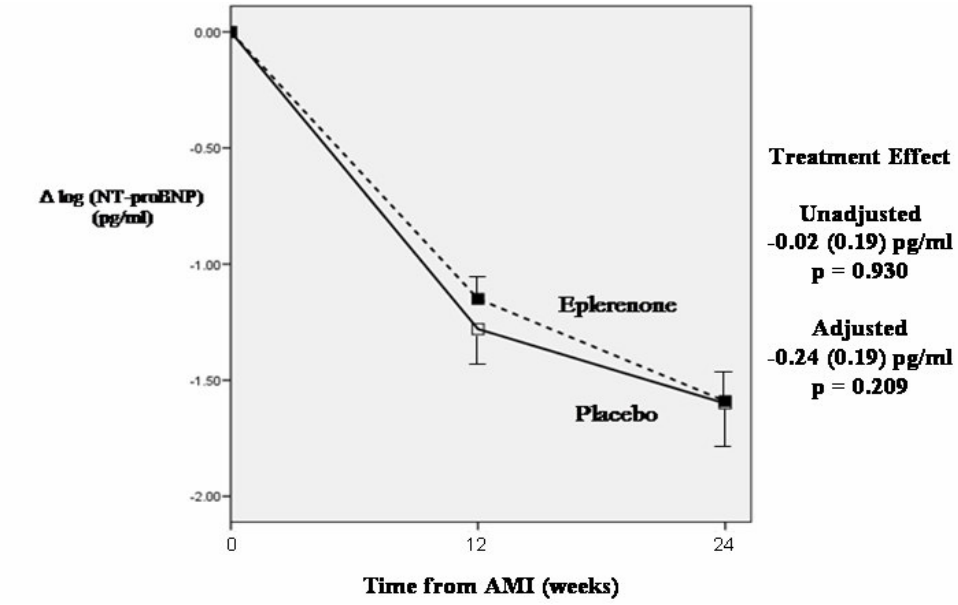


Figure 4.2 Mean (±SEM) changes from baseline in $\log_{10}$NTproBNP (at 12 and 24 weeks from randomisation) according to treatment group. Unadjusted and covariate-adjusted treatment effect listed.

The unadjusted treatment effect of eplerenone on NTproBNP compared to placebo was non-significant, and remained non-significant after adjustment for the covariates

listed in Table 3.2: unadjusted treatment effect (eplerenone -1876.1 pg/ml v placebo -1616.3 pg/ml, p=0.512); adjusted treatment effect (SD) -124.9 (317.5) pg/ml, p=0.695. Similarly, using the logarithmic scale, the unadjusted treatment effect of eplerenone on $\log_{10}$(NTproBNP) was non-significant and remained so after covariate adjustment: unadjusted treatment effect (eplerenone -0.69 units v placebo -0.68 units, p=0.930); adjusted treatment effect (SD) -0.24 (0.190), p=0.209 (Figure 4.2).

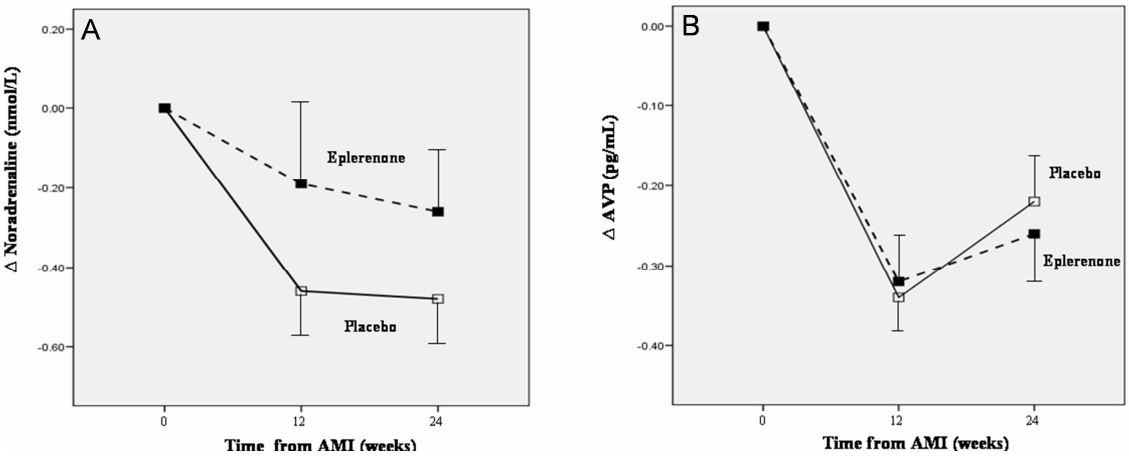


Figure 4.3 Mean (±SEM) changes from baseline in (A) noradrenaline and (B) AVP at 12 and 24 weeks from randomisation according to treatment group.

Noradrenaline (NA)

NA levels were at the upper limit of normal at baseline; there was no significant baseline imbalance between treatment groups (Table 4.1; normal range <4nmol/l). Within both groups, NA levels fell over time (Figure 4.3 A). In the placebo group, mean NA decreased significantly from 3.18 nmol/l to 2.74 nmol/l (p=0.013), while in the eplerenone group it fell from 3.03 nmol/l to 2.82 nmol/l (p=0.348). Eplerenone did not exert a significant treatment effect on unadjusted NA levels over the 24 week follow-up (eplerenone -0.21 [1.49] nmol/l v placebo -0.44 [1.86] nmol/l, p=0.174). Following covariate adjustment, this treatment effect remained non-significant (+0.24 [0.23] nmol/l, p=0.309).

AVP

Serum AVP levels were within normal limits at baseline and were well-matched between treatment groups (Table 4.1; normal range 0.2-2.2pg/ml). AVP decreased significantly over the 24 week follow-up in both groups (Figure 15B). Within the placebo group mean AVP decreased from 0.87 pg/ml to 0.65 pg/ml (p=0.001), and from 0.88 pg/ml at baseline to 0.62 pg/ml at 24 weeks in the eplerenone group (p=0.004). Eplerenone had no significant treatment effect on serum AVP levels either before or after adjustment: unadjusted (eplerenone -0.26 [0.55] pg/ml v placebo -0.21 [0.72] pg/ml, p=0.789); adjusted treatment effect (SD): +0.14 (0.13) pg/ml, p=0.294

4.3.2 The effects of eplerenone on hormones of the RAAS:

	Baseline Values		
	Placebo (n=50)	**Eplerenone (n=50)**	**p**
Plasma Renin Concentration (uIU/ml)	122.7 (155.1)	169.5 (240.2)	0.25
Aldosterone (pmol/l)	242.7 (235.8)	184.7 (149.0)	0.15
Aldosterone:Renin Ratio	4.77 (6.00)	2.81 (2.81)	0.04

Table 4.2 Plasma concentrations of plasma renin concentration, aldosterone and aldosterone:renin ratio at baseline (mean 3.7 days after AMI) according to randomisation group. Data are expressed as mean (SD), and represent the results of **all** participants in the study pre-randomisation (n=100).

PRC and aldosterone

At baseline there were no significant differences in PRC or plasma aldosterone between treatment groups (Table 4.2). PRC increased in both treatment groups over the study period (Figure 4.4 A); the change within each group approached significance. In the placebo group, mean PRC increased from 117.7 uIU/ml to 215.2 uIU/ml (p=0.064) over 24 weeks; in the eplerenone group PRC increased from 156.7 uIU/ml to 305.8 uIU/ml (p=0.071). The unadjusted treatment effect of eplerenone on PRC was non-significant (eplerenone +149.2 [547.0] uIU/ml v placebo +97.5 [352.6] uIU/ml, p=0.589) and failed to achieve significance after covariate adjustment (+98.2 [121.7] uIU/ml, p=0.423).

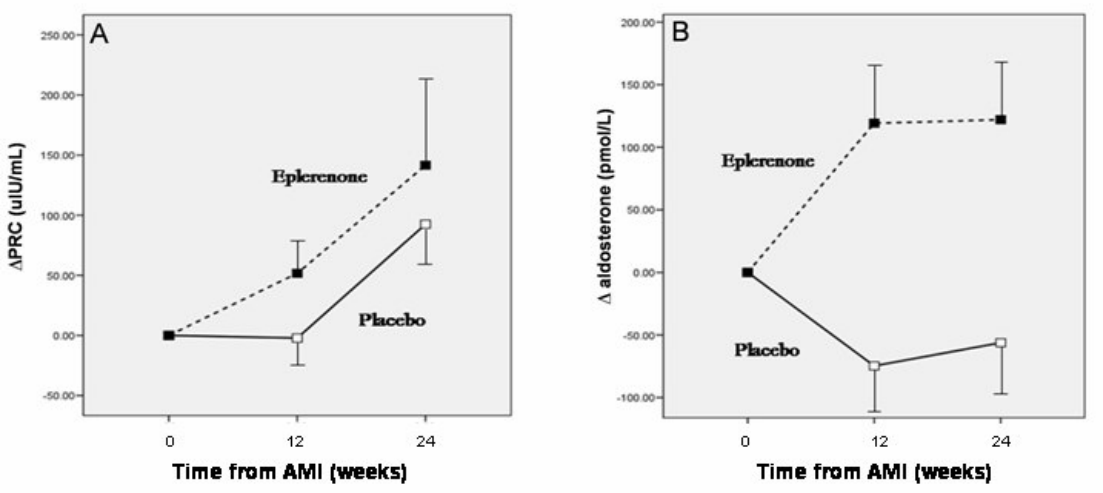


Figure 4.4 Mean (±SEM) changes from baseline in (A) plasma renin concentration (PRC) and (B) plasma aldosterone at 12 and 24 weeks from randomisation according to treatment group.

Conversely, plasma aldosterone did not change significantly within the placebo group although there was a trend towards a decrease over time (from 235.3 pmol/l to 186.5 pmol/l, p=0.238) but rose significantly within the eplerenone group, from 180.0 pmol/l to 287.9 pmol/l (p=0.006) over the study period (Figure 4.4 B). There was a

significant treatment effect of eplerenone on serum aldosterone (eplerenone +107.9 [248.3] pmol/l v placebo -48.8 [279.4] pmol/l, p=0.006) which remained significant after covariate adjustment (+130.0 [52.2] pmol/l, p=0.015).

Aldosterone to renin ratio (ARR)

ARR was significantly lower in patients in the eplerenone group compared to the placebo group at baseline (Table 4.2). There were no significant changes in ARR over time within each group. In the placebo group, mean ARR was 4.85 at baseline and 5.89 at 24 weeks (p=0.523), and in the eplerenone group it was 2.89 at baseline and rose to 5.74 at 24 weeks (p=0.103). Eplerenone had no significant treatment effect either before (eplerenone +2.85 [11.3] v placebo +1.04 [11.1], p=0.445) or after (+0.43 [3.0], p=0.888) covariate adjustment.

4.3.3 The effects of eplerenone on MMPs / TIMPs:

Each of the sampled MMPs and TIMPs was elevated at baseline compared to a reference control population (data kindly provided by Professor LL Ng, Leicester Royal Infirmary, who performed the MMP and TIMP assays); changes in each biomarker over time are shown in Figure 4.5.

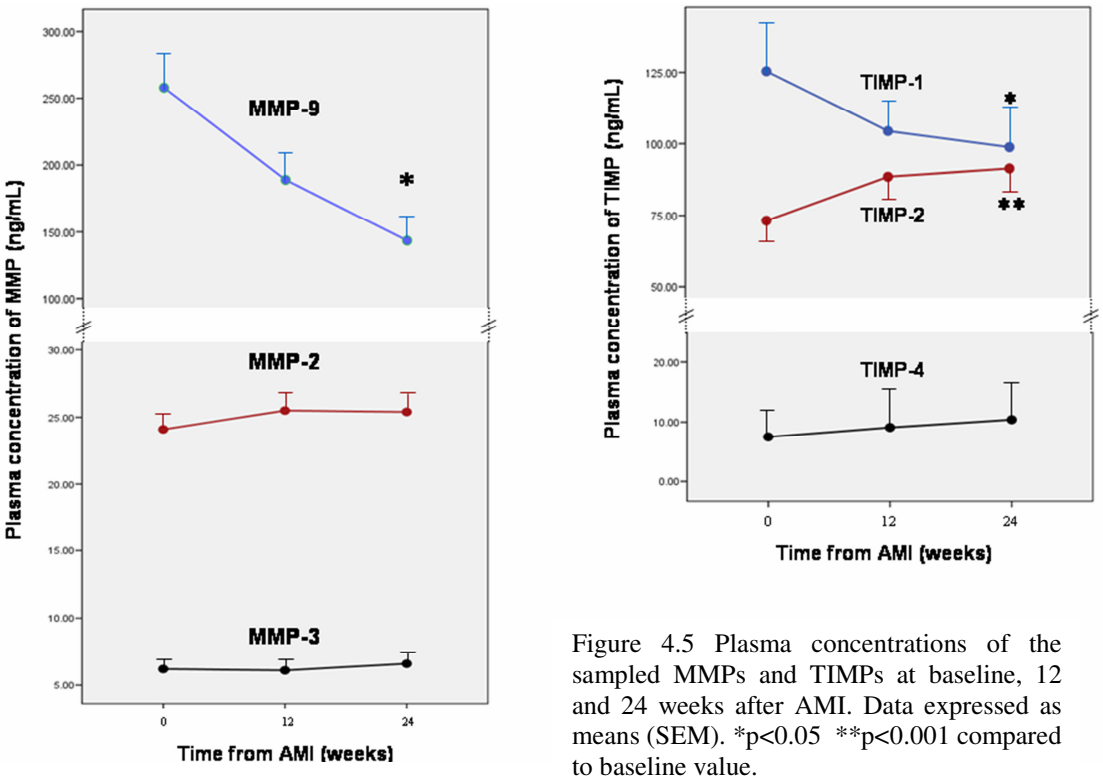


Figure 4.5 Plasma concentrations of the sampled MMPs and TIMPs at baseline, 12 and 24 weeks after AMI. Data expressed as means (SEM). *p<0.05 **p<0.001 compared to baseline value.

Plasma MMP-2, MMP-3, MMP-9 and TIMP-1, TIMP-2 and TIMP-4 levels were well-matched between treatment groups at baseline (Table 4.3).

Biomarker	Normal Range*	Eplerenone group mean (SD)	Placebo group mean (SD)	p
MMP-2	10-20	24.0 (11.4)	24.2 (11.7)	0.745
MMP-3	2.0-5.0	6.07 (2.80)	6.32 (3.40)	0.669
MMP-9	30-60	243.2 (247.1)	271.6 (295.9)	0.782
TIMP-1	3-10	132.8 (88.4)	118.5 (108.8)	0.532
TIMP-2	20-60	72.4 (22.1)	73.8 (93.0)	0.821
TIMP-4	1.0-3.0	3.38 (3.72)	11.3 (58.7)	0.352

Table 4.3 Plasma concentrations of each MMP and TIMP at baseline, by randomisation group. Values are expressed as mean (SD) and represent the results of **all** participants in the study pre-randomisation (n=100). All units are ng/mL. Reference (control) population values kindly donated by Professor LL Ng, Leicester Royal Infirmary.

MMP-2 and MMP-3 levels were modestly elevated at baseline and remained high at 24 weeks (Table 4.3, Figure 4.5). MMP-9 concentration was highly elevated at baseline, and although it fell significantly over time, remained high at 24 weeks. TIMP-1 concentration was elevated at baseline and decreased significantly over time; TIMP-2 concentration was modestly elevated and increased significantly, while TIMP-4 remained elevated throughout with no significant change over time (Table 4.3, Figure 4.5). Correlations between MMPs, TIMPs and LV parameters are shown in Table 4.4.

	MMP-2	MMP-3	MMP-9	TIMP-1	TIMP-2	TIMP-4
Baseline:						
LVESVI	0.12	0.19	0.10	-0.01	0.20	0.21*
LVEDVI	0.12	0.08	0.05	-0.05	0.20*	0.16
LVEF	-0.09	-0.27**	-0.10	-0.08	-0.10	-0.18
LVMI	0.17	0.03	0.08	-0.17	-0.11	-0.07
Infarct volume	0.03	0.28**	0.28**	-0.07	0.08	0.02
24 weeks:						
LVESVI	0.11	0.28**	-0.01	-0.05	0.33**	0.39**
LVEDVI	0.12	0.21**	-0.03	-0.08	0.31**	0.38**
LVEF	-0.13	-0.32**	0.02	-0.01	-0.19	-0.21*
LVMI	0.07	0.08	-0.02	-0.06	0.03	0.06
Infarct volume	0.05	0.31**	0.31**	0.03	0.16	0.16
Change over time:						
ΔLVESVI	0.03	0.18	-0.09	-0.06	0.26*	0.34**
ΔLVEDVI	0.04	0.19	-0.06	-0.04	0.22*	0.36**
ΔLVEF	-0.08	-0.12	0.09	0.06	-0.13	-0.09
ΔLVMI	-0.14	0.05	-0.11	0.19	0.20	0.19
ΔInfarct volume	-0.01	-0.20	0.05	0.15	0.04	0.14

Table 4.4 Pearson correlation co-efficients for each MMP/TIMP (baseline sample only) and parameters of LV function / infarct volume at baseline and 24 weeks, following Bonferroni correction. Correlation co-efficients are also listed for the relationship between each biomarker at baseline and change in each measured ceCMR parameter. *p<0.05 **p<0.01

Eplerenone was associated with a fall in MMP-2 in addition to a greater increase in MMP-3 and a lesser reduction in MMP-9 compared to placebo-treated patients, but these effects were non-significant (Table 4.5). Following covariate adjustment, however, the effects of eplerenone on MMP-2 (-6.22 [3.00] ng/ml, p=0.039) and MMP-9 (+72.6 [37.1] ng/ml, p=0.046) were significant (Table 4.5). There was no

significant treatment effect of eplerenone on TIMP-1, TIMP-2 or TIMP-4 over the 24 weeks follow-up either before or after covariate adjustment (Table 4.5).

Biomarker	Eplerenone group Δ marker (SD)	Placebo group Δ marker (SD)	p	Adjusted treatment effect	p*
MMP-2	-0.35 (14.8)	+2.82 (13.0)	0.275	-6.22 (3.00)	0.039
MMP-3	+0.67 (3.21)	+0.07 (2.83)	0.342	+0.04 (0.64)	0.947
MMP-9	-83.2 (304.5)	-143.7 (301.1)	0.338	+72.6 (37.1)	0.046
TIMP-1	-32.0 (94.4)	-21.6 (122.1)	0.648	+10.1 (17.1)	0.555
TIMP-2	+17.2 (22.3)	+19.2 (33.0)	0.739	-5.1 (7.0)	0.464
TIMP-4	+1.47 (2.91)	+4.25 (21.00)	0.381	+0.36 (1.25)	0.771

Table 4.5 Change between baseline and 24 weeks in sampled MMPs/TIMPs. All measurements in ng/ml. [Δ marker (SD) – change over 24 weeks in each marker; p* - p value for covariate-adjusted treatment effect of eplerenone on each biomarker]

4.3.4 The effects of eplerenone on cytokines:

Cytokines	Baseline value mean (SEM)		p	Change between baseline and 24 weeks mean (SEM)		p	Covariate-adjusted treatment effect mean (SEM)	p
	Eplerenone	Placebo		Eplerenone	Placebo			
Eotaxin	134.5 (8.8)	159.0 (10.5)	0.078	55.0 (9.7)	46.8 (9.0)	0.538	-0.33 (15.73)	0.983
RANTES	178904 (44107)	178441 (144152)	0.994	-24536 (44220)	48673 (67474)	0.367	-15876 (92651)	0.864
IL-1Ra	157.6 (55.9)	798.0 (380.6)	0.099	-35.0 (15.0)	-167.3 (72.1)	0.076	-3.4 (38.3)	0.930
IL-2R	62.2 (4.6)	113.0 (47.3)	0.288	-4.4 (2.5)	-24.1 (21.2)	0.358	-0.32 (3.90)	0.934
IL-4	15.2 (0.3)	16.9 (0.3)	0.306	-0.02 (0.30)	-0.96 (0.79)	0.280	0.21 (0.29)	0.461
IL-5	1.09 (0.05)	6.57 (5.10)	0.286	-0.11 (0.0.05)	-0.20 (0.14)	0.522	-0.006 (0.056)	0.918
IL-6	33.9 (4.3)	61.1 (18.1)	0.148	-26.8 (4.3)	-30.6 (7.1)	0.647	-7.3 (6.2)	0.243
IL-7	55.1 (2.4)	59.8 (5.3)	0.419	6.4 (3.5)	3.5 (3.9)	0.585	7.78 (4.93)	0.119
IL-8	40.5 (9.6)	40.6 (7.4)	0.992	-7.5 (10.5)	-7.7 (7.9)	0.988	2.32 (2.47)	0.349
IL-10	26.6 (10.8)	29.4 (10.4)	0.851	-11.6 (10.6)	-0.2 (5.2)	0.335	-18.3 (8.6)	0.036
IL-12p40	152.0 (12.8)	324.2 (107.8)	0.116	45.2 (18.3)	-9.7 (57.5)	0.365	-15.4 (29.5)	0.603
IL-15	14.1 (1.6)	70.3 (36.3)	0.126	-2.8 (1.1)	-31.0 (18.8)	0.138	-0.37 (1.57)	0.814
IP-10	37.7 (7.6)	37.4 (9.2)	0.977	11.6 (13.1)	-1.8 (6.5)	0.363	15.1 (14.0)	0.287
MIG	52.5 (6.9)	81.5 (21.0)	0.194	-7.0 (8.1)	-8.5 (10.4)	0.908	-6.3 (11.3)	0.576
MIP-1α	39.5 (1.8)	81.6 (27.0)	0.123	-2.8 (1.6)	-19.1 (11.6)	0.167	-0.65 (1.54)	0.676
MIP-1β	119.7 (14.4)	226.4 (73.9)	0.160	-13.1 (7.7)	-47.6 (23.9)	0.177	-4.1 (10.0)	0.685
MCP	695.7 (43.3)	893.8 (101.4)	0.075	197.1 (53.5)	187.2 (65.9)	0.907	-127.0 (80.0)	0.117
TNFα	1.61 (0.43)	17.60 (10.92)	0.147	-0.54 (0.37)	-9.38 (6.59)	0.184	-0.51 (1.50)	0.733

Table 4.6 Comparison of serum cytokines at baseline, and change in each cytokine between 0 and 24 weeks, according to randomisation group. The treatment effect of eplerenone, adjusted for the covariates listed in Table 4 using the statistical model described in the Methods Section (Chapter 3.2.3), is also shown. All data are expressed as mean (SEM).

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Baseline levels of serum cytokines were well-matched between treatment groups (Table 4.6). Eplerenone had no significant effect on the change in any of the measured cytokines over 24 weeks, compared to placebo. The only significant effect of eplerenone after covariate adjustment was on the anti-inflammatory chemokine IL-10, in which it attenuated the reduction over 24 weeks relative to placebo ($p=0.036$).

Of note, a number of cytokines sampled at the same time-points (baseline: 3.7 ± 1.8 days, 12 weeks, 24 weeks) were consistently below the lower limits of detection of the assays in both groups, including IL-13, IL-17, interferon γ, interferon α and GM-CSF.

4.3.5 The effects of eplerenone on markers of haemostasis:

Biomarker	Normal range	Eplerenone group mean (SD)	Placebo group mean (SD)	p
vWF (iu/dl)	50-200	222.7 (55.9)	212.0 (54.8)	0.18
tPA Ag (ng/ml)	1-20	7.19 (3.38)	7.52 (4.43)	0.77

Table 4.7 Comparison of plasma haemostatic biomarkers at baseline according to randomisation group. Data are expressed as mean (SD)

Initial vWF and tPA antigen (Ag) were well-matched between both groups (Table 4.7). vWF was elevated in both treatment groups at baseline and decreased significantly within each group over the 24 weeks follow-up (Figure 4.6 A). Within the placebo group, mean vWF fell from 212.0 iu/dl to 144.2 iu/dl ($p<0.001$); in the eplerenone group mean vWF fell from 222.7 iu/dl to 160.5 iu/dl ($p<0.001$). The unadjusted treatment effect of eplerenone on vWF was non-significant (eplerenone -62.2 [42.7] iu/dl v placebo -67.8 [51.8] iu/dl, p=0.597) as was the covariate-adjusted effect (+4.1 [11.2] iu/dl, p=0.718)

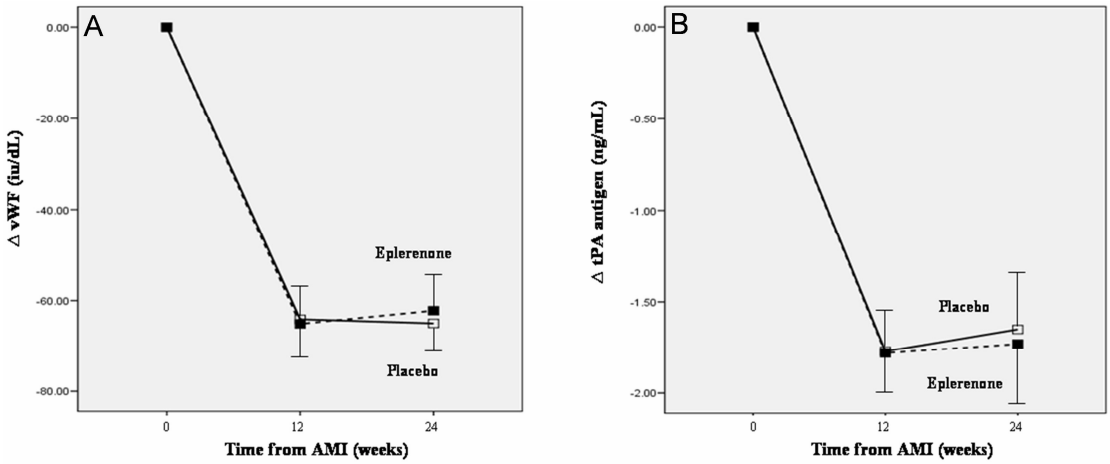

Figure 4.6 Mean (±SEM) changes from baseline in (A) plasma vWF and (B) tPA antigen at 12 and 24 weeks from randomisation according to treatment group.

tPA Ag was within normal limits in both groups at baseline and fell significantly within each group over the course of the 24 weeks follow-up (Figure 4.6 B). In the placebo group mean tPA Ag fell from 7.52 ng/ml to 5.95 ng/ml ($p<0.001$) and in the eplerenone group it fell from 7.19 ng/ml to 5.46 ng/ml ($p<0.001$). Eplerenone had no significant treatment effect on tPA Ag (eplerenone -1.72 [2.14] ng/ml v placebo -1.57 [3.02] ng/ml, $p=0.781$), even after covariate adjustment (-0.36 [0.66] ng/ml, $p=0.584$).

4.4 Biomarker predictors of LV remodelling

Baseline biomarker	ΔLVESVI	ΔLVEDVI	ΔLVEF
Peptide neurohormones			
NTproBNP†	+0.30**	+0.29**	-0.16
BNP†	+0.22**	+0.15	-0.21*
Noradrenaline	+0.05	+0.02	-0.09
AVP	+0.12	+0.14	-0.08
PRC	+0.23*	+0.15	-0.19
Aldosterone†	+0.21*	+0.19	-0.21*
Collagen matrix markers			
MMP-2	+0.03	+0.05	-0.08
MMP-3	+0.18	+0.19	-0.12
MMP-9	-0.09	-0.06	+0.09
TIMP-1	-0.06	-0.04	+0.06
TIMP-2	+0.26*	+0.22*	-0.13
TIMP-4	+0.34**	+0.36**	-0.09
Haemostatic markers			
tPA antigen	+0.38**	+0.32**	-0.23*
vWF	+0.27**	+0.19	-0.28**
Cytokines			
Eotaxin	-0.26*	-0.12	+0.27**
RANTES	-0.13	-0.18	+0.09
IL-1Ra	-0.03	-0.01	+0.08
IL-2R	-0.08	-0.09	+0.05
IL-4	-0.04	-0.05	+0.02
IL-5	-0.09	-0.09	+0.05
IL-6	+0.09	+0.12	+0.05
IL-7	-0.23*	-0.17	+0.23*
IL-8	+0.05	+0.09	-0.03
IL-10	+0.06	+0.04	-0.08
IL-12p40	-0.06	-0.06	+0.05
IL-15	-0.03	-0.02	+0.03
IP-10	-0.08	-0.08	+0.06
MIG	-0.10	-0.11	+0.11
MIP-1α	-0.07	-0.07	+0.05
MIP-1β	-0.09	-0.09	+0.06
MCP	-0.17	-0.12	+0.15
TNFα	-0.04	-0.05	+0.02

Table 4.8 Pearson's correlation co-efficients for each sampled biomarker at baseline with change in (Δ) LV volumes and LVEF from baseline to 24 weeks across the entire study cohort (n=100). *p<0.05 **p<0.01 † log transformed

Correlation co-efficients were computed for all biomarkers sampled at baseline with serial change in LV volumes and LVEF from baseline to 24 weeks, and are shown in

Table 4.8. Non-normally distributed biomarker concentrations including BNP and NTproBNP were log-transformed prior to parametric correlation.

Increased baseline concentrations of NTproBNP, BNP, PRC, aldosterone, TIMP-2, TIMP-4, tPA antigen and vWF, and decreased concentrations of eotaxin and IL-7, were significantly associated with increasing LVESVI over time. Increased BNP, aldosterone, tPA antigen and vWF, and decreased eotaxin and IL-7 at baseline, correlated with worsening LVEF over time.

Multivariable analysis of biomarkers in LV remodelling

A multivariable model adjusted for clinical predictors of LVESVI at 24 weeks, and fitted with those baseline biomarkers selected as univariate predictors of ΔLVESVI, was constructed. Significant clinical predictors of LVESVI at 24 weeks were chosen by inserting all baseline clinical variables listed in Table 3.1 into a stepwise selection model; this selected the following baseline variables as independent predictors of LVESVI at 24 weeks: baseline LVESVI and infarct volume, prior MI, age, smoking status, thrombolysis, dyslipidaemia, serum urea, and ACE inhibitor/ARB prescription on discharge.

The multivariable analysis of a model fitted with these clinical variables and the baseline biomarkers selected as univariate predictors of LV remodelling revealed that age, baseline LVESVI, infarct volume (all $p<0.001$), thrombolysis ($p=0.007$) and dyslipidaemia ($p=0.009$) were all independent predictors of LVESVI at 24 weeks. Of the selected biomarkers, only TIMP-4 and tPA antigen remained in the model as

significant independent predictors of LVESVI at 24 weeks, and thus LV remodelling (Table 4.9).

Variable	Multivariable β co-efficient	p
Clinical		
Age	0.34	<0.001
Smoker	-0.003	0.96
LVESVI	0.69	<0.001
Infarct Volume	0.39	<0.001
Thrombolysed	-6.2	0.007
Prior MI	0.09	0.08
ACE inhibitor/ARB	-0.07	0.24
Hypercholesterolaemia	-6.8	0.009
Urea	0.09	0.14
Serological		
Log_{10} NTproBNP	-0.02	0.80
Log_{10} BNP	-0.02	0.81
PRC	0.05	0.44
Log_{10} aldosterone	0.03	0.64
TIMP-2	-0.09	0.21
TIMP-4	0.07	0.028
tPA antigen	0.78	0.019
vWF	0.006	0.92
Eotaxin	-0.09	0.11
IL-7	-0.1	0.075

Table 4.9 Multivariable predictors of LVESVI at 24 weeks, separated into clinical and serological categories.

4.5 Discussion

4.5.1 Prediction of LV function after AMI

LVEF is a powerful predictor of survival after AMI. Even a single measurement of LVEF in patients who have sustained AMI predicts major cardiovascular outcomes including development of CHF, recurrent AMI and death.[22-27] Moreover, persistence of a depressed LVEF for >40 days following AMI is now an indication for consideration of implantable cardioverter defibrillator therapy (ICD).[124, 125] Selection of patients who would potentially qualify for ICD insertion due to persistent LVSD after AMI is difficult in practice, due to a number of factors. These include the lack of robust schemes for serial cardiac imaging after AMI to re-quantify LV function, marked inter-modality differences in LVEF measurement, difficulties in predicting at baseline the patients in whom LVEF will improve and those in whom it will fail to improve and/or progressively deteriorate and the lack of biomarkers that closely track serial change in LV function over time. Our study design provided serial follow-up data regarding LV function following AMI, but additionally a number of circulating biomarkers of relevance to the pathophysiology of post-infarction LV remodelling were sampled. In addition to analysing the effects of eplerenone on serial change in each biomarker, I also examined the significance and potential use of a single measurement of each biomarker early after AMI in the prediction of medium-term LV function.

BNP and NTproBNP

BNP and NTproBNP are powerful prognostic markers after AMI.[40, 152] Plasma concentrations of both natriuretic peptides were highly elevated at baseline across the

entire study population and fell significantly over time (although the mean concentration of each remained above the normal range even at 24 weeks). NTproBNP had weak correlations with baseline and 24-week LVESVI and infarct volumes and inverse correlations with LVEF at these time-points; it also correlated with 24-week LVEDVI and ΔLVESVI. BNP displayed a weak inverse correlation with 24-week LVEF and ΔLVEF but not with any measure of LV volume; it did correlate weakly with baseline and 24-week infarct volume. Neither BNP nor NTproBNP were independent predictors of ΔLVESVI in a model fitted with clinical variables of statistical importance to LV remodelling.

These findings agree with previous studies of natriuretic peptides and remodelling. While both BNP and NTproBNP are strong independent predictors of mortality in acute coronary syndromes (NTproBNP more so than even LVEF), their relationship with LV function and serial change therein after AMI is very inconsistent.[152] This may relate in part to the timing of neurohormone sampling. In a long-term follow-up CMR study, serial NTproBNP levels sampled at a mean 3.3 days after AMI (similar to our sampling interval) then again at 1 month, 1 year and >4 years were correlated with LV volumes and LVEF at least 4 years remote from the infarct.[157] Plasma NTproBNP at 3.3 days showed a weak correlation with chronic LVEF only (not with LV volumes), although much stronger correlations were noted between the NTproBNP samples at the 3 later sampling time-points and chronic LV volumes and LVEF. Similarly, serial measurements of plasma BNP over the first 30 days after AMI in a small study (n=30) revealed modest correlations only between day 2 BNP and ΔLVESVI, ΔLVEDVI and ΔLVEF over one month but much stronger correlations between BNP measurements sampled at later time-points in the first

month (strongest at day 7) and these parameters.[156] Significant correlations between BNP and infarct size but not with LV volumes or LVEF were seen in a nuclear imaging study of 54 patients with either recent (<2 month) or remote (>6 month) AMI.[153]

It is conceivable that the strength of the relationship between natriuretic peptides and LV structure and function is somewhat reduced in the first few days after AMI, and that delaying the timing of sampling to at least day 7 might increase the accuracy of the natriuretic peptides in predicting remodelling, although this hypothesis is speculative. It is noteworthy, however, that NTproBNP correlates more powerfully with LVEF in patients with stable or unstable angina than in those with AMI, with the suggestion that the process of infarction blunts the relationship between LVEF and NTproBNP by influencing the production and release of natriuretic peptides.[158]

Eplerenone had no significant effect on BNP or NTproBNP over time in our study despite covariate adjustment. Interestingly, despite the significant difference in baseline LVEF between treatment groups, baseline natriuretic peptides were well-matched which again suggests a dissociation between these peptides and LV function early after AMI. Infarct volume was well-matched between treatment groups and both natriuretic peptides correlated with this parameter at baseline and 24 weeks. That the effect of eplerenone on remodelling was at best modest overall, and that the relationships between the natriuretic peptides and parameters of LV function were weak, it is perhaps unsurprising that we did not demonstrate a treatment effect of eplerenone on either BNP or NTproBNP. Consistent with these findings, ramipril

significantly attenuated LV remodelling after AMI in the HEART trial but in the neurohormonal sub-study it had no effect on BNP.[40]

Noradrenaline

Plasma concentrations of NA at baseline were within the upper limits of normal, and fell significantly over the 24-week study period. Inverse correlations were observed between baseline NA and baseline and 24 week LVEF but no relationship with LV volumes or change in any LV functional parameter over time was seen.

NA is an important effector molecule of the sympathetic nervous system, and circulating levels act as a marker of sympathetic activation. Experimental evidence suggests that NA may play an important role in triggering and potentiating remodelling after AMI through direct toxic effects on myocytes, activation of the RAAS and stimulation of pro-inflammatory cytokines and natriuretic peptides.[40, 41, 43, 159] We sampled *circulating* NA only, and only at one time-point in the early post-infarction period, thus we have no data on local tissue NA nor on any fluctuations in circulating NA that may have occurred prior to the first sample (mean day 3.7). Nonetheless, that NA was shown to correlate with lower LVEF both acutely and remote from the infarct does provide a weak link between circulating NA and LV function, although there was no association between NA and change in any parameter of LV function, and it had no predictive effect on remodelling on multivariable analysis.

Eplerenone had no effect on plasma NA. As NA was not significantly elevated at baseline sampling, and as it does not track serial change in LV volumes or LVEF, we

would not expect to observe a treatment effect of eplerenone on plasma concentrations of this neurohormone. Similarly, ramipril failed to influence circulating NA despite attenuating remodelling in the HEART neurohormonal sub-study.[40]

AVP

AVP levels remained within the normal range but declined over the course of the study. In keeping with prior studies I found no association between AVP and LV function.[166, 167] Eplerenone had no effect on plasma AVP concentration.

Marked increases in plasma AVP have been documented within 6 hours of AMI which then decrease over time (irrespective of progression to heart failure).[163-165] The timecourse of activation of AVP appears to be very variable following AMI, however, with no relationship to activation of any other neurohormonal system (including NA, renin and atrial natriuretic peptide [ANP]) when sampled pre-discharge in patients with post-infarction LVSD.[20] Suggestions that persistent activation of AVP in such patients who do not display clinical heart failure following AMI (akin to our study cohort) may indicate those at higher risk of developing clinical complications including heart failure have never been supported by any robust evidence and remain speculative. The novel biomarker copeptin (the C-terminal component of the AVP pre-cursor) is believed to be more stable than AVP *ex vivo*, and this surrogate marker of AVP release may well supersede AVP assay in time, but on the strength of our data using AVP I found no relationship between AVP and LV remodelling.[287]

MMPs and TIMPs

Each MMP and TIMP was only measured at one time-point (mean 3.7 days) in the early post-infarction period. I observed a number of temporal trends in MMP and TIMP activation which will be discussed separately. Each biomarker was well-matched at baseline between treatment groups and was unaffected by eplerenone over time. Following covariate-adjustment, however, eplerenone was found to attenuate significantly the increase in MMP-2 and to limit the decrease in MMP-9 seen in the placebo group.

In the study cohort, MMP-2 concentration was elevated throughout the 24 weeks, with no significant change over time. Baseline MMP-2 did not have any correlation with any LV parameter or change in LV volumes/LVEF and did not predict ΔLVESVI. A number of studies of ventricular function and MMP:TIMP balance after AMI have provided variable results regarding the prognostic and functional significance of MMP-2 in the early post-infarction phase. Consistent with our findings, elevated MMP-2 concentrations out to 96 hours after AMI, bearing no relationship to LV volumes or LVEF, were reported in 91 patients admitted with STEMI (59% thrombolysed) with resultant LVSD.[181] Smaller studies, however, have reported inverse correlations between very early MMP-2 and LV volumes, while MMP-2 measured 14 days after infarction has been shown to predict progressive ventricular dilatation.[179, 288, 289] MMP-2 may therefore have a biphasic role, protective in the first few days, during early remodelling, but detrimental if it remains high over the ensuing weeks and months. Consistent with this MMP-2 correlated inversely in a previous study with NTproBNP in the first few days after AMI but this relationship was lost by 30 days.[180] Persistent MMP-2 activation therefore appears to have adverse

consequences for ventricular function following AMI; although I found no relationships between MMP-2 and LV volumes/LVEF, I have shown that eplerenone reduces this biomarker over 24 weeks, albeit after covariate-adjustment.

MMP-9 concentrations were highly elevated throughout the study although they decreased significantly over time. Baseline MMP-9 correlated with baseline and 24-week infarct volume but not with any other LV parameter and did not predict remodelling. MMP-9 has been extensively investigated in man and has a very interesting early activation temporal profile following AMI. Peak circulating MMP-9 occurs within 12 hours of infarction but then falls rapidly to a plateau phase, which persists for several months, during which MMP-9 concentration remains higher than normal controls.[181] A secondary peak at c.96 hours was reported by one group but this has not been consistently demonstrated.[179] Peak MMP-9 correlates with lower LVEF at baseline and greater remodelling over a mean 6 month period, but interestingly higher plateau MMP-9 concentrations appear protective, predicting higher LVEF and less change in LV volumes over time.[181] In keeping with this, peak but not plateau MMP-9 correlates with NTproBNP and predicts the occurrence of late-onset CHF after AMI.[180, 182]

The timing of our baseline MMP-9 sample most likely coincides with the plateau phase. I found that this relates to higher infarct volumes at baseline and 24 weeks, findings that are novel. I postulate that increased MMP-9 activity at a mean 3.7 days after AMI is related to wound healing. In view of the lack of correlation with LV volumes/LVEF it is difficult to determine whether this elevated MMP-9 activity represents a reparative response to a large infarct or a proteolytic consequence of

extensive myocardial damage leading to destabilisation. MMP-9 null mice experience less early LV rupture and late remodelling after experimental AMI but many findings from rodent models of MMP:TIMP activation have failed to find parallels in human studies.[177, 178] That eplerenone, which exerted an albeit modest anti-remodelling effect on this population, reduced the fall in MMP-9 over time relative to placebo suggests that elevated MMP-9 in this (prolonged) plateau phase is beneficial, although in the absence of any relationship with serial LV volume/LVEF change this view is speculative.

MMP-3 was modestly elevated at each time-point but did not vary significantly over time. Baseline MMP-3 correlated inversely with LVEF and positively with infarct volume at baseline and 24 weeks, and additionally correlated with LVESVI and LVEDVI at the latter time-point although it did not predict ΔLVESVI or change in any other LV parameter. MMP-3 specifically targets a number of key ECM proteins including aggrecan, fibronectin and several fibrillar collagens (mainly type III), and has also been demonstrated to activate several pro-MMPs (including pro-MMP-1) in experimental *in vitro* studies, yet it has been less extensively studied in humans than the gelatinases.[174, 290, 291] A recent TTE study of 382 patients admitted with AMI with resultant low LVEF, followed up over a mean of 5 months, has revealed several insights into the activity profile of MMP-3.[188] MMP-3 concentrations progressively increase between AMI and hospital discharge but fall to normal levels by 5 months. The authors found that the peak pre-discharge MMP-3 concentration had no relationship with baseline LV volumes or LVEF, but did have weak but significant correlations with increasing LV volumes and decreasing LVEF at follow-up. They also reported a predictive effect of peak pre-discharge MMP-3 on LV remodelling.

Complementary to these data, and using a much more reproducible and accurate imaging modality, I have found that elevated MMP-3 concentration on a single sample measured at a mean 3.7 days after AMI does have a significant relationship to lower baseline LVEF and, as our imaging technique allows measurement of infarct volumes, I have for the first time shown a relationship between increased MMP-3 and higher infarct volume both acutely and at 24 weeks. In keeping with prior data, I again found that baseline MMP-3 predicts greater LV volumes and lower LVEF at 24 weeks. That MMP-3 did not have any effect on serial change in LV parameters (including ΔLVESVI) might reflect the aggressive invasive and pharmacologic management of this study population (which effectively abolished remodelling across the cohort), the smaller number of patients in this study, or the fact that I used a solitary pre-discharge MMP-3 measurement rather than peak pre-discharge MMP-3; these may also explain the lack of treatment effect of eplerenone. Nevertheless I have shown that a single pre-discharge MMP-3 measurement does have a predictive effect on medium-term LV volumes, LVEF and infarct volume, but not remodelling *per se*.

The role of TIMPs in humans after AMI has been less thoroughly investigated than that of the MMPs. I found that, in comparison to a reference control population, plasma TIMP-1 concentration was elevated throughout but fell significantly over time, TIMP-2 concentration was elevated throughout and increased significantly over time, while TIMP-4 concentration was elevated throughout and increased non-significantly between baseline and 24 weeks. TIMP-1 had no relationship with LV volumes or LVEF and did not predict remodelling. In contrast, baseline measures of TIMP-2 and TIMP-4 correlated with several LV parameters. Increased baseline TIMP-2 correlated with greater baseline LVEDVI, greater 24-week LVESVI and LVEDVI, and higher

ΔLVESVI and ΔLVEDVI. Baseline TIMP-4 correlated with baseline LVESVI, 24-week LVESVI, LVEDVI and (inversely) LVEF in addition to greater ΔLVESVI and ΔLVEDVI. In the multivariable model fitted with clinical variables of importance to remodelling, TIMP-4 but not TIMP-2 remained as an independent predictor of ΔLVESVI. Eplerenone had no effect on any TIMP.

Our findings of a persistently elevated TIMP-1 over 24 weeks with no relationship to LV volumes or LVEF are consistent with prior human studies.[288, 292] TIMP-1 deficiency amplifies adverse remodelling following experimental MI in mice.[183] Its precise role in human ventricular remodelling remains unclear, although a correlation between TIMP-1 and NTproBNP has previously been reported when both biomarkers were simultaneously sampled 30 days after AMI.[180]

I observed that TIMP-2 levels increased over time. A smaller study of 32 patients with AMI found identical temporal trends in circulating TIMP-2 concentrations but no relationship with any parameter of LV function.[292] TIMP-4 levels were found to be reduced in that study throughout the first 6 months and to have no relationship to LV structure or function. In contrast I found that TIMP-4 was mildly increased throughout our study. No groups have found any relationship between either TIMP-2 or TIMP-4 and LV volumes, LVEF or remodelling after AMI in humans. I present for the first time data that suggest that a single measure of TIMP-2 or TIMP-4 early after AMI can predict LV volumes and, in the case of TIMP-4, LVEF at 24 weeks, and moreover that a single measure of TIMP-4 early after AMI independently predicts LV remodelling.

The mechanism of this predictive effect is unclear. That MMPs and TIMPs have very variable temporal profiles of release and activity particularly in the first few days after AMI is well documented. I cannot comment on circulating concentrations of any of the biomarkers in our study during the interval between infarction and first sampling which was at a mean 3.7 days after AMI. However, I propose that elevated concentrations of TIMP-2 and TIMP-4 at the time of first sampling may inactivate certain MMPs which have protective properties towards the integrity of the ECM at that time-period, rather than switching off aggressively proteolytic MMPs, hence the associations with adverse ventricular function over time.

Cytokines

Several families of cytokines were analysed in this study. Only two of the sampled cytokines had any relationship to remodelling. Eotaxin and IL-7, when measured at baseline, correlated with ΔLVEF and inversely with ΔLVESVI suggesting an association between these two cytokines and improved LV function over time after AMI. Eplerenone had no effect on either cytokine.

Eotaxin (CCL11) is an eosinophil-specific chemokine produced by a variety of cell types and acts as a potent eosinophil chemoattractant.[293] The eotaxin receptor (CCR3) has been identified on a number of cell types other than eosinophils including macrophages, mast cells and neutrophils, ie. cells that are involved to varying extents in post-infarction remodelling.[294] The observation that circulating eotaxin concentrations were higher in patients with chronic stable angina admitted for elective PCI compared to control patients, and increased transiently after PCI, led to further studies to investigate the potential significance of eotaxin as a vascular risk factor.[295]

The results of such studies have been inconsistent to date. No difference in circulating eotaxin was found between patients with or without atherosclerosis or prior MI, and eotaxin had no relationship to severity of coronary artery disease at angiography.[296, 297] However, a large population study (n=1014) of patients with known CHD showed that a low level of circulating eotaxin is an independent predictor of future cardiovascular events.[298] Our findings complement this last study, as lower eotaxin concentrations early after AMI related to greater remodelling and lower LVEF over time in our patients, although the predictive efficacy of the chemokine was lost on multivariable analysis.

IL-7 is predominantly a haemopoetic factor necessary for the production and release of various cells of the immune response, including B- and T-cells and natural killer cells. There are very few published data on IL-7 concentrations after AMI in man. A study of patients with angina (stable or unstable) showed higher plasma IL-7 levels in both angina sub-groups compared to normal controls, and further reported on associations between IL-7 and other pro-inflammatory cytokines suggesting that elevated IL-7 levels were deleterious and promoted atherosclerotic plaque (and thus clinical) instability.[299] Our findings are at odds with this, as high IL-7 levels predicted better functional recovery of the LV (albeit we measured this cytokine in a different patient population), although this predictive value was lost on multivariable analysis.

The significance of our findings regarding IL-7, and indeed eotaxin, is unclear, particularly when the following are taken into consideration. I sampled plasma cytokines, and thus have no information on local (tissue) effects of each cytokine class. Also, venepuncture was performed once only in the early post-infarction period,

and by the mean time of sampling (3.7 days), the early phase of cytokine stimulation (which occurs mainly in the first 24-48 hours after infarction) would have passed. This may explain our rather disappointing yield from the analysis of multiple classes of pro- and anti-inflammatory cytokines. Further study in the field of cytokine activation after AMI should focus on very early initial measurement and serial sampling in the first few post-infarction days.

Haemostatic biomarkers

Across the study population, vWF was elevated at baseline tPA antigen (a surrogate for the tPA-PAI-1 complex) just within the normal range; both decreased significantly over time. Baseline concentrations of both biomarkers correlated with serial change in LV structure and function. vWF correlated with ΔLVESVI and inversely with ΔLVEF while tPA antigen had significant correlations with both of these parameters and with ΔLVEDVI. Moreover, on multivariable analysis tPA antigen remained in the model as an independent predictor of ΔLVESVI.

tPA is produced predominantly by vascular endothelial cells and promotes fibrinolysis via the conversion of plasminogen to plasmin, but as described in Chapter 1 (1.10), several studies have confirmed that tPA antigen acts mainly as a marker of the tPA-PAI-1 complex *in vivo*, and therefore acts as a surrogate marker of plasma PAI-1 activity (and thus reduced fibrinolytic activity) rather than free tPA.[217, 218, 222]

I have shown that tPA antigen concentration is associated with increasing LV volume after AMI in this study. A number of studies have shown strong associations between tPA antigen levels and adverse cardiovascular outcomes across the spectrum of

atherosclerosis.[217, 218, 221] Epidemiologic data suggest a link between plasma tPA antigen concentrations and the development of CHD.[217] In patients with angiographically-confirmed CHD and established angina pectoris, tPA antigen concentrations predict the 2-year occurrence of acute coronary syndromes.[218] Following STEMI, tPA antigen predicts 10-year cardiovascular mortality; PAI-1 is a strong independent predictor of 30-day mortality in this setting.[220, 221] Plasma PAI-1 concentrations also predict 30-day occurrence of heart failure after STEMI, correlate (weakly) with decreasing LVEF, and PAI-1 knockout mice display less myocardial fibrosis (a key component of remodelling) after experimental AMI.[220, 224] To the best of my knowledge, no associations have been published previously between tPA antigen concentrations and serial LV volume change after AMI in man.

Circulating vWF is recognised as a plasma index of endothelial damage and dysfunction.[300] Consistent with this I found that circulating vWF concentrations were elevated at baseline but fell back to within the normal range over time. vWF has previously been shown to predict development of CHD, and the occurrence of heart failure 30 days after STEMI (although less strongly than tPA antigen), and independently predicts 30-day death, re-infarction and revascularisation following non-ST-elevation ACS.[217, 219, 220] I found a relationship between baseline vWF concentration and LVESVI at 24 weeks on univariate but not multivariate analysis; previous groups have also failed to demonstrate a consistent relationship between vWF and LV structure and function after AMI.[220]

An association has recently been found between mineralocorticoid receptor activation by aldosterone and production of a variety of haemostatic factors in cultured human

vascular endothelial cells, suggesting a possible link between the RAAS and the coagulation-fibrinolysis system.[301] My findings did not provide any further evidence towards such a link as eplerenone had no effect on either haemostatic biomarker over time.

In summary, I have shown for the first time that tPA antigen, as a surrogate marker of plasma PAI-1 activity, predicts LV volumes and LVEF 24 weeks after AMI in patients with LVSD but neither heart failure nor diabetes mellitus. Further studies should focus on mechanistic links between the coagulation-fibrinolysis cascade and systems of pathophysiological importance to the process of LV remodelling, in particular MMP:TIMP balance as plasmin has been shown to activate pro-MMPs; these two systems may therefore be linked following AMI.

4.5.2 Limitations

As outlined in Chapter 3 (3.6.4), the major limitation of this study is the dependence of the results on covariate adjustment, made necessary by the significant baseline imbalances in LV volumes, mass and function between the treatment groups.

I designed the study to analyse the effects of eplerenone on structural and functional LV change, assessed by serial LGE-CMR, and on serial change in a number of plasma biomarkers. However, each biomarker was sampled at only one time-point at baseline; during the interval from infarct to venepuncture (mean 3.7 days), the process of 'early' remodelling (which occupies the first 72 hours after infarction) would have ceased and 'late' remodelling would have begun. Plasma concentrations of several of the sampled biomarkers fluctuate, particularly in the first few days after AMI. I cannot

comment on such fluctuations, nor indeed on plasma concentrations of each biomarker in the very early post-infarction phase.

Regarding the biomarker sub-studies, it is also relevant that I measured plasma concentrations of each biomarker only, and thus have no data on local tissue activity. However, one of our aims was to determine, for potential use on a practical (ward-based) level, whether a single measure of any of the plasma biomarkers could predict LV functional recovery post-MI thus local tissue activity was beyond the scope of this thesis.

It is noteworthy that very little adverse remodelling occurred over the course of the study in this well-treated cohort (LVESVI did not change significantly over 24 weeks across the cohort as a whole), thereby reducing the likelihood of detecting strong correlations between biomarkers and change in LV volumes. In the primary study (Chapter 3) eplerenone had no effect on LV remodelling prior to covariate adjustment, hence it is perhaps unsurprising that it failed to influence any of the biomarkers (other than plasma aldosterone) prior to covariate-adjustment in this study.

Finally, I have reported correlations between measured biomarkers and LGE-CMR parameters; such correlations do not imply any definite biologic interaction but are hypothesis-generating. This must be borne in mind when interpreting the results of this study.

4.5.3 Conclusions

Eplerenone had no effect on any measured biomarker other than plasma aldosterone prior to statistical adjustment. A covariate-adjusted treatment effect of eplerenone was demonstrated on two of the MMPs known as the gelatinases, which have gained popularity as key enzymes in ECM turnover and matrix remodelling. Specifically, eplerenone decreased MMP-2 and attenuated the drop in MMP-9 that occurred in the placebo group. Both of these treatment effects are theoretically beneficial and provide support for an anti-remodelling effect of eplerenone.

In the prediction of LV functional recovery following AMI, I found that, based on a single sample taken on (mean) day 3 after AMI, an elevated plasma BNP, NTproBNP, TIMP-2, TIMP-4, tPA antigen and vWF, and a reduced plasma eotaxin and IL-7, correlated with poorer LV function over time. Furthermore, I found that TIMP-4 and tPA antigen were independent predictors of LV remodelling, findings that are novel and may provide further pathophysiological insights into the mechanisms that underlie this process.

Chapter 5

A study of the role of aldosterone in post-infarction remodelling

5.1 Introduction

The mineralocorticoid hormone aldosterone, synthesised in the zona glomerulosa of the adrenal cortex, exerts a number of adverse effects on the cardiovascular system as described in Chapter 1. Aldosterone also possesses pro-fibrotic properties, and is strongly implicated in the development of myocardial fibrosis, a key component of the pathophysiological process of post-infarction remodelling. Such pro-fibrotic effects occur only in the presence of a high-salt environment in animal studies, although equivalent data from human studies are lacking.[103]

Through a variety of mechanisms aldosterone production is upregulated early after AMI, and aldosterone levels correlate directly with mortality in heart failure.[95] Significant reductions in cardiovascular morbidity and mortality have been confirmed with the use of aldosterone antagonists in patients with advanced CHF (RALES), and in survivors of AMI with LVEF <40% and heart failure or diabetes mellitus (EPHESUS).[121, 123] These data might suggest that aldosterone antagonism should have an anti-remodelling effect, but this had not been demonstrated convincingly when I began my research project.

The aim of this study was to describe in detail the role of aldosterone in post-infarction remodelling. This was performed by examining not just circulating aldosterone concentrations following AMI, but additionally using urinary steroid metabolite excretion rates, and genetic markers. As described in Chapter 1.5, mutations in the gene that encodes *aldosterone synthase* significantly influence aldosterone excretion rates – I therefore assessed the influence of a common mutation in *aldosterone synthase* on plasma aldosterone concentrations and remodelling

outcomes following AMI. I also examined the potential interactions between circulating aldosterone, urinary sodium excretion (a marker of total body sodium state) and remodelling in order to determine whether the pro-fibrotic/pro-remodelling effects of aldosterone were influenced by salt-status in humans.

5.2 Methods

5.2.1 Study patients and trial protocol

All 100 patients enrolled in the primary study of this thesis (Chapter 3) were included in this study examining the role of aldosterone in post-infarction remodelling. The screening, recruitment, randomisation process and trial outline are described in detail in Chapter 2 (2.1 – 2.3), and baseline demographic data of the study cohort are shown in Table 3.1.

Plasma aldosterone and renin concentrations were measured at baseline (mean 3.7 [SD 1.8] days after AMI) and again at 12 and 24 weeks. Detailed methods of collection, storage and analysis are described in Chapter 2.8.3.

24-hour urinary collections were performed at baseline (prior to randomisation to placebo or eplerenone) and again at 12 weeks; urinary sodium concentration was multiplied by total volume of urine produced over 24 hours to provide a measurement of 24-hour urinary sodium excretion; a 20ml aliquot was withdrawn from each 24-hour urine collection and stored for later measurement of urinary steroid metabolite excretion rates, as described in Chapter 2.8.9.

A whole blood sample taken at trial recruitment was used for genetic analysis, as described in Chapter 2.8.10.

LGE-CMR imaging was performed at baseline (pre-randomisation), 12 and 24 weeks; the methods of imaging and analysis are described in detail in Chapter 2 (2.5 – 2.6).

5.2.2 Statistical methods

Biomarkers exhibiting logarithmic-normal distribution were log-transformed prior to parametric correlation. Bivariate correlations between aldosterone and LGE-CMR parameters were examined across the entire study cohort, and then by treatment group. Inter-group comparisons were made using paired sample *t*-tests or Mann-Whitney-U tests as appropriate for continuous variables and Chi-squared test for categorical variables. Paired *t*-tests were used to detect changes in LGE-CMR measurements and biomarkers within each treatment group over the 24 weeks of the study, and differences between these changes were analysed using an unpaired *t* test. A probability value of $p<0.05$ was considered significant.

***Urinary steroid analysis*:**

Prior to analysing the predictive effect of urinary steroid sub-groups on serial change in LV parameters, bivariate correlation was undertaken with all sub-groups inserted into the model, producing Pearson correlation co-efficients. Paired sub-groups that were highly correlated were analysed in separate regression equations. Multicollinearity was assessed using variance inflation factors (VIF) for each independent variable in each regression equation; if two variables had VIF >10 it was

deemed that the variance of each of the two variables had been inappropriately increased by the other due to collinearity, and such variables were analysed separately.

The treatment effect of eplerenone on urinary steroid sub-groups, and the influence of this effect on serial change in LV parameters and infarct volume, was examined using interaction quotients. For each steroid sub-group the baseline and 12 week values were multiplied by either '0' if on placebo or '1' if on eplerenone. In this way interaction quotients were created for each steroid sub-group at baseline and 12 weeks, and were then used in linear regression analysis equations to examine their influence on serial change in LVESVI, LVEDVI, LVEF, LVMI and infarct volume. Interaction quotients were also created in this manner to investigate the influence of urinary sodium excretion on the effects of eplerenone on LV parameters.

***Genetic analysis of SNPs in aldosterone synthase gene*:**

The distribution of allelic frequencies within the study population was analysed for Hardy-Weinberg equilibrium using an online Hardy-Weinberg calculator (www.oege.org/software/hardy-weinberg.shtml). Inter-genotypic comparison of LV parameters, infarct volume and plasma aldosterone was performed using one-way ANOVA. The effect of the -344T/C allele status on LVESVI, LVEDVI, LVEF, LVMI and infarct volume was examined with multiple linear regression analysis after adjustment for the following common clinical variables: age, sex, hypertension history, smoking status and previous MI.

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) under the guidance of Professor Ian Ford.

5.3 Aldosterone in post-infarction LV remodelling

Aldosterone and its potential role in LV remodelling was examined using three separate indices: plasma concentration, urinary metabolite excretion rates and genetic markers. Each will be described separately.

5.3.1 Plasma studies

Across the entire study cohort (n=100), PRC and aldosterone were sampled at baseline, 12 and 24 weeks. PRC was elevated at baseline and plasma concentrations rose significantly over time (Figure 5.1 A). Plasma aldosterone concentration was within normal (supine) limits at baseline and although it did not change significantly, there was a trend towards an increase over time (Figure 5.1 B).

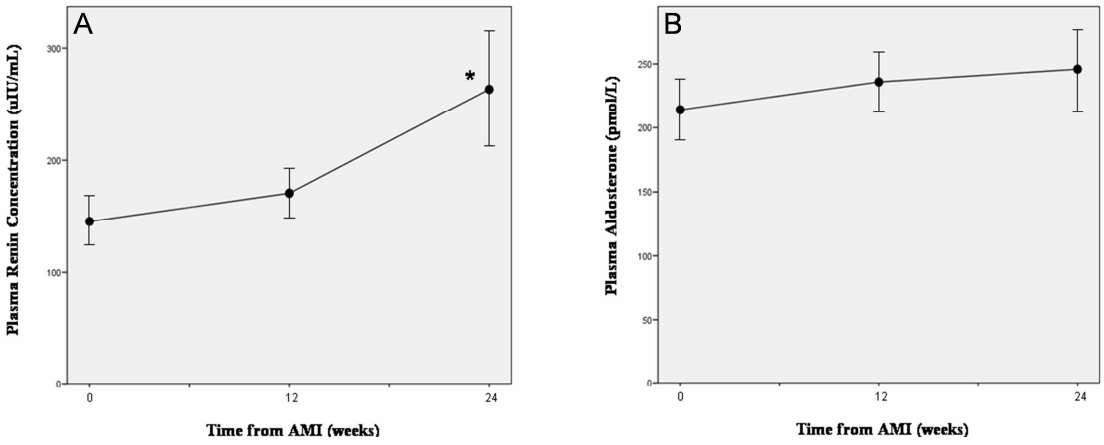

Figure 5.1 Mean plasma renin concentration (A) and aldosterone (B) at baseline, 12 and 24 weeks for the entire study cohort. Data are expressed as mean (SEM). * p=0.011 for difference between baseline and 24 week PRC; there was no significant change in plasma aldosterone over the 24 week follow-up.

Baseline aldosterone concentrations were non-normally distributed and were thus log transformed. Correlations between logarithmic baseline aldosterone concentration and LV parameters at baseline, 24 weeks and change in these parameters between baseline

and 24 weeks are shown in Table 5.1. Baseline aldosterone concentration had weak but significant correlations with parameters of deteriorating LV function over time. Baseline log-aldosterone correlated with greater LVESVI, larger infarct volume and lower LVEF at baseline and 24 weeks, and also with greater LVEDVI at 24 weeks although not at baseline. Baseline log-aldosterone also correlated with a greater increase in LVESVI (ie. greater degree of LV remodelling) and a greater decrease in infarct volume between baseline and 24 weeks. The *change* in plasma aldosterone (Δ aldosterone) from baseline to 24 weeks, however, had a weak *inverse* correlation with ΔLVEDVI over the same time period (r= -0.23, p = 0.02) and a positive correlation with Δ infarct volume (r = 0.23, p = 0.03) but had no significant correlations with ΔLVESVI, ΔLVEF or ΔLVMI.

	Baseline	24 weeks	Δ 0-24 weeks
LVESVI	0.26**	0.32**	0.21*
LVEDVI	0.15	0.24*	0.20
LVEF	-0.31**	-0.31**	-0.10
LVMI	0.24*	0.16	-0.15
Infarct Volume	0.39**	0.37**	-0.43**

Table 5.1 Pearson correlation co-efficients for (logarithmic) baseline plasma aldosterone concentration with LGE-CMR parameters at baseline and 24 weeks across the entire study cohort. Δ 0-24 weeks represents the correlation between aldosterone and change in each ceCMR parameter between baseline and 24 weeks. *p<0.05 **p<0.01

Plasma aldosterone by treatment group

Mean (SD) baseline aldosterone concentration was not significantly different in the placebo group (in which baseline LV function was significantly poorer) than in the eplerenone group: placebo 242.7 (235.8) pmol/l vs. eplerenone 184.7 (149.0) pmol/l; p = 0.15.

Placebo group:

In placebo-treated patients, there was a trend towards a decrease in mean plasma aldosterone over time from 242.7 (235.8) pmol/l to 186.5 (168.2) pmol/l at 24 weeks, but this was not significant (p = 0.24) – Figure 4.4. Correlations between logarithmic baseline aldosterone and LV parameters by treatment group are shown in Table 5.2. Baseline aldosterone in the placebo group correlated significantly with baseline infarct volume but not with any baseline LV functional parameter. There was however significant positive correlation between baseline aldosterone and ΔLVESVI (ie. remodelling) and ΔLVEDVI.

In the placebo group Δaldosterone (baseline to 24 weeks) correlated inversely with ΔLVEDVI (r = -0.38, p = 0.008) but not with ΔLVESVI, ΔLVEF, ΔLVMI or Δinfarct volume.

Eplerenone group:

Eplerenone-treated patients experienced a significant increase in plasma aldosterone concentration over time, from 184.7 (149.0) pmol/l to 306.6 (266.0) pmol/l at 24 weeks, p = 0.006 (Figure 4.4). Although there was significant correlation between logarithmic baseline aldosterone and baseline LVESVI, LVEDVI, infarct volume and (inversely with) LVEF, there was no correlation between logarithmic baseline aldosterone and change in any LV functional parameter over time including ΔLVESVI (Table 5.2).

In the eplerenone group Δaldosterone (baseline to 24 weeks) did not correlate with change in any parameter (ΔLVESVI, ΔLVEDVI, ΔLVEF, ΔLVMI or Δinfarct volume) over time.

	PLACEBO GROUP			EPLERENONE GROUP		
	Baseline	24 weeks	Δ parameter from 0-24 weeks	Baseline	24 weeks	Δ parameter from 0-24 weeks
LVESVI (ml/m^2)	0.18	0.32*	0.29*	0.39**	0.35*	0.15
LVEDVI (ml/m^2)	0.08	0.24	0.31*	0.29*	0.18	-0.02
LVEF (%)	-0.26	-0.28	-0.11	-0.32*	-0.41**	-0.26
LVMI (g/m^2)	0.21	0.20	-0.11	0.25	0.07	-0.18
Infarct volume (ml/m^2)	0.32*	0.40**	-0.31*	0.60**	0.36*	-0.68**

Table 5.2 Pearson correlation co-efficients for (logarithmic) baseline plasma aldosterone concentration with ceCMR parameters at baseline and 24 weeks by treatment group. Correlation co-efficients are also displayed for (logarithmic) baseline plasma aldosterone and change in (Δ) each ceCMR parameter between 0 and 24 weeks. *p<0.05 **p<0.01

5.3.2 Urinary sodium excretion

24-hour urinary sodium excretion (expressed as median, interquartile range) was 61.8 (36.0 – 105.7) mmol at baseline and 127.9 (91.4 – 182.2) mmol at 12 weeks, p<0.001. There was no interaction between eplerenone therapy, urinary sodium excretion rate (baseline or 12 weeks) and any parameter of LV function. For the entire study cohort, division of baseline and 12 week 24-hour urinary sodium excretion rates into tertiles, and comparison of highest versus lowest 24-hour urinary sodium excretion rate revealed no significant inter-tertile difference in any parameter of LV function. Similarly, comparison of highest versus lowest 24-hour urinary sodium excretion rates within each treatment group revealed no significant inter-tertile difference in change

in any LV functional parameter. These analyses were limited, however, by very small patient numbers within each tertile.

Interaction between baseline plasma aldosterone, baseline 24-hour urinary sodium excretion and remodelling (ΔLVESVI) was assessed by creating an interaction coefficient, defined as the product of baseline plasma aldosterone and baseline 24-hour urinary sodium excretion. This interaction coefficient was then used in a linear regression analysis model for each treatment group, and also for the study cohort as a whole. No significant interactions were observed between these two quantities and ΔLVESVI in either treatment group, or in the entire study population.

5.3.3 Urinary steroid metabolites

At baseline (pre-randomisation) and at 12 weeks, 24-hour urinary collections were undertaken in all patients. The excretion rates of the following steroid metabolites were measured: tetrahydrodeoxycorticosterone (THDOC), tetrahydroaldosterone (THAldo), tetrahydrodeoxycortisol (THS) and total cortisol metabolites (Figure 1.2: biosynthetic pathway).

Across the entire study cohort, urinary excretion of THDOC did not vary significantly between baseline and 12 weeks ($p = 0.80$) but urinary excretion of THAldo, THS and total cortisol metabolites fell significantly over time (all $p<0.001$). There were no significant differences in any of these urinary steroids between treatment groups at either sampling time-point (Table 5.3). Of note, total cortisol metabolites were very highly elevated at baseline.

	BASELINE			12 WEEKS		
	Placebo (n=50)	Eplerenone (n=50)	p	Placebo (n=47)	Eplerenone (n=47)	p
THDOC (ug/24hr)	83.4 (97.0)	85.0 (127.1)	0.94	80.1 (71.8)	86.2 (96.4)	0.73
THAldo (ug/24hr)	131.6 (127.6)	117.9 (101.6)	0.56	71.1 (49.5)	71.7 (64.4)	0.96
Total cortisol metabolites (ug/24hr)	10867 (8698)	10133 (6659)	0.64	5414 (3367)	4913 (3147)	0.46
THS (ug/24hr)	93.6 (112.3)	96.7 (109.3)	0.89	63.1 (68.7)	62.7 (79.8)	0.98

Table 5.3 Urinary steroid metabolites according to treatment group, at baseline (ie. pre-randomisation) and after 12 weeks of therapy with either placebo or eplerenone. Data are presented as mean (SD). Inter-group comparisons were made using paired sample *t*-tests. 12-week urinary collections were available in 94 of the 100 patients at baseline.

In order to determine whether either (or both) aldosterone or cortisol are implicated in LV remodelling following AMI, the urinary steroids were divided into those produced predominantly during the biosynthesis of aldosterone (THAldo) and those produced during cortisol biosynthesis (THS and total cortisol metabolites). THDOC is not only an intermediate in aldosterone biosynthesis but also in cortisol biosynthesis thus its usefulness as a marker of remodelling in this analysis is ambiguous.

Baseline urinary steroids and serial change in LV function

Many of the baseline urinary steroids were highly correlated (Figure 5.2), particularly THS and THDOC ($r = 0.93$, $p<0.001$). Due to this high inter-correlation, inclusion of

all four groups of urinary steroids in a single regression equation (to determine their influence on serial change in LV volumes, mass and function) led to excessively high VIFs (>>10) as described in Chapter 5.2.2, which would reduce the accuracy of the regression analysis. As THS and THDOC were the most powerfully correlated, two separate regression equations were constructed for each LV parameter: THS, total cortisol metabolites and THAldo were analysed in one; THDOC, total cortisol metabolites and THAldo in the other (ie. THS and THDOC were analysed separately).

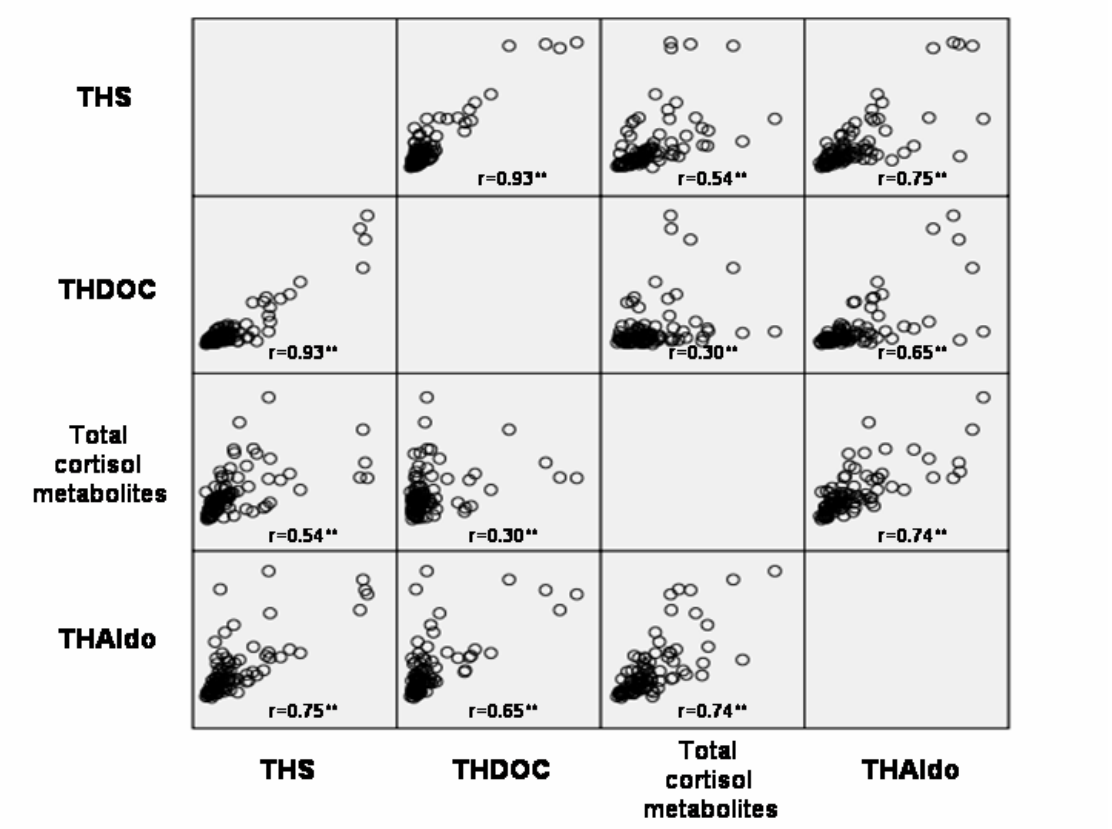

Figure 5.2 Inter-correlations between the urinary steroids at baseline. Pearson correlation coefficients are displayed for each interaction. ** p<0.01

The results of multiple paired linear regression analysis equations, constructed to determine the predictive value of baseline urinary steroids on 24-week LVESVI, LVEDVI, LVEF, LVMI and infarct volume are shown in Table 5.4. Although the value of the data is limited by the relatively small sample numbers involved (n=100),

certain trends are evident. None of the baseline urinary steroids had any predictive effect on 24-week LVESVI, LVEF or LVMI. However, decreased urinary THDOC and increased THAldo at baseline predicted increased 24-week LVEDVI, while increased THAldo also predicted greater 24-week infarct volume (irrespective of whether THS or THDOC was excluded from the model). These data provide some support for the plasma aldosterone findings, in which baseline plasma aldosterone also correlated significantly with 24-week LVEDVI and infarct volume (Table 5.1). The data additionally suggest that it is the aldosterone limb of the cholesterol metabolic pathway (Figure 1.2) rather than the cortisol limb that is related to serial change in LV structure (and infarct volume), as the only significant predictors of such change were THDOC and THAldo; THS and total cortisol metabolites had no predictive value.

	THS Excluded		THDOC Excluded	
	Multivariable β co-efficient	**p**	**Multivariable β co-efficient**	**p**
LVESVI				
THDOC	-0.03	0.16	-	-
THS	-	-	-0.02	0.43
Total cortisol metabolites	0.00	0.93	0.00	0.68
THAldo	0.04	0.11	0.03	0.24
	[Model R^2 = 0.55]		**[Model R^2 = 0.56]**	
LVEDVI				
THDOC	-0.04	0.05	-	-
THS	-	-	-0.04	0.13
Total cortisol metabolites	0.00	0.69	0.00	0.80
THAldo	0.06	0.04	0.05	0.09
	[Model R^2 = 0.54]		**[Model R^2 = 0.53]**	
LVEF				
THDOC	0.01	0.29	-	-
THS	-	-	0.01	0.66
Total cortisol metabolites	0.00	0.73	0.00	0.47
THAldo	-0.01	0.45	-0.01	0.74
	[Model R^2 = 0.34]		**[Model R^2 = 0.33]**	
LVMI				
THDOC	-0.01	0.27	-	-
THS	-	-	-0.01	0.68
Total cortisol metabolites	0.00	0.18	0.00	0.30
THAldo	0.02	0.15	0.02	0.32
	[Model R^2 = 0.59]		**[Model R^2 = 0.58]**	
Infarct Volume				
THDOC	-0.02	0.08	-	-
THS	-	-	-0.01	0.30
Total cortisol metabolites	0.00	0.24	0.00	0.50
THAldo	0.04	0.009	0.03	0.028
	[Model R^2 = 0.69]		**[Model R^2 = 0.68]**	

Table 5.4 Multivariable predictors of LV parameters and infarct volume at 24 weeks performed using linear regression analysis. Baseline urinary steroid metabolites were used as independent variables. Due to collinearity between THS and THDOC, these two steroid sub-groups could not be inserted into the same regression equation. Each regression equation was therefore computed separately using either THS or THDOC. The multiple correlation co-efficient for each regression equation is shown; all metabolites $\log_{10}$-transformed prior to analysis.

Effects of eplerenone on urinary steroid sub-groups

Eplerenone had no effect on serial change in any urinary steroid sub-group compared to placebo (Table 5.3). The treatment effect of eplerenone on urinary steroid sub-

groups was analysed using interaction quotients as described in the Statistical Methods section (5.2.2) There were no relationships between eplerenone therapy, urinary steroid sub-groups and serial change in LVESVI, LVEDVI, LVEF, LVMI or infarct volume. It is noteworthy that such analysis involved <50 patients per sub-group and was thus vastly under-powered to detect any significant treatment effect.

5.3.4 Genetic studies: influence of *aldosterone synthase* polymorphisms on plasma aldosterone and LV function:

Seven of the 100 whole blood samples obtained for genetic analysis at randomisation were destroyed due to storage issues outwith the control of the principal investigator. Thus stored whole blood samples were available for 93 patients in the study, from which DNA was successfully extracted. The allelic frequencies of the aldosterone synthase -344T/C SNP for these patients are shown in Box 5.1, and were in Hardy-Weinberg equilibrium (χ^2 0.14, p = 0.71).

All patients (n = 93):	
TT genotype	26 (28.0%)
CT genotype	48 (51.6%)
CC genotype	19 (20.4%)

Box 5.1 Allelic frequencies of -344T/C SNP

Baseline and 24-week LV parameters, infarct volume and plasma aldosterone sub-divided by genotype are displayed in Table 5.5. Although the absolute number of patients involved in this genetic sub-study was very small, some significant inter-genotypic differences in certain LV functional parameters were observed. Baseline LVEF was significantly higher in the presence of the TT genotype compared to the CC genotype (p = 0.040). At 24 weeks, LVESVI was significantly lower (p = 0.018)

and LVEF significantly higher (p = 0.007) in TT than CT or CC genotypes. There were no significant differences between CT and CC genotypes in any parameter listed in Table 5.5. No significant inter-genotypic difference in plasma aldosterone was observed at either time-point.

	BASELINE			24 WEEKS		
Genotype	TT (n=26)	CT (n=48)	CC (n=19)	TT (n=24)*	CT (n=47)*	CC (n=16)*
LVESVI (ml/m^2)	39.3 (12.0)	43.5 (14.2)	46.0 (12.6)	33.8 (16.4)††	43.1 (18.0)††	47.8 (19.0)††
LVEDVI (ml/m^2)	80.2 (15.1)	83.3 (17.2)	84.6 (13.3)	79.4 (17.0)	88.5 (23.1)	90.3 (17.1)
LVEF (%)	51.4 (8.2)†	48.6 (8.1)	46.3 (7.8)†	59.3 (11.0)††	52.5 (9.7)††	48.0 (13.6)††
LVMI (g/m^2)	69.6 (13.1)	73.9 (14.6)	73.0 (11.2)	63.7 (13.6)	66.1 (12.4)	65.9 (9.5)
Infarct volume (ml/m^2)	25.6 (16.7)	34.3 (22.7)	36.4 (17.6)	19.5 (14.0)	19.5 (11.7)	23.4 (11.8)
Plasma aldosterone (pmol/l)	180.8 (177.6)	215.2 (193.7)	220.4 (185.1)	308.3 (325.9)	248.3 (228.9)	194.0 (145.5)

Table 5.5 Comparison of inter-genotypic variations in ceCMR parameters and plasma aldosterone at baseline and after 24 weeks. Data are presented as mean (SD). Continuous data compared with the use of ANOVA. TT, CT and CC represent single nucleotide polymorphisms in the *aldosterone synthase* gene at a site 344 base pairs upstream of the transcription initiation site.
[**Key**: † p<0.05 TT v CC only; †† p<0.05 TT v CC, TT v CT (but not CT v CC); *over 24 week period 2 patients with CC genotype and one with TT genotype died, and one patient of each of the three genotypes withdrew from follow-up]

Baseline and 24-week LVESVI, LVEDVI, LVEF and plasma aldosterone data and change in each of these parameters by treatment group are shown graphically in Figure 5.3.

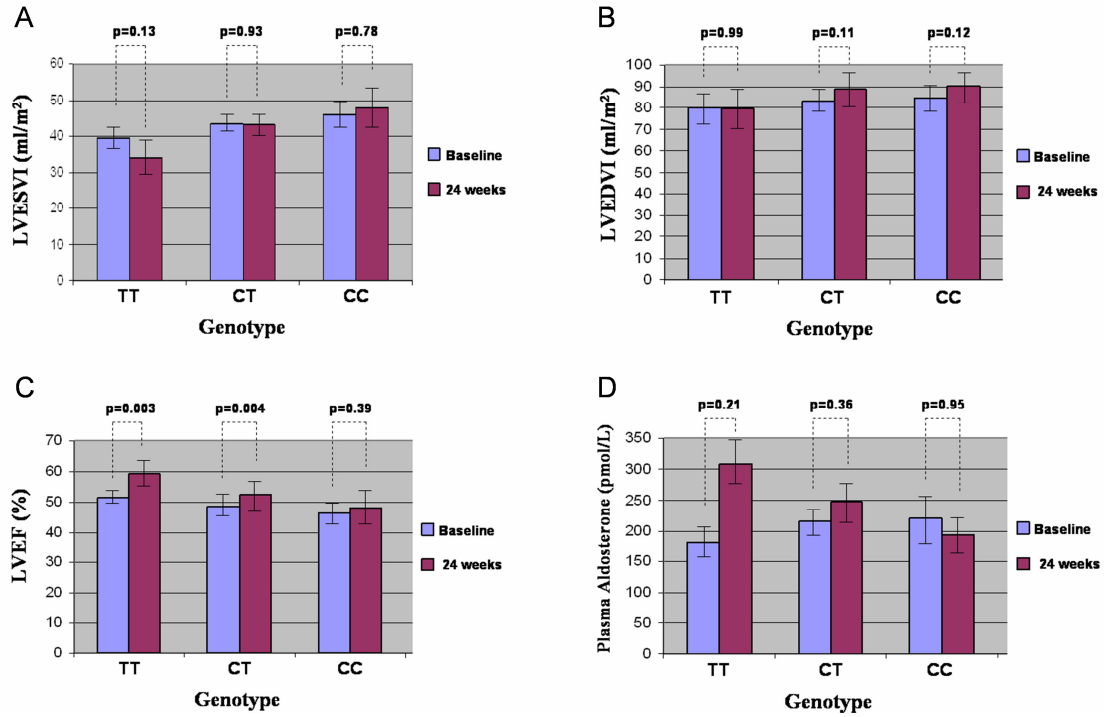

Figure 5.3 Baseline and 24-week LVESVI (A), LVEDVI (B), LVEF (C) and plasma aldosterone (D) by genotype for the 93 patients included in the genetic sub-analysis. Data are presented as mean (SEM). Significance values for comparison of baseline and 24-week value for each parameter within each genotype are shown.

There were no significant changes over time in ΔLVESVI, ΔLVEDVI or Δaldosterone within any of the three genotype sub-groups (Figure 5.3 A,B,D) although there was a trend towards a reduction in LVESVI and an increase in plasma aldosterone, in TT patients compared to the two other genotype sub-groups. LVEF increased significantly in both TT (ΔLVEF +7.9 [11.3] %, p = 0.003) and CT (ΔLVEF +3.9 [8.1]%, p = 0.004) sub-groups but not significantly in the CC sub-group (ΔLVEF +1.7 [12.4] %, p = 0.39).

The influence of genotype on serial change in LV structure, function and infarct volume was then analysed using multiple linear regression analysis following adjustment for age, sex, hypertension history, smoking status and prior MI as described in the statistical methods section (Chapter 5.2.2) – Table 5.6. There did

appear to be a weak association between the TT genotype and greater final LVEF although the small patient numbers and the low multiple correlation co-efficient of the model ($R^2 = 0.32$) limit the significance of this finding. A significant association was also noted between the TT genotype and final infarct volume. No significant relationships were observed between the CT or CC genotype and any ceCMR parameter.

Genotype	**TT** (n=26)			**CT** (n=48)			**CC** (n=19)		
	β co-efficient	p	R^2	β co-efficient	p	R^2	β co-efficient	p	R^2
LVESVI (ml/m²)	-5.74	0.09	0.44	4.31	0.16	0.43	1.72	0.67	0.42
LVEDVI (ml/m²)	-5.92	0.12	0.45	4.42	0.21	0.45	2.01	0.66	0.44
LVEF (%)	5.50	**0.02**	0.32	-3.35	0.12	0.29	-2.27	0.43	0.27
LVMI (g/m²)	1.23	0.47	0.68	-0.03	0.98	0.68	-0.90	0.65	0.68
Infarct volume (ml/m²)	4.09	**0.02**	0.67	-2.21	0.17	0.66	-0.31	0.88	0.65

Table 5.6 Multiple linear regression analysis assessing the predictive value of -344T/C SNPs on serial change in LV parameters and infarct volume, adjusted for age, sex, prior MI, hypertension history and smoking status. The multiple correlation co-efficient (R^2) for each model is shown.

5.4 Discussion

5.4.1 The role of aldosterone in post-infarction remodelling

Aldosterone has a number of detrimental effects on the cardiovascular system, including promotion of myocardial interstitial and perivascular fibrosis, stimulation of myocyte apoptosis, mediation of baroreceptor dysfunction, prevention of myocardial neuronal re-uptake of norepinephrine, increase in sympathetic drive, and potentiation of fluid overload and electrolyte imbalance.[84-89, 302] Furthermore, elevated aldosterone levels correlate directly with mortality following (ST-elevation) AMI and in CHF.[95, 303] Robust clinical trial evidence supports beneficial effects on cardiovascular morbidity and mortality of aldosterone antagonists in both CHF and AMI.[121, 123] There has been debate recently, however, as to whether the effects of aldosterone antagonists on the heart are due to mineralocorticoid receptor blockade independently of aldosterone effects, ie. that circulating aldosterone is a "bystander".[304]

I have shown, taking our cohort as a whole, that aldosterone was within normal supine limits at baseline with a trend towards increase over time, in keeping with the well-documented phenomenon of aldosterone escape.[118] I found a direct correlation between baseline aldosterone and measures of adverse LV remodelling over 24 weeks, and also found a significant relationship between aldosterone and infarct volume. Analysis of the treatment groups revealed that plasma aldosterone did not change over time in the placebo group but increased significantly in the eplerenone group. Baseline plasma aldosterone correlated with 24-week LVESVI, ΔLVESVI and ΔLVEDVI in the placebo group, and Δaldosterone correlated inversely with ΔLVEDVI. Thus a high baseline aldosterone in the placebo group correlated with

adverse remodelling, but surprisingly a fall in aldosterone over time correlated with progressive ventricular dilatation. In the eplerenone group baseline aldosterone correlated with baseline and 24-week LVESVI, baseline and 24-week LVEF (inversely) and baseline LVEDVI but not with *change* in any of these parameters over time, unlike in the placebo group. The association between Δaldosterone and ΔLVEDVI, seen in the placebo group, was lost in eplerenone-treated patients.

Before discussing the possible interpretation of these results, it is important to re-affirm that the absolute number of patients in this study was small as the study was powered for CMR end-points, not fluctuations in plasma RAAS hormones.

In eplerenone-treated patients, in whom we can hypothesise that a significant percentage of mineralocorticoid receptors were occupied by the study drug, although a high baseline aldosterone did correlate with some parameters of poorer LV function at 24 weeks, it did not specifically predict remodelling, ie. change over time in LVESVI (or indeed change over time in LVEDVI or LVEF). Moreover, even though plasma aldosterone rose over time, this had no bearing on LV remodelling either. In contrast to this, in placebo-treated patients, in whom mineralocorticoid receptors would not be occupied by the study drug, the LV continued to dilate even though plasma aldosterone tended to decrease over time. These data may suggest that in the early stages of AMI, circulating aldosterone acts to select a particular remodelling pathway for each patient but that over time it is the mineralocorticoid receptor blockade that is key to modifying the remodelling pathway. Although plasma aldosterone had a tendency to decrease in the placebo group, the mineralocorticoid receptors were not occupied by study drug, thus circulating cortisol (the concentration

of which was highly elevated) could activate these receptors, thereby potentiating adverse LV remodelling independently of circulating aldosterone. Conversely in the eplerenone group, although plasma aldosterone rose over time, the mineralocorticoid receptors were occupied by eplerenone thus neither aldosterone nor cortisol could bind to and activate these receptors. Thus aldosterone may have an adverse effect in the acute post-infarction phase but then takes more of a 'bystander' role in the sub-acute phase: our data suggest that it is mineralocorticoid receptor activation that drives remodelling rather than circulating aldosterone.

The predictive value of metabolites of corticosteroid catabolism, measured in urine, was assessed in an attempt to analyse further the relative roles of aldosterone and cortisol in LV remodelling. Of the urinary steroids produced predominantly during the aldosterone limb of the pathway (Figure 1.2), THAldo excretion rate fell significantly over the 12 weeks between urinary collections. Total cortisol metabolite and THS excretion rates, both predominantly metabolites of cortisol biosynthesis, fell significantly over time. Total cortisol metabolites were very highly elevated at baseline, presumably reflecting the acute stress response following large AMI across the patient population. Multiple linear regression analysis was complicated by multiple correlations between excretion rates of the steroid groups. Removing those with the strongest inter-correlation revealed trends towards a relationship between a lower baseline THDOC and a higher baseline THAldo with greater 24-week LVEDVI. THAldo also predicted 24-week infarct volume.

Although the study is insufficiently powered to detect significant differences between urinary steroid sub-groups, these results show that, although the cortisol pathway is

highly activated early after infarction, the only significant correlation with LV function occurred in the aldosterone pathway. This suggests that aldosterone rather than cortisol is linked with the pathophysiology of LV dilatation and dysfunction early after AMI. The speculated relationship of THAldo, the principal metabolite of aldosterone, with ΔLVEDVI and infarct volume supports the baseline plasma aldosterone findings and again suggests that aldosterone in the *early* post-infarction period is a key factor in determining subsequent LV remodelling.

Given the power of the study it is unsurprising that no interaction was observed between eplerenone therapy, steroid sub-group excretion rates and LV function. Adequately-powered future studies in the field of urinary steroids and their predictive effect on LV function after AMI in the presence of potential anti-remodelling medication would shed more light in this area. Nevertheless, the urinary steroid findings do support some of the plasma aldosterone results and again suggest that aldosterone in the first few days after AMI is a key determinant of subsequent remodelling.

Following AMI, fibrotic change occurs at the site of acute myocardial injury over time, and reactive fibrosis develops in non-infarct myocardium in a number of animal models.[37, 104, 105] Fibrosis can be induced in the rat heart experimentally by infusing aldosterone in salt-loaded conditions (although aldosterone had no pro-fibrotic effect in salt-deplete conditions).[103, 106] Our sub-groups are too small to allow any major insights into the association between aldosterone and fibrosis, but the correlations between baseline plasma aldosterone and infarct volumes in both treatment groups and between THAldo and infarct volume suggests a relationship between aldosterone

and infarct zone fibrosis that is independent of mineralocorticoid receptor occupation. Contrary to the rat model, I found no interaction between urinary sodium excretion (a marker of total body sodium), eplerenone therapy and LV function/infarct volume over time although again the study was not powered to detect such a relationship.

The genetic sub-study into the relationships of the -344T/C SNP in *aldosterone synthase* and LV function was limited to 93 patients, thus any results should be treated as hypothesis-generating only. I observed that LVEF at baseline was higher in patients with TT genotype (n=26) than in those with either CT or CC. This probably represents the play of chance given the small numbers involved. At 24 weeks, LVESVI was significantly lower and LVEF significantly higher in TT patients than in CT or CC. Although neither ΔLVESVI nor ΔLVEDVI differed significantly between genotype sub-groups, there was a trend towards reduction in both in TT patients compared to CT or CC. That the TT sub-group had superior baseline LV function suggests that these patients would be more likely to improve/reverse remodel in comparison to those with poorer LV function in the CT and CC sub-groups, thus these results may also be spurious and related to the chance baseline difference in LV function. However, LVEF increased significantly in not only TT but also CT patients, while there was no significant change in LVEF in CC patients. Moreover, in a multivariable model adjusted for clinical variables, the TT genotype remained a weak predictor of superior 24-week LV function, and also of infarct volume. Despite the small numbers, the -344T allele in our population did appear to be associated with improved LV function.

Aldosterone excretion is influenced by this SNP in *aldosterone synthase*, although there appears to be significant inter-racial variability, with higher excretion rates of aldosterone seen with the -344T allele in Caucasian populations and with the -344C allele in African American populations.[134, 305] In this small study I found no significant differences in plasma aldosterone concentrations between the genotypes at baseline or 24 weeks, although 24-week plasma aldosterone and Δaldosterone were non-significantly higher in TT patients than CT or CC. As 99% of the study cohort was Caucasian, these trends would support the previously-reported association between the -344T SNP and higher circulating aldosterone in such a population.[134, 305] It is noteworthy, however, that a study of 216 European hypertensive patients found higher aldosterone excretion rates in those with the -344C allele, while a separate European epidemiologic study of 562 patients found no association between -344T/C allele status and plasma aldosterone.[306, 307]

I found a relationship between the -344T allele and improved LV function, occurring despite a trend towards increase over time in plasma aldosterone in this sub-group. I also observed an association between the -344T allele and greater infarct volume. The significance of these findings is unclear. I hypothesised earlier that circulating aldosterone in the first few days after AMI is detrimental and may select a particular remodelling pathway, but that over the ensuing weeks and months circulating aldosterone is no longer integral to ongoing remodelling, but rather mineralocorticoid receptor activation (by aldosterone or cortisol) is the main driving force behind progressive remodelling. That LV function improved in the TT sub-group despite a trend towards increasing aldosterone is consistent with the latter hypothesis, that of the "bystander" role for aldosterone in the subacute phase. However, in view of the

small number of patients in each of the genotype sub-group, further speculation on the relative importance of the -344T/C SNP in this population is unwarranted. A more appropriately-powered study showed no difference in allele frequency in patients 5 years after AMI than in the general population, while in a separate study of 226 patients with incident anterior AMI, no association was found between -344T/C and LV remodelling out to 1 year after AMI.[138, 139]

5.4.2 Limitations

That each plasma biomarker was sampled at only one time-point at baseline (mean 3.7 days), by which time the process of 'early' remodelling (which occupies the first 72 hours after infarction) would have ceased and 'late' remodelling would have begun, and that plasma concentrations of several of the sampled biomarkers fluctuate, particularly in the first few days after AMI and thus cannot be commented upon in this study, have been discussed earlier (Chapter 4.5.2). Additionally, plasma concentrations of each biomarker were measured only; no data on local tissue activity of each biomarker (which was beyond the scope of this thesis) are available.

Finally, the primary study on which this sub-study was based was powered for LGE-CMR end-points only. Results of the genetic, plasma and urinary aldosterone analysis must be interpreted in this light and as such are hypothesis-generating only. Where appropriate, results of adequately-powered studies in the literature have been cited.

5.4.3 Conclusions

The role of aldosterone in LV remodelling was examined in detail. Combining results from genetic analysis, plasma aldosterone and urinary steroid metabolite excretion rates, I hypothesise that there exists a temporal variation in the cardiac effects of aldosterone after AMI, specifically that circulating aldosterone in the first few days after infarction is key in selecting a remodelling pathway but that over the following weeks and months circulating aldosterone is less influential in the pathogenesis of ongoing remodelling – mineralocorticoid receptor activation (by aldosterone or cortisol) potentiates remodelling over time.

I also demonstrated a relationship between plasma aldosterone and greater infarct volume that was unaffected by eplerenone therapy, which provides support for the theory that aldosterone antagonists do not influence reparative fibrosis after AMI; no evidence of reactive fibrosis was found in our optimally-treated population.

Finally, analysis of the -344T/C SNP in *aldosterone synthase* in the study population revealed an association between the -344T allele and superior LV function, contrary to published studies in this field. I acknowledge however that this study was significantly under-powered to detect any definite relationships in any of the neurohormonal or genetic data, and that the hypotheses above are purely speculative.

Chapter 6

A study of the predictive value of infarct characteristics determined by contrast-enhanced cardiac magnetic resonance imaging in post-infarction left ventricular remodelling

6.1 Introduction

Prognosis after AMI is directly related to the extent of myocardial injury that occurs during coronary occlusion.[308] CMR, through delayed contrast-enhanced imaging techniques as described in Chapter 1, allows *in vivo* visualisation of infarcted myocardium. A number of infarct characteristics can then be described and/or quantified. Previous studies have confirmed that total infarct size, endocardial extent and transmurality predict adverse cardiovascular outcomes.[255, 309] MVO has also been shown to confer an adverse prognosis, although this has been questioned in a recent study.[257]

The cohort examined in the primary study (Chapter 3) represents a population of patients who underwent LGE-CMR imaging serially over 24 weeks after AMI. The aims of this study were to examine the infarct characteristics on delayed enhancement imaging in the study cohort at baseline, and to assess the utility of these characteristics in the prediction of LV remodelling over 24 weeks after AMI.

6.2 Methods

6.2.1 Study patients and trial protocol

All 100 patients enrolled in the primary study of this thesis (Chapter 3) were included in this study examining infarct characteristics and their potential role in the prediction of post-infarction remodelling. The screening, recruitment, randomisation process and trial outline are described in detail in Chapter 2 (2.1 – 2.3), and baseline demographic data of the study cohort are shown in Table 3.1.

LGE-CMR imaging was performed at baseline, 12 and 24 weeks. Detailed descriptions of imaging techniques and methods of analysis (including definitions of the various infarct characteristics measured in this study) are provided in Chapter 2 (2.5 – 2.6).

6.2.2 Statistical methods

All data are expressed as mean (SD) for continuous variables and number (%) for categorical variables unless otherwise stated. Comparisons between sites of infarction were made using paired sample *t*-tests or Mann-Whitney-U tests as appropriate for continuous variables and Chi-squared test for categorical variables. Pearson's and Spearman's correlation co-efficients were used for parametric and non-parametric data respectively.A probability value of $p<0.05$ was considered significant.

6.3 Results

6.3.1 Site of infarction

The site of the acute infarct on baseline ceCMR, defined using the 17-segment AHA model, was anterior in 53 (53.0%), inferior in 24 (24.0%) and lateral in 4 (4.0%). Infarcted myocardium was equally distributed between anterior and lateral segments in 2 (2.0%) and between inferior and lateral segments in 17 (17.0%) – such infarcts were classified as anterolateral and inferolateral respectively. Baseline infarct characteristics according to site are shown in Table 6.1.

	Total (n=100)	Anterior ± lateral (n=55)	Inferior ± lateral (n=41)	Lateral (n=4)	p†
Infarct vol (ml/m^2)	33.2 (20.7)	36.1 (22.1)	30.2 (18.8)	23.7 (16.2)	0.17
Endocardial extent (%)	36.8 (11.5)	40.2 (11.0)	33.1 (11.1)	27.4 (5.8)	0.003
Transmurality Score	3.3 (0.6)	3.2 (0.6)	3.5 (0.5)	3.1 (0.6)	0.031
Early MVO	69 (69%)	35 (35%)	32 (32%)	2 (2%)	0.13
Late MVO	56 (56%)	29 (29%)	26 (26%)	1 (1%)	0.30

Table 6.1 Baseline infarct characteristics on LGE-CMR. Infarcts classified according to the 17-segment AHA model. Anterior and anterolateral infarcts were combined as "anterior ± lateral" while inferior and inferolateral were combined as "inferior ± lateral". 4 infarcts involved only lateral segments with no anterior or inferior extension and are hence classified as lateral only. Data are presented as mean (SD) for continuous variables and number (%) for categorical variables unless otherwise stated. Two sample t-test used to compare continuous variables and chi-square test for categorical variables. †Comparison made between "anterior ± lateral" and "inferior ± lateral" only due to small numbers (n=4) in "lateral" group.

There was no significant difference in infarct volume between combined anterior/anterolateral, inferior/inferolateral and lateral infarct sites. Anterior and anterolateral infarction was of greater endocardial extent than inferior and inferolateral infarction, while the latter group had mildly but significantly higher transmurality scores. Of note, there was no significant difference in the extent of LV remodelling between anterior/anterolateral and inferior/inferolateral infarcts

(ΔLVESVI was +0.06 [15.4] ml/m^2 in anterior vs. -0.9 [12.1] ml/m^2 in inferior AMI, p=0.76)

6.3.2 Transmurality score and endocardial extent

Across the entire study cohort, there were significant correlations between transmurality score, endocardial extent and LV volumes/LVEF at baseline and 24 weeks (Table 6.2). Both infarct parameters, particularly transmurality score, correlated with ΔLVESVI (Figure 6.1).

	Transmurality Score	**Endocardial extent (%)**
Baseline		
LVESVI	0.21*	0.45**
LVEDVI	0.20*	0.28**
LVEF	-0.15	-0.55**
24 weeks		
LVESVI	0.43**	0.50**
LVEDVI	0.37**	0.37**
LVEF	-0.46**	-0.55**
ΔLVESVI (0-24 weeks)	0.47**	0.26*

Table 6.2 Spearman correlation coefficients for baseline infarct transmurality score, endocardial extent and LGE-CMR parameters of LV function and remodelling. Units: LVESVI, LVEDVI: ml/m^2; LVEF: % Key: *p<0.05 **p<0.01

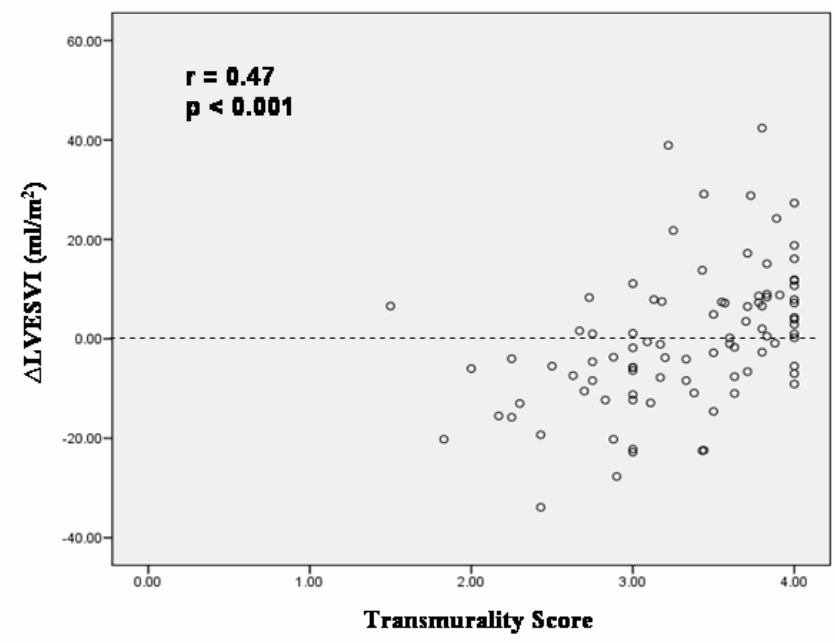

Figure 6.1 Plot of change in LVESVI from baseline to 24 weeks against mean transmurality score at baseline. A statistically significant correlation exists between the measured quantities.

6.3.3 Microvascular obstruction

MVO was present in 69% of the study cohort and persisted on delayed contrast-enhanced images (ie. late MVO) in 56% (Table 6.1). A representative example is shown in Figure 6.2. The presence of late MVO was significantly correlated with adverse LV remodelling whereas the absence of late MVO was associated with reverse remodelling (ΔLVESVI +4.0 [13.4] ml/m^2 in patients with late MVO vs. -6.4 [12.7] ml/m^2 without late MVO, p<0.001); Figure 6.3.

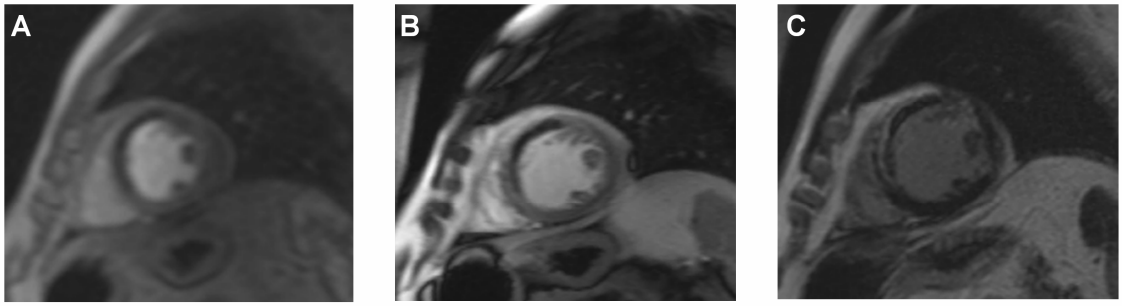

Figure 6.2 Short-axis LGE-CMR images (at mid-ventricular level) of a 48 year-old male patient admitted with an anteroseptal STEMI, performed 3.5 days after AMI. (A) First-pass

perfusion image depicting an extensive hypoperfused segment occupying the septum and anterior wall (black area); note the bright appearance of the blood pool in the LV cavity, and less so in the RV cavity, as these images were acquired within seconds of IV injection of gadolinium-DTPA. (B) Single-shot steady-state free precession sequence with a non-selective inversion pulse acquired 2 minutes after contrast injection (non-breath-hold) confirms lack of penetrance of contrast into infarcted segment (black area) – this represents "early MVO". (C) Contrast-sensitive segmented inversion recovery sequence (with breath-hold) acquired 15 minutes after contrast injection. Infarcted tissue appears bright as contrast ultimately penetrates slowly into the hypoenhanced segment seen in (A) and (B), but the hypoenhanced core within the bright anteroseptal infarct depicts "late MVO".

The absolute volume of late MVO significantly correlated with total infarct volume ($r=0.65$, $p<0.001$). Although there were weak correlations between late MVO volume and 24 week LVESVI ($r=0.30$, $p=0.029$) and LVEDVI ($r=0.32$, $p=0.023$), there was no significant relationship between MVO volume and ΔLVESVI ($r=0.25$, $p=0.07$).

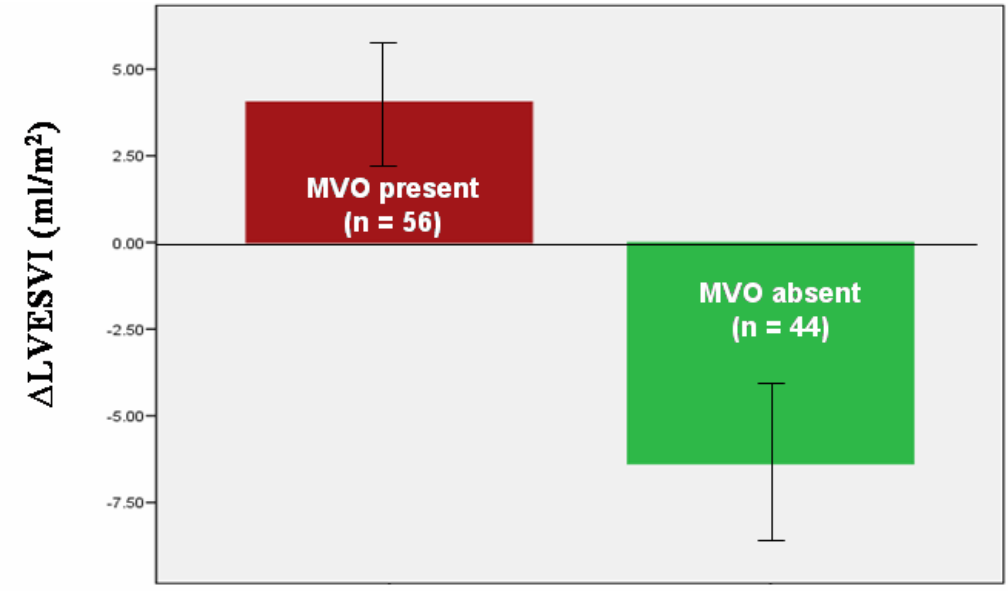

Figure 6.3 Mean change in LVESVI from baseline to 24 weeks in patients grouped according to presence or absence of late MVO on baseline LGE-CMR scan. Error bars represent SEM. A statistically significant difference in ΔLVESVI exists between the patient groups ($p < 0.001$).

Coronary angiography was performed in 48/56 (85.7%) patients with late MVO on baseline LGE-CMR and in 37/44 (84.1%) with no evidence of late MVO. TIMI flow rates within the IRA at the end of angiography (with or without PCI) are shown in Table 6.3.

			TIMI flow within IRA			
	n	**Angio performed**	**3**	**2**	**1**	**0**
MVO present	56	48 (85.7%)	42 (75.0%)	2 (3.6%)	0	4 (7.1%)
MVO absent	44	37 (84.1%)	32 (72.7%)	3 (6.8%)	0	2 (4.5%)

Table 6.3 Final TIMI flow rates within the IRA at completion of angiography +/- PCI according to presence or absence of late MVO on baseline (pre-discharge) LGE-CMR.

6.4 Discussion

6.4.1 Infarct characteristics and the prediction of LV function after AMI

Using LGE-CMR images, I analysed the predictive value of a number of characteristics of the acute infarct in LV remodelling. Unsurprisingly total infarct volume, endocardial extent and transmurality score predicted adverse remodelling in keeping with previous studies, as all three quantities indicate more substantial infarction.[255, 309] One of the aims of this thesis, however, was to examine the phenomenon of late MVO and its predictive value as recent trial data have been contradictory. I found that late MVO occurred in 56% of the population and was evenly distributed between (predominantly) anterior and (predominantly) inferior anatomical location. Importantly, the presence or absence of late MVO divided patients into two distinct groups: those with late MVO adverse remodelled while those without late MVO reverse remodelled, with a strongly significant difference in mean ΔLVESVI of around 10 ml/m^2 between these two groups.

MVO represents abnormal microvascular perfusion within an infarcted segment thus it is predictable that it is associated with deteriorating LV function. Several prior CMR studies have produced similar results, with incidences of MVO of 45-55% amongst patients admitted with AMI, the majority of whom had low LVEF, and associations between MVO and adverse remodelling.[255, 257, 265] One recent study in which 57.5% of infarcts displayed late MVO reported that, although those with MVO had larger infarct volumes, greater elevation in cardiac biomarkers and lower LVEF at baseline than their counterparts without late MVO there was no difference between these two groups in LV remodelling over 4 months.[257] This appears counterintuitive,

and the authors argue that the aggressive treatment of their patients abolished the deleterious effect of late MVO on serial LV function (all patients underwent primary PCI and the discharge prescription of β blockers and ACE inhibitors/ARBs was 100% and 80% respectively), but it is more likely to relate to the small patient numbers involved (total population n = 40, 23 of whom had late MVO). In comparison with recent CMR studies, we have shown that not only is late MVO common despite a very high uptake of acute reperfusion therapies, but also it remains ominous in terms of LV functional recovery despite a higher uptake of contemporary anti-remodelling pharmacotherapy than in any other post-infarction remodelling trial to date.

A key aspect of post-MI care is the prediction of those patients in whom LV function may progressively deteriorate, as such patients warrant more stringent follow-up and may be candidates for ICD therapy. Many studies (and guidelines) use LVEF as the criterion upon which such decisions are made. However, early post-infarction LVEF measurement is not as powerful a predictor of adverse remodelling and major adverse cardiac events as might be anticipated.[8] Significant myocardial stunning may lead to under-estimation of LVEF, while compensatory hyperkinesis of non-infarcted myocardium may 'falsely' suggest a higher LVEF despite significant myocardial damage; variations in afterload may also influence LVEF acutely. It has been suggested that LVEF not be used as an end-point in early post-infarction studies.[310]

The data provided in this thesis suggest that the presence or absence of MVO may be of use in predicting remodelling outcomes. Previous criticisms of the use of MVO as a predictor of adverse outcome were based on the theory that MVO simply related to larger infarction, and that it was infarct size that determined outcome rather than

presence of MVO. While there is undoubtedly a relationship between MVO and larger infarct size (as demonstrated in this thesis and previous studies), presence of MVO has been shown to remain an indicator of adverse prognosis even after controlling for infarct size.[255, 257, 311] Likewise, MVO predicts development of a fibrous, transmural scar after AMI even when adjusted for infarct size.[255] It therefore appears that although MVO is related to infarct size, it also provides independent prognostic information rather than simply acting as a marker of the magnitude of infarcted myocardium.

A number of angiography-based trials have used patency of and TIMI flow rates within the IRA as end-points (Appendix IX). In such trials, TIMI 3 flow is generally accepted as the optimal result following AMI, irrespective of the means of reperfusion. It is therefore of considerable interest that MVO has been shown to be a stronger predictor of post-infarction death, re-infarction, CHF and stroke than patency of the IRA.[255] Moreover, MVO is frequently present despite TIMI 3 flow within the IRA; in the cohort on which this sub-study is based, (late) MVO was present in 56/100 patients (56%), of whom 48 underwent coronary angiography and 42 (i.e. 75% of those with late MVO) ultimately had TIMI 3 flow restored within the IRA despite subsequent detection of MVO on pre-discharge ceCMR imaging. This finding is of considerable significance, as it implies that TIMI 3 flow alone does not equate to normal microcirculatory perfusion, and further suggests that ceCMR-measured infarct characteristics, particularly MVO, might be a more appropriate end-point in clinical studies assessing the "success" of reperfusion therapies. MVO may indeed be the missing pathophysiological link between reperfusion, remodelling and cardiovascular outcome after AMI.

6.4.2 Limitations

The measurement of baseline infarct size in the study cohort was undoubtedly an over-estimation of the amount of myocardium genuinely infarcted. I included all abnormal myocardium (using standardised contrast and brightness settings) during the *post hoc* analysis of the delayed enhancement images: this will have included the infarct plus the surrounding peri-infarct zone, consisting of oedema, haemorrhagic change (particularly as the majority of infarcts were acutely reperfused) and acutely inflamed tissue within and around the fresh infarct. This is an almost inevitable limitation in LGE-CMR imaging of large acute infarcts, and although trials using "oedema imaging" are ongoing, this process is not used in clinical practice at the time of writing this thesis.

In comparison to previous studies examining infarct characteristics following AMI, there are a number of methodological issues that lead to marked inter-study variability. These include partial volume effects, variability in gadolinium-DTPA dose and time to delayed enhancement imaging between studies, variations in the wash in/wash out profiles of different gadolinium-DTPA preparations and in the definition of the boundary zones of the infarct.[246, 263, 312, 313] Such methodological issues provide compelling evidence for the need for a universal consensus on infarct size measurement on LGE-CMR.

Finally, it must be acknowledged that the patients in this study were required to have LVSD, thus the findings cannot be applied to all AMI patients. MVO is less likely to occur in association with smaller infarcts, and the predictive potential of MVO in small-to-medium infarcts cannot be commented upon from the data in this thesis.

6.4.3 Conclusions

The findings of this study suggest that the presence or absence of late MVO on pre-discharge LGE-CMR imaging divides patients presenting with AMI into two groups with very different remodelling outcomes and may assist in the risk stratification process. The detection of (late) MVO, with its adverse effects on prognosis, in patients in whom angiography (with or without follow-on PCI) had ultimately confirmed TIMI 3 flow in the IRA, suggests that patency of and flow within the IRA are not necessarily markers of optimal outcome after AMI. Infarct characteristics on LGE-CMR, and in particular MVO, may be more appropriate end-points in future studies assessing the success of reperfusion therapies.

Chapter 7

A study characterising plasma apelin concentrations after acute myocardial infarction in man

7.1 Introduction

Apelin, the endogenous ligand for the APJ receptor (a G-protein coupled receptor found in many organs including heart, kidney, lung, vasculature and adipose tissue) has a putative role in cardiovascular physiology and homeostasis, as discussed in Chapter 1.7.4. The demonstration in both animal and human studies of potent inotropic, diuretic and (nitric-oxide dependent) vasodilator properties, in addition to antagonistic effects on angiotensin II, have stimulated interest in the role of apelin as an endogenous mediator, which would be of particular relevance in the failing heart.[169, 314-316]

Data regarding blood apelin concentrations in patients with heart failure are inconsistent. Plasma apelin concentrations were similar in patients with and without acute heart failure in one study evaluating the predictive value of biomarkers in this condition.[317] Studies in CHF, generally limited by small numbers of patients, showed a lower concentration of apelin in patients with both ischaemic and non-ischaemic CHF compared to controls, across a wide range of disease severity.[171, 318, 319] Interventions such as cardiac resynchronisation therapy and left ventricular assist device implantation increase LVEF, which is associated with a rise in plasma apelin concentrations over time in small studies of patients with advanced CHF.[172, 320]

That apelin has been demonstrated to possess such inotropism suggests a potential role for this peptide in LV remodelling following AMI. At the time of my research project, there were no published data on plasma apelin concentrations following AMI in man. The aim of this study was therefore to characterise for the first time the temporal profile of plasma apelin that occurs following AMI in man, and to determine

whether apelin had any relationships with parameters of LV function/remodelling or with a selection of established biomarkers thought to be of pathophysiological significance in post-infarction remodelling.

7.2 Methods

7.2.1 Study patients

All 100 patients enrolled in the primary study of this thesis (Chapter 3) were included in this study examining the role of apelin in post-infarction remodelling. The screening, recruitment, randomisation process and trial outline are described in detail in Chapter 2 (2.1 – 2.3). Separate from the AMI cohort, 38 patients on no regular prescribed medications, with no history of cardiac events, and with a normal ECG and TTE, acted as controls (data on controls kindly donated by Dr. KS Chong, Scottish National Advanced Heart Failure Service, Glasgow Royal Infirmary).

7.2.2 Trial outline

LGE-CMR imaging was performed at baseline (pre-randomisation), 12 and 24 weeks; the methods of imaging and analysis are described in detail in Chapter 2 (2.5 – 2.6).

Plasma apelin was measured twice in patients in the AMI cohort: at baseline (pre-randomisation to placebo or eplerenone) and again at 24 weeks. In the 38 normal controls, plasma apelin was measured only once. Details of apelin collection, storage and analysis are described in Chapter 2.8.8.

In order to elucidate further the role (if any) of apelin in LV remodelling after AMI, I also assessed its relationships with NTproBNP (a prognostic marker after AMI), NA (a marker of sympathetic activation and of potential pathophysiological significance in post-infarction remodelling) and AVP (which has been shown to antagonise apelin in animal models).[321] For the purposes of this study, only the baseline and 24 week measurements of each of these three biomarkers were included; detailed methods of collection, storage and analysis are described in Chapter 2.8.

7.2.3 Statistical methods

Plasma apelin concentrations were analysed across the entire study population (n=100) and compared to control plasma apelin concentrations (n=38). Plasma apelin concentrations within the study cohort were also analysed by treatment group. Inter-group comparisons were made using paired sample *t*-tests or Mann-Whitney-U tests as appropriate for continuous variables and Chi-squared test for categorical variables. Paired *t*-tests were used to detect changes in ceCMR measurements and biomarkers within each treatment group over the 24 weeks of the study, and differences between these changes were analysed using an unpaired *t* test.

Pearson's and Spearman's correlation coefficients were used for parametric and non-parametric variables respectively. Comparison of median plasma apelin concentrations within quartiles of LVESVI and LVEF was then performed using separate Kruskal-Wallis tests. A probability value of $p<0.05$ was considered significant. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

7.3 Apelin following AMI in man

7.3.1 Demographic data of AMI cohort patients and controls

Comparison of demographic data between the study cohort and the control population is shown in Table 7.1. The mean age of patients admitted with AMI (n = 100) was 58.9 ± 12 years and that of controls (n = 38) was 56.4 ± 11.4 years (p = 0.24). Significantly more patients were of male gender in the AMI cohort (77%) than in the control population (59.4%); p = 0.03.

	AMI COHORT (n=100)	**CONTROLS (n=38)**	**p**
Patient demographics:			
Mean age (SD) years	58.9 (12.0)	56.4 (11.4)	ns
% male	77.0	59.4	0.03
Haemodynamics:			
Mean (SD) blood pressure			
Systolic (mmHg)	113.0 (16.3)	119.9 (14.4)	ns
Diastolic (mmHg)	69.9 (11.6)	77.3 (8.1)	ns
Mean (SD) heart rate (bpm)	65.8 (14.3)	63.5 (10.9)	ns
Renal function:			
Mean (SD) creatinine (μmol/l)	100.1 (21.2)	91.2 (13.0)	0.03
Mean (SD) eGFR (ml/min/m^2)	70.2 (17.5)	74.0 (10.6)	0.04
Neuropeptides			
NTproBNP (pg/ml)	2588 (2732)	32 (28)	<0.001
Apelin (ng/ml)	0.54 (0.25)	3.22 (3.01)	<0.001

Table 7.1 Baseline characteristics of AMI patients (n=100) and controls (n=38). Unless otherwise stated, continuous data are expressed as mean (SD), while categorical data are expressed as percentages of the relevant treatment group. eGFR was calculated using the MDRD formula.

7.3.2 Serial change in plasma apelin concentrations after AMI

Baseline plasma apelin concentrations, sampled on average 2 days after AMI, were significantly lower than in the control population: mean ± SD apelin was 0.54 ± 0.25 ng/ml in AMI patients v. 3.22 ± 3.01 ng/ml in controls (p<0.001). Plasma apelin concentration was lower in smokers at baseline (0.50 ± 0.18 ng/ml v. 0.64 ± 0.36 ng/ml in non-smokers, p=0.014) but had no relationship with age, sex, prior MI, history of hypertension, dyslipidaemia or stroke.

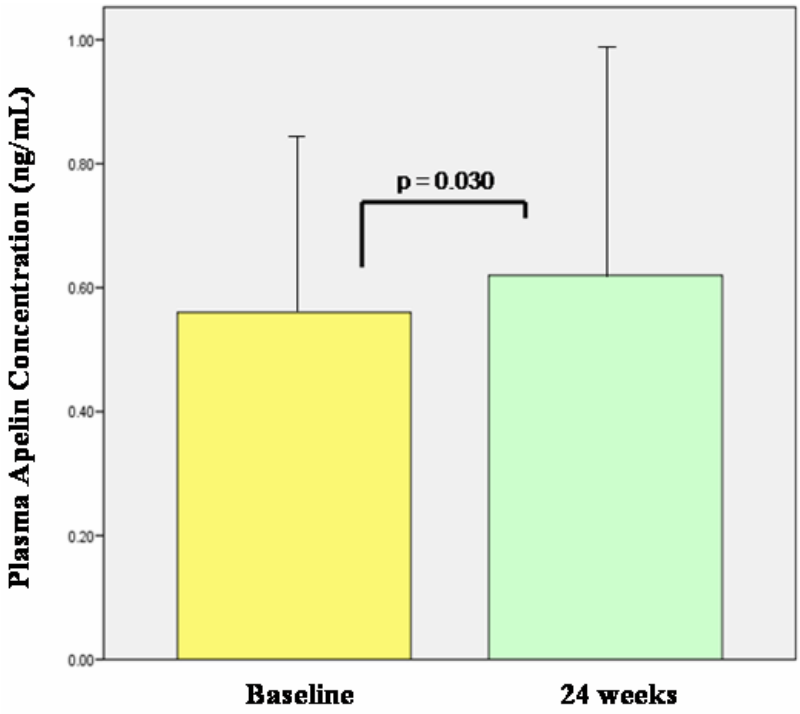

Figure 25. Mean (SD) plasma apelin concentration at baseline and 24 weeks in study cohort. The increase in plasma apelin concentration from baseline to 24 weeks is statistically significant.

Plasma apelin concentrations rose significantly over time (0.54 ± 0.25 ng/ml at baseline v. 0.62 ± 0.36 ng/ml at 24 weeks, p=0.030) – Figure 7.1, Table 7.2 – but even at 24 weeks remained significantly lower than the control apelin concentrations (p<0.001).

Peptide hormone	Baseline	24 weeks	p
Apelin (ng/ml)	0.54 (0.25)	0.62 (0.36)	0.030
NTproBNP (pg/ml)	2588 (2732)	841 (1983)	<0.001
Noradrenaline (nmol/l)	3.19 (1.81)	2.74 (1.23)	0.013
AVP (pg/ml)	0.87 (0.83)	0.65 (0.72)	0.001

Table 7.2 Baseline and 24 week comparative plasma concentrations of peptide hormones for entire study (post-MI) cohort (n = 100).

7.3.3 Relationships between apelin and parameters of LV function

Baseline plasma apelin had no significant relationship with initial LVESVI ($r = 0.01$, $p = 0.92$), nor did it correlate with baseline LVEDVI, LVMI, LVEF or infarct volume index (Table 7.3). There was no relationship between baseline apelin and ΔLVESVI ($r=0.06$, $p=0.55$). Similarly there were no associations between baseline apelin and ΔLVEDVI, ΔLVEF, ΔLVMI or Δinfarct volume.

I divided study patients into quartiles of baseline LVESVI (Figure 7.2 A) and LVEF (Figure 7.2 B); no interquartile differences in baseline apelin concentrations were demonstrated. Similarly, baseline apelin bore no relationship to ΔLVESVI or ΔLVEF over 24 weeks within each quartile.

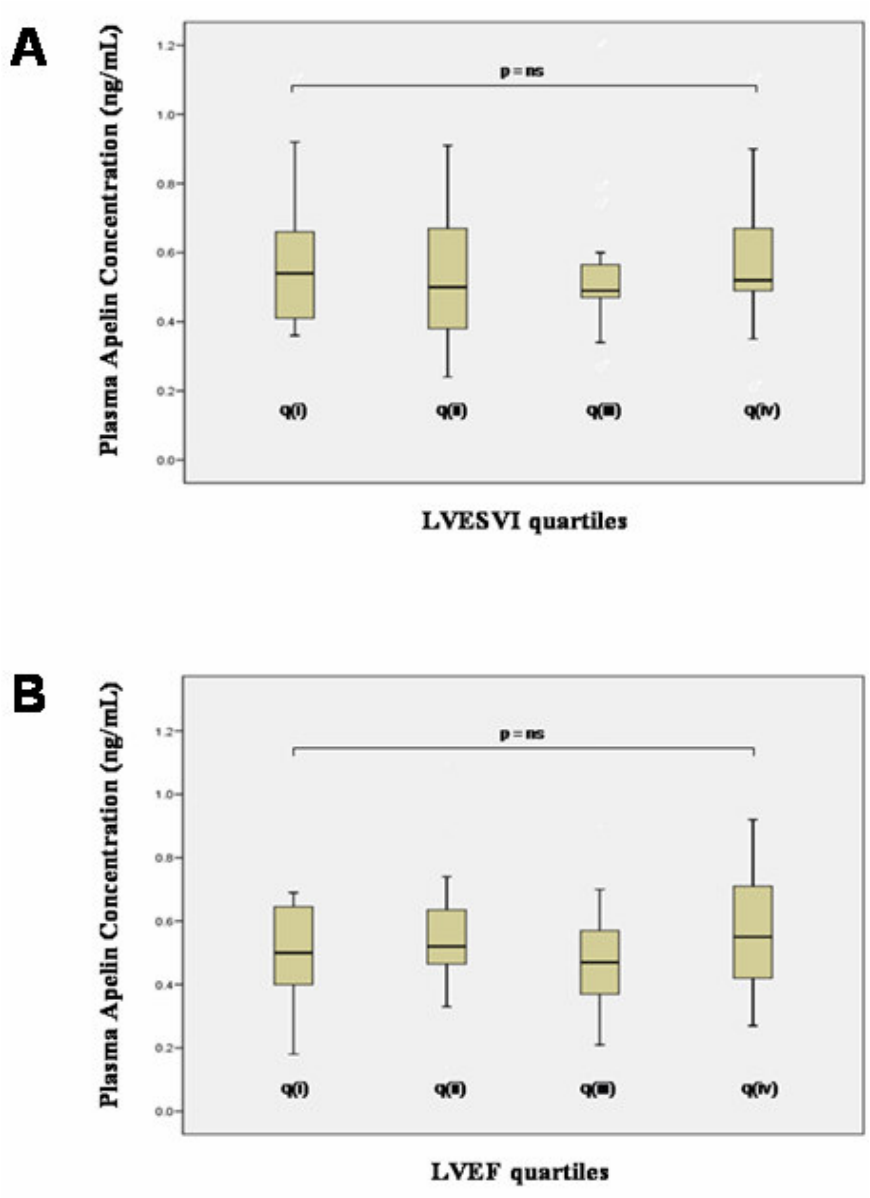

Figure 7.2
(A) Boxplot graph depicting plasma apelin concentrations in the AMI cohort (n = 100) according to quartiles of baseline LVESVI: q(i) ≤33.5 ml/m^2; q(ii) 33.6 – 42.4 ml/m^2; q(iii) 42.5 – 50.1 ml/m^2; q(iv) ≥50.2 ml/m^2. There were no significant inter-quartile differences in apelin concentrations.

(B) Boxplot graph depicting plasma apelin concentrations according to quartiles of baseline LVEF: q(i) ≤43.4%; q(ii) 43.5 – 49.2%; q(iii) 49.3 – 54.2%; q(iv) ≥54.3%. There were no significant inter-quartile differences in apelin concentrations.

	Apelin		NT-proBNP		Noradrenaline		AVP	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
Baseline								
LVESVI	0.010	-0.116	0.325**	0.338**	0.172	0.048	0.066	0.004
LVEDVI	0.089	-0.089	0.191	0.235**	0.100	-0.004	0.028	-0.039
LVEF	0.064	0.050	-0.393**	-0.368**	-0.223*	-0.129	-0.143	-0.105
LVMI	0.003	-0.064	0.055	0.163	0.228*	0.208*	-0.036	0.075
Inf Volume	-0.071	-0.115	0.262**	0.359**	0.167	0.111	0.115	-0.023
24 weeks								
LVESVI	0.104	0.018	0.431**	0.577**	0.227*	0.169	0.137	-0.042
LVEDVI	0.114	-0.077	0.301**	0.476**	0.087	-0.037	0.091	-0.066
LVEF	-0.077	-0.102	-0.448**	-0.549**	-0.312**	-0.317**	-0.155	-0.028
LVMI	0.069	-0.059	0.024	0.247*	0.162	0.069	-0.139	-0.184
Inf Volume	-0.117	-0.126	0.335**	0.447**	0.174	0.090	0.094	-0.032
ΔceCMR parameter (0 – 24 weeks)								
ΔLVESVI	0.063	-	0.265*	-	0.101	-	0.156	-
ΔLVEDVI	-0.002	-	0.191	-	0.001	-	0.156	-
ΔLVEF	-0.137	-	-0.191	-	-0.147	-	-0.103	-
ΔLVMI	0.004	-	-0.056	-	-0.194	-	-0.140	-
ΔInf Volume	0.006	-	-0.079	-	-0.139	-	-0.146	_

Table 7.3 Spearman correlation coefficients for neurohormones (sampled at baseline and 24 weeks) and LGE-CMR parameters of LV remodelling. Units: LVESVI, LVEDVI, Infarct Volume: ml/m^2; LVEF: %; LVMI g/m^2 Key: *p<0.05 **p<0.01

7.3.4 Relationships between apelin and sampled biomarkers

The relationships between baseline plasma apelin and NTproBNP, NA and AVP are shown in Figure 7.3 A-C. Baseline apelin correlated weakly but significantly with baseline NA (r = 0.26, p = 0.008). There were no significant correlations between baseline apelin and baseline NT-proBNP or AVP. Baseline apelin had no association with ΔNA (r = -0.04, p = 0.70), ΔNT-proBNP (r = 0.01, p = 0.98) or ΔAVP (r = -0.03, p = 0.81) over the 24 week follow-up.

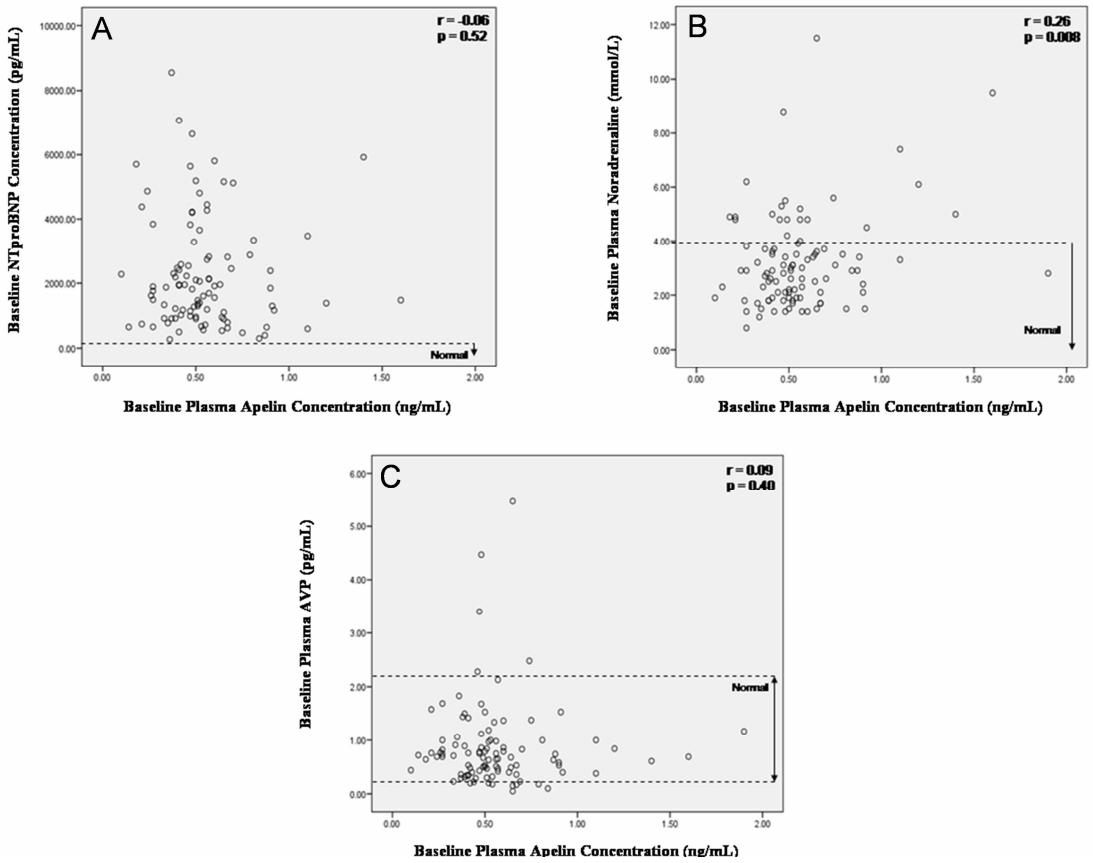

Figure 7.3 Scatter plots showing the relationships between apelin and baseline NT-proBNP (A), NA (B) and AVP (C). Spearman correlation coefficients are shown for each group. Normal reference ranges are depicted by dotted lines.

7.3.5 Treatment effect of eplerenone on plasma apelin concentration

Baseline plasma apelin concentration was similar within both treatment groups (0.58 ± 0.33 ng/ml in the placebo group vs. 0.54 ± 0.21 ng/ml in the eplerenone group, p=0.45). Over 24 weeks, plasma apelin concentrations were significantly higher in the eplerenone group than in the placebo group: the change in plasma apelin concentration was -0.01 ± 0.28 ng/ml in the placebo group and +0.17 ± 0.38 ng/ml in the eplerenone group (p=0.016).

7.4 Discussion

7.4.1 Plasma apelin and post-infarction LV remodelling

There are no published data on plasma apelin concentrations early after AMI in humans. This study has shown for the first time that plasma apelin concentration is reduced compared to healthy controls following AMI, and remains low out to 24 weeks. A small but significant increase over time in apelin concentration was observed, although there was no association between apelin and LV remodelling, quantified using ceCMR. The lack of any relationship between apelin and LV function, either acutely or remote from the infarct, is perhaps counter-intuitive. It is relevant, however, that very little adverse ventricular remodelling occurred in this well-treated group of patients: LVESVI remained virtually unchanged although LVEF improved significantly, by 4.1%, over the course of the study.

The source of circulating apelin in humans is unclear. While APJ receptor-like immunoreactivity (IR) and apelin-like IR have been demonstrated in endothelial and smooth muscle cells from major conduit vessels, the predominant source of plasma apelin is thought to be myocardial tissue, in particular the atria; plasma and atrial apelin levels are positively correlated.[319, 322] Changes in LV shape after large AMI and consequent elevated filling pressures may therefore be partly responsible for the reduced plasma apelin concentrations noted in this study. Rat myocytes, when subjected to mechanical stretch in culture, or chronic pressure overload in vivo, significantly down-regulate apelin mRNA synthesis.[316] In order to meet the criteria for recruitment, each patient in this study was required to have LVSD and thus had necessarily suffered an acute change in LV geometry. Such changes in LV geometry

may well suppress apelin synthesis via apelin mRNA down-regulation. Also, stretching and expansion of the infarcted segment and consequent morphological change in the non-infarcted ventricle is an ongoing process, and may contribute to the persisting depression in plasma apelin concentration at 24 weeks. In addition, animal models of CHF have shown that activation of RAAS hormones may promote the down-regulation of myocardial apelin levels seen in this syndrome.[323] Activation of the RAAS early after AMI may have contributed to the reduced plasma apelin levels in our patients, while powerful pharmacologic inhibition of the RAAS, which was undertaken in almost all of our study patients, may be partly responsible for the significant increase in apelin over time, although as the effects of such pharmacological interventions on the apelin-APJ system in humans are currently unknown, these views are purely speculative. It is noteworthy that measurement of plasma apelin provides no information on local activity of the peptide at tissue level; indeed the lower plasma concentrations seen in the AMI group may reflect an atherosclerosis-related effect not applicable to the control group. Additionally, there are no published data on apelin levels following AMI in patients with preserved LVEF. If my theory that change in LV geometry promotes down-regulation of cardiac apelin synthesis is correct, we might anticipate higher plasma apelin levels in patients with preserved LVEF compared to our cohort, although this view is speculative.

Evidence from a number of animal and human studies suggests that apelin possesses a range of properties that would be beneficial early after AMI. Apelin directly increases sarcomeric shortening and promotes enhanced contractility in cultured normal and failing rat myocytes.[314-316] Apelin knockout mice develop progressive LVSD over time.[324] Infusions of apelin into rats following experimental AMI promote

improvements in stroke volume in the context of unchanged LVEDV, suggesting an antiremodelling effect; similar inotropic effects are seen in healthy mice in response to apelin infusion.[170, 325] In humans with CHF, plasma apelin levels are reduced across the spectrum of disease severity, with weak but significant correlations to LVEF, right ventricular ejection fraction (RVEF) and peak VO_2, and interventions such as cardiac resynchronisation therapy and left ventricular assist device implantation promote improvements in LV function and increases in plasma apelin levels.[171, 172, 320]

These data suggest an inotropic role for apelin. Although I have shown that apelin did increase over time, plasma concentrations remained lower than in healthy controls remote from the infarction phase and bore no relation to LV function. The reasons for this are unclear but may relate to the lack of adverse remodelling seen in the study population and possible inter-species differences in myocardial response to apelin. In response to experimental AMI and systemic hypoxaemia in murine models, myocardial apelin expression is modestly increased, although this upregulation tends to diminish over time.[326] Apelin expression after experimental AMI is thus variable and possibly time-dependent. That plasma apelin was reduced compared to healthy controls when measured early after AMI may relate to such time-dependent expression, although we have no data on myocardial apelin expression in our patients. Plasma apelin has been demonstrated to be elevated in early/mild CHF but reduced in advanced symptomatic CHF; my findings, in a cohort of patients with coronary artery disease and LVSD, are consistent with these prior human studies.[171, 172]

NA is an important effector molecule of the sympathetic nervous system, and may play an important role in the pathogenesis of LV remodelling.[40, 41] I found significant

inverse correlations between NA and LVEF early and late after AMI. That apelin concentrations were greatest in those with the highest NA – i.e. those displaying the highest sympathetic stress response (likely to represent the most unwell) – suggests indirectly a possible protective role for apelin early after AMI, although this requires further investigation.

Eplerenone reduces cardiovascular morbidity and mortality in patients with LVSD and heart failure (or diabetes) early after AMI although the mechanism of this effect is unclear.[123] 94% of the patients in this study were treated with an ACE inhibitor, thus the majority of the patients in the eplerenone group were receiving two antagonists of the RAAS. Angiotensin II concentrations were not measured in this study, but it is feasible that aldosterone antagonism may have promoted feedback upregulation of angiotensin II production. An interaction exists between apelin and angiotensin II in animal models.[323] The elevated apelin concentrations seen after 24 weeks of eplerenone therapy compared to placebo in this study may reflect this interaction although, in the absence of angiotensin II concentrations, these opinions are speculative. That eplerenone therapy was associated with less remodelling and greater apelin concentrations over time, however, supports a potential protective effect of eplerenone following AMI.

I have therefore shown for the first time that plasma apelin concentration is reduced after AMI in patients with LVSD but without heart failure, and that although it increases over time, it remains low at 24 weeks. The plasma concentration of apelin has no relationship with LV volumes, mass or function and does not appear to influence remodelling although I cannot comment on local tissue effects of apelin. A

weak relationship exists between plasma concentrations of apelin and NA early after AMI which may suggest a possible inotropic role for apelin but further studies are required to investigate these findings. It is, however, unlikely that apelin will be of use as a prognostic marker after AMI.

7.4.2 Limitations

The lack of remodelling in this cohort of patients has already been mentioned. The parent study was powered for CMR end-points, but a larger population would allow adequate power to determine conclusively the role, if any, of apelin after AMI. Patients in the control group were age-matched but more frequently female (p=0.071) than the AMI patients. Only apelin was measured in controls; NTproBNP, NA and AVP were sampled in the AMI group alone. The apelin assay measured apelin-12, -13 and -16 but not other members of the apelin family (including apelin-17), which may have influenced the results. Finally, it is possible that apelin levels fluctuate after AMI; I collected samples once in the first few days then again at 24 weeks and therefore have no data on serial change in circulating apelin concentrations in the acute phase. I cannot therefore comment on any possible temporal variations during the sampling interval.

7.4.3 Conclusions

I have shown for the first time that plasma apelin concentration is reduced after AMI in patients with LVSD but without heart failure compared to healthy controls, and that although it increases over time, it remains low at 24 weeks. I have also shown for the first time in humans that eplerenone therapy was associated with higher circulating apelin levels. The plasma concentration of apelin has no relationship with LV

volumes, mass or function and does not appear to influence remodelling although I cannot comment on local tissue effects of apelin.

Chapter 8

A study characterising serum soluble ST2 concentrations after acute myocardial infarction in man

8.1 Introduction

Risk stratification of patients admitted with acute coronary syndromes is increasingly dependent on measurement of prognostically significant cardiac biomarkers, as discussed in Chapter 4. Serum ST2 may be such a biomarker. ST2 is a member of the interleukin-1 receptor (IL-1R) family and exists in two isoforms – ST2L and soluble ST2 (sST2) – as described in Chapter 1.9. sST2 is thought to function as a decoy receptor which neutralises IL-33, which has recently been demonstrated to be the ligand for this receptor.[210] A possible cardioprotective role for the IL-33/ST2 signalling pathway has been suggested by animal studies, and in human studies correlations have been found between sST2 and cardiac biomarker release (such as creatine kinase), NTproBNP and (pre-discharge) LVEF, as discussed in Chapter 1.9. These observations suggest that sST2 may be related to, or even play a role in, post-infarction LV remodelling but this had not previously been evaluated at the time of my research project.

The aims of this study were therefore to examine for the first time the relationships between serum sST2, parameters of LV function, and a variety of circulating mediators of pathophysiological significance in post-infarction remodelling in my trial cohort of 100 AMI patients with LVEF <40% but neither heart failure nor diabetes mellitus, using LGE-CMR as the imaging modality.

8.3 Methods

8.2.1 Study patients and trial protocol

All 100 patients enrolled in the primary study of this thesis (Chapter 3) were included in this study examining the role (if any) of sST2 in post-infarction remodelling. The screening, recruitment, randomisation process and trial outline are described in detail in Chapter 2 (2.1 – 2.3), and baseline demographic data of the study cohort are shown in Table 3.1.

Each patient underwent LGE-CMR imaging at baseline, 12 and 24 weeks; detailed methodological protocols and analytic techniques are described in Chapter 2 (2.5 – 2.6). Measurement of sST2 was performed at baseline (mean 3.7 [1.8] days after AMI) and at 12 and 24 weeks. In order to examine further the role (if any) of sST2 in post-infarction remodelling, I also assessed its relationships with NTproBNP, NA and aldosterone (each measured at the same three time-points), biomarkers related to remodelling as described in Chapter 1. Detailed methods of collection, storage and measurement of each of these biomarkers are described in Chapter 2.8. Finally, I assessed whether eplerenone affected sST2 concentrations over time.

8.2.2 Statistical methods

The relationships between sST2 and parameters of LV remodelling were examined, in addition to the correlations between ST2 and NTproBNP, aldosterone and NA. All baseline biomarker measurements were taken prior to randomisation, and serial biomarker data were initially analysed regardless of parent study treatment allocation, then by treatment group. Inter-group comparisons were made using paired sample *t*-

tests or Mann-Whitney-U tests as appropriate for continuous variables and Chi-squared test for categorical variables. Paired *t* tests were used to detect changes in ceCMR measurements and biomarkers within each treatment group over the 24 weeks of the study, and differences between these changes were analysed using an unpaired *t* test.

Non-normal data were logarithmic-transformed prior to analysis; Pearson's and Spearman's correlation coefficients were used for parametric and non-parametric variables respectively. Comparison of mean ceCMR parameters within tertiles of serum sST2 was then performed using separate Kruskal-Wallis tests. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

8.3 sST2 following AMI in man

8.3.1 Changes in sST2 concentration after AMI

Serum sST2 concentration, expressed as median [interquartile range], decreased significantly from 263.3 [139.4-491.5] pg/mL at baseline to 140.0 [83.0-196.2] pg/mL at 24 weeks ($p<0.001$). This decrease occurred between baseline and 12 weeks – from 12 to 24 weeks there was no significant change in serum sST2 concentration (Figure 8.1).

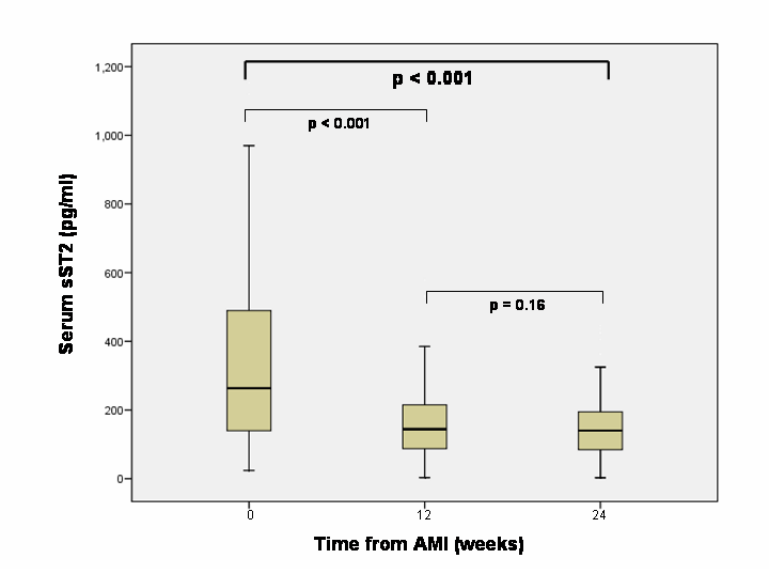

Figure 8.1 Boxplot depicting median serum sST2 concentrations sampled at baseline, 12 and 24 weeks after AMI. Boxes represent interquartile ranges.

8.3.2 sST2 concentrations and LV function after AMI

Serum sST2 concentrations were non-normally distributed and accordingly were logarithmic-transformed prior to analysis. Baseline sST2 correlated inversely with baseline LVEF (r = -0.30, p = 0.002) and baseline infarct volume index (r = 0.26, p = 0.005) but not with baseline LVESVI, LVEDVI or LVMI (Table 3). Baseline sST2 correlated significantly with 24-week LVEF (r = -0.23, p = 0.026) and infarct volume index (r = 0.22, p = 0.037) but not with 24-week LV volumes or LVMI. Baseline sST2 correlated inversely with change in (Δ) infarct volume index (r = -0.28, p = 0.009) but not with change in any other LGE-CMR parameter.

ΔsST2 from baseline to 24 weeks correlated significantly with ΔLVEDVI (r = -0.24, p = 0.023) and with Δinfarct volume index (r = 0.21, p = 0.043) but not with ΔLVESVI, ΔLVEF or ΔLVMI (Table 8.1).

Table 8.1 Spearman correlation coefficients for circulating biomarkers, sampled at baseline, and LGE-CMR parameters of LV remodeling. Units: LVESVI, LVEDVI, infarct volume index (Inf Vol): ml/m^2; LVEF: %; LVMI g/m^2 Key: *p<0.05 **p<0.01 †Change in each biomarker between baseline and 24 weeks

	Baseline log_{10} (serum sST2)		**Baseline log_{10} (NTproBNP)**		**Baseline log_{10} (noradrenaline)**		**Baseline log_{10} (aldosterone)**	
Baseline								
LVESVI	0.17		0.33**		0.17		0.25*	
LVEDVI	0.01		0.19		0.10		0.15	
LVEF	-0.30**		-0.39**		-0.22*		-0.31**	
LVMI	0.05		0.05		0.23*		0.22*	
Inf Volume	0.26**		0.26**		0.17		0.39**	
24 weeks								
LVESVI	0.17		0.43**		0.23*		0.34**	
LVEDVI	0.06		0.30**		0.09		0.18	
LVEF	-0.23**		-0.45**		-0.31**		-0.41**	
LVMI	0.03		0.02		0.16		0.22*	
Inf Volume	0.22*		0.33**		0.17		0.41**	
ΔceCMR parameter (0 – 24 weeks)		**ΔsST2†**		**ΔNTproBNP†**		**ΔNoradrenaline†**		**ΔAldosterone†**
ΔLVESVI	0.11	-0.13	0.26**	-0.14	0.10	0.08	0.28**	-0.23*
ΔLVEDVI	0.18	-0.24*	0.19	-0.06	0.01	-0.02	0.20	-0.33**
ΔLVEF	-0.01	0.01	-0.19	0.08	-0.15	-0.12	-0.21*	0.07
ΔLVMI	0.03	-0.01	-0.06	0.12	-0.19	0.07	-0.07	-0.07
ΔInf Volume	-0.28**	0.21*	-0.08	0.10	-0.14	0.15	-0.34**	0.19

The change over time in LV volumes, LVEF, LVMI and infarct volume index was compared according to tertiles of baseline serum sST2 (Figure 8.2). Compared to patients in the highest tertile of baseline serum sST2, those in the lowest tertile displayed a significantly lower ΔLVEDVI and less reduction in infarct volume index over time; there was a trend towards a lower ΔLVESVI but this was not significant. There were no significant inter-tertile differences in ΔLVEF or ΔLVMI.

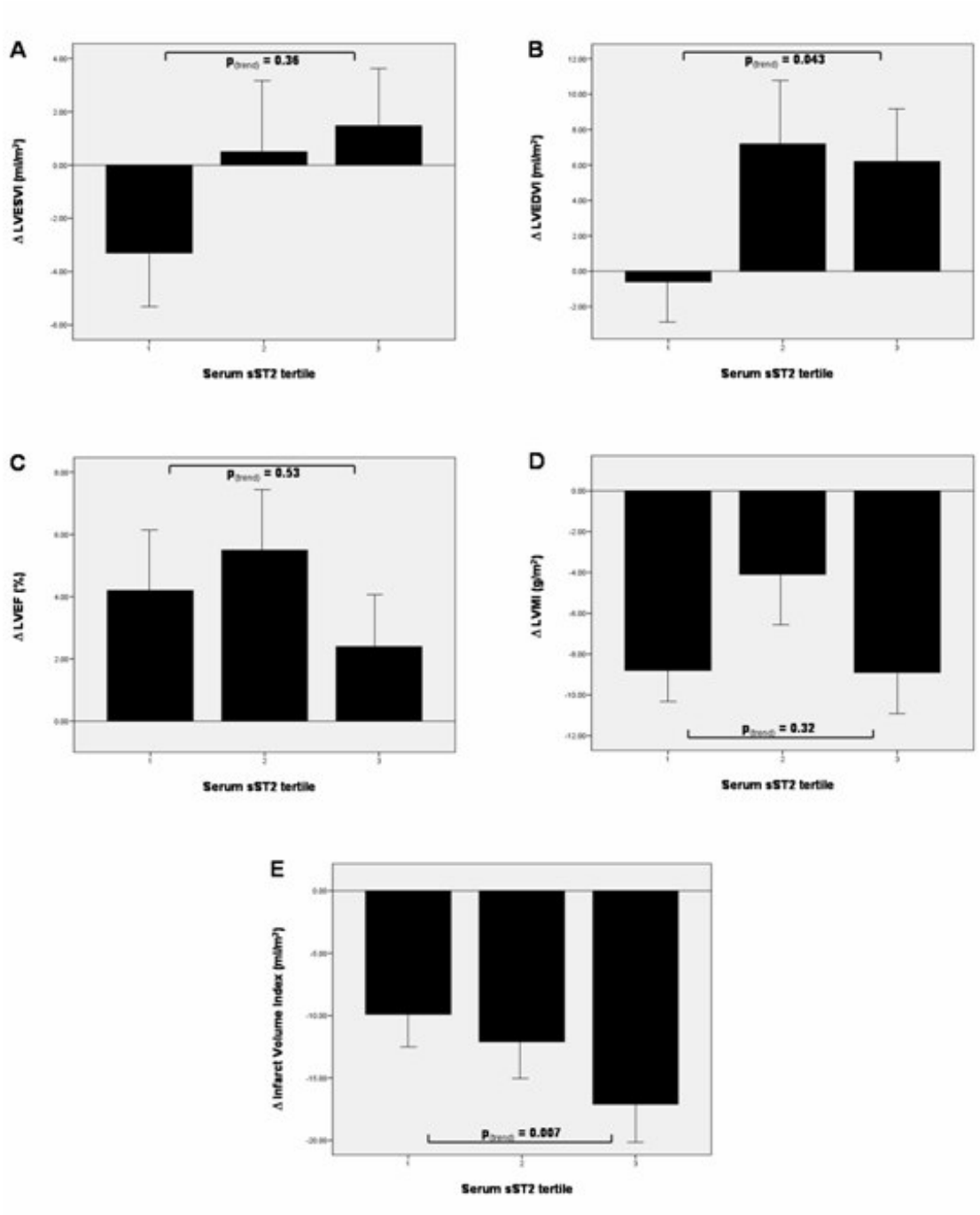

Figure 8.2 Comparison of change in LGE-CMR parameters between 0 and 24 weeks according to tertiles of baseline serum sST2: (A) ΔLVESVI (B) ΔLVEDVI, (C) ΔLVEF, (D) ΔLVMI, (E) ΔInfarct volume index. [Serum sST2 tertile concentration ranges: tertile 1 – 0-174.4 pg/ml; tertile 2 – 174.41-372.3 pg/ml; tertile 3 – ≥372.3 pg/ml]

8.3.3 sST2 concentrations and infarct characteristics after AMI

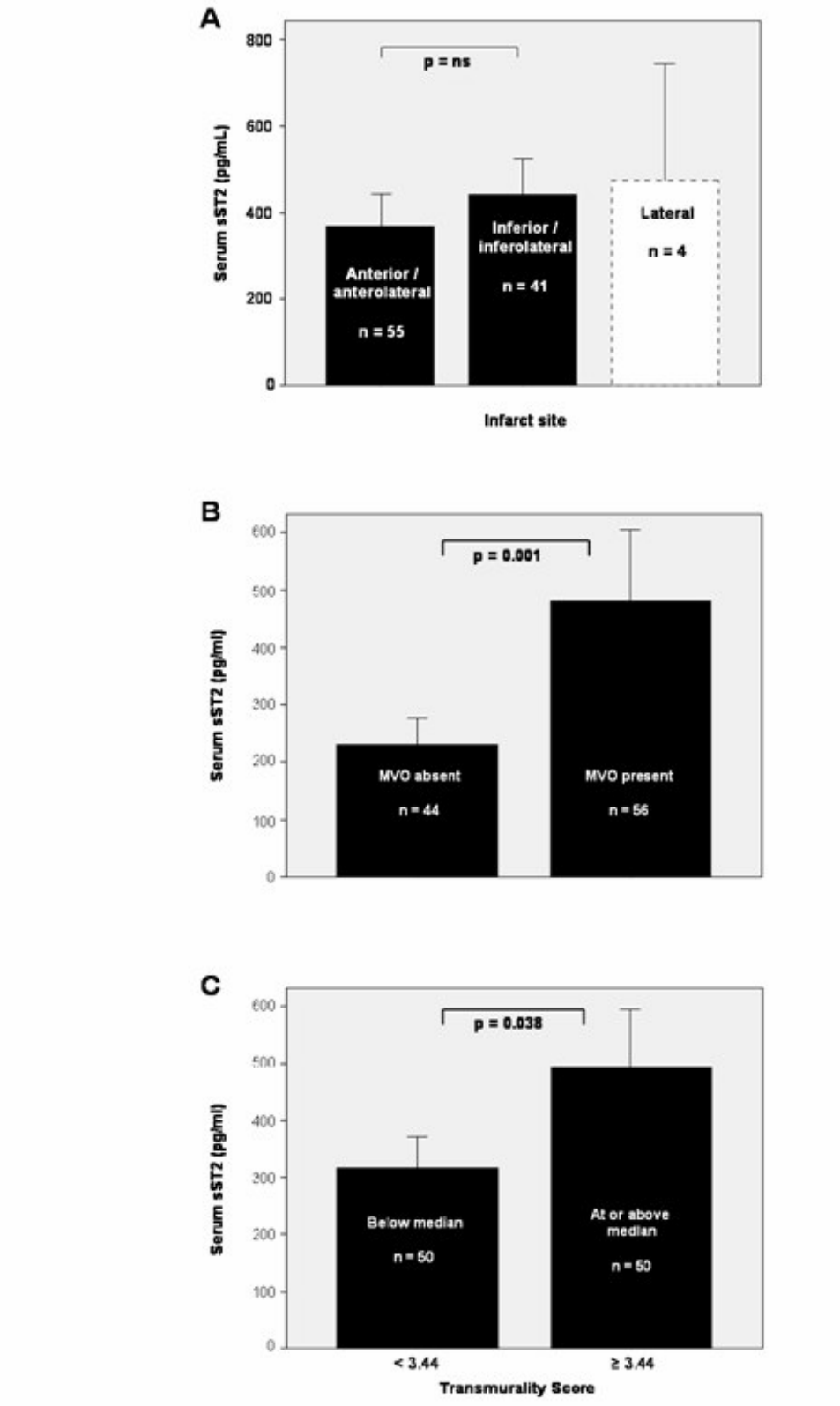

Figure 8.3 Comparison of mean serum sST2 concentrations according to (A) LGE-CMR-measured anatomical infarct site (note: lateral infarct site not used in comparative analysis with other infarct sites as n = 4), (B) presence of late microvascular obstruction, (C) median transmurality score. Error bars indicate SEM. Median transmurality score in (C) was 3.44

Baseline infarct characteristics are shown in Table 6.1. There were no significant differences in serum sST2 between anterior/anterolateral (n = 55, mean 367.4 [335.3] pg/mL), inferior/inferolateral (n = 41, mean 441.9 [372.2] pg/mL) and lateral (n = 4, mean 474.5 [638.0] pg/mL) sites of infarction (Figure 8.3 A). Serum sST2 correlated significantly with the endocardial extent of the infarct (r = 0.26, p = 0.008). The presence of late MVO was associated with a significantly higher serum sST2 (520.1 [512.5] vs. 253.0 [206.9] pg/mL without MVO, p=0.001) – Figure 8.3 B. Serum sST2 was significantly higher in patients with a transmurality score at or above the median compared to those below the median (493.2 [537.4] vs. 316.4 [261.1] pg/mL, p=0.038) – Figure 8.3 C

8.3.4 sST2 and neurohormones after AMI

The relationships between (log-transformed) plasma NTproBNP, NA and aldosterone at baseline and parameters of LV function and infarct volume over time are shown in Table 8.1. Baseline sST2 correlated significantly with baseline NA (r = 0.21, p = 0.034) and aldosterone (r = 0.28, p = 0.006) but not with NTproBNP (r = 0.14, p = 0.15) – Figure 8.4. Baseline sST2 also correlated with ΔNA (r = -0.25, p = 0.014) but not with Δaldosterone or ΔNTproBNP.

ΔsST2 correlated significantly with Δaldosterone (r = 0.26, p = 0.012) and ΔNA (r = 0.23, p = 0.024) but not with ΔNTproBNP (r = 0.15, p = 0.14).

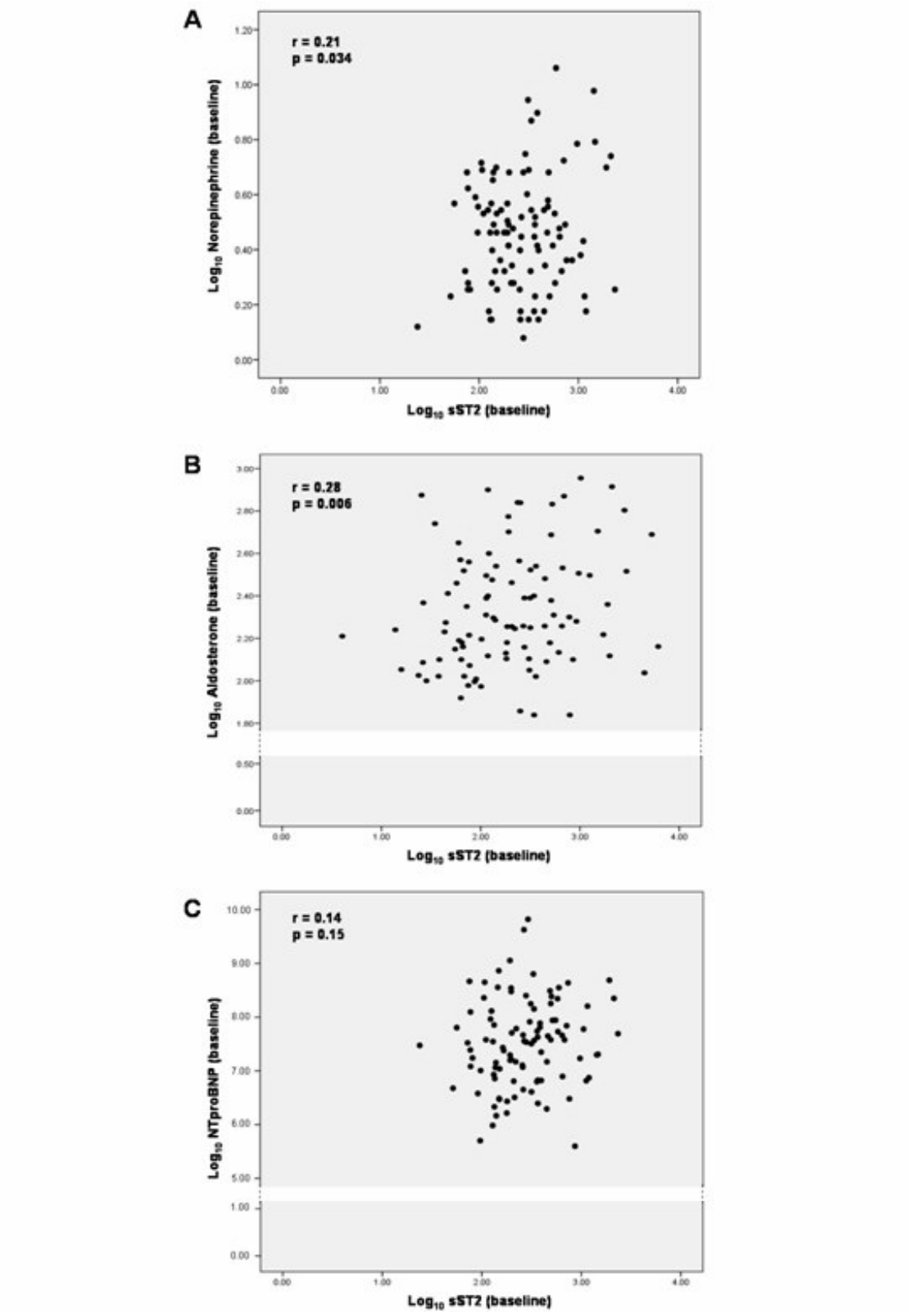

Figure 8.4 Scatterplots depicting the relationship of baseline (logarithmic-transformed) serum sST2 with baseline noradrenaline (A), aldosterone (B) and NTproBNP (C). Correlation coefficients and significance values are shown.

8.3.5 Treatment effect of eplerenone on sST2

Eplerenone, the study drug in the primary trial on which this thesis is based, had no significant effect on serum sST2 concentration over time. Baseline serum sST2 concentration, expressed as mean ± SD, was similar within both randomisation groups (394.3 ± 322.6 pg/mL in the placebo group vs. 411.8 ± 514.0 pg/mL in the eplerenone group, p = 0.84). Over 24 weeks, the change in serum sST2 concentration was -251.7 ± 323.9 pg/mL in the placebo group and -208.1 ± 448.0 pg/mL in the eplerenone group (p = 0.59).

8.4 Discussion

8.4.1 Serum sST2 and LV function after AMI

In this study, I not only confirmed that there is a relationship between sST2 and LV function early after AMI, but also showed that sST2 is associated with medium-term LV function, and additionally that sST2 is related to infarct magnitude and infarct remodelling over time. I also provided novel data on potential interactions between sST2, aldosterone and noradrenaline, and suggest that sST2 may play a pathophysiological role in post-infarction remodelling.

Both sST2 isoforms – ST2L and sST2 – are transcriptionally induced by the application of biomechanical strain to rodent cardiomyocytes and cardiac fibroblasts *in vitro*.[209, 212] Not only is sST2 elevated *in vivo* following experimental AMI in mice, it has also been shown to be elevated in the serum of human subjects following AMI, in acute and chronic heart failure.[209, 211-216] When measured early after AMI, significant associations have been found between sST2, higher creatine kinase concentrations and lower pre-discharge LVEF.[209, 214] Moreover, baseline sST2 predicts 30-day occurrence of heart failure or death following STEMI and, when combined with NTproBNP, significantly improves risk stratification in this condition.[213, 214] sST2 is also of prognostic benefit in both acute and chronic heart failure, in which it is associated with poorer LV function and higher NYHA class.[211, 215, 216] Prior to this study, the relationship between sST2 and *serial* change in LV function following AMI had not been characterised. Using a gold-standard technology for LV functional assessment, LGE-CMR, which additionally affords detailed infarct

examination, I report for the first time on the relationship between sST2, LV remodelling and infarct characteristics following AMI.

In the study cohort, all of whom were required to have depressed LVEF, I found a significant correlation between sST2 (measured in the first few days after AMI) and pre-discharge LVEF, in keeping with recent clinical studies. In the largest published study examining sST2 in AMI, a correlation (albeit weak) was found between sST2 and lower LVEF early after STEMI in 551 patients enrolled in the CLARITY-TIMI 28 trial.[214] A similar relationship was seen in 69 predominantly STEMI patients enrolled in the HEART study.[209] My findings, that baseline sST2 correlates with medium-term (24-week) LVEF, and moreover that greater adverse LV remodelling was seen in patients with the highest baseline sST2, are novel. Whether sST2 has a pathophysiological role in post-infarction remodelling or whether it simply identifies a population with poorer LV function who would be more likely to remodel, is unclear; data from infarct characteristics and biomarker analysis provide further insights.

I assessed the relationship between sST2, baseline infarct characteristics and infarct evolution over time. Although I did not find any relationship with infarct location, I have shown that sST2, measured early after AMI, was associated with greater baseline infarct volume, correlated with the endocardial extent of infarction and was higher in patients with greater infarct transmurality, all parameters of large infarction. I also found that sST2 was higher in patients displaying MVO, which is known to be associated with greater baseline LV volumes, more significant remodelling and adverse cardiovascular outcome after AMI.[254, 255] Over time, I found a correlation

between sST2 and 24-week infarct volume, and an inverse relationship with Δ infarct volume which occurs as peri-infarct oedema resolves and the infarcted myocardium remodels.[309] These data suggest not only a link between the magnitude of infarcted myocardium and serum sST2 release, but also point towards a possible role for sST2 in infarct healing/scar formation.

A role for serum sST2 in ventricular (and infarct) remodelling is further suggested by its observed correlation with plasma aldosterone. Aldosterone, as described previously in this thesis, has strong pro-fibrotic effects on the heart – both reparative fibrosis in the infarct zone and reactive fibrosis in non-infarct myocardium – some of which are thought to be mediated by mineralocorticoid receptor activation on cardiac fibroblasts.[84, 101] Aldosterone antagonism can attenuate or even reverse myocardial fibrosis, with concomitant improvement in LV function.[106, 107] Significant improvements in cardiovascular outcomes have been demonstrated using aldosterone antagonists in survivors of AMI with resultant LVSD and heart failure (or diabetes mellitus) and in advanced CHF.[121, 123] Statistically significant associations between plasma aldosterone and parameters of LV remodelling were found in my cohort.

The mechanism of a possible relationship between sST2 and circulating aldosterone is unclear. That significant interplay occurs between cytokines and other systems integral to LV remodelling, including the RAAS, is well-documented.[81, 207] IL-33, recently demonstrated to be the ligand for serum sST2, antagonises angiotensin II- and phenylephrine-induced hypertrophy in cardiomyocytes; sST2 blocks these effects.[210, 212] Serum sST2 may therefore function as a soluble decoy receptor for IL-33, and it appears likely that the IL-33:sST2 *ratio* determines IL-33-mediated

signalling.[212] This theory may be relevant to the observed correlation between sST2 and aldosterone seen in this study – as sST2 rises, it may disinhibit the effects of angiotensin II on cardiomyocytes by reducing the concentration of free, unbound IL-33, resulting in cardiomyocyte hypertrophy, greater aldosterone biosynthesis and more significant remodelling, although in the absence of simultaneous IL-33 measurement these views are purely speculative. That change in sST2 over time correlated inversely with ΔLVEDVI is surprising, but may simply reflect my earlier findings – that although sST2 falls over time, those with the highest sST2 at baseline are more likely to remodel adversely and thus undergo progressive ventricular dilatation.

I found that a higher sST2 was associated with higher baseline noradrenaline and a greater reduction in noradrenaline over time. A similar relationship between sST2 and noradrenaline has been reported in a cohort of patients with advanced CHF.[215] Noradrenaline is a surrogate marker of neurohormonal activation and can stimulate the release of pro-inflammatory cytokines.[42] I was unable to assess whether noradrenaline has any effect on the IL-33:sST2 ratio in this study; no published studies on IL-33 to date have analysed the effects of noradrenaline on its release. It is theoretically possible, however, that a significantly elevated noradrenaline may influence the balance between sST2 and its ligand, perhaps reducing the concentration of unbound IL-33 and diminishing its protective effects on cardiomyocytes. Noradrenaline was associated with increasing LV volumes and poorer LV function over the course of our study.

I did not find any correlation between sST2 and NTproBNP. While I acknowledge that, in a more appropriately powered study, an association was found between sST2 and natriuretic peptides – a significant correlation between sST2 and NTproBNP was observed in more than 1200 patients following STEMI in the CLARITY-TIMI 28 trial – this correlation was weak, and indeed no significant correlation was observed between sST2 and BNP in a smaller study of 810 STEMI patients.[213, 214] sST2 has been shown to correlate with natriuretic peptides in acute and chronic heart failure although again these associations were weak.[215, 216] Rather than replacing natriuretic peptides, perhaps the most promising future role for sST2 following AMI and in heart failure is the enhancement of risk stratification when combined with natriuretic peptides.[214, 216]

That median baseline sST2 in the study cohort (263 pg/mL) was higher than that seen in two recent studies of AMI (80 pg/mL and 235 pg/mL) but lower than in several recent AHF studies (490, 500 and 570 pg/mL) may relate to inter-study variation in timing of sST2 sampling and analysis kit used.[211, 213, 214, 216, 327] However, unlike in the quoted AMI trials, each patient in this study was required to have LVSD, which presumably contributed to the higher sST2 concentrations given the correlation between sST2 and LVEF, and reinforces my (and others') findings that sST2 and LV function are related following AMI.

8.4.2 Limitations

The above findings with respect to sST2 must be considered in light of some important limitations. The parent study was powered for CMR end-points, hence the relatively small sample size (n=100). Where relevant, findings from more

appropriately powered studies examining sST2 in AMI have been acknowledged. These results pertain only to patients with LVSD after AMI, as all patients were required to have a reduced LVEF prior to study inclusion. Serum sST2 was sampled only once in the early post-infarction phase, at a mean 46 hours after AMI. The precise temporal profile of serum sST2 concentration following AMI in humans has yet to be characterised, but data from the CLARITY-TIMI 28 trial show a small but significant decrease in sST2 between admission with STEMI and angiography 96 hours later.[214] I cannot assume that I measured "peak" sST2 following AMI, merely a "pre-discharge" serum concentration. I did not simultaneously measure IL-33 and thus cannot comment on the IL-33:sST2 ratio, but this is a clear focus for future research in both AMI and heart failure. Finally, serum sST2 concentrations are elevated in non-cardiac conditions including asthma, trauma, sepsis, malignancy and autoimmune disease; while I did not adjust for these conditions, they are only applicable to a very small number of the trial cohort and would thus be unlikely to influence the results.[328]

8.4.3 Conclusions

I have shown that measurement of sST2 early after AMI assists in the prediction of medium-term LV functional recovery following AMI, and that direct relationships exist between sST2, infarct magnitude and infarct remodelling. I also, for the first time, report on a relationship between sST2 and circulating aldosterone, and suggest that the IL-33:sST2 signalling system and the RAAS may be interlinked, raising the possibility of a direct role for the IL-33:sST2 system in the pathogenesis of post-infarction remodeling. Further studies to examine in more detail the potential role of this system are warranted, as it may not only serve as a prognostic marker but also provide a therapeutic target in conditions of myocardial injury and/or strain.

Chapter 9

Additional intracardiac and extracardiac abnormalities detected by pre-discharge ceCMR imaging following acute myocardial infarction

9.1 Introduction

Following AMI, 25 to 60% of patients will have sustained sufficient myocardial damage to cause LVSD, thus it is predictable that European and American guidelines recommend that all AMI patients should undergo a formal evaluation of LV function, ideally pre-discharge.[11, 127, 128] TTE is the most commonly used imaging modality for this purpose. However, TTE carries significant inter- and intra-observer variability that limit its applicability, and for a variety of reasons CMR is now considered the gold-standard means of assessment of LV volumes and ejection fraction, as discussed in Chapter 1.11. CMR also allows imaging of the thorax and upper abdominal viscera, and through the use of contrast additionally also allows assessment of myocardial viability and perfusion (LGE-CMR).

The aim of this observational study was to assess the influence of pre-discharge LGE-CMR imaging on patient management in the 100 patients admitted with AMI with resultant LVSD that constitute the patient cohort for the studies on which this thesis is based. Each patient had undergone unenhanced TTE prior to LGE-CMR, i.e. each patient had had an assessment of cardiac function as occurs in ‘standard’ post-MI management.

9.2 Methods

9.2.1 Patients and imaging protocols

All 100 patients enrolled in the primary study of this thesis (Chapter 3) were included in this observational study. Screening, recruitment and the trial outline are described in detail in Chapter 2 (2.1 – 2.3), and baseline demographic data are shown in Table 3.1.

Each patient underwent a single screening TTE as per trial protocol, although a minority underwent repeat TTE imaging for clinical indications – these are detailed below where relevant. LGE-CMR imaging took place at baseline, 12 and 24 weeks. Details of the imaging techniques and analysis are provided in Chapter 2 (2.4 – 2.6).

9.2.2 Statistical analysis

All discrete variables are expressed as percentages within the study population. Continuous variables are expressed as means with standard deviations. All statistical analysis was performed using SPSS version 15.0

9.3 Additional findings detected by ceCMR

1.4.1 Intracardiac abnormalities

The addition of LGE-CMR as part of the pre-discharge investigations in patients admitted with AMI in the study cohort afforded the detection of a variety of additional abnormalities, which have been divided into ‘intracardiac’ and ‘extracardiac’.

LV thrombus

Thrombus within the LV apex was detected or strongly suspected on screening TTE in 5/100 patients (5%). Using LGE-CMR, LV apical thrombus was definitely present in 15/100 (15%). All cases with LV thrombus had presented with anterior AMI; within this subgroup (of 55 patients) the incidence of LV thrombus was 27.3%. TTE was deliberately repeated after LGE-CMR in 8 of the 10 patients who were "TTE negative, LGE-CMR positive" for LV thrombus (2 were transferred back to the referring hospital before repeat TTE could be performed): in 3 out of 8 the thrombus had become apparent on unenhanced TTE but in the remaining 5 there was still no echocardiographic evidence of thrombus (note: echocardiographic contrast agents were not used). All patients with LV thrombus were formally anticoagulated with warfarin for a period of time decided by the admitting cardiology team. In no case was there any evidence of thromboembolism during the 6 month follow up period for each patient. An extreme example of a LV thrombus developing late during the admission is shown in Figure 9.1.

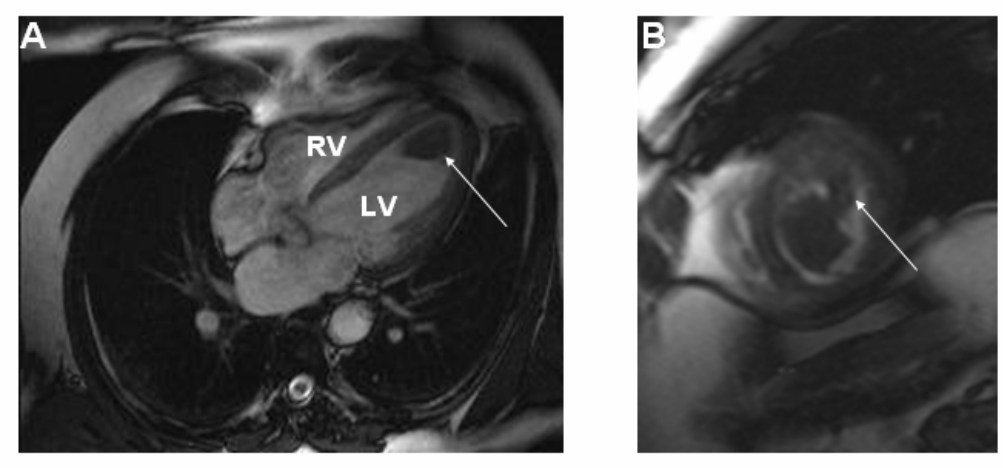

Figure 9.1 CMR scan performed 4 days after anterior AMI displaying LV apical thrombus. (A) Horizontal long-axis view showing extensive apical thrombus formation (arrow) within LV cavity. (B) Short-axis view through LV apex in Figure 28A reveals a thin pedicle (arrow) by which the thrombus inserts onto the endocardial aspect of the anteroapical wall.

Right ventricular infarction

Right ventricular infarction (RVI) was detected on TTE in 10 patients (10%), all of whom had been admitted with inferior STEMI (n=45); the incidence within this group was therefore 22.2%. In only 4 cases was RVI clinically apparent (raised jugular venous pulse, hypotension, oliguria with no evidence of pulmonary rales). LGE-CMR confirmed RVI (defined as RV regional hypokinesis with congruent delayed hyper-enhancement after gadolinium DTPA administration [Figure 9.2]) in these 10 patients but also detected RVI in an additional 11 patients, thus overall 21% of the study population suffered RVI (19/45 [42.2%] inferior STEMI, 2/55 [3.6%] anterior AMI). In 6 of these 11 additional patients with significant RVI on LGE-CMR, loop diuretics (which had been commenced due to relative oliguria in the context of echocardiographic LVSD by the ward staff) were with-held following the LGE-CMR findings of both RVSD and LVSD. Cautious volume loading with intravenous crystalloids was used successfully in 4 of these 6 patients to improve urine output without precipitating clinical heart failure.

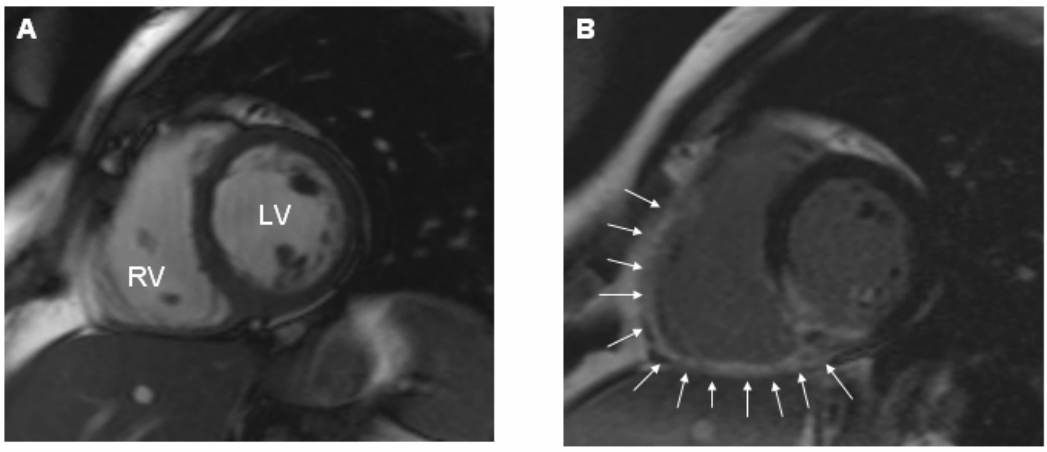

Figure 9.2 Right ventricular infarction. (A) CMR short-axis view of a patient admitted with an inferior STEMI. The RV is slightly dilated on this still image, while the cine showed significant RV dysfunction in addition to inferoseptal hypokinesia of the LV. (B) Delayed-enhancement CMR image (slice position copied from 29A) 20 minutes after gadolinium-DTPA injection. LV inferoseptal infarct visible (with involvement of the posteromedial papillary muscle); extensive late contrast uptake within the RV (arrows) consistent with RV infarction.

Intracardiac mass

An intracardiac mass was detected in one patient, a 67-year-old female admitted with an inferoposterolateral STEMI on ECG in whom screening TTE was rendered difficult by both kyphoscoliosis and a minor anterior chest wall deformity. Apical views were acceptable but parasternal views obscured. LGE-CMR revealed not only extensive infarction of the lateral wall of the LV and both papillary muscles (Figure 9.3 A) but also identified a small mass within the left atrium, adherent to the interatrial septum (Figure 9.3 B) that had not been detected on TTE. Subsequent investigation by transoesophageal echocardiography confirmed the presence of a small atrial myxoma (2.8 x 2.0 cm) which was ultimately surgically resected 6 weeks after the AMI.

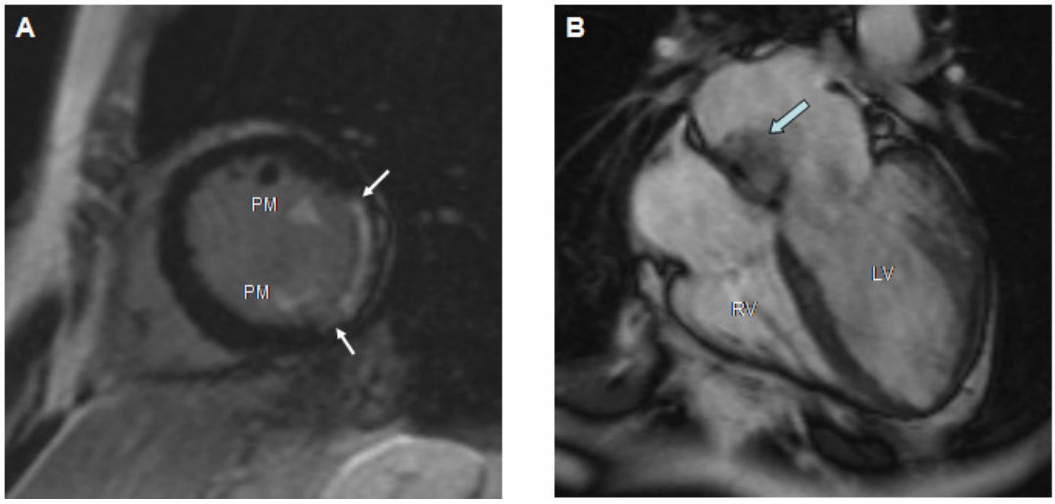

Figure 9.3 Left atrial myxoma. (A) CMR short-axis slice at mid-ventricular level, 20 minutes after gadolinium-DTPA injection, of a 67-year-old female patient presenting with an inferolateral STEMI. Obvious infarction of the lateral wall of the LV (arrows), and both papillary muscles (PM) visible. (B) CMR horizontal long-axis view of same patient showing mass adherent to the interatrial septum, confirmed after excision as a benign myxoma (arrow).

9.3.2 Extracardiac abnormalities

The thorax and upper abdomen are necessarily imaged during CMR scanning. In 5 patients (5% of the cohort) abnormalities of varying clinical relevance were detected on CMR. These included: mesothelioma in a 69-year-old smoker whose chest X-ray had shown probable consolidation at the right lung base, but respiratory opinion and subsequent diagnosis were prompted by the abnormal CMR appearances (Figure 9.4 A); prominent bullae within the lung fields of a 48-year-old non-smoker (Figure 9.4 B,C) with subsequent diagnosis of mild airflow obstruction on spirometry and a low-normal alpha-1-antitrypsin level of 102 mg/ml (normal range 100-300 mg/ml); a pancreatic pseudocyst in a 50-year-old man with chronic pancreatitis but no previous pseudocyst, who described weight loss and early satiety prior to his admission with AMI (Figure 9.4 D,E); and asymptomatic renal cysts in two elderly patients whose management was not directly influenced by this discovery.

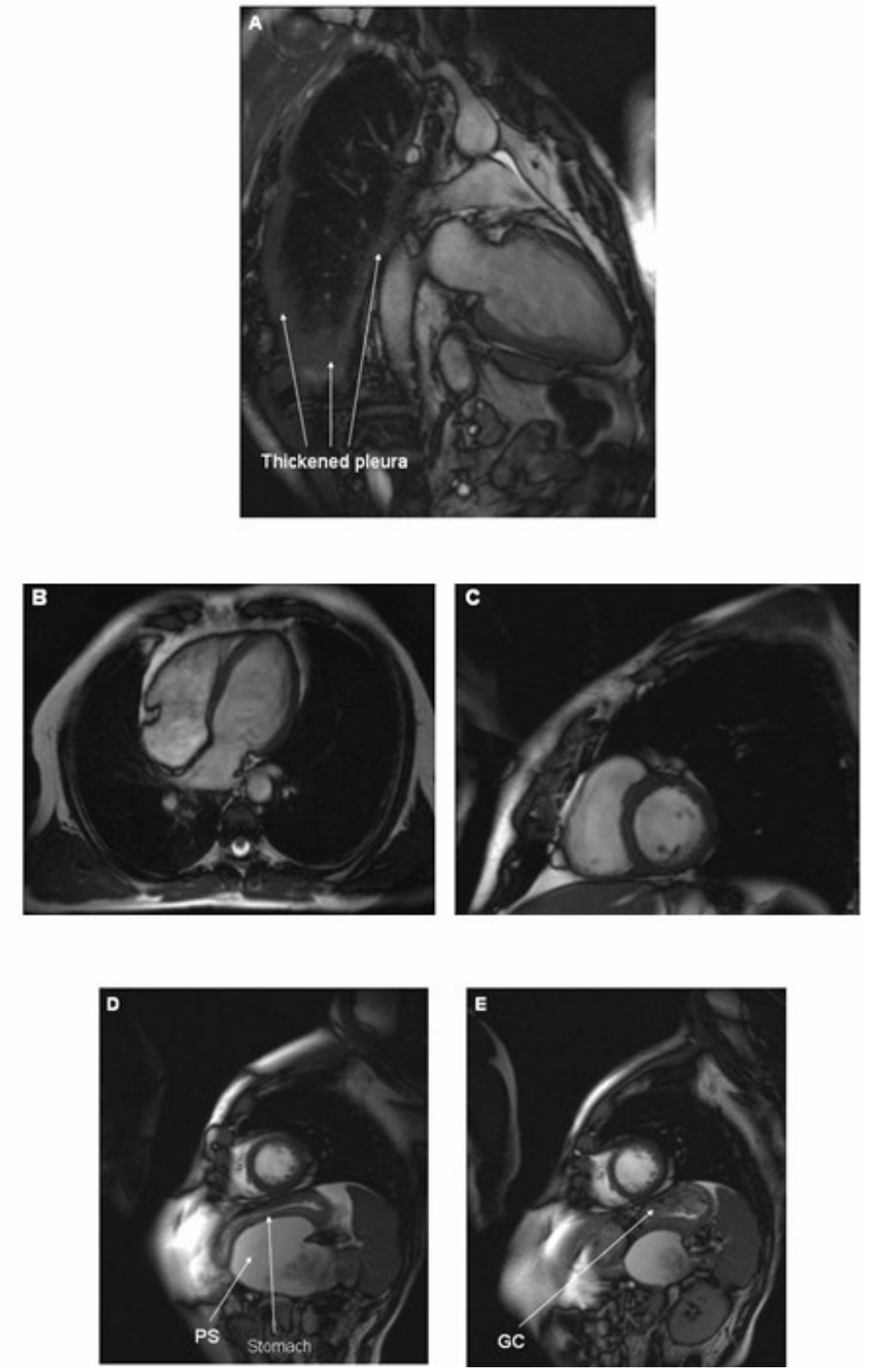

Figure 9.4 Extracardiac abnormalities. (A) Oblique vertical long-axis CMR view of a 69-year-old patient admitted with an inferior STEMI. Admission chest X-ray suggested consolidation at the right lung base, but CMR suggests diffuse pleural thickening consistent with mesothelioma (arrows). (B) Horizontal and (C) short-axis CMR views of a 48-year-old non-smoker admitted with an anterior NSTEMI. Bullous hyper-expansion of both lung fields apparent (more marked on the left), with medial displacement of the mediastinum and its contents. (D) CMR short-axis view of 50-year-old patient with chronic pancreatitis, admitted with an inferior STEMI. A large pancreatic pseudocyst (PS) is present, visibly compressing the stomach. (E) After 3 months of conservative therapy, there is a marked reduction in the size of the PS, with less compression of the stomach and visible gastric contents (GC).

9.4 Discussion

9.4.1 The benefits of pre-discharge ceCMR imaging following AMI

All patients should undergo assessment of ventricular function following AMI. I compared standard practice – unenhanced TTE – with standard practice plus pre-discharge CMR imaging in 100 patients with extensive AMI and resultant LVSD. The clinical management of 19% of the cohort (ie. the combined number of patients with LV thrombus absent on initial TTE, RVI on LGE-CMR resulting in with-holding of diuretic and/or commencement of intravenous fluids, and undiagnosed atrial myxoma, emphysema and mesothelioma) was directly influenced by the LGE-CMR findings. An additional 5% were found to have sub-clinical RVI on LGE-CMR that did not directly influence *acute* management but which led to a more cautious approach to up-titration of ACE inhibitors and β blockers, thus in almost one-quarter of this post-AMI population the LGE-CMR scan added significant therapeutic and prognostic information that would have been unavailable if the "standard care" approach had been taken.

Studies from the pre- and early reperfusion eras reported that LV thrombus occurred in 15-56% of anterior AMIs, while contemporary studies suggest a lower overall incidence in general (c. 4%), skewed towards the anterior AMI subgroup within which the incidence approaches 10%.[329-334] I found that the overall incidence of LV thrombus in AMI patients with LVSD was 15% in this study cohort. Within the subgroup of patients with anterior AMI and LVSD the incidence was 27.3%. More than half of LV thrombi undetected on initial TTE remained undetected on repeat TTE. While there has been debate as to the actual incidence of embolic events in

cases of LV thrombus after AMI, its detection is of relevance as it is associated with poorer LV function, adverse remodelling and greater mortality.[333-336] Our departmental policy was to anticoagulate formally all patients with identified LV thrombus; in all cases the thrombus had resolved by the 3 month follow-up scan, and in no case was there evidence of an embolic event during the 6 month follow-up. I therefore suggest that LV thrombus formation remains common after anterior AMI, particularly in the context of LVSD, and should be excluded by *serial* TTE and, in cases of uncertainty, LGE-CMR.

RVI complicates up to 50% of inferior AMI yet remains subclinical in the majority.[337] RVI, whether clinically apparent or not, remains a significant independent risk factor for increased mortality after AMI, even following primary PCI.[338] Many imaging methods struggle to visualise the RV, including TTE. LGE-CMR not only allows formal qualitative and quantitative assessment of RV function, but also allows accurate identification and quantification of RV infarction.[226, 233, 234] Identification of RV dysfunction in patients with LV infarction aids risk stratification and targeted secondary preventive therapy, while in the acute phase knowledge of RVI may assist in the management of these high-risk patients.[337] I found that TTE failed to detect more than 50% of those patients with RVI on LGE-CMR.

One patient in the study cohort had an atrial myxoma which was only detected on CMR. I acknowledge that the overall incidence of primary intracardiac tumours is low (incidence of 0.02% on post-mortem studies) thus routine CMR imaging for the detection of such neoplasms is not justified.[339] I simply report the incidental detection of an intracardiac myxoma in this observational retrospective report.

The wider imaging field of view afforded by CMR subtends further benefits over TTE. I detected extracardiac abnormalities in 5% of our cohort, of which 3/5 (60%) were clinically significant and led to targeted investigation and appropriate referral. These data are similar to the non-cardiac abnormal findings in a recent series of patients with suspected coronary artery disease, undergoing CMR (2% of 108 patients had 'significant' non-cardiac abnormalities, defined as requiring further clinical or radiological follow-up, with 'non-significant' non-cardiac abnormalities detected in a further 6%).[340] Cardiologists in training in the field of non-invasive imaging need pay particular attention to the non-cardiac abnormalities that modalities such as CMR and computed tomography will reveal, from both a medical and legal standpoint. Multidisciplinary reporting sessions involving cardiologists and experienced radiologists are to be encouraged.

9.4.2 Limitations

The predominant limitation in this study is the time delay between TTE and CMR. The primary study of this thesis (Chapter 3) mandated that the screening TTE be performed prior to randomisation but did not stipulate that repeat TTE be performed on the same day as the CMR. This would have allowed more direct comparison of the two separate imaging techniques in terms of volumetric and functional measurements, and additional abnormalities detected. However, the purpose of this report is to highlight the overall benefit to patient care of adding a pre-discharge LGE-CMR rather than to compare directly TTE and LGE-CMR.

9.4.3 Conclusions

Through the detection of both intra- and extra-cardiac abnormalities, LGE-CMR significantly enhanced the management of 24% of patients with AMI and LVSD. In no case was patient safety compromised by transfer from the coronary care unit to the CMR department early after AMI. With faster, more refined imaging sequences, and increasing availability of CMR, greater numbers of AMI patients will be able to undergo CMR scanning pre-discharge. I suggest, based on this cohort of patients, that a solitary TTE performed in the first few days after AMI is insufficient for formal cardiac assessment following AMI, and that at the very least a repeat TTE should be performed prior to discharge; where possible LGE-CMR should be used as an adjunct to routine post-AMI care.

Chapter 10

General discussion and conclusions

10 General discussion

10.1 Eplerenone – an anti-remodelling agent?

Aldosterone has a number of detrimental effects on the cardiovascular system in general, demonstrated directly through experimental *in vitro* and *in vivo* (predominantly animal) studies and indirectly through the beneficial effects of aldosterone antagonism in humans with hypertension, AMI or CHF. The specific role of circulating aldosterone in LV remodelling following AMI is unclear, however, and recent trial data have been inconsistent. The primary aim of this thesis was to determine the effect of selective mineralocorticoid receptor blockade with eplerenone compared to placebo on LV remodelling over 24 weeks in a cohort of patients admitted with extensive AMI resulting in LVSD but without clinical or radiological heart failure (or diabetes mellitus) using LGE-CMR, the gold standard imaging modality for assessment of LV function. In comparison to EPHESUS, I found that eplerenone continued to exert a treatment effect in a less unwell population with a greater revascularisation rate and higher uptake of ACE inhibitors/ARBs and beta blockers (although statistical adjustment of the primary results was necessary); the greater clinical stability of the patients in this study allowed earlier commencement of eplerenone (4.8 versus 7.3 days in EPHESUS) which was safe and well-tolerated. These CMR-based results were supported by a covariate-adjusted treatment effect of eplerenone on MMP-2 and MMP-9, enzymes of critical importance in ECM turnover and matrix remodelling. This is, to the best of my knowledge, the first time that eplerenone has been shown to exert an anti-remodelling effect in 'asymptomatic' LVSD after AMI.

Although eplerenone confers significant reductions in cardiovascular morbidity and mortality upon EPHESUS-like patients, its prescription in such patients at the time of writing this thesis has been surprisingly low in the West of Scotland, and indeed worldwide. Concerns over the safety of dual inhibition of the RAAS early after AMI, and cost issues, compounded by a general impression that spironolactone (an off-patent and thus substantially cheaper drug) would be just as effective in AMI patients with reduced LVEF and heart failure, have contributed to this under-prescription.

The primary results of this thesis, as detailed in Chapter 3, require to be confirmed in larger studies powered to detect an improvement in clinical outcomes in 'asymptomatic' LVSD after AMI prior to the possible use of eplerenone in a broader patient population than that enrolled in EPHESUS, i.e. irrespective of heart failure status or diabetes. These results, in which the addition of eplerenone on day ~5 after AMI attenuated remodelling to an extent, taken together with previous data confirming the safety and anti-remodelling efficacy of aldosterone blockade (with spironolactone) on day 1 after AMI, suggest that aldosterone antagonism should be instituted as early as possible after AMI in order to maximise its anti-remodelling potential.[94] While these data provide information that would be of use in the design of a large-scale clinical study assessing eplerenone in 'asymptomatic' LVSD after AMI, such a study appears unlikely at present for a variety of reasons, not least of which is the current global financial climate. Eplerenone, a Pfizer-produced and marketed drug, will be off-patent in 2011 and it is unlikely that the company will sponsor further large-scale trials using eplerenone in view of the relatively poor global uptake of eplerenone despite the overwhelmingly positive results of EPHESUS, and in light of the disappointing provisional results of the REMODEL study, in which eplerenone

was not associated with an anti-remodelling effect in stable CHF.[130] The ongoing EMPHASIS-HF study will assess the effects on major clinical end-points of eplerenone in CHF but the results have yet to be reported. Additionally, the previously discussed non-significant trend towards a higher occurrence of stroke in eplerenone-treated patients in EPHESUS has led to concerns over its safety; EMPHASIS-HF should provide further information in that regard in a patient population at higher risk of stroke than that enrolled in EPHESUS.

10.2 Predictors of post-infarction remodelling

Prediction of LV functional recovery following AMI is of critical importance, as patients with a persistently reduced LVEF represent a high-risk population that not only warrants stringent monitoring but may also be considered for ICD therapy. I found that, based on a single sample taken on (mean) day 3 after AMI, an elevated plasma BNP, NTproBNP, TIMP-2, TIMP-4, tPA antigen and vWF, and a reduced plasma eotaxin and IL-7, correlated with poorer LV function over time. Moreover I found that TIMP-4 and tPA antigen were independent predictors of LV remodelling, findings that are novel. Additionally, I detected correlations between MMP-3 and the haemostatic biomarkers tPA antigen and vWF. While correlations do not imply definite biologic effect, these results nonetheless suggest a potential pathophysiological link from the coagulation-fibrinolytic system to collagen matrix turnover and consequent adverse remodelling after AMI.

The studies described in Chapter 4 were not powered to determine the predictive efficacy of biomarkers in post-infarction remodelling, thus the results would require confirmation in larger, more appropriately-powered studies. That TIMP-4 and tPA

antigen were independent predictors of remodelling was surprising, however, and merits further investigation.

TIMP-4 has been relatively under-studied, particularly post-AMI, but might be expected to inhibit certain MMPs which, in theory, should limit ECM turnover and remodelling. One hypothesis to explain the results in Chapter 4, that an elevated TIMP-4 level predicted *greater* remodelling, is that the enzyme switches off certain MMPs (and other enzymes) that are protective against ECM turnover in the early post-infarction period, but has no effect on some of the more aggressive proteolytic MMPs. Previous attempts to limit remodelling using oral MMP inhibition have been disappointing; if the findings regarding TIMP-4 in this thesis are confirmed in larger-scale trials, then future attempts to modulate the MMP:TIMP system to limit remodelling may focus on TIMPs in addition to or even instead of targeting MMPs.

Previous studies have shown that vWF and PAI-1 are elevated and of considerable prognostic significance following acute coronary syndromes.[219, 220] The findings of this thesis, detailed in Chapter 4, reinforce these data: tPA antigen (a surrogate marker of PAI-1 complexed to free tPA) predicted remodelling, and furthermore was related to MMP-3. Additional investigations are now needed to determine whether tPA antigen (and vWF) are simply markers of infarcted/necrotic myocardium, indices of ischaemia or reperfusion, markers of endothelial-platelet interaction or simply representative of the general pro-coagulant state that exists after AMI. Whether these haemostatic biomarkers may represent potential pharmacological targets in the future is unclear, although interestingly studies of ACE inhibitors in AMI have shown a

reduction in PAI-1 in association with improved outcome, although whether this is coincidence as opposed to a definite biologic effect is unclear.[341]

10.3 Aldosterone in post-infarction remodelling

The role of aldosterone in LV remodelling following AMI was examined in detail across the study population as a whole, and according to treatment group. I analysed the relationships between aldosterone and serial change in LV structure and function at three levels: genetic polymorphisms in *aldosterone synthase*, plasma aldosterone activity and urinary steroid metabolites. Through such detailed examination of aldosterone production, activity and degradation, and utilising the current gold standard means of LV functional assessment in patients with LVSD, the results detailed in Chapter 5 provide further insights into the actions of this important hormone in the pathophysiology of short-to-medium term post-infarction LV remodelling.

There has been considerable debate as to whether the effects of aldosterone antagonism on the heart are due to mineralocorticoid receptor blockade independently of aldosterone effects, in other words that circulating aldosterone is a bystander.[304] The results detailed in Chapter 5 suggest that circulating aldosterone exhibits a temporal variation in activity. Aldosterone, when measured at an average 3 days after AMI (prior to randomisation to placebo or eplerenone but in most cases after the commencement of a small dose of ACE inhibitor) correlated directly with parameters of adverse remodelling. Aldosterone then rose in the eplerenone-treated group but this change over time did not relate to adverse remodelling, suggesting that occupation of the mineralocorticoid receptor was preventing aldosterone (and cortisol) from

activating the receptor and promoting remodelling. Conversely, aldosterone tended to decrease in placebo-treated patients, but this change correlated with increasing ventricular dilatation and more significant remodelling. The reasons for this are unclear, but I propose that, as 11β-hydroxysteroid dehydrogenase is not expressed to any great extent in the heart, the cardiac mineralocortocoid receptors in placebo-treated patients were likely to be occupied and activated by cortisol, and that it was cortisol rather than aldosterone that potentiated the remodelling process via mineralocorticoid receptor activation.[99]

One hypothesis that arises from these results is that circulating aldosterone has a role in selecting a remodelling pathway in the first few days after AMI, but that subsequently it is mineralocorticoid receptor activation, largely by cortisol, that is the key determinant of progressive remodelling. The finding that, although under-powered as a genetic study, the presence of the -344T allele in CYP112B in the study patients was associated with a trend towards greater aldosterone but also superior LV function indirectly supports this hypothesis. If our results (and hypothesis) were to be confirmed, this would provide further evidence for the very early initiation of mineralocorticoid receptor antagonists following (large) AMI.

10.4 Micovascular obstruction as a determinant of LV outcomes after AMI

The presence of MVO after non-reperfused AMI strongly predicts adverse cardiovascular outcome and ventricular remodelling. A recent small (n=40) study in reperfused AMI suggested that MVO in the presence of an open IRA did not predict adverse remodelling.[257] The vast majority of infarcts in the study population investigated in this thesis were reperfused, and through the use of LGE-CMR I was

able not only to determine the presence of MVO but also to quantify MVO volume. I therefore examined the predictive value of presence of late MVO on medium-term LV remodelling.

Despite a very high uptake of acute reperfusion therapy and a very high prescription of contemporary secondary preventative therapies, late MVO remained common, occurring in 56% of the study cohort. Additionally, the presence or absence of late MVO divided patients into two distinct groups with very different remodelling outcomes. Moreover, three-quarters of patients with late MVO on baseline LGE-CMR had TIMI 3 flow within the IRA, an observation of considerable significance as TIMI 3 flow is accepted as the optimal angiographic result from coronary artery reperfusion therapy. From the results detailed in Chapter 6, I propose that the demonstration of late MVO on a pre-discharge LGE-CMR in patients admitted with AMI, should serve as a serious indicator of impending adverse remodelling irrespective of final angiographic result, and is of considerable potential as a predictor of remodelling. Indeed, MVO (and infarct characteristics such as magnitude, transmurality and endocardial extent) may be more appropriate end-points in clinical studies assessing success of reperfusion therapy in AMI than angiographic appearances, or even LVEF.[310]

The issues surrounding measurement of infarct volume on LGE-CMR were discussed in detail in Chapter 6, and the need for a global consensus highlighted. Through the Magnetic And eLectrical Technologies (MALT) society, incorporating colleagues in USA, Canada, Sweden, the Netherlands and France, the study population investigated

in this thesis is currently being used as part of an international collaboration, with the goal of standardising infarct volume measurement on LGE-CMR.

10.5 Novel biomarkers: apelin and serum sST2

The study population which provides the focus of this thesis represents a contemporary, thoroughly investigated and aggressively treated cohort of AMI patients, and as such provides an excellent population in which to study the (potential) roles of novel mediators in acute coronary syndromes. Two such mediators are plasma apelin and serum sST2.

Since its discovery a decade ago, a number of actions have been demonstrated that suggest that apelin may be of theoretical benefit in humans following AMI, as described in Chapter 1. In my study population, I found that plasma concentrations of apelin were reduced after AMI, and that although apelin concentration increased over time, it remained lower than in healthy controls even at 24 weeks. I observed no relationship between apelin and any parameter of LV function over time. There was a weak relationship between apelin and NA, a potent inotrope, which may provide some support for the inotropic activity demonstrated in animal models, and eplerenone significantly increased plasma concentrations of apelin over time, possibly due to its known interaction with angiotensin II. However, from these results it would appear unlikely that apelin will be of use as a prognostic marker after AMI.

The results in Chapter 7 are based on measurement of apelin at only two time-points: at baseline (~4 days after AMI) and at 24 weeks. Future studies should include serial measurement of apelin, in order to characterise whether circulating apelin displays a

temporal profile in activity. Also, measurement of apelin in a broader population of AMI patients would be of interest, in particular those with preserved LVEF, which would allow testing of the hypothesis made in Chapter 7 – that geometric change following large AMI acts as a trigger for variations in apelin expression. Simultaneous measurement of angiotensin II would also allow greater insight into the inter-relations of apelin and RAAS hormones after AMI.

Serum sST2 is elevated after AMI and in heart failure, and is gaining popularity as a predictor of major adverse cardiovascular events in these conditions, as described in Chapter 1. The findings described in Chapter 8 build upon previous data regarding the relationship between sST2 and LV function after AMI. Specifically, novel relationships were detected between sST2 and medium-term (24 week) LV function, and interestingly between sST2 and parameters of large infarction. Moreover, a potential link to aldosterone, a hormone of considerable importance in post-infarction remodelling, was suggested.

There are ongoing studies examining the predictive value of sST2 in cardiovascular disease and in a variety of conditions, including chronic lung disease, rheumatological disease, sepsis, trauma and autoimmune disease. The results of this thesis suggest that further studies specifically designed to determine whether sST2 has a role in the pathophysiology of remodelling, rather than simply serving as a marker of adverse prognosis, are warranted. The possible link between sST2 (and thus the IL-33:sST2 signalling pathway) and the RAAS merits particular attention – if a biologic relationship between sST2 and aldosterone is confirmed, it may represent a potential therapeutic target in the treatment and limitation of post-infarction remodelling.

10.6 'Routine' use of LGE-CMR in patients admitted with AMI

The 'standard care' approach to cardiac imaging in patients admitted with AMI at the time of writing this thesis (and during the recruitment phase of the study) consists of at most a single TTE, usually in the early stages of the acute admission and often performed in suboptimal conditions in non-expert hands. Through description of additional intra- and extra-cardiac abnormalities of variable clinical significance detected using LGE-CMR scanning in almost one-quarter of the study population that would otherwise have been missed had the 'standard care' approach been followed, I suggest that LGE-CMR be used where possible as an adjunct to the standard management of AMI.

It is noteworthy that these results, outlined in Chapter 9, have contributed to the greater and more frequent use of LGE-CMR imaging in patients admitted with AMI in Glasgow since completion of the clinical work pertaining to this thesis.

10.7 Conclusions

- Eplerenone did not attenuate LV remodelling in patients with reduced LVEF but without heart failure or diabetes mellitus after AMI, although there was, by chance, a significant imbalance in baseline LV function between randomisation groups. Following pre-specified covariate-adjustment of the results, eplerenone did, however, exert a significant anti-remodelling effect in this population.
- Eplerenone exerted a (covariate-adjusted) effect on MMP-2 and MMP-9, supporting an anti-remodelling effect for this drug.
- Larger studies powered to detect a benefit on clinical outcomes using eplerenone in patients with 'asymptomatic' LVSD after AMI would be valuable.
- A single measurement of tPA antigen or TIMP-4 early after AMI may be of use in the prediction of medium-term LV remodelling after AMI.
- Aldosterone appears to display a temporal variation in its cardiac effects after AMI, playing a role in the selection of a remodelling pathway in the early phase, but thereafter it seems that cardiac mineralocorticoid receptor activation (probably by cortisol rather than aldosterone) potentiates the remodelling process. This hypothesis would support the very early initiation of aldosterone antagonists in patients with AMI and LVSD.
- The presence of (late) MVO on a pre-discharge covariate-adjustment of the results was necessary scan is a portent of adverse remodelling after AMI irrespective of angiographic TIMI III flow within the IRA.
- Plasma apelin is reduced (compared to healthy controls) after AMI and, although it increases over time, remains low at 24 weeks. Apelin has no relationship to LV function/remodelling.

- Serum sST2 correlated with baseline and pre-discharge LVEF and infarct volume, and also with aldosterone, suggesting a possible role for this mediator in post-infarction remodelling.
- The performance of a pre-discharge covariate-adjustment of the results was necessary scan positively influenced patient management in 24% of the study cohort and is a useful and safe adjunct to the standard management and investigation of patients admitted with AMI.

Appendix I-IX

Appendix I Consent Form

WEST ETHICS COMMITTEE

FORM OF CONSENT FOR PATIENTS IN CLINICAL RESEARCH PROJECT

Title of Project:	The effects of eplerenone on left ventricular remodelling post-acute myocardial infarction: a double-blind placebo-controlled cardiac-MR based study
Investigators:	Dr. Robin Weir, Clinical Research Fellow, Western Inf. Prof. HJ Dargie, Professor of Cardiology, Western Inf. Prof. JJV McMurray, Professor of Cardiology, Western Inf.

By signing this form you give consent to your participation in the project whose title is at the top of this page. You should have been given a complete explanation of the project to your satisfaction and have been given the opportunity to ask questions. You should have been given a copy of the patient information sheet approved by the West Ethics Committee to read and to keep. Even though you have agreed to take part in the research procedures you may withdraw this consent at any time without the need to explain why and without any prejudice to your care.

Consent:

I,…….(PRINT)

of……

give my consent to the research procedures above, the nature, purpose and possible consequences of which have been described to me

by……...

Patient's signature……

Doctor's signature……

Appendix II Patient Information Sheet

All patients were provided with the following document prior to formal recruitment into the trial:

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Study Title
The effects of the drug eplerenone on left ventricular remodelling post-acute myocardial infarction (a double-blind, placebo-controlled, cardiac MRI study)

What does this title mean?
Following a heart attack, patients are usually prescribed a number of tablets to help the heart recover. What we want to know is whether taking an extra tablet (a drug called eplerenone) – in addition to these other tablets – further reduces or prevents the ongoing damage to the heart muscle that can occur following the heart attack. We plan to compare the drug eplerenone against a dummy pill (placebo) to see if this is the case, with neither you nor us knowing which of the two you are taking.

What is the purpose of the study?
Coronary artery disease or "hardening of the arteries" is very common worldwide, especially in the West of Scotland. Despite all the advances in medicine, we still see large numbers of patients who develop angina and/or heart attacks.

Our understanding of heart attacks has come on leaps and bounds in the last few decades. We now know that the best current treatment involves a combination of attempting to improve the blood flow to the heart muscle as quickly as possible (either by clot-dissolving drugs straight into the veins – "thrombolysis" – or by directly inserting stents into narrowed or blocked arteries – "angioplasty") and putting patients on appropriate tablets to help the heart heal and reduce the chances of another heart attack.

Unfortunately, despite us putting patients who have had heart attacks on a combination of 3 or 4 (sometimes more) tablets – usually including aspirin, a beta-blocker drug, a cholesterol-lowering drug and an ACE inhibitor, which protects the heart and helps it heal (usually ends in –pril) – there are some patients whose heart muscle does not recover as well as we would hope. Indeed despite these tablets, in some people the heart muscle continues to get weaker with time, the heart swells up and ultimately its pumping function deteriorates. This is called "remodelling" and leads to fluid retention (known as "heart failure"), poorer quality of life, and premature death.

The drug we are using, eplerenone, has been shown in big trials already to reduce this remodelling process in patients who have significant enough damage to the heart muscle following a heart attack that the muscle is struggling to cope – these patients have developed heart failure. What we plan to do is to look at how eplerenone affects this remodelling process in patients who, like yourself, have had a heart attack which has caused heart muscle damage but who have not developed heart failure.

If you agree to be involved in this study, the maximum planned study duration is 6 months for each patient.

Why have I been chosen?
You have been chosen because you have had a heart attack which we know (based on the ultrasound scan of your heart – the "echo") has caused damage to the heart muscle, but we also know that you have not had heart failure (fluid retention). You fall into exactly the group of patients that we would like to study, to see if the eplerenone drug has any further beneficial effect in helping the heart to recover.

We plan to study a total of 100 patients. We are selecting patients by performing ultrasound scans on all new heart attack patients in the Coronary Care Units within the Western Infirmary, Southern General and Royal Alexandria Hospital, and looking out for those who have heart muscle damage (like yourself).

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
If you take part, we will perform some extra tests while you are still in hospital recovering from the heart attack (this will <u>in no way</u> delay your discharge from hospital), and then ask you to come back for a follow-up visit to the Cardiology Department at the Western Infirmary at two separate times – 3 months on from the heart attack and 6 months on. The 3 month visit will actually involve a short (less than 1 hour) visit one morning, then a slightly longer (c. 2.5 hour) visit the following morning. The 6 month visit will take one morning only and will last around 3 hours, after which your involvement in the trial will be finished.

In addition, we will ask you to attend for a blood test on 2-3 separate occasions (usually around week 4 and week 5 after the heart attack). Each visit will take only 5 minutes, and travel expenses or taxis will be provided free of charge if necessary.

If I volunteer, what is the first thing that will happen?
While you are still in hospital (within the first few days after your heart attack), the following will occur. Please note that, as soon as you agree to the trial, you will be randomly assigned a number which will be unique to you, and the number (<u>not</u> your name etc.) will be used on all blood tests and other tests planned for trial purposes.

(i) An extra sample of around 50ml (10 teaspoons) of blood will be taken from a vein in the arm. This blood will be stored prior to analysis at the end of the study. We will look at lots of different blood components that are known to be involved in the "remodelling" process.

Note: A proportion of the blood will be used to look at one of your genes, as some people might naturally be less susceptible to eplerenone than others – this is "DNA analysis". The sample will only have your unique number (not your name etc.) and we plan to keep all genetic analysis anonymous. Please note that we are looking at very minor differences between patients in this one gene, and there should be no impact to other members in the family based on our studies. If we wanted to identify you, there is a master copy of the key-code which would allow us to do this, but it is not planned.

(ii) You will be asked to wear a 24 hour ECG monitor. This is basically a belt that holds a small recording box, from which a few wires run to the chest. They are attached to sticky labels on the chest (just like when you get a heart tracing or ECG). You will be expected to wear this while you are on the ward for 24 hours, although it should not restrict your movements nor activities at all. After 24 hours, the device will be removed. It tells us how fast or slow the heart has been going over the 24 hour period.

(iii) You will be given a plastic bottle into which we would ask you to collect <u>all</u> urine that you pass over a 24 hour period. We will try to synchronise this with the 24 hour ECG to minimise inconvenience. We are looking at salts and hormone levels in the urine.

(iv) A cardiac MRI scan will be performed. MRI scans give us very clear pictures of the heart (much better than any other test which we have available at present). Unlike X-rays and CAT scans, MRI scans do not use any radiation – they use magnets to allow excellent quality pictures. As they use magnets, you should not have a scan if you have any metallic implants (eg. pacemakers, hip replacements, metal plates, aneurysm clips etc.) nor any history of any potential eye injuries that may involve metal objects. All of this will be discussed with you before the scan.

You will be taken from the ward of your hospital to Level 4 Cardiology at the Western Infirmary, where the scan is performed. All you will be asked to do is lie flat on your back and follow requests to breathe in, hold, then breathe out while we take pictures. You will be lying partly in a cylindrical tube (the magnet) while we take the pictures, and the whole scan will last around 45 minutes although may last up to 1 hour. We will also inject a chemical dye (called gadolinium) into the veins via a plastic tube (cannula or venflon) in the arm, which should show up the area of damaged heart muscle very clearly.

Once these tests have been performed, you will be given the trial drug. It is a once-daily tablet (usually taken in the morning). This type of trial is a randomised, double-blind trial involving a placebo – these terms are defined as follows:

Randomised Trial

Sometimes because we do not know which way of treating patients is best, we need to make comparisons. People will be put into groups and then compared. The groups are selected by a computer which has no information about the individual – i.e. by chance. Patients in each group then have a different treatment and these are compared. You will have a 50:50 chance of receiving the study drug (ie. 1 in 2).

Blind trial

In a blind trial you will not know which treatment group you are in. If the trial is a double blind trial, neither you nor your doctor will know in which treatment group you are (although, if your doctor needs to find out he/she can do so).

Placebo

A placebo is a dummy treatment such as a pill which looks like the real thing but is not. It contains no active ingredient.

Eplerenone can occasionally cause an upset to the salts in your blood and the kidney function (much like some of the other tablets you will be on following a heart attack), so we plan to keep an eye on these by performing a single blood test on a few occasions, namely just before you are discharged from hospital following the heart attack, then at 4 weeks after starting the tablet. The reason we check the salts in the blood at 4 weeks is because we plan to increase the dose of the trial drug at that time. For the same reason we will also check a single blood test 1 week after the dose increase (ie. 5 weeks after starting the drug). Each of these two visits will only take around 5 minutes in the hospital, and travel expenses and/or free taxis are available if needed.

Your GP will be informed in writing of your participation in the trial.

The 3 month follow-up visit

You will be asked to come up for a short period of time on two successive mornings around 3 months after the heart attack.

On the first morning, you will be asked to attend for at most 1 hour. We will ask you to come up to Western Infirmary (Level 4) fasted from midnight the previous night, and will simply repeat some of the tests you had done when you first joined the trial:

(i) your blood pressure will be taken
(ii) around 40ml of blood (8 teaspoons) will be removed from a vein in the arm
(iii) you will have a heart tracing (ECG)
(iv) a 24 hour ECG monitor will again be attached
(v) you will be given another plastic bottle and asked to start collecting all the urine you pass over the next 24 hours.

As soon as the blood test is done, you can eat whatever you want! You can then leave the hospital and are asked to return the next day (no fasting necessary). This visit includes a second MRI scan of the heart and will last around 2.5 hours. At this visit:

(i) the 24 hour tape will be removed
(ii) you can hand in the 24 hour urine collection
(iii) we will perform the MRI scan (exactly as above) – again, 45-60 minutes are set aside for this

You will then be free to leave. As before, travel expenses and/or free taxis are available if needed.

The 6 month follow-up visit
This takes one morning only, and is expected to last around 3 hours. You are asked to turn up fasted from midnight the night before, and the following will occur:

(i) your blood pressure will be checked
(ii) 40ml of blood (8 teaspoons) will be removed from a vein in the arm
(iii) you will have another heart tracing (ECG)
(iv) you will have a third and final MRI scan of the heart (identical to above)

Finally you will be asked to complete a short questionnaire about what symptoms (if any) you have had over the course of the study. The study will then be finished, and the trial drug will be stopped. Your GP will be informed that you have completed the trial.

Summary of your involvement

During your hospital admission	Recruitment, blood test, MRI, 24hr tape, 24hr urine
4 weeks	Single blood test
5 weeks	Single blood test
3 months	Day 1: blood test, 24hr tape, 24hr urine, ECG Day 2: remove 24hr tape, return urine, MRI scan
6 months	Blood tests, ECG, MRI scan

END OF TRIAL

What are my commitments? Will the trial affect my lifestyle?
There are no new restrictions on your lifestyle while in the trial other than the general lifestyle advice and restrictions on driving/working/heavy exercise which you will be given by the medical team looking after you following your heart attack. Eplerenone can occasionally cause dizziness, and driving should be avoided if you are experiencing this symptom.

We ask you simply to take the trial drug as a once-daily tablet (it is important that you take it every day unless advised by us to the contrary) and attend for the blood tests and follow-up visits as above.

What is eplerenone?
Eplerenone is a drug that blocks the activity of a naturally-occurring hormone called aldosterone. Following a heart attack, aldosterone levels are increased and are known to contribute to the detrimental "remodelling" process. It has mild diuretic ("water tablet") properties also.

Eplerenone is already licensed in the UK for people with heart attacks, heart muscle damage and fluid retention or heart failure, but not (yet) for those who, like you, who have heart muscle damage but no heart failure. It is a once daily tablet with a dose of 25mg daily for the first month, and then 50mg daily for the rest of the trial. Occasionally we have to reduce the dose of the trial drug, or even stop it for a short while, depending on the salt levels in your blood and your kidney function – this is why we ask you to attend for the blood tests and follow-up visits.

What are the alternatives to eplerenone?
We are not changing any of the other tablets that you would normally be taking after your heart attack. We simply plan to add an extra tablet to these, not replace or substitute any tablet.

Does eplerenone have any side-effects that I should know about?
Every tablet has potential side-effects. Most of eplerenone's side-effects pertain to your blood salts/kidney function which we will be monitoring closely with blood tests. Side-effects that are not uncommon include dizziness, nausea, loose bowel motions and low blood pressure (often asymptomatic). Rare side-effects include dehydration, headache, sweating, flatulence, vomiting, leg cramps, irregular heart beats, and damage to the heart muscle (only in very high doses – around 10x higher than we are using).

Patients with pre-existing significant kidney disease or severe liver disease should not take eplerenone.

As it is a relatively new drug there may be some as yet unknown side-effects.

What are the possible disadvantages or risks of my taking part in the trial?

- Although blood sampling is quick and straight-forward, it is a little uncomfortable and some patients feel faint. There is a minor risk of bleeding, bruising or infection at the puncture site.
- Some patients find the MRI machine claustrophobic although most can tolerate the scan.
- Eplerenone can cause the side-effects listed above.
- The "dye" that we inject (gadolinium) can occasionally cause headache, nausea, injection site coldness and, rarely, allergic reactions.
- It is possible that if the treatment is given to a pregnant woman it will harm the unborn child. Pregnant women must not therefore take part in this study, neither should women who plan to become pregnant during the study. Women who are at risk of pregnancy may be asked to have a pregnancy test before

taking part to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

- If you hold private medical insurance, you should check with your company if involvement in this trial will affect your contract.

What are the possible benefits of taking part*?*

The purpose of this study is to find out if eplerenone gives added benefit to patients like yourself who have had a heart attack and have heart muscle damage. We strongly suspect it will do, and moreover this study will give insight into exactly how eplerenone does exert this predicted beneficial effect. Obviously half of the patients in this trial will receive the placebo, so we cannot say that we guarantee to improve the well-being of all patients over the 6 month period. What we can say is that the information from this trial will be used ultimately to decide whether patients like yourself (indeed, including yourself) should routinely be prescribed eplerenone in the longer term.

We also monitor your blood tests very closely, which is beneficial for you as not only eplerenone but also some of the other drugs you are likely to be taking can cause subtle upset to your blood tests.

What if new information about the drug or my condition becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information we might consider it to be in your best interests to withdraw you from the study. If this occurs, we will explain the reasons and arrange for your care to continue.

What happens when the research study stops?

At the end of the 6 months, the trial drug will be withdrawn. Neither you nor us will find out which of the two (eplerenone or placebo) you were taking. It will be left to the discretion of your GP and/or any doctors who see you at hospital out-patient clinics as to whether eplerenone is formally prescribed in the longer-term.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Will my taking part in the trial be kept confidential?
If you consent to take part in the research any of your medical records may be inspected by the company sponsoring (and/or the company organising) the research for purposes of analysing the results. They may also be looked at by people from the company and from regulatory authorities to check that the study is being carried out correctly. Your name, however, will not be disclosed outside the hospital/GP surgery. Please note that your GP will be made aware of your participation in the trial.

What will happen to the results of the research study?
We plan to publish the results of this study in a variety of medical journals, all of which are generally available to the public on the internet. Please note that you will not be identified in any report/publication.

Who is funding the study?
The study is funded by the drug company Pfizer, who produce eplerenone. Pfizer will pay money into Professor Dargie's funding account for including you in this study. The principal investigator (Dr. Robin Weir) will not receive any additional payment for including you in the study.

Has this study been reviewed?
The West Ethics Committee, who meet twice monthly, have reviewed the study protocol.

Thank you very much for your participation in this study. You will be given a copy of this document and a copy of the signed consent form for your records.

<u>Contacts for further information</u>

Principal Investigator	Dr. Robin Weir
Working hrs (9am-5pm)	0141-211-2000 and ask for page no. 3295 0141-211-8527 (Direct Line)
Out-with working hrs	0141-211-2594 (Coronary Care Unit)

If you are unable to contact Dr. Weir, a message can be left on his answering machine on his direct line as above. Failing this, a message can be left with Professor Dargie's secretary on 0141-211-2803

Appendix III MRI Safety Questionnaire

CRI MRI UNIT

Safety Checklist and Investigation Details - *PATIENTS*

Patient Name	Date of Scan:
Address: Postcode:	Referring Clinician: Location:
Date of Birth: Hospital No:	Weight (kg): Height (cms):

SAFETY QUESTIONS

	YES	NO
Have they:		
A cardiac pacemaker	___	___
An artificial heart valve	___	___
An aneurysm clip	___	___
A bladder implant	___	___
An ear implant	___	___
A pain relief implant	___	___
A time release drug dispenser	___	___
An eye prostheses	___	___
Any other implants	___	___
Have they ever:		
Done welding or grinding?	___	___
Had metal enter their bodies	___	___
Had heart trouble?	___	___
Had an operation on their head?	___	___
Had any artificial joints or screws or pins or plates for broken bones?	___	___
Had any other surgical operations?	___	___

List.....

	YES	NO
Have they:		
Any dentures or hearing aids?	___	___
Any hairclips and jewellery?	___	___
Any tattoos or permanent makeup?	___	___
Any body piercing?	___	___
Any hairpieces?	___	
Removed their watch, credit cards and emptied their pockets?	___	___
Ladies only:		
Could you be pregnant or breast feeding?	___	___
Have you been sterilised or have you an IUD fitted ?	___	___
Do you have asthma hayfever or any allergies that you know of?		

I confirm that the answers to the above safety questions are correct
I also confirm that I will accept a contrast agent injection if required.

Signature of patient*parent guardian _____ Date __/__/__
***Patient only if over 16**

Signature of Authorised Scanning Staff Member

If the patient is comatose, young or confused :

- have you confirmed with the Supervising Radiologist that they are safe to image? (please ring) YES NO
- If the patient is unable to sign please state why. _____

The Supervising Clinician should countersign here if no clear history of implants or operations, can be obtained.

- Note the implant or operation here:

_____

The Supervising Clinician should sign here if they now consider the scan to be completely safe.

Appendix IV Letter For General Practitioner

Glasgow Cardiac Magnetic Resonance Unit,
Level 4 Cardiology,
Western Infirmary,
Glasgow G11 6NT

[date]

Dear Dr.,

Patient: DOB: Address:

The above-named patient was recently admitted to hospital with an acute MI. Although a separate discharge summary will be sent to you by the hospital medical team detailing the admission, the purpose of this letter is to inform you that Mr(s) ***** has kindly agreed to participate in a drug trial, in which I am the principal investigator, using the new selective mineralocorticoid receptor antagonist eplerenone ("Inspra").

Eplerenone reduces morbidity and mortality in patients with left ventricular systolic dysfunction (LVSD) **and** heart failure **or** diabetes after a recent MI. We plan to examine the effect of eplerenone on LV remodelling post-MI using sequential cardiac MRI scans, compared with placebo.

Your patient has LVSD following his/her recent MI. He/she has consented to be involved in a double-blind placebo-controlled study in which he/she will participate for a total of 6 months. We plan to perform three CMR scans, together with a host of bloods pertaining to remodelling, at baseline, 3 months and 6 months.

After the first CMR scan, the patient is randomised to eplerenone or placebo. The patient is asked to continue the study drug (a once daily tablet) for the duration of the 6 month follow-up. The dosage regime is as follows: **25mg daily for 4 weeks then increased to a target 50mg daily for the rest of the study**. All U&Es monitoring and dosage alterations will be performed by the research team at the Western. GPs should not have to perform any extra blood analysis for the purposes of this research project. Likewise all monitoring of the trial will be performed by the Chief Investigator (Dr. R. Weir).

The patient will take eplerenone or placebo in addition to standard post-MI medications decided upon by the hospital medical team and yourself (including ACE inhibitors, β-blockers, ARBs). Caution is advised in its concurrent use with trimethoprim, lithium, α-blockers, tricyclics, neuroleptics, NSAIDs and steroids, while it is absolutely contra-indicated with K-sparing diuretics, clarithromycin, cyclosporin, tacrolimus, itraconazole, ketoconazole, nefazodone, ritonavir and nelfinavir. Eplerenone contains lactose thus should be avoided by those who are lactose-intolerant. The maximum daily dose of eplerenone should be 25mg (not 50mg) in those on amiodarone, verapamil or diltiazem. The effect of eplerenone is

reduced by phenytoin, carbamazepine, rifampicin and St. John's Wort thus these should be avoided if possible. Your patient has been given a "Trial Card" summarising the above.

Relatively common side-effects include **dizziness, hypotension, diarrhoea** and **nausea** in addition to **electrolyte disturbance/renal dysfunction**. Uncommon side-effects include headache, insomnia, pruritus, sweating, dehydration, hypercholesterolaemia, leg cramps, pyelonephritis, pharyngitis and flatulence/vomiting. As eplerenone should be used with caution in pregnancy, as must CMR scanning, **we have excluded pregnant females from the study**. As there is a lack of information on the penetration of eplerenone into breast milk, **breast-feeding should be discouraged.**

At the end of the 6 month study the trial drug will be withdrawn and you will be informed in writing of the patient's completion of the trial. It will be left to the discretion of yourself and/or any hospital medical team looking after the patient at that time as to whether eplerenone be prescribed regularly.

If you have any queries about the contents of this letter, or if any issues arise during the trial, please do not hesitate to contact me using one of the modes below; if within office hours the best way to contact me is via the Western Infirmary switchboard.

The patient is aware that I have informed you of his/her inclusion in the trial.

Direct line: 0141-211-8527
Page : #3295 via switchboard (0141-211-2000)
E-mail: robinweir75@hotmail.com

Yours sincerely,

Dr. Robin Weir

Appendix V Adverse Events – Definitions/Reporting Forms

An adverse event is any undesirable experience associated with the use of a medical product in a patient. The event is SERIOUS and should be reported when the patient outcome is:

Death

Report if the patient's death is suspected as being a direct outcome of the adverse event.

Life-Threatening

Report if the patient was at substantial risk of dying at the time of the adverse event or it is suspected that the use or continued use of the product would result in the patient's death.

Hospitalization (initial or prolonged)

Report if admission to the hospital or prolongation of a hospital stay results because of the adverse event.

Disability

Report if the adverse event resulted in a significant, persistent, or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life.

Congenital Anomaly

Report if there are suspicions that exposure to a medical product prior to conception or during pregnancy resulted in an adverse outcome in the child.

Requires Intervention to Prevent Permanent Impairment or Damage

Report if you suspect that the use of a medical product may result in a condition which required medical or surgical intervention to preclude permanent impairment or damage to a patient.

Unexpected Adverse Event

This is an adverse drug experience that has not been previously observed, for example an adverse reaction that does not appear on the list of known adverse reactions of eplerenone but which is suspected to be related to its use, or else a reaction that is much more severe than would be expected from the list of “common” and “uncommon” adverse events.

Serious Adverse Event form

THE EFFECTS OF EPLERENONE ON LEFT VENTRICULAR REMODELLING POST-ACUTE MYOCARDIAL INFARCTION: A DOUBLE-BLIND PLACEBO-CONTROLLED CARDIAC MR-BASED TRIAL
Protocol no. Pfi-RW-2005-01 Eudract no. 2004-004399-35 Version 2

1. Date reported to Chief Investigator:

2. Patient details: Initials: _____ Date of birth: ___/____/_____
   Sex: M/F (circle as appropriate)

3. Details of investigational medicinal product (IMP), ie. the study drug:
   - dose: _____
   - time last taken: _____
   - start date: _____
   - concomitant medications/doses:

     _____
     _____
     _____
     _____
     _____

4. Serious adverse event (SAE) reporting criteria (check all appropriate to the event)

   ☐ Resulted in death
   ☐ Was life-threatening
   ☐ Required or prolonged in-patient hospitalisation
   ☐ Resulted in persistent or significant disability/incapacity
   ☐ Was a congenital anomaly/birth defect
   ☐ Was important medical event jeopardising the patient or requiring intervention to prevent serious outcome

5. Onset of SAE: _____ _____
   date *time (if applicable)*

6. SAE(s) in medical terms (diagnosis if possible):

7. Description of the above SAE(s) – include related symptoms/signs, course, relevant treatment of the event etc.:

8. Findings and lab values relevant to the above SAE(s) [if applicable]:

9. Outcome of the SAE(s) described in [5] above:

   a. Resolved on _____
   b. Improved
   c. Unchanged
   d. Deteriorated
   e. Died of _____ on _____
   f. Other, please specify: _____

10. GP: Dr. _____
    Practice address: _____
    Phone no.: _____

11. Chief Investigator details:

    Chief Investigator: _____
    Address: _____
    _____
    _____
    Phone no.: _____
    e-mail address: _____
    Fax no.: _____

    Chief Investigator's comments:

For suspected SAE, above form to be filled out as completely as possible by the Chief Investigator (Dr. Robin Weir, Level 4 Cardiology, Western Infirmary, Glasgow G11 6NT, fax no. 0141-211-1791, phone no. 0141-211-8527) within 24 hours of receipt of the information and sent as soon as possible (maximally 7 working days) to the MHRA.

Appendix VI Pharmacovigilance

1. Eplerenone was produced in film-coated tablets (pharmaceutical form).

2. Although both 25mg and 50mg tablets were available, we used 25mg tablets only, to reduce the risk of accidental over- or under-dosing if patients have both strengths of tablet in their possession. Thus when the dose was up-titrated from 25mg to 50mg, two 25mg tablets were taken to constitute the latter dose.

3. Absolute contraindications to eplerenone:

   (a) hypersensitivity to eplerenone or one of its excipients
   (b) serum potassium > 5.0mmol/l at initiation
   (c) moderate/severe renal impairment (creatinine >220μmol/l)
   (d) severe hepatic insufficiency
   (e) concurrent use of potassium supplements, potassium-sparing diuretics or strong inhibitors of the enzyme CYP3A4 – clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, ritonavir, nelfinavir.

4. Major pharmacodynamic interactions with other drugs:

   (a) lithium toxicity has been reported in patients also taking diuretics and ACE inhibitors, but direct interaction studies have never been performed with eplerenone. Nonetheless, concomitant use of lithium and eplerenone should be avoided. If absolutely necessary, serum lithium levels should be monitored.
   (b) due to the potential nephrotoxic effects of tacrolimus and cyclosporin, their use with eplerenone should be avoided. If absolutely necessary, serum levels of tacrolimus and cyclosporin should be monitored.
   (c) concomitant use of trimethoprim and eplerenone increases the risk of hyperkalaemia thus U&Es monitoring is necessary.
   (d) α-1 blockers (eg. prazosin, doxazosin) increase the risk of postural hypotension when combined with eplerenone, thus lying/standing BP measurements should be performed in these patients, with similar measurements being made in those who take tricyclic antidepressants or neuroleptics with eplerenone.

5. Major pharmacokinetic interactions with other drugs:

   (a) strong inhibitors of CYP3A4 potentiate the effects of eplerenone, thus the drugs listed in 3(e) above should be avoided.
   (b) less powerful inhibitors of CYP3A4 can increase the bioavailability of eplerenone. It is therefore recommended that 25mg be the maximum dose of eplerenone when administered concomitantly with such drugs – mainly amiodarone, diltiazem, verapamil, erythromycin.

(c) strong inducers of CYP3A4 can lead to reduced efficacy of eplerenone and should thus be avoided. These include rifampicin, carbamazepine, phenytoin and St. John's Wort.

(d) systemic exposure to digoxin is increased by 16% when co-administered with eplerenone, therefore caution is warranted at doses near the upper limit of the normal range.

(e) NO interaction with warfarin has been demonstrated.

Pregnancy / Lactation

Insufficient data exist on the use of eplerenone in these situations, thus its use should be avoided.

Recognised adverse effects

The overall incidence of adverse events in the EPHESUS trial (**ref**) was similar to placebo (78.9% v 79.5%) and the discontinuation rate was 4.4% for the eplerenone group and 4.3% for the placebo group.

The following recognised side effects are sub-divided into common (ie. between 1:10 and 1:100) and uncommon (between 1:100 and 1:1000):

Common	Hyperkalaemia
	Dizziness
	Hypotension
	Abnormal renal function
	Diarrhoea, nausea
Uncommon	Headache
	Insomnia
	Postural hypotension
	Flatulence, vomiting
	Sweating, pruritus
	Back pain, leg cramps
	AF, MI, heart failure
	Pharyngitis
	Eosinophilia
	Pyelonephritis

Appendix VII Angiographic Data

Diagnostic coronary angiogram (n = 85):

Single vessel disease:	50/85 (58.8%)
≥2 vessel disease:	35/50 (41.2%)

Culprit vessel:

Left main stem (LMS)		1/85	1.2%
Left anterior descending artery (LAD)		45/85	52.9%
Circumflex artery (Cx)		10/85	11.8%
Right coronary artery (RCA)		22/85	25.9%
Diagonal branch		1/85	1.2%
Obtuse marginal		4/85	4.6%
CABG graft:	Left internal mammary artery	0/85	0%
	Vein graft to RCA	2/85	2.4%

PCI during acute admission (n = 74):

Single vessel PCI:	67/74 (90.5%)

Target of single vessel PCI:

LMS	1/74	1.4%
LAD	31/74	41.9%
Cx	8/74	10.8%
RCA	21/74	28.4%
Diagonal (no LAD PCI)	1/74	1.4%
Obtuse marginal (no Cx PCI)	4/74	5.3%
Vein graft to RCA	1/74	1.4%

Multivessel PCI:	7/74 (9.5%)

Vessels with at least 50% luminal stenosis included in the above analysis, as described in the Statistical Methods section (Chapter 2.7).

Appendix VIII Safety Data

Renal function:

	EPLERENONE			PLACEBO		
	Baseline	24 weeks	p	Baseline	24 weeks	p
Serum potassium (mmol/l)	4.10 (0.35)	4.12 (0.72)	0.84	4.17 (0.35)	4.02 (0.67)	0.15
Serum creatinine (μmol/l)	101.7 (18.7)	100.1 (19.0)	0.44	98.1 (23.6)	97.5 (24.1)	0.77
eGFR (ml/min/m^2)	67.3 (16.0)	68.6 (16.0)	0.39	73.3 (18.9)	73.9 (18.1)	0.74

There were no significant changes in serum potassium, creatinine or eGFR between baseline and 24 weeks in either randomisation group (data expressed as mean [SD]).

Serious adverse events:

	Eplerenone (n=50)	**Placebo** (n=50)
Death	3 (6%)	0 (0%)
Heart failure hospitalisation	1 (2%)	1 (2%)
Chest pain admission (biomarker negative)	3 (6%)	3 (6%)
Recurrent AMI	1 (2%)	1 (2%)
Stroke	3 (6%)	0 (0%)
Hospitalisation with possible arrhythmia	2 (4%)	3 (6%)

Appendix IX TIMI Grade Scale for angiography

A grading scale for coronary blood flow based on visual assessment of the rate of contrast opacification of the IRA at angiography.[342]

TIMI 0 flow - absence of antegrade flow beyond a coronary occlusion

TIMI 1 flow - faint antegrade coronary flow beyond an occlusion, with incomplete filling of distal coronary bed

TIMI 2 flow - delayed or sluggish antegrade flow with complete filling of the distal coronary bed

TIMI 3 flow - normal flow which fills distal coronary bed completely

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