

Aortic stenosis: pathophysiological effects on the myocardium and predictors of clinical events

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During my time in OCMR I and my colleagues have not only pushed back the frontiers of science (!), but also founded the OCMR running club, the OCMR fine dining society, OCMR

pudding club, OCMR wine tasting society resulting in innumerable memorable evenings out.

I hope the future OCMR fellows continue these noble societies.

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Declaration

The work in this thesis is my own. I recruited, scanned and followed up all the asymptomatic aortic stenosis patients in the study. I analyzed all the CMR scans, interpreted the data, carried out the statistics and wrote the script. I collaborated with Dr. Steven White from the Heart Hospital, University College London for work presented in chapters 4 and 5; he provided the data for all the patients with severe *symptomatic* aortic stenosis. Dr. Andrew Flett from the Heart Hospital analyzed the histology samples and this data is presented in chapters 4 and 5. Dr. Stefan Piechnik helped with the processing of the T1 mapping data, provided scan data from normal volunteers and helped with statistical analysis for chapter 5. Dr. Theo Karamitsos helped with statistical analysis for chapters 3 and 6. Dr White, Dr Piechnik and Dr Flett have given permission for this data to be presented in the thesis.

Abbreviations

ACE	Angiotensin Converting Enzyme
AS	Aortic Stenosis
ARB	Angiotension Receptor Blocker
AV	Aortic Valve
AVA	Aortic Valve area
AVR	Aortic Valve Replacement
BNP	B-type Natriuretic Peptide
CRF	Coronary Flow Reserve
CS	Circumferential Strain
CAD	Coronary Artery Disease
CMR	Cardiac Magnetic Resonance
CO	Cardiac Output
CVF	Collagen Vascular Fraction
ECG	Electrocardiogram
Echo	Echocardiography
EDV	End Diastolic Volume

EF	Ejection Fraction
ESV	End Systolic Volume
FT	Feature Tracking
HLA	Horizontal Long Axis
LGE	Late Gadolinium Enhancement
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LMI	Left Ventricular Mass Index
LS	Longitudinal Strain
LVOT	Left Ventricular Outflow Tract
LVOT coro	Left Ventricular Outflow Tract coronal
MACE	Major Adverse Cardiac Events
MPR	Myocardial Perfusion Reserve
MPRI	Myocardial Perfusion Reserve Index
PET	Positron Emission Tomography
RAS	Renin-Angiotensin-System
RIAS	Ramipril In Aortic Stenosis trial

SA	Short Axis
SD	Standard Deviation
ShMOLLI	Shortened Modified Look- Locker Inversion Recovery
SSFP	Steady State Free Precession
SV	Stroke Volume
TE	Echo Time
TR	Repetition Time
VLA	Vertical Long Axis

Thesis summary

The management of the asymptomatic patients with severe aortic stenosis (AS) is challenging; clinicians have to balance the risks of early surgery against the risk that irreversible myocardial damage may occur with a conservative management strategy.

It has become increasingly apparent that prognosis in asymptomatic AS depends not only on the degree of valvular stenosis, but also on the myocardial response to pressure overload and understanding the mechanisms of myocardial decompensation may help to guide management in the future. The degree of myocardial fibrosis, microvascular dysfunction, hypertrophy and left ventricular (LV) geometry may all play important roles. However, current guidelines for management of asymptomatic AS limit assessment of the myocardium to the measurement of ejection fraction with echocardiography. More advanced techniques may provide greater information that could be clinically useful.

This thesis seeks to further our understanding of the mechanisms of the myocardial response to AS, using Cardiac Magnetic Resonance (CMR) in patients with moderate and severe AS. Myocardial perfusion in AS is examined in chapter 3. The results show that CMR first pass perfusion can be carried out safely and is well tolerated by AS patients. Microvascular dysfunction in these patients was associated with age, exercise time and markers of diastolic dysfunction. Myocardial strain is examined in chapter 4, utilizing a new software tool to look at strain throughout the left ventricle, and also to explore the relationship between strain and myocardial fibrosis. The results show that there are significant variations in circumferential strain measurements, depending on slice position in

the LV, and also that there was no relationship found between strain and the degree of LV fibrosis.

In chapter 5, the potential of CMR T1 mapping to identify fibrosis is examined using a new shortened non-contrast sequence (ShMOLLI - Shortened Modified Look-Locker Inversion) developed in our unit. CMR T1 values were validated against histological quantification of myocardial fibrosis in a large group of moderate and asymptomatic AS. A good correlation was found between ShMOLLI derived T1 values, with T1 values increasing with the severity of AS.

The clinical value of measuring myocardial perfusion and LV global strain is examined in chapter 6 by linking these to prognosis. Measurement of circumferential strain could predict prognosis in asymptomatic AS, but myocardial perfusion showed poor ability to predict events.

In conclusion, this thesis offers further insights into the changes that occur in the myocardium of patients with asymptomatic moderate and severe AS, using established and new CMR techniques. The clinical value of measuring these CMR parameters to aid risk stratification is shown, and the future potential for monitoring new therapies in these patients is discussed in the final chapter.

Chapter 1: Introduction

1.1 Overview of aortic stenosis

Aortic stenosis (AS) is common, occurring in 2% of the population over 65 years of age, rising to 7% in men over 85 years.¹ These figures are set to rise (as a result of increasing life expectancy in developed countries) as highlighted by a recent editorial in *Heart*² which identified valvular heart disease as the “next cardiac epidemic”. When AS is classed as severe and symptoms are present, aortic valve replacement (AVR) surgery is recommended.³ The median survival without surgery in this group is 2-4 years depending on the symptoms.^{4, 5}

For asymptomatic patients however, the management is less clear. Early valve replacement exposes patients to the risks of surgery and the on-going risks of complications from prosthetic aortic valves for a longer period. Waiting for symptoms to occur, however, risks the development of left ventricular (LV) dysfunction and there is a small risk (0.4% /year) of sudden death.⁶ A recent study by Rosenhek et al identified a high risk group of “very severe asymptomatic AS” (jet velocity of > 5.5 m/s) with exceptionally poor event free survival rate (3% at 6 years) as a subgroup of patients that would benefit from surgery before symptoms develop.⁷ Kang et al showed that early surgery in patients with very severe AS (defined as AVA < 0.75 and valve peak aortic velocity >4.5 m/s) is associated with improved long-term survival and decreased cardiac mortality compared to conventional management strategies in those with low operative risk.⁸

The current European Society of Cardiology guidelines for management of asymptomatic AS are shown in Figure 1.1.

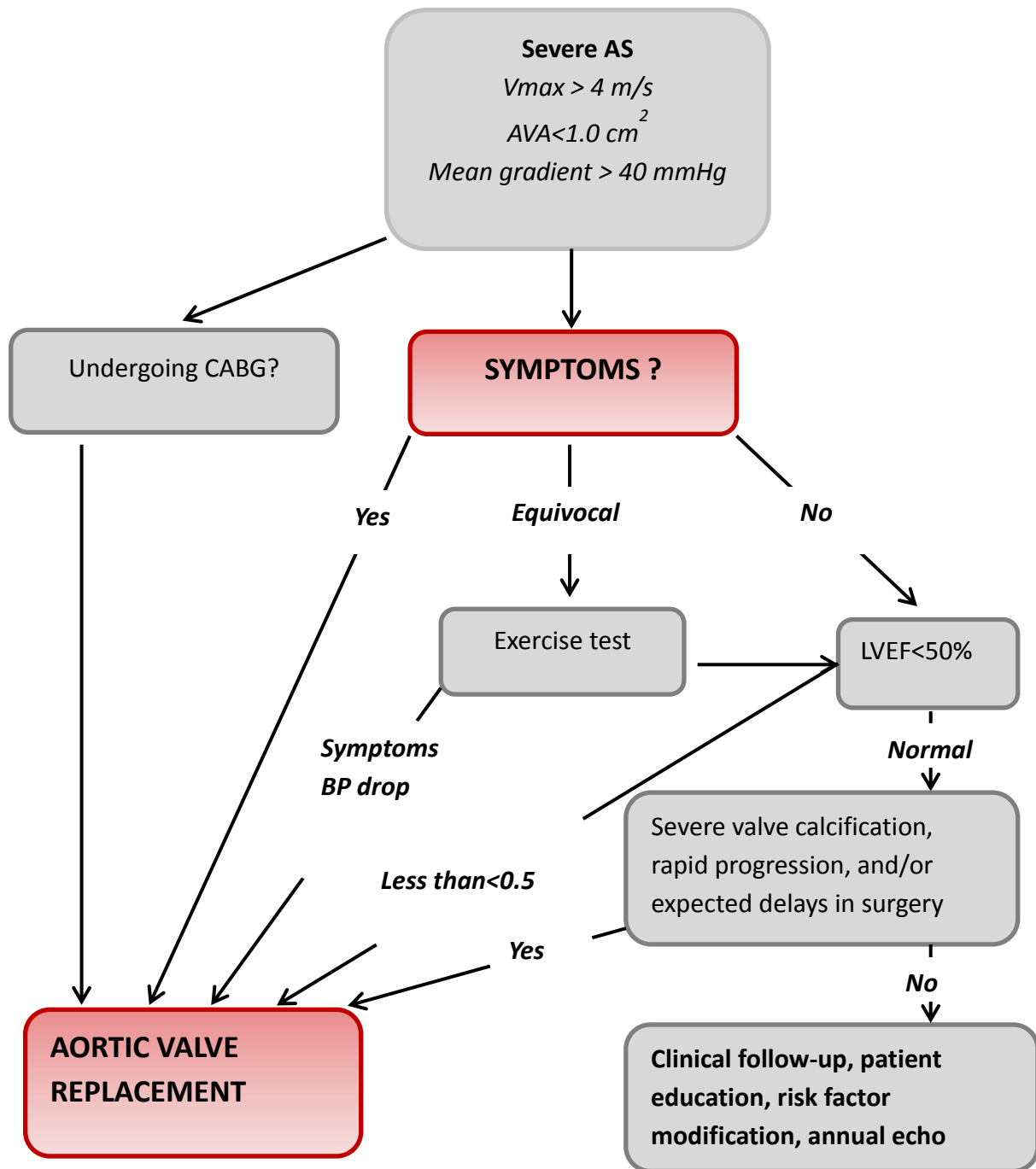


Figure 1.1. European Society of Cardiology Guidelines for management of the asymptomatic AS patient. (Figure adapted from review by Lung et al).⁹ Echocardiography is the recommended imaging modality for assessment of the degree of valve stenosis.

In both the American Heart Association/American College of Cardiology¹⁰ and the European Society of Cardiology Guidelines¹¹ for management of the asymptomatic AS patient, the emphasis is placed on documenting progression of the aortic valve gradient by echocardiography; assessment of the myocardium is limited to measurement of EF only. However, a reduction in LV ejection fraction (LVEF) is a late development in the natural history of the disease and associated with a dismal prognosis (survival < 2 years)¹² and higher operative risk (mortality up to 50%) in those with no contractile reserve.¹³

There is increasing evidence to support the idea that the *physiological response of the myocardium* (ie: changes in LV geometry,¹⁴ fibrosis¹⁵ and strain¹⁶) to the valve obstruction is an important factor determining the prognosis of asymptomatic AS patients as highlighted by Gould et al in a recent editorial.¹⁷

The development of advanced cardiac imaging methods has enabled increasingly sophisticated assessments of myocardial function and tissue composition to be carried out. It is important, however, to identify which cardiac imaging parameters will prove useful for highlighting changes in the myocardium of asymptomatic AS patients before irreversible myocardial damage occurs. Myocardial factors currently identified as being associated with either an adverse prognosis in AS or poor recovery of the ventricle post AVR include a high degree of left ventricular hypertrophy(LVH);¹⁸⁻²⁰ a high percentage of fibrosis as measured by histology and cardiac magnetic resonance (CMR) late gadolinium enhancement(LGE)²¹ and reduced longitudinal strain.¹⁶ Some studies have shown impaired microvascular perfusion²² in AS, but prognostic significance has not been shown.²³

1.2 Left ventricular hypertrophy in aortic stenosis

Until recently, left ventricular hypertrophy (LVH) was classically interpreted as the compensated physiological response to pressure overload. This hypothesis stated that as AS progresses and the intra-ventricular pressure rises, the LV wall thickness increases (due to an increased number of contractile elements at a cellular level) to normalize wall stress according to the law of Laplace as shown in Figure 1.2.²⁴

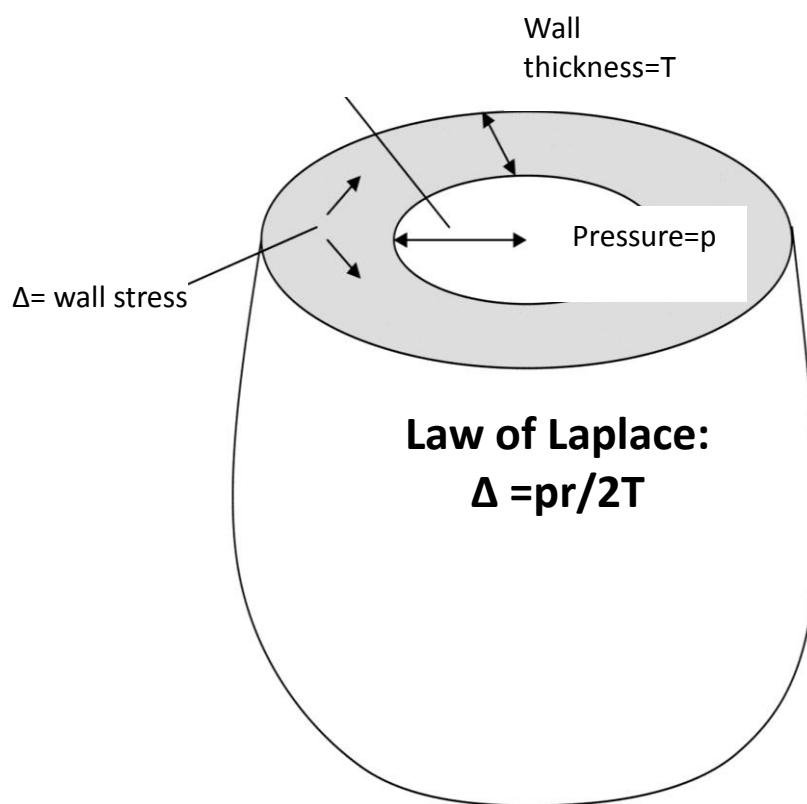


Figure 1.2. The Law of Laplace. (Figure adapted from review article by Scott et al²⁵) The change in wall stress is directly proportional to the intra-ventricular pressure and radius and inversely proportional to LV wall thickness.

This paradigm is being challenged by emerging clinical²⁶ and experimental work²⁷ showing that normalization of wall stress does not prevent the natural history of progressive systolic dysfunction and dilatation; hence the question posed by Yotti et al²⁶ “Left ventricular hypertrophy in aortic valve stenosis: friend or foe?”

Several clinical studies support the idea of LVH, in particular concentric LVH, as a “foe”. One of the most recent studies in this area comes from the SEAS (Simvastatin and Ezetimibe in Aortic Stenosis Study)²⁸ study investigators who looked at a large patient cohort of asymptomatic patients with mild to moderate AS; both ECG left ventricular strain pattern and LVH (as measured by echocardiography) were found to be independent predictors of poor prognosis in this patient cohort.¹⁸ Other studies have also shown that severe concentric LVH in AS patients is associated with earlier onset of symptoms (independent of valve gradient),²⁹ is an independent risk factor for cardiovascular events in asymptomatic patients²⁰ and is associated with an adverse prognosis early and late post AVR surgery.^{30 31} LVH in AS has also been shown to impair coronary blood flow reserve, reduce diastolic function and is associated with increased mortality in other disease states.^{23, 32-34}

It is clear from several studies that not only is the overall LV mass of importance, but LV geometry also plays a powerful role in determining clinical outcome in these patients. The patterns of LV remodeling include concentric remodeling (normal LV mass with increased regional wall thickness)³⁵; concentric hypertrophy (increased LV mass with increased relative wall thickness)³⁵; eccentric hypertrophy (increased LV mass with normal regional wall thickness)³⁵ and asymmetric hypertrophy (disproportionate thickening of the anterior ventricular septum relative to the posterior LV free wall).³⁶ See Figure 1.3.

In hypertension induced LVH there is a gradation of cardiovascular risk with concentric hypertrophy conferring the highest risk, followed by concentric remodeling and then eccentric hypertrophy.³⁷ In AS, most of the information that we have about LV geometry comes from small studies of patients with severe AS where up to a 90% prevalence of *concentric* LVH is reported.^{38, 39}

There are many complex factors which govern the LV remodeling response to pressure overload; gender,⁴⁰ co-morbidities,⁴¹ genetic,⁴² molecular and cellular⁴³ mechanism all play a role.

Women with AS develop smaller LV cavity sizes and greater wall thickness than men;⁴⁴ regression of myocardial hypertrophy also occurs faster in women post AVR.⁴⁰

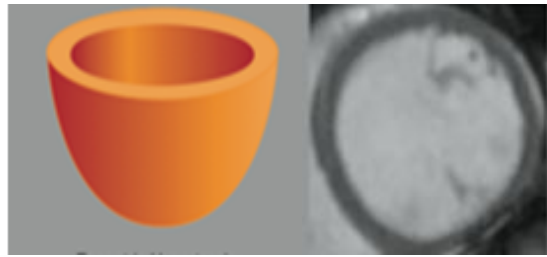
The presence of co-morbidities impacts on the degree of LVH; greater degrees of LVH in AS patients have been found in those with diabetes⁴¹ and metabolic syndrome.⁴⁵

A number of genetic factors – but in particular the gene polymorphisms encoding the Angiotensin-Converting Enzyme (ACE) influence the degree of LVH and the extent to which LVH remodeling occurs post-surgery in AS patients.^{42, 46}

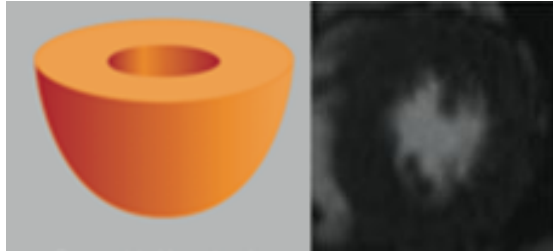
The molecular and cellular mechanisms involved in driving LVH are multiple and complex. Circulating members of the transforming growth factor beta (TGF β) superfamily,⁴³ matrix metalloproteinases (MMPs⁴⁷), activin A⁴⁸ and growth-differentiation factor 15 (GDF-15),⁴³ amongst others, are thought to play an important roles in the development of LVH at a molecular and cellular level.

In summary, whilst accepting that LVH may lead to normalizing of wall stress according to Laplace, the majority of the clinical evidence points to a deleterious effect of extreme concentric LVH in AS.

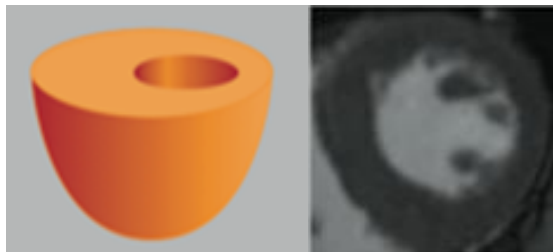
Indeed, a recent study proposed the incorporation of left ventricular mass index (amongst other parameters) in a new proposed risk score for predicting mortality in patients with asymptomatic mild to moderate AS;⁴⁹ this clearly highlights the value added of assessing the myocardium as well as the aortic valve.



Eccentric Hypertrophy



Concentric Hypertrophy



Asymmetric Hypertrophy

Figure 1.3. Different patterns of left ventricular hypertrophy. (Figure created by Dr Marc Dweck from the University of Edinburgh in unpublished work. Adapted by the author. Dr Dweck has given permission for this figure to be used in this thesis)

Eccentric hypertrophy is characterized by an increase in LV diameter. Concentric hypertrophy is characterized by a thickening of the LV wall in symmetrical fashion with overall reduction in the size of the LV cavity. Asymmetric hypertrophy shows thickening of one (or more but not all) LV walls.

1.3 Myocardial fibrosis in aortic stenosis

Clinical studies have shown that a high degree of fibrosis in the ventricles of asymptomatic AS patients is an independent predictor of mortality, and is also associated with poor prognosis in “low-gradient” flow AS and surgical outcomes post AVR.^{15, 50, 51} The mechanisms by which fibrosis is thought to lead to adverse prognosis are multiple; it leads to increased ventricular stiffness, abnormal cardiac remodelling, worse left ventricular function⁵²⁻⁵⁴ and is thought to serve as a structural substrate for arrhythmia.¹⁵ Given the clinical deleterious consequences of myocardial fibrosis in AS, is important to understand what factors govern the fibrotic process in the myocardium and to consider how the degree of fibrosis could be quantified non-invasively.

Three different types of myocardial fibrosis have been identified: *infiltrative* interstitial fibrosis, *reactive* interstitial fibrosis and *replacement* fibrosis.⁵² Infiltrative interstitial fibrosis occurs in conditions such as amyloid and results from the deposition of insoluble proteins in the cardiac interstitium; this does not occur in AS.⁵⁵ Reactive diffuse interstitial fibrosis is found in AS and a range of other conditions including hypertensive heart disease,⁵⁶ idiopathic dilated cardiomyopathy⁵⁷ and diabetes.⁵⁸ This type of fibrosis is reversible and precedes *irreversible* replacement fibrosis; for this reason it would be of great clinical interest to identify reactive fibrosis non-invasively in the myocardium before irreversible replacement fibrosis occurs. Replacement fibrosis occurs when myocardial cells are damaged and replaced with type I collagen;⁵⁹ it is the final endpoint of both infiltrative and reactive fibrosis.⁵²

The molecular mechanisms driving the fibrosis cascade process in AS (see Figure 1.4) are complex, and understanding the many pathways involved is the subject of on-going research. There are a number of mechanical triggers that up-regulate the fibrosis process such as increased pressure (in AS) which leads to local cellular damage;⁶⁰ as a consequence a variety of chemokines (including transforming growth factor beta (TGF β), angiotensin II, matrix metalloproteinases (MMPs) and collagen pre-cursors) are released and endothelial integrin expression is triggered which attracts inflammatory cells into the extracellular matrix, which binds to integrin.⁶¹ One pathway of particular importance involves angiotensin II and the profibrotic cytokine TGF β – which up-regulates pro collagen 1 and 3 production;⁶² 80% of cardiac collagen is type 1 collagen and this crosslinks with glycoproteins such as fibronectin. Matrix metalloproteinases (regulated by metalloproteinase tissue inhibitors) are involved in collagen degradation⁶³ and the resulting

collagen degradation products can be measured in blood although their use as a surrogate marker for diffuse interstitial fibrosis is limited due to confounding factors such as chronic disease.⁶⁴

Currently the gold standard for quantification of diffuse myocardial fibrosis in AS is histological biopsy, which is invasive and subject to sampling error.

Extensive research is underway in all cardiac imaging modalities to find reliable, reproducible and accurate techniques for accurate quantification of diffuse interstitial fibrosis.⁶⁵

The CMR techniques currently available to do this are presented in chapter 2, Section 2.10.1. In chapter 5 the results of fibrosis mapping using a new T1 mapping CMR sequence, Shortened Modified Look-Locker Inversion recovery (ShMOLLI)⁶⁶ will be presented.

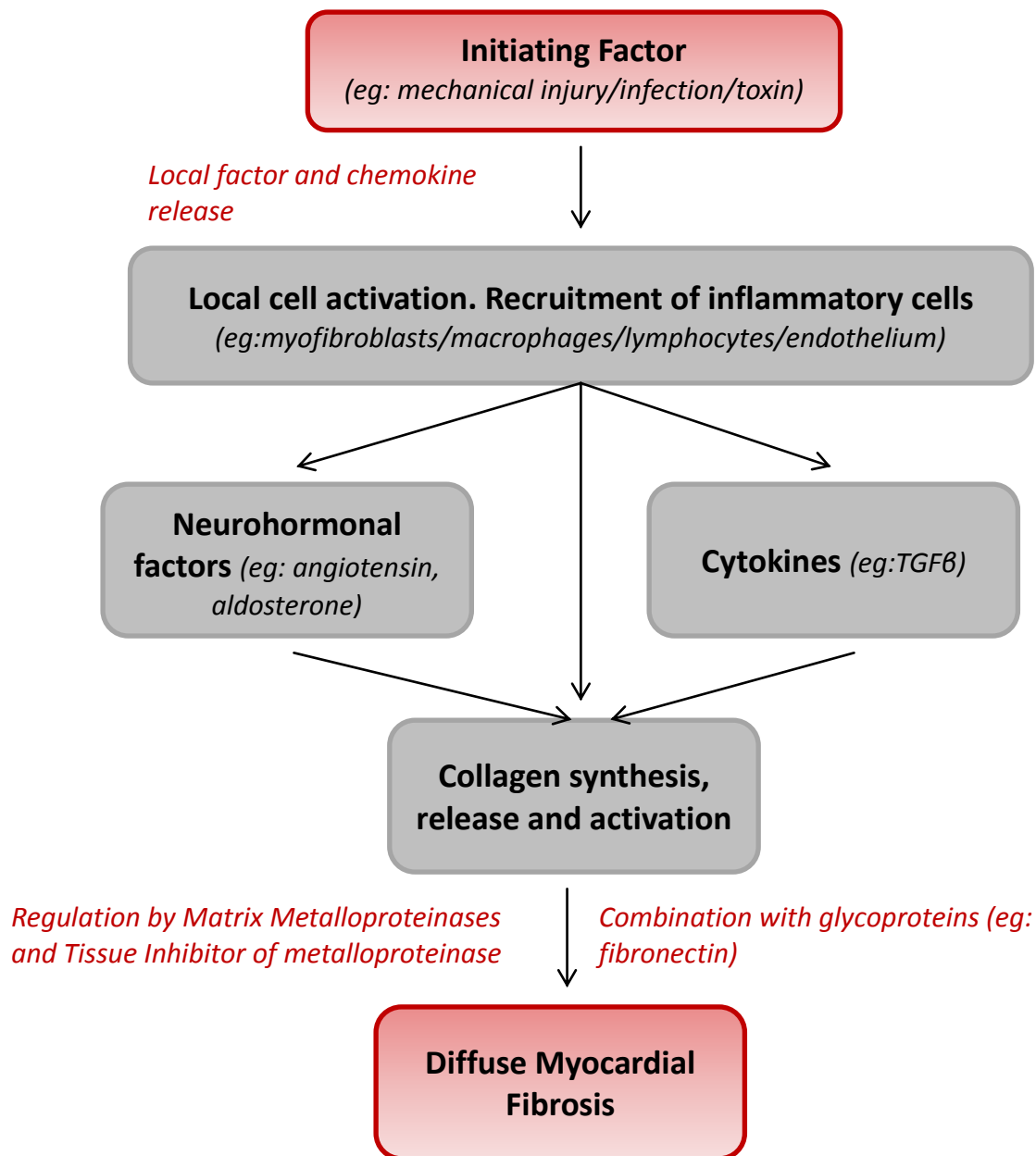


Figure 1.4. Fibrosis Cascade. (Figure recreated from a review by Sado et al).⁶¹ This shows the fibrosis cascade and the mediating factors

1.4 Hypertrophy, fibrosis and renin-angiotensin

There is good evidence for involvement of the renin-angiotensin system (RAS) and in particular the local cardiac tissue RAS in pressor induced LV hypertrophy.^{67, 68} Increased RAS

activity can be shown, with upregulation of RAS genes in the heart of sheep with experimental aortic stenosis,⁴⁶ and the human angiotensin-converting enzyme (ACE) gene polymorphism influences the degree of hypertrophy in patients with AS.⁴² Increased cardiac levels of mRNA for renin and angiotensinogen are seen under conditions of chronic pressure overload,⁶⁹ and cardiac ACE activity and collagen formation were increased 1.5 to 2-fold in humans with aortic stenosis.⁷⁰ In a rat AS model, a 4-fold increase in ACE activity was associated with diastolic dysfunction, which was normalised acutely with infusion of an ACE inhibitor.^{71, 72}

The main effector peptide of the RAS, angiotensin II, can increase proto-oncogene transcription, growth factor expression and protein synthesis.^{67, 73} Increased myocardial fibrosis and collagen synthesis in response to angiotensin II have also been shown, an effect which may be mediated via aldosterone and/or inhibition of the collagen-degrading enzyme metalloproteinase-1.⁷⁴ Angiotensin II can also induce apoptosis in cardiomyocytes,^{75, 76} which contributes to the progression from cardiac hypertrophy to heart failure. Angiotensin II binds to two functionally distinct receptors - AT₁ and AT₂, with the majority of the known effects mediated via the AT₁,⁷⁷ a G-protein coupled receptor.⁷⁸ While in control hearts, the AT₁ receptor is the dominant subtype, in hypertension-induced LV hypertrophy, both receptor subtypes are up-regulated. In an aortic banding model, the AT₂ subtype predominates, which may represent adaptive receptor responses to prevent further hypertrophy, given the potential growth inhibitory function of the AT₂ receptor.^{79, 80}

In addition to increased RAS activity in LV hypertrophy, RAS inhibition can affect the mechanisms by which persistent pressure overload may lead to LV dysfunction. A reduction in excitation contraction coupling, with decreased Ca²⁺-ATPase mRNA levels, has been

observed in pressor induced heart failure, and was attenuated by ACE inhibitors.⁸¹ A direct inhibitory effect of ACE inhibitors on metalloproteinases has also been seen⁸² which may lead to a reduction in the degree of fibrosis. Chronic ACE inhibition in rat models of aortic stenosis causes regression of LV hypertrophy without altering LV systolic pressures, reduces myocardial collagen content, reduces end-diastolic pressure, and perhaps most importantly, reduces mortality.⁸³⁻⁸⁶

TGF β is induced by angiotensin II and the ameliorative effects of ACE-I and ARB (angiotensin receptor blockers) therapy in cardiac remodelling are partly attributed to the reduction in extracellular matrix protein accumulation that is mediated by TGF β signalling.⁸⁷

The angiotensin II pathway is therefore a key therapeutic target for intervention to reduce fibrosis and LV hypertrophy. A recent large retrospective study of over 2000 patients points to a beneficial effect of ACE inhibition in AS patients; those on ACE has an improved survival and a lower risk of cardiovascular events.⁸⁸ The results from the first double-blinded, prospective, placebo controlled trial of ACE inhibition in asymptomatic AS - the RIAS (Ramipril In Aortic Stenosis) trial, are due out later this year and will shed more light on how the LV responds to ACE inhibition in AS.

1.5 Microvascular dysfunction in aortic stenosis

The cardiac microcirculation (arterioles and capillaries) in humans cannot be visualized directly and therefore studies in this area have relied on measurements of myocardial blood flow (MBF) and coronary flow reserve (CFR) using positron emission tomography (PET)²³ and, more recently, CMR perfusion imaging.²² CFR is an integrated measure of flow through the large coronary arteries and the microcirculation.⁸⁹

In AS patients, the development of LVH is associated with abnormalities in microcirculatory function; whilst resting myocardial blood flow in the heart is increased proportional to mass, CFR is reduced (see Figure 1.5).^{23, 90} This may explain why some AS patients experience angina on exertion despite having normal coronary arteries on angiography.⁹¹

The reduction in CFR in other causes of LVH (e.g. secondary to hypertension or hypertrophic cardiomyopathy) occurs due to massive medial hypertrophy of the coronary arterioles, leading to increased vascular resistance. These vascular remodelling changes are however not seen in LVH due to AS,⁹² although the exact reason for this is unclear; although arteriolar structure remains intact in AS, there are increased fibroblasts and myofibroblasts.⁹³ Coronary circulation changes in AS are caused by a decrease in the overall capillary density in the myocardium; capillary growth cannot match the increased volumes of myocytes in the hypertrophied myocardium and this leads to an increase in the minimum coronary resistance per gram of tissue.⁹⁴ Perivascular fibrosis in AS increases minimum coronary resistance further.⁹⁵

Extravascular changes in hypertension and AS leading to a reduction in CFR include elevated LV diastolic pressures, which lead to mechanical impairment of blood flow in the subendocardium, particularly during physical stress.^{90, 96} It is thought that extravascular mechanisms, in particular extramural compression and reduction in diastolic perfusion time, are primarily responsible for microvascular dysfunction in AS.⁹² This is supported by clinical studies showing improvement in CFR post-AVR correlates with valve area and diastolic perfusion time, but only weakly with LV mass.^{23, 90}

However, more recent clinical CMR perfusion study did find LV mass index to be independently associated with reduced myocardial perfusion reserve;²² the exact

relationship between LVH and perfusion in AS therefore remains unclear but will be explored further in chapter 3.

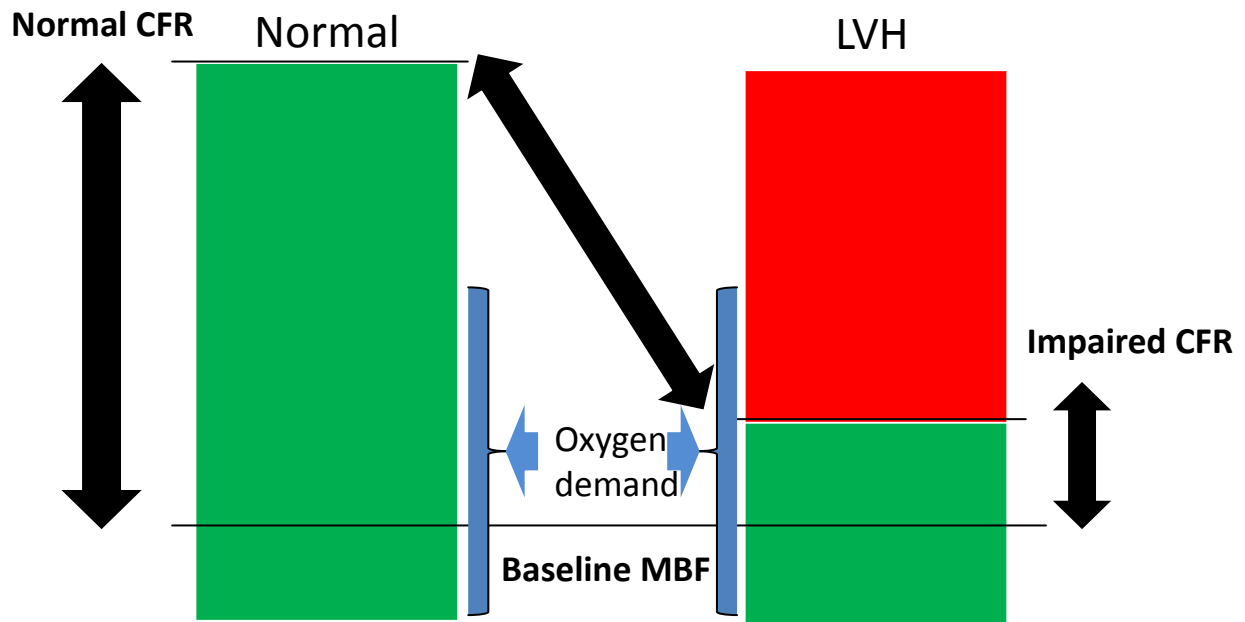


Figure 1.5. Coronary Flow Reserve (CFR) in normal myocardium and in LVH (Figure adapted from review by Camici et al).⁹⁷ In normal myocardium, the coronary microvasculature can increase myocardial blood flow to meet the oxygen demand (left). In patients with LVH, the coronary flow reserve is impaired and this causes microvascular ischaemia in situations when increased oxygen demand cannot be met (right). MBF=Myocardial Blood Flow.

1.6 CMR for assessment of the myocardium in aortic stenosis

CMR is an ideal tool for examining the physiology of the myocardium in AS because it has the ability to assess many of the relevant physiological aspects, combined with excellent image quality and a lack of ionising radiation.

There are an increasing number of studies relating to aortic valve disease that have used CMR as the primary imaging modality of choice. A search of Pubmed using the terms

“cardiovascular magnetic resonance and aortic stenosis” elicited 959 results today on the 5th of May 2012. Using the same search terms, the following numbers of publications were found: 1995-2000(120); 2000-2005(247); 2005-2010 (424); 2010-2012 (180) confirming the trend that CMR is increasingly used for research studies in AS.

This increase in the number of CMR publication relating to aortic valve disease is linked to the many advances made in the development of new CMR sequences for clinical applications. Early (1995-2000) CMR studies in aortic valve disease used CMR for assessment of cardiac metabolism and LV torsion in AS.^{98,99} In the period 2000-2005, CMR studies started to focus on CMR flow quantification,¹⁰⁰ and comparison of CMR aortic valve area measurements to echocardiography.^{101, 102} CMR measurement of LV mass was used in the earliest studies measuring perfusion in AS using positron emission topography (PET) pre and post AVR²³ and in a trial of 2 different aortic valve prosthesis to monitor LV mass regression.¹⁰³ The most recent publications have investigated CMR perfusion imaging in AS,²² compared CMR and CT (Computed Tomography) measurements of aortic valve area,¹⁰⁴ and shown the prognostic significance of LV fibrosis imaging in AS using CMR.¹⁵

To date, CMR has been shown to be the most accurate and reproducible technique for assessment of LV mass and function;¹⁰⁵⁻¹⁰⁷ aortic valve area can be determined with great accuracy.¹⁰⁸

Likely future developments in this area are the application of sequences such as four dimensional flow for assessment of aortic valve flow¹⁰⁹ (see Figure 1.6) and increasingly sophisticated sequences (such T1 and T2 mapping sequences described in chapter 2)⁶⁶ for detailed myocardial assessment.

The individual CMR sequences used in this work will be discussed in greater detail in chapter

2.

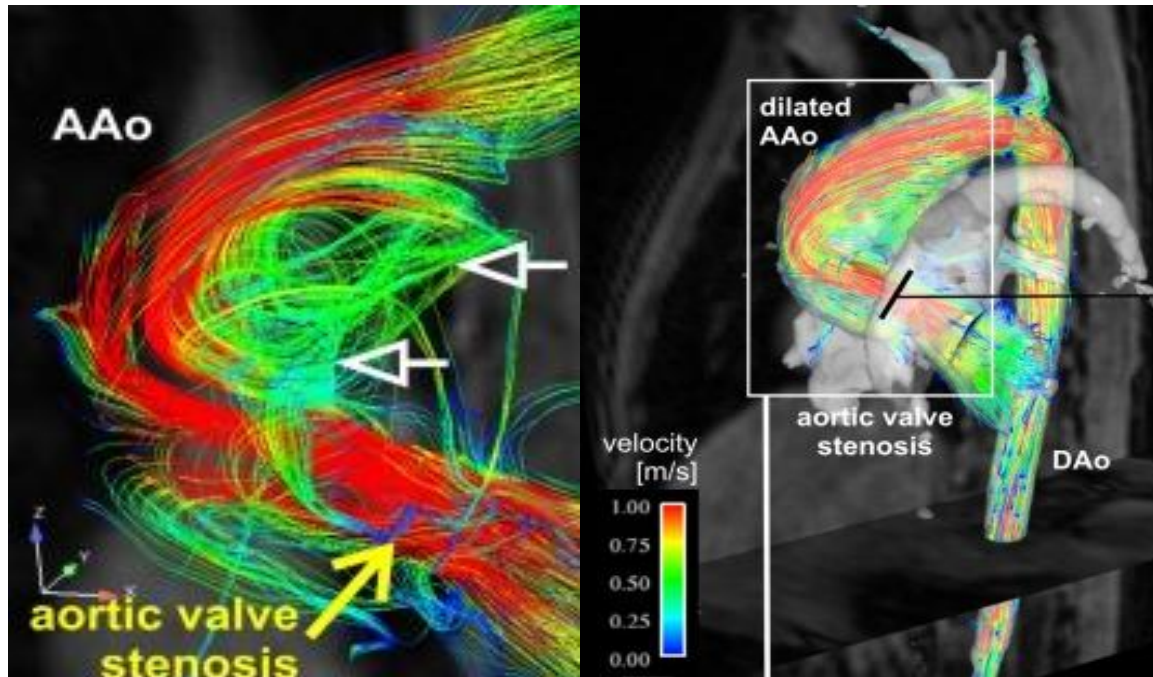


Figure 1.6. Example of CMR Four Dimensional Flow in a patient with aortic stenosis.
(Figure adapted from a review by Markl et al)¹⁰⁹ The patient had a valve area of 1.2 cm^2 .
Vortices form in the ascending aorta as illustrated by the white arrows.

1.7 Aims of this research

The broad focus of this research was to investigate the physiological changes in the myocardium of patients with AS using advanced cardiac imaging modalities.

The main aims were:

1. To investigate the physiological properties of the myocardium of patients with moderate and severe asymptomatic AS using CMR.
2. To examine the clinical value and feasibility of a new CMR sequence developed in our unit Shortened Modified Look-Locker Inversion recovery (ShMOLLI)⁶⁶ as a new method for non-invasive fibrosis mapping in AS.
3. To establish whether CMR imaging parameters can aid risk stratification of the asymptomatic AS population and to consider whether these could be used to monitor potential therapies in future clinical trials.

Chapter 2: General Methods

2.1 Rational for selection of the patient group

All subjects with asymptomatic AS were recruited to take part in a double blinded, placebo controlled clinical trial (International Standardized Randomized Controlled Trial Number 24616095) of Ramipril in asymptomatic AS; the RIAS trial. The data presented in this thesis is a sub-study of this trial.

These patients are a particularly interesting study group because they were recruited according to their asymptomatic status and both moderate and severe AS patients were included in the study. It is precisely in this *asymptomatic* AS group that interrogation of the myocardium using advanced cardiac imaging is of interest to identify subclinical early deterioration of LV function. The close clinical follow up required of the trial patients made them an ideal subgroup to study because measures of myocardial function could be directly linked to clinical outcome.

One of the main disadvantage of this trial sub-study is that power calculations were based on the primary end-point of the clinical trial (change in LV mass in those on Ramipril) and not on the outcomes of this study *per se*.

The initial number of patients recruited to the RIAS trial was based the power calculation for anticipated Ramipril mediated changes in LV mass. A CMR study looking at patients with severe AS showed that mean baseline LV mass in using CMR was 142g/m^2 with a standard deviation of 35g/m^2 .¹¹⁰ The mean reduction in LV mass with ACE inhibitors from a large meta-analysis of antihypertensive treatments was 15%, which equates to 21.3g/m^2 .¹¹¹ Using these figures, and a one-sided paired test (only including reduction in LV mass) with

85% power (β error) and 95% confidence (α error), the number needed in each study group is 43. By including 50 in each group, a 15% drop-out rate was accounted for.

From these power calculations, the aim was to include 100 patients in the clinical trial. In the end, only 96 patients were recruited to the trial for the following reasons: 2 withdrew on the day for personal reasons; one patient was so claustrophobic that he could not tolerate the CMR scan: one patient who was found to have been prescribed an ACE inhibitor (exclusion criteria for entry into the trial) by his general practitioner the day before he started the trial.

The symptomatic AS group data from 24 patients presented in chapters 4 and 5 came from collaborative work carried out with Dr Steven White from the Heart Hospital in London (see Declaration).

Inclusion of this data in the thesis enabled comparisons to be made between CMR measured changes in the myocardium of symptomatic and asymptomatic AS patients.

This group of symptomatic patients from the Heart Hospital underwent CMR scanning CMR with a 1.5T clinical scanner (Avanto, Siemens Healthcare, Erlangen, Germany) scanner using the same CMR methods described under section 2.6.2 (volumes and function), 2.6.3 (aortic valve area assessment), 2.6.4 (late gadolinium imaging) and 2.8 (T1 mapping using ShMOLLI sequence) and biopsy at the time of AVR as described in section 2.9.

2.1.1 Patient recruitment, inclusion and exclusion criteria

Asymptomatic patients (n=96) with moderate to severe AS (based on Doppler echocardiographic demonstration of peak aortic valve gradient ≥ 36 mmHg or valve area < 1.5 cm², according to established criteria)¹¹² were prospectively recruited from October 2008 to

April 2011 from outpatient clinics at the John Radcliffe and surrounding hospitals in Oxford according to pre-specified trial inclusion criteria listed below.

Twenty-four symptomatic patients with severe AS awaiting AVR were prospectively enrolled from the Heart Hospital Imaging Centre in London, UK between July 2010 and March 2012. The inclusion and exclusion criteria also applicable to this study group are noted by * in the list below.

Subjects were eligible for inclusion in the study if they fulfilled the following criteria:

- Aged 18 and above*
- Had given informed consent*
- Had asymptomatic AS of at least moderate degree (defined by peak velocity > 3m/s or aortic valve area > 1.5 cm² by Echocardiography)
- Had an LV ejection fraction >40% without regional wall motion abnormalities

Subjects were **excluded** if any of the following were present:

- Any other significant (> mild) valvular heart disease*
- Those who have been prescribed ACE-I or ARBs in the previous 3 months
- Patients with a sustained systolic blood pressure measurements <100mmHg or > 200mmHg.*
- Any contraindications to MR scanning (metal implants, pacemakers, claustrophobia, previous head or eye surgery).*
- History to suspect pregnancy OR a positive pregnancy test OR breastfeeding.*
- Impaired renal function (defined as Creatinine > 200 pmols/L)*

Each participant gave written informed consent to be involved. This study was approved by the Oxfordshire Research Ethics Committee (07/H0606/139) and was carried out in accordance with the declaration of Helsinki.

2.2 Clinical assessment

On the day of scanning, all asymptomatic subjects underwent a clinical assessment. This included recording a full medical history and documenting:

- The presence or absence of exclusion criteria
- The presence or absence of cardiovascular symptoms (in particular chest pain, shortness of breath and syncope)
- The past medical history (with particular attention paid to past cardiovascular history)
- Drug history, including allergies
- Alcohol and smoking history
- Family history, with particular reference to cardiovascular problems

A full physical cardiovascular examination was also performed and the following recorded:

- Height (cm) and weight (kg) using calibrated scales
- Blood pressure measurements using a manual sphygmomanometer
- Pulse rate, volume, regularity
- The presence/absence of added heart or chest sounds and ankle oedema
- Abnormalities of the abdominal, neurological or respiratory system

The following investigations were performed:

- 12 lead electrocardiogram, (ECG)

- Blood tests for serum total cholesterol, BNP, Liver function tests, Urea, Creatinine and Electrolytes.
- Exercise testing using the Naughton protocol
- Cardiac imaging: Echocardiography and a full cardiac MRI scan

2.3 Exercise testing

Exercise testing was carried out to as an objective measure of exercise capacity and to ensure that the patients included in the study were truly asymptomatic; previous studies have shown that exercise testing unmasks symptoms in up to 29% of apparently “asymptomatic” AS patients.¹¹³ We used the Naughton protocol exercise test¹¹⁴ because it has been shown to be safe to use in AS patients;^{115, 116} further, its slow and gradual increment of work load made it more suitable for use in elderly patients than a standard Bruce protocol stress test.¹¹⁷ (See Figure 2.1 for Naughton Protocol).

The test was carried out under medical supervision in an exercise testing laboratory with a continuous 12-lead electrocardiogram recording and computerized data analysis. Blood pressure was recorded manually every 3 minutes during exercise, at the point the exercise test was terminated and after 5 minutes of recovery time. The test was terminated at completion of the protocol or if the patient developed any one of the following:

- Symptoms of angina, excessive dyspnoea, syncope or pre-syncope
- A drop in systolic blood pressure of >10 mm Hg,
- Muscular exhaustion
- Ventricular arrhythmias on the ECG
- Down-sloping ST depression of > 1 mm.

Stage	Time (minutes)	Speed (km/h)	Grade (%)
1	3	3.2	0.0
2	3	3.2	3.5
3	3	3.2	7.0
4	3	3.2	10.5
5	3	3.2	14.0
6	3	3.2	17.5
7	3	3.2	21.0
8	3	4.8	21.0
Recovery	5	0	0.0

Figure 2.1. The Naughton Exercise Protocol. The speed and % gradient increase are lower than in the Bruce protocol making this test suitable for use in patients with moderate and severe AS.

2.4 Rational for using echocardiography

Transthoracic echocardiography remains the gold standard for clinical investigation for patients with aortic valve disease and has been used for assessment of patients with in landmark studies looking at prognosis^{7, 118} and clinical trials of medical treatments for AS.^{28,}

119

There are many advantages to using echocardiography for cardiac imaging; it is non-invasive, low cost, versatile, reliable, repeatable, reproducible and has excellent temporal resolution compared to CMR (2 ms versus 30-50 ms) which makes it an excellent choice for assessment of parameters of diastolic dysfunction.¹²⁰

However, echocardiography also has some limitations. Image quality may be poor in some patients due to poor acoustic windows which may lead to inaccurate measurement of the LV outflow tract diameter; the Doppler beam can be miss-aligned leading to underestimation of aortic valve flow.¹²¹ Further, aortic valve area measurements in echocardiography are derived from the continuity equation¹¹² which makes an assumption

that the left ventricular outflow tract (LVOT) is a perfect circle, when it is in actual fact an ellipse. This can also lead to discrepancies between aortic valve area measurements between transthoracic echocardiography and CMR.¹²¹

Nevertheless echocardiography remains the gold standard imaging modality for assessment of patients with valvular heart disease and for this reason all patients included in this study underwent echocardiography.

2.5 Echocardiography scan protocol

Transthoracic echocardiography was carried out using a Philips iE33 advanced echo system (Philips Medical Systems, Best, The Netherlands) equipped with 2.5-3.5 MHz transducers.

A full echo study was performed according to the European and American Society for Echocardiography guidelines for assessment and classification of aortic stenosis¹²² and chamber quantification.¹²³ Left ventricular volumes and ejection fraction were calculated by Simpson's rule.

LV diastolic function was assessed in line with the American Society of Echocardiography guidelines.¹²⁴ Pulse wave tissue Doppler imaging to acquire mitral annular velocities was performed from the apical 4-chamber view. A 1.5-mm sample volume was placed 1 cm within the septal and lateral insertion sites of the mitral valve leaflets sequentially¹²⁵ and analysis was performed for the systolic (S'), early (E') and late (A') diastolic velocities. The ratio of early to late annular velocity (E'/A') was determined as a parameter of diastolic function.¹²⁴

2.6 Cardiac magnetic resonance imaging

The rationale for using CMR in this study was discussed in chapter 1, section 1.6. To recap briefly, the main advantages of using CMR for this study are that image quality is not limited; it can therefore be used in all patients for myocardial tissue characterization, measures of LV mass and function and accurate measurements of aortic valve area and flow.¹²⁰

The disadvantages of using CMR are that claustrophobic patients cannot tolerate the scan; further, CMR scanning is time consuming and necessitates the administration of contrast for certain scan sequences. It is more expensive and has lower temporal resolution than echocardiography and is therefore poorer at measurements of diastolic function. Nevertheless, CMR remains the best non-ionising, radiation free imaging modality for multi-parametric myocardial assessment and was therefore the principal imaging modality of choice for this study.

2.6.1 CMR imaging scan protocol

All scanning was conducted on a Siemens 1.5 T Avanto MR system (Erlangen, Germany). Average scan time was approximately 55 minutes. In cases where the patient was judged to be too frail to undergo a full scan at the discretion of the investigator certain sequences were omitted. All subjects underwent a comprehensive CMR scan (see Figure 2.2) protocol which measured the following:

- (i) Cardiac mass and function
- (ii) Aortic valve area and peak blood flow velocity through the aortic valve
- (iii) Strain in the basal, mid and apical slices using “tagging” sequence
- (iv) T1 values using the T1 mapping sequence “ShMOLLI” (Shortened Modified Look-Locker Inversion recovery) sequence, on a mid ventricular slice
- (v) Rest and adenosine stress perfusion imaging
- (vi) Late gadolinium enhancement, (LGE) to assess fibrosis

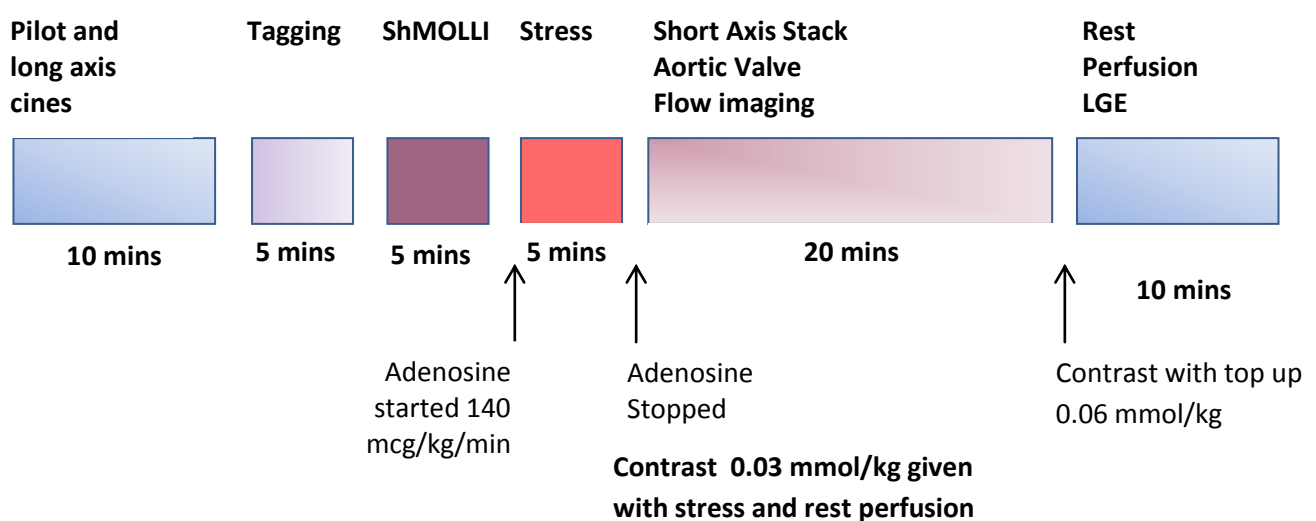


Figure 2.2. Scan Protocol Timeline. The average time taken for this protocol was 55 minutes.

2.6.2 Cardiac mass, volumes and function

CMR has been shown to be an extremely accurate, reproducible and well validated method for measuring left ventricular volumes and mass with the Steady State Free Precession (SSFP) sequence.^{126, 127} Cardiac volumes were acquired using pilot images, horizontal long axis, vertical long axis, and short axis stack images with the patient in the supine position, in line with standard CMR imaging protocols.¹²⁸ Each slice was 7mm thick with a 3mm gap and was retrospectively gated. The slices were obtained during a breath-hold at the end of normal expiration to minimize the effects of respiratory motion.

LV short axis epicardial and endocardial borders were automatically contoured using the software Q Mass (version 7.2, Medis, Netherlands-see Figure 2.3). The contours were adjusted manually at end diastole and end systole to calculate the end diastolic volume (EDV); end systolic volumes (ESV); stroke volume (SV). Ejection fraction (EF) and cardiac output (CO) were calculated from these measures. Myocardial mass was calculated based on a prior knowledge of calculated by myocardial specific gravity (1.05 g/cm^3) by subtracting the endocardial volume from the epicardial volume.

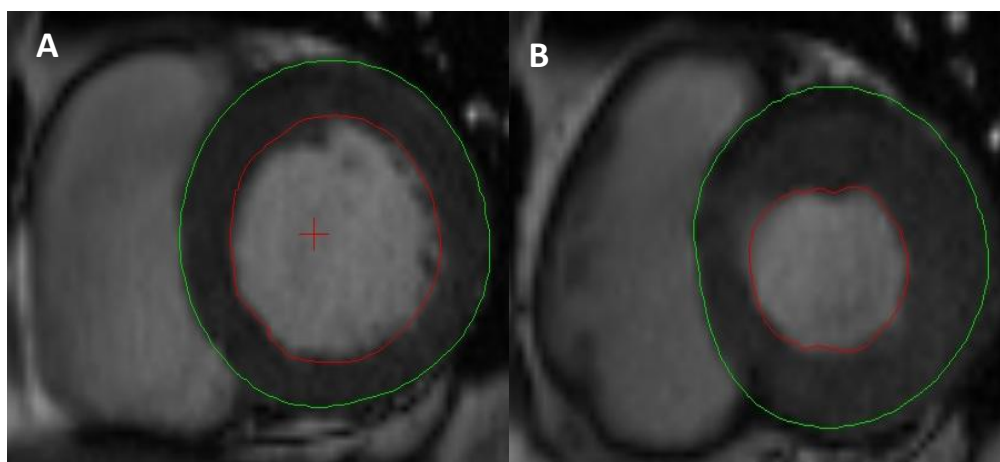


Figure 2.3. Measurement of LV mass and ejection fraction. Panel A shows the LV contoured in diastole and B shows LV contoured systole.

2.6.3 Aortic valve assessment

CMR was used to assess aortic valve morphology, aortic valve area (AVA) and flow using methods validated in a number of previous studies.^{129, 130} This technique has been previously validated, is highly reproducible and shows good correlation with AVA as measured by transoesophageal echocardiography (TOE).^{108, 131} A known limitation of the method is that AVA can be overestimated if the CMR slice position is not positioned directly at the aortic valve tips.¹²⁰

The aortic valve morphology and AVA were determined by application of a thin slice (4-5 mm) radial SSFP sequence through the tips of the aortic valve as determined from the LVOT coro and LVOT views. The AVA was then determined by direct planimetry of the orifice using Argus software (Siemens Inc., Erlangen, Germany). Aortic flow was determined using velocity flow mapping, a reproducible CMR technique for quantification of flow; a known drawback of the methodology is that it systematically underestimates peak velocity due to lower temporal resolution.^{130, 132, 133}

Aortic in-plane flow was determined by applying the flow sequence in the LVOT and LVOT coronal views. See Figure 2.4. The aortic valve radial cine image position was copied and the in-plane velocity flow maps used to determine the location of maximal velocity (vena contracta) and to plan “through-plane” (perpendicular to flow) flow sequence to calculate the peak and mean blood flow velocity through the AV. In instances of alias the imaging was repeated at a higher velocity. See Figure 2.5.

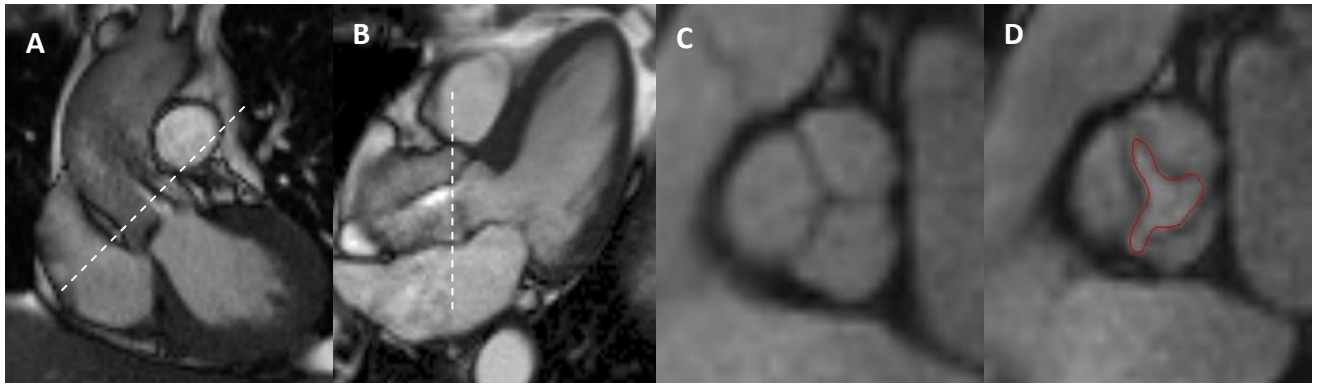


Figure 2.4. Measurement of aortic valve area. Panel A shows LVOT coro view used to plan aortic valve cine by cutting through the aortic valve tips. Panel B shows LVOT view used to plan aortic valve cine. Panel C shows aortic valve cine (radial sequence) in diastole. Panel D shows how the aortic valve area is measured using planimetry.

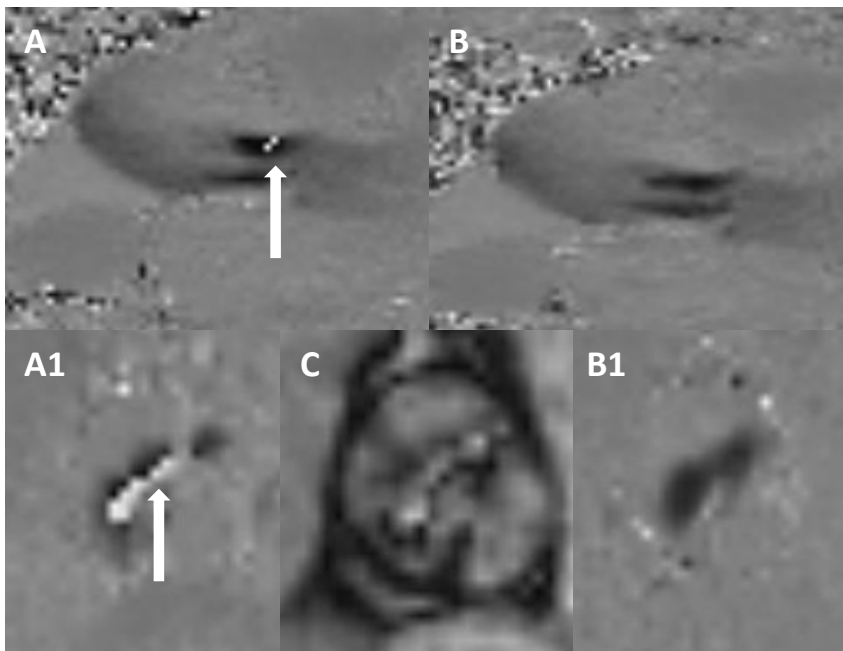


Figure 2.5. CMR measurement of blood flow through the aortic valve. Panel A shows LVOT view in- plane flow measurement with alias (velocity set at 200 m/s – note white spots resulting from alias). A1 shows through-plane image with alias. B shows in-plane flow with no alias (velocity 400 m/s). B1 shows through plane flow with no alias. C shows the aortic valve anatomy.

2.6.4 Perfusion and late gadolinium imaging

CMR adenosine perfusion and late gadolinium enhancement (LGE) imaging has been carried out in a number of other studies in AS using CMR and has been shown to be a safe technique that is well tolerated by patients, even in those with severe AS.^{15, 22, 134} The recently published cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC) study showed that CMR perfusion imaging has superior sensitivity and negative predictive values than SPECT (Single Photon Emission Computed Topography).¹³⁵

Adenosine stress CMR perfusion imaging was performed to assess myocardial perfusion reserve and identify endocardial perfusion defects from the AS-induced LV hypertrophy. Adenosine was administered at a rate of 140 µg/kg/min for 3 minutes before perfusion imaging. Gadolinium-based contrast (gadodiamide [Omniscan]; Nycomed Amersham, Little Chalfont, UK) was administered intravenously as a bolus at a dose of 0.03 mmol/kg (injection rate, 6 ml/s; concentration, 0.5 mmol/ml), to maintain a linear relationship between signal intensity and absolute perfusion. The gadolinium was followed by a saline flush of 15 ml at the same rate. Perfusion imaging was performed every heartbeat during the first pass of the contrast bolus with the use of a T1-weighted fast (spoiled) gradient echo sequence with saturation-recovery magnetisation preparation, as described previously.¹³⁶ Perfusion images were acquired in 3 short-axis sections at basal (between LV outflow tract and the papillary muscles), mid-ventricular, and apical levels chosen according to recommended guidelines.¹³⁷ To allow sufficient contrast washout, rest perfusion imaging was performed at least 20 minutes after the stress study.

After rest perfusion imaging, an additional dose of 0.1 mmol/kg gadodiamide was administered (total dose: 0.16 mmol/kg). The LGE images were acquired after an additional 10 minute delay with the use of an inversion-recovery prepared, segmented gradient echo sequence, as previously described.¹³⁸ In all patients, imaging was repeated for each short-axis image in 2 separate phase-encoding directions to exclude artifact. Mid-wall LGE was only deemed to be present when the area of signal enhancement could be seen in both phase-swapped images and in a cross-cut long-axis image by the independent observers.¹³⁹

The perfusion images were processed by tracing endocardial and epicardial contours (QMass software, version 7.2, Medis, Netherlands). A region of interest was drawn in the LV blood pool, avoiding any papillary muscles therein, to permit the derivation of an arterial input function. The myocardium was divided into equiangular segments based on the American Heart Association segmentation model.¹³⁷ Rest and stress myocardial perfusion upslopes were calculated using 5 point linear fit model of signal intensity vs. time and normalized to the LV blood pool upslope.¹⁴⁰ Myocardial perfusion reserve index (MPRI) was derived from the combined average of the 16 segments and per subject and is defined as the ratio of stress to rest normalized myocardial perfusion upslopes. See Figures 2.6 and 2.7.

All patients with an estimated glomerular filtration rate (eGFR) $< 60\text{mls/min/1.73m}^2$ were excluded from LGE and perfusion imaging to negate the risk of contrast induced nephrogenic systemic fibrosis.

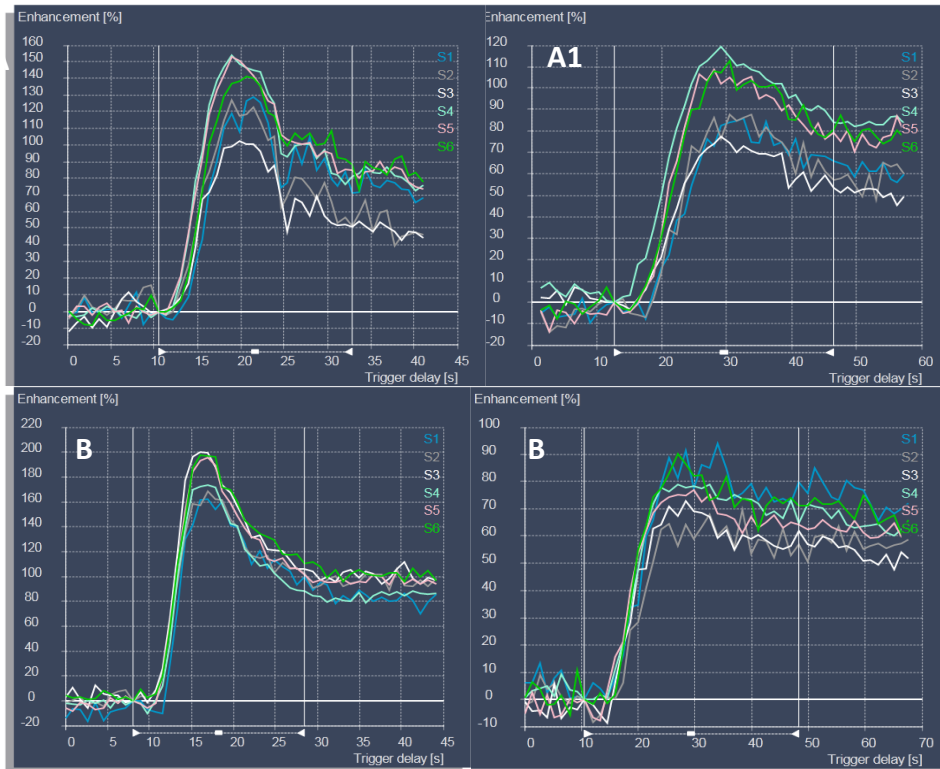


Figure 2.6. Myocardial intensity slope curves. A and A1 show intensity slope curves for a patient with AS at rest and stress (respectively). B and B1 show intensity slope curves for a normal subject at stress and rest respectively. During stress, note the less steep upslope in A1 for segments 1, 2, 3 during stress compared to B1. This indicates a perfusion defect seen in anterior and anterolateral region.

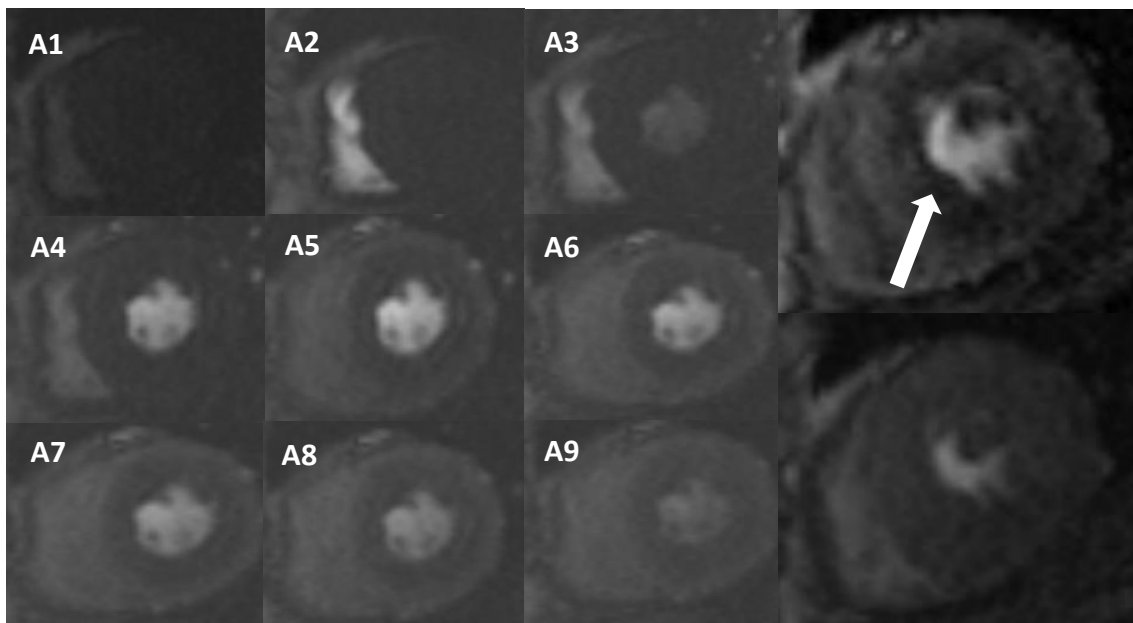


Figure 2.7. First pass myocardial CMR perfusion imaging. A1-A9 show sequential perfusion images during adenosine stress in a patient. Initially, no contrast (A1), then it enters the right ventricle (A2,A3), then left ventricle (A4-6) and flushes through the myocardium (A7-9). Note the circumferential perfusion defect in the stress image (dark rim shown by arrow, right upper panel) which is not present in the rest image (immediately below).

2.7 Strain imaging

A number of recent studies have highlighted that measurement of LV strain, in particular longitudinal strain, using echocardiography in AS may identify early impairment of myocardial function.¹⁴¹⁻¹⁴³

Strain is a dimensionless quantity of myocardial deformation defined as the change of myocardial fibre length during stress at end-systole (L) compared to its original length during stress at end-diastole (L_0) and is normally expressed as a percentage ($\text{Strain} = L - L_0 / L_0$). Strain rate (SR) is the change in strain per unit time.¹⁴⁴ Strain can be measured in longitudinal, circumferential and radial directions and partially reflect sub-endocardial, mid-wall and sub-epicardial myocardial fibre function respectively.¹⁴⁵ (See Figure 2.8).

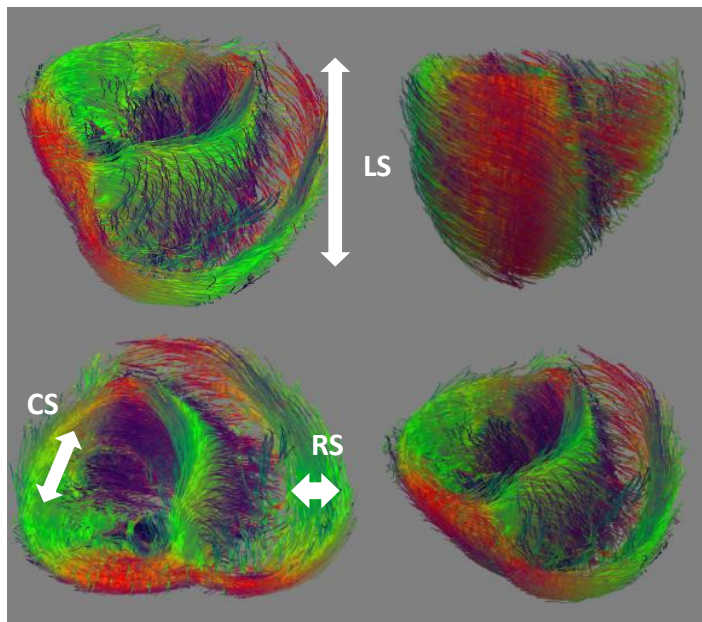


Figure 2.8. Myocardial fibre architecture from Tensor MRI. (Figure adapted from Heart-Muscle Fiber Reconstruction from Diffusion Tensor MRI Leonid Zhukov Alan H. Barr Department of Computer Science, California Institute of Technology; Proceedings of the 14th IEEE Visualization Conference VIS'03). Black shows longitudinal subendocardial muscle fibres, LS the longitudinal strain motion plane; green shows mid-wall fibres, CS the circumferential strain motion plane, red shows sub-epicardial fibre, RS the radial strain motion plane.

LV torsion represents the difference between rotation of the apex and rotation of the base; the base and apex rotate in opposite directions secondary to co-ordinated contraction of sub-endocardial and sub-epicardial fibres.¹⁴⁶ (See Figure 2.9).

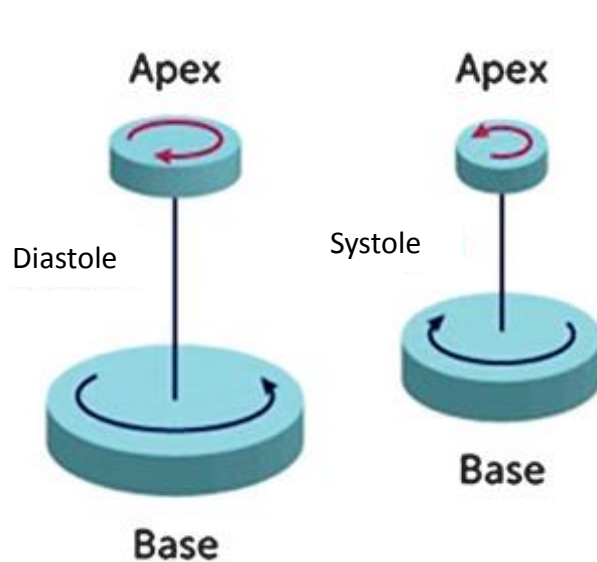


Figure 2.9. Left Ventricular Torsion in systole and diastole (Figure adapted from review by Blessberger et al)¹⁴⁷ LV torsion is the net difference between apical and basal rotation (calculated from two short axis cross-sectional planes of the LV).

Measurement of multi-directional myocardial strain with echocardiography uses a technique developed in 2004 called “speckle tracking”¹⁴⁸ which uses speckle artifacts in the echogram image as natural acoustic markers to track myocardial motion. This technique can be employed at the bedside and is cost-efficient making it attractive for use in clinical studies. However, the accuracy of speckle tracking strain measures depends on the 2D image quality and frame rates, as highlighted in a review by Argyle and Ray.¹⁴⁹

CMR has the advantage over echocardiography that measurement of strain is not limited by image quality. Poor acoustic windows were not exclusion criteria in this study; to maximise the amount of strain information that could be included in the study, we used opted to use CMR for measurement of LV strain in this study.

2.7.1 CMR tagging for LV strain assessment

CMR is a well validated tool for measurement of multidirectional strain using a CMR sequence known as “tagging”,¹⁵⁰ which has been validated against myocardial tissue tracking markers.¹⁵¹ The disadvantages of using tagging are those associated with additional long breath-holds for the patient, prolonged post-processing and “tag-fading” (particularly at 1.5 T field strength) in diastole;¹⁵¹ for these reasons is not used routinely in clinical practice.

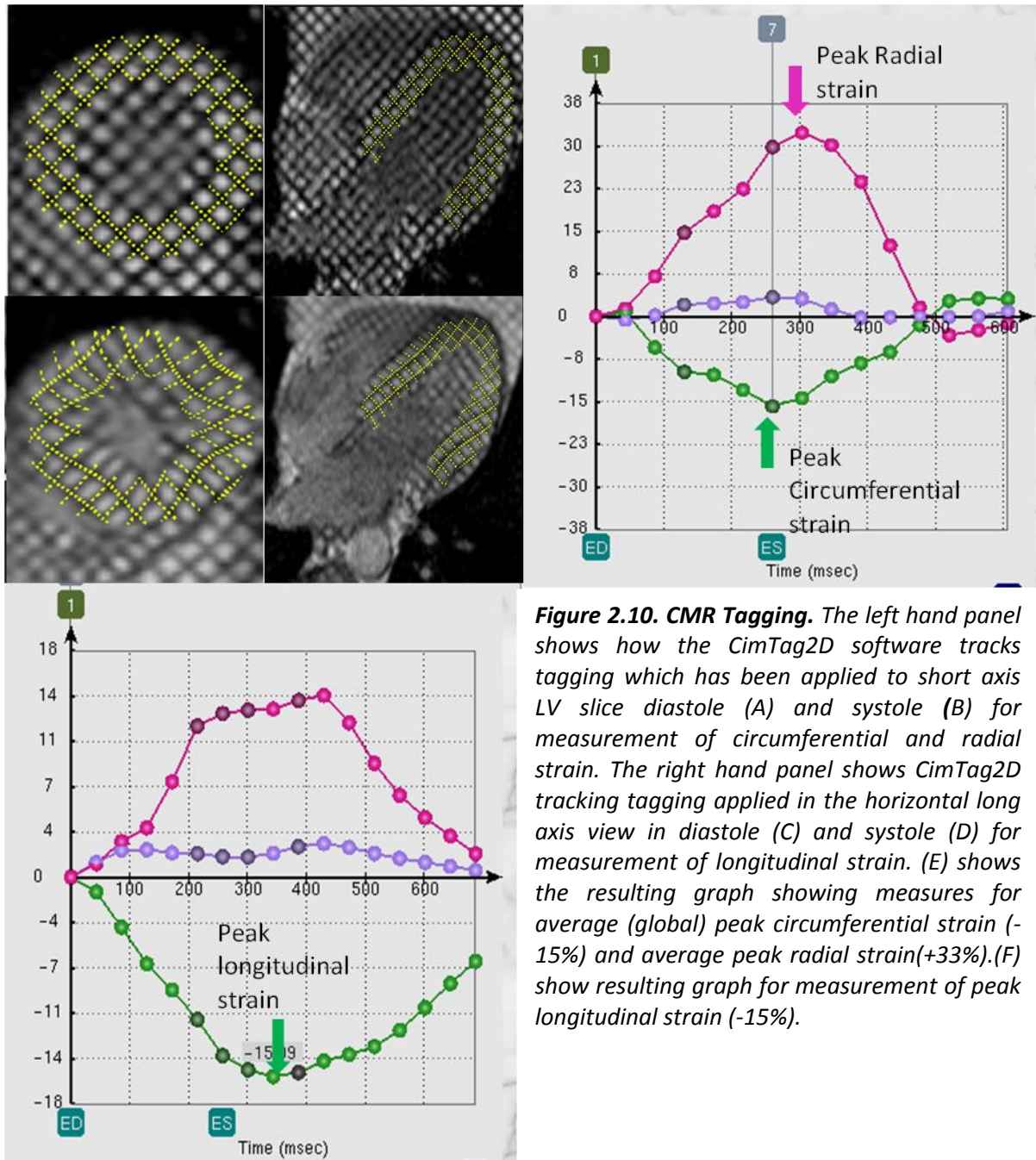
In all patients, a gradient echo-based tagging pulse sequence was performed in the long axis (horizontal long axis) and in the basal, mid ventricular and apical short axis slices with a segmented k-space, multi-shot sequence (repetition time 25ms, echo time 7.4ms, and flip angle 25°).¹⁵²

The basal slice position was defined as first slice to show circumferential myocardium at both systole and diastole. The apical slice was the last slice to show intracavity blood pool in all phases over the cardiac cycle. The mid ventricular slice was positioned half-way between the basal and apical slice.

Spatial modulation of magnetization¹⁵³ produced images with a grid-based pattern of horizontally and vertically modulated ‘stripes’ 1cm apart, acquired during a single breath hold. The temporal resolution was <40 ms in all data sets, and 15–25 frames per cardiac cycle were recorded, depending on heart rate.

Tagged images were analysed using CimTag2D version 7 (Auckland Medical Research, Auckland, New Zealand); this software uses non-rigid registration on the original images and tracks features (tags in this case). CimTag2D is a manual corrected tracking procedure;

errors in the tracking are corrected by visual feedback by the user, previously validated against displacement encoding using stimulated echoes (DENSE) and deformable gel phantoms.¹⁵⁴ Global peak strains (averaged from all myocardial segments) were measured in the circumferential and longitudinal directions in systole from the short axis views and horizontal long axis views respectively; radial strain was not recorded due to high variations in this parameter reported in previous studies.¹⁵⁵ See Figure 2.10.



2.7.2 CMR feature tracking for LV strain assessment

Until recently, LV strain measurement using CMR could only be carried out if tagging sequences were acquired. However, newly developed software, Tomtec myocardial feature tracking software (Diogenes MRI:Tomtec: Unterschleissheim, Germany) allows measurement of myocardial strain from routine SSFP CMR images using a technique analogous to echocardiographic speckle tracking.¹⁵⁶

CMR feature tracking (CMR-FT) allows tracking of a tissue voxel of motion in SSFP CMR images which allows circumferential and radial strain to be assessed from short axis images; longitudinal strain can be derived from the short axis and long axis images respectively. (See Figure 2.11).

There are already a small number of publications showing good correlations and low measurement variability between CMR-FT derived measures of LV strain (in particular circumferential strain) and tagging.¹⁵⁷⁻¹⁵⁹

In our unit, work by Augustine et al analysed strain by tagging using CIMTag2D version 7 (Auckland Medical Research, Auckland, New Zealand) and CMR- FT from SSFP sequences in normal controls; they found good correlation in strain measurements with the two techniques with the most reproducible measure being average mid-circumferential strain (CS) both for inter-observer variability (limits of agreement -3.27% to 0.39%) and for agreement with tagging derived strain data (-5.6% to 4.3%). There was poor agreement between feature tracking and tagging for global longitudinal (-14.6% to 5.34%) and radial strain (-1.43 % to 22.56%). (See abstract by Augustine et al. in Appendix 1).

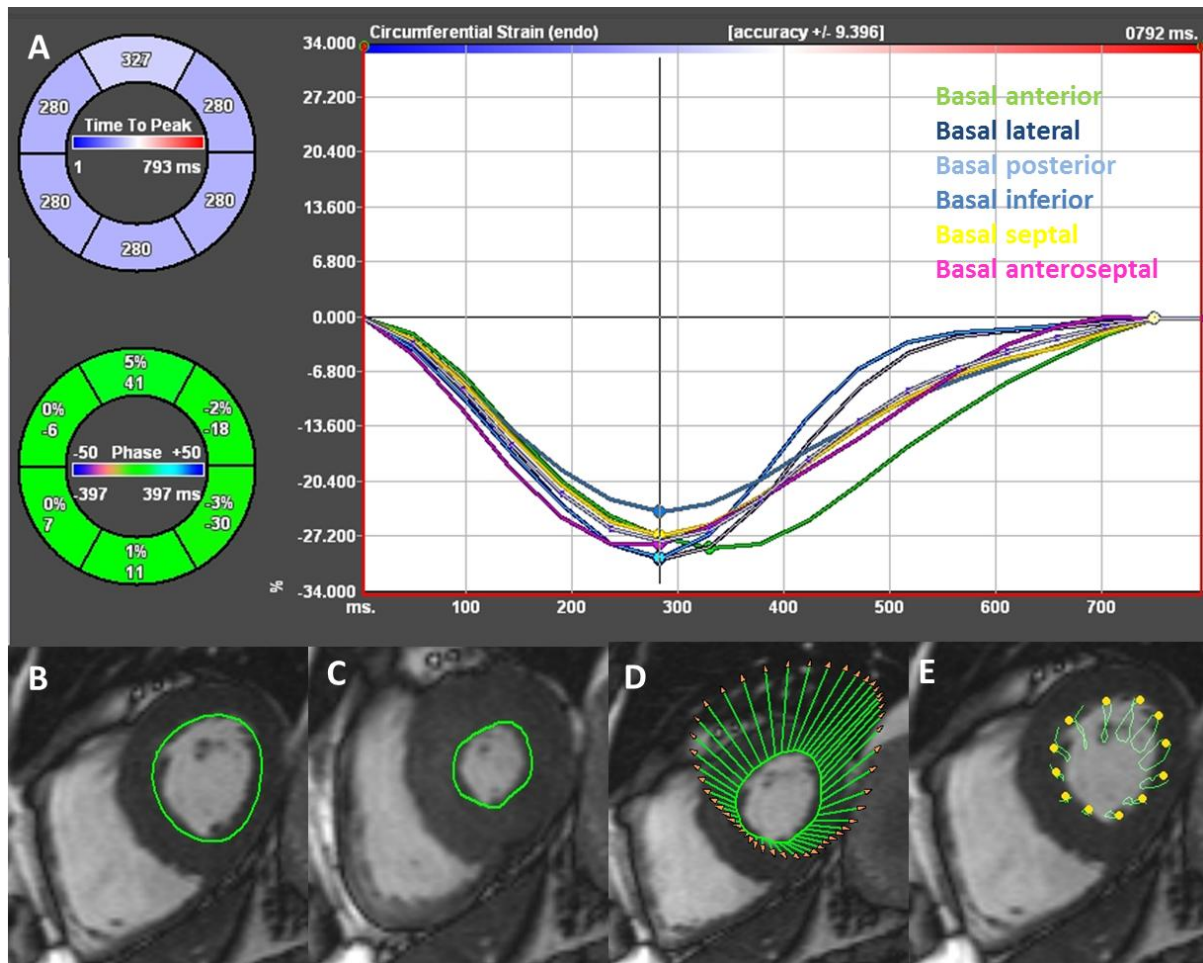


Figure 2.11. CMR-FT Software. Panel A shows the circumferential strain curve derived for all 6 segments in this basal short axis LV slice and the resulting strain curve; each colour represents a different myocardial segment. Panel B shows the endocardial contouring in diastole; Panel C the endocardial contouring in systole, Panel D the motion vectors and Panel E the 12 marker tracking points from which the strain measurement is derived.

Given the fact that CS had the lowest inter-observer variability and good agreements with tagging data, we opted to investigate this parameter in more detail in our AS study population using CMR-FT and these results are presented in chapter 4.

The author investigated her own intra-observer variability of global mid-CS measurements using CMR-FT in 47 patients with AS. All the cases were re-analysed 4 weeks after the first analysis. The limits of agreement are shown below in Figures 2.12.

The limits of agreement for the author were -3.9 to 3.9%; considerably higher than the inter-observer variability demonstrated by Augustine et al (see Appendix 1).

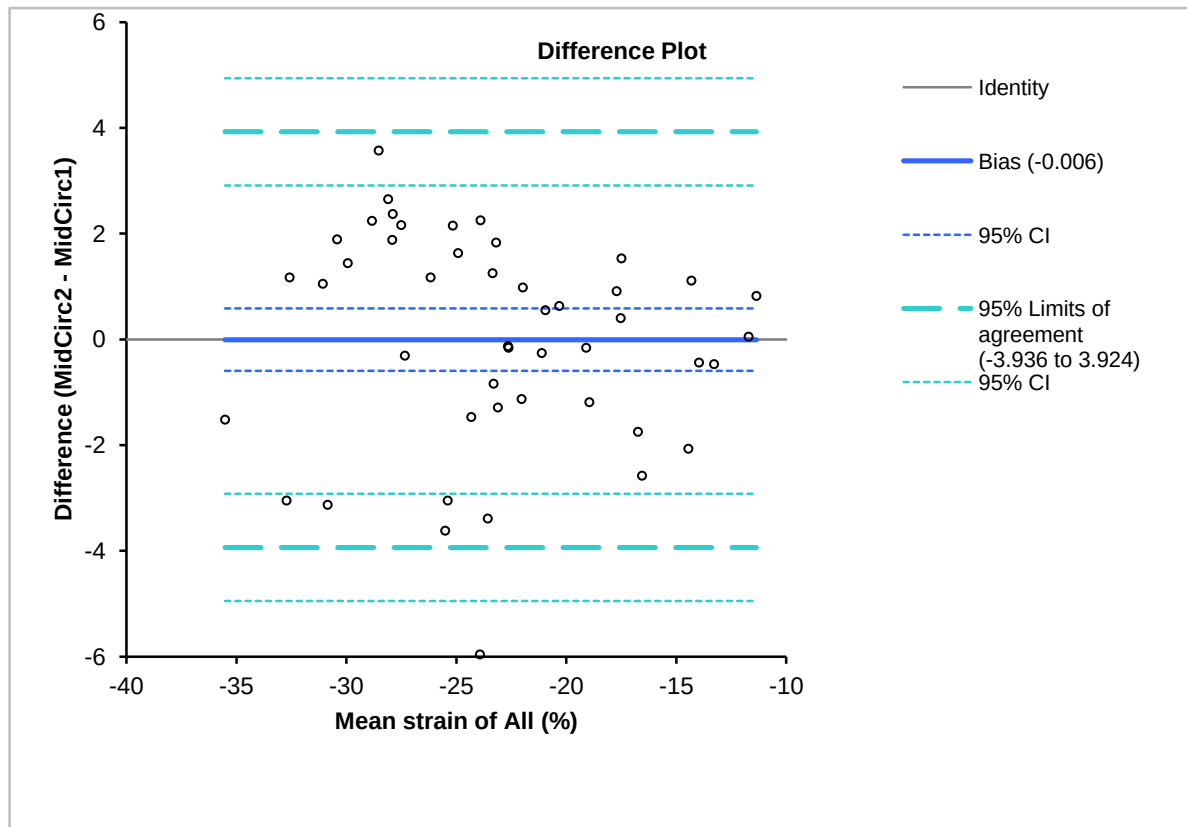


Figure 2.12. Circumferential Strain Bland-Altman plot. Mid-CS was re-analyzed 4 weeks after the first analysis in 47 patients; the limits of agreement were (-3.9 % to 3.9%). This intra-observer variability was worse than the inter-observer variability (-3.27 % to 0.39%) found by Augustine et al. (See Appendix 1).

2.8 *CMR T1 mapping technique*

In CMR imaging, the pixel signal intensity depends on the relaxation time of hydrogen nuclei (protons) in the static magnetic field after a radiofrequency pulse is applied.

There are two proton relaxation parameters: 1) T1 (or spin-lattice relaxation time) - the time taken for the proton to recover 2/3 of the *longitudinal* magnetisation value and 2) T2 (spin-spin time) which is the time taken for the proton to recover 2/3 of the *horizontal* magnetisation value.

At particular magnetic field strengths, different tissue types exhibit a characteristic range of T1 relaxation times when an inversion pulse is applied which depend on the intrinsic properties of the tissue; T1 values and can therefore be used for tissue characterisation.⁶⁵ (See Figure 2.13).

Changes in T1 values measured by CMR have been reported in a number of myocardial diseases including myocardial infarction,¹⁶⁰ valvular heart disease,¹⁶¹ and heart failure.⁵⁴

Currently this CMR technique is not used in routine clinical practice, however there is a large and expanding body of research investigating the clinical application of CMR T1 mapping;⁵² the technique is attractive because it has the potential to detect minute changes in cardiac tissue composition, in particular oedema and fibrosis which may be helpful for monitoring disease progression.

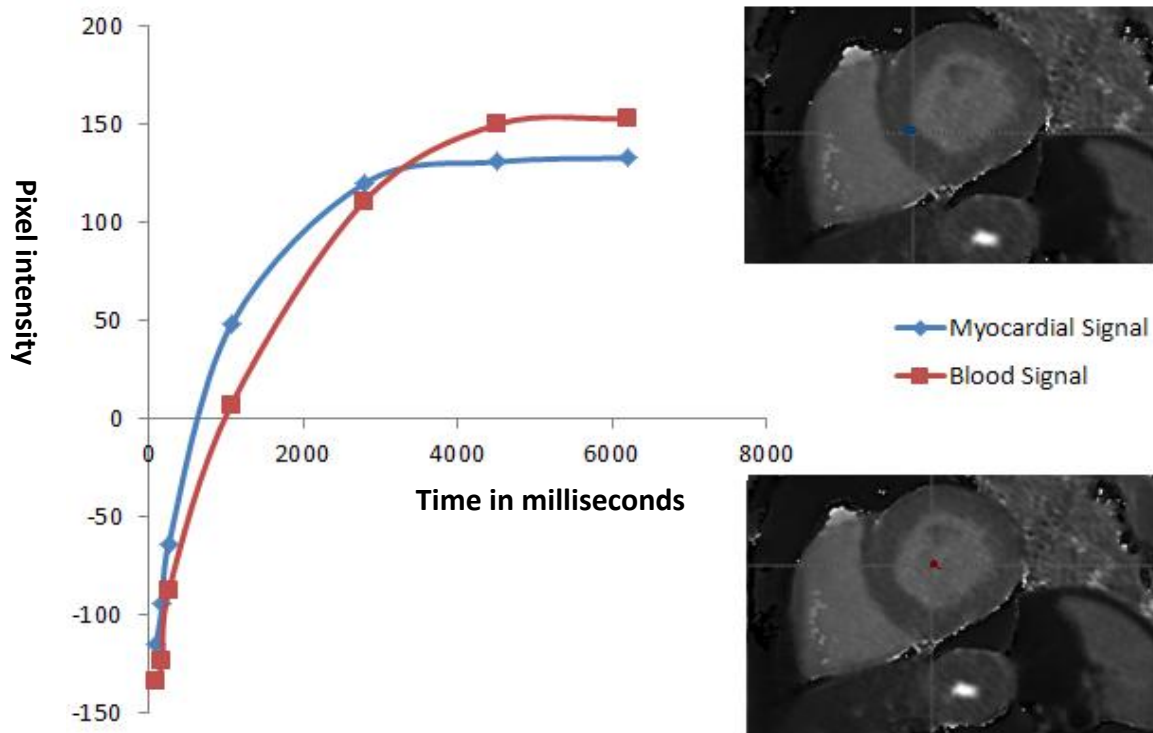


Figure 2.13. T1 relaxation time in blood and myocardium. T1 relaxation time is longer for blood than for myocardium (point at which the lines cross the x-axis); on the final image blood appears brighter than the myocardial tissue.

2.8.1 T1 mapping sequences MOLLI and ShMOLLI

New T1 mapping sequences have increased the capability of CMR to measure myocardial T1 robustly and in a single breath-hold. The most commonly used T1 mapping sequence is called MOLLI (Modified Look-Locker Inversion recovery);¹⁶² when combined with the extracellular contrast agent gadolinium, CMR MOLLI can determine the myocardial extracellular volume by comparing the ratio of signal change (T1 pre and post-contrast) in blood and myocardium after contrast.¹⁶³

However, the breath-hold times for MOLLI sequences are relatively long (15-20 seconds; 17 heart beat breath hold time to acquire a single slice) which can be difficult for many patients

with AS; further, it cannot be applied to patients with severe renal impairment as this is a contra-indication to gadolinium contrast administration.

A modified T1 mapping technique- ShMOLLI (Shortened Modified Look-Locker Inversion recovery) technique using a 9 heart beat breath hold time was recently developed in our unit. This technique is robust, reproducible and breath hold times are 50% less than using the MOLLI sequence.⁶⁶ (See Figure 2.14). We investigated pre-contrast T1 values in AS in chapter 5.

Following standard planning the ShMOLLI sequence was applied to a mid-ventricular short axis slice (definition half way between the basal and apical slices) in end- expiration as described in the methods paper by Piechnik et al.⁶⁶ ShMOLLI T1-maps are based on 5 images with specific T1=100-5000ms, collected using SSFP readouts in a single breath-hold, typically: TR/TE=201.32/1.07 ms, flip angle=35°, matrix=192x144, 107 phase encoding steps, interpolated voxel size=0.9x0.9x8mm, cardiac delay time TD=500ms; 206ms acquisition time for single image.

Short axis ShMOLLI images were manually contoured to outline the endo- and epicardium, and the average T1 value over this whole slice calculated using an in-house software (MyoCardial Regions Of Interest, MC-ROI programmed by Dr Stefan Piechnik in IDL (Interface Definition Language) v.6.1, ITT Exelis, Mclean, Virginia, US) as described by Piechnik et al.⁶⁶

T1-maps derived using ShMOLLI are subject to off-resonance artifacts, breathing motion and poor T1 model fit which can lead to incorrect T1 measurement. To exclude breathing artefacts, the raw T1 SSFP images were examined; to assess how well T1 model fitting was

achieved for each T1-map, a parametric map of the “goodness of fit” (R^2 maps) was generated. This pixel-wise indicator of the quality of the T1 map aids visual detection of artifacts, with many dark pixels indicating artifact. (See Figure 2.14).

Segments that had obvious areas of artefact were rejected from the analysis. Global T1 values per short axis slice were calculated as the average of the T1 values of all pixels in that slice. See Figure 2.15.

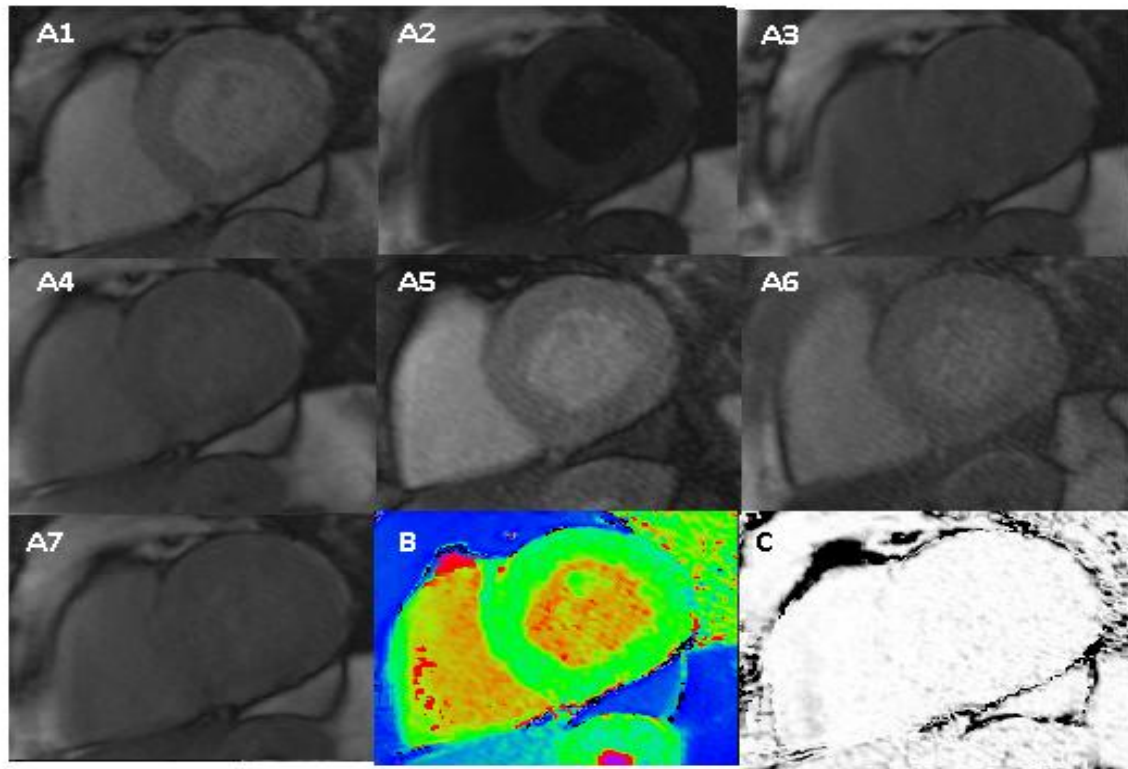


Figure 2.14. T1 mapping using ShMOLLI CMR sequence. A1-A7 shows the raw data T1 data collected in one single breath hold at differing time points; B shows the colour map generated from the raw data for colour representation of T1 values (see figure 2.15 for colour scale); C shows the R^2 used to identify artefacts; in this case there are no artefacts as no black pixels are seen within the R^2 map.

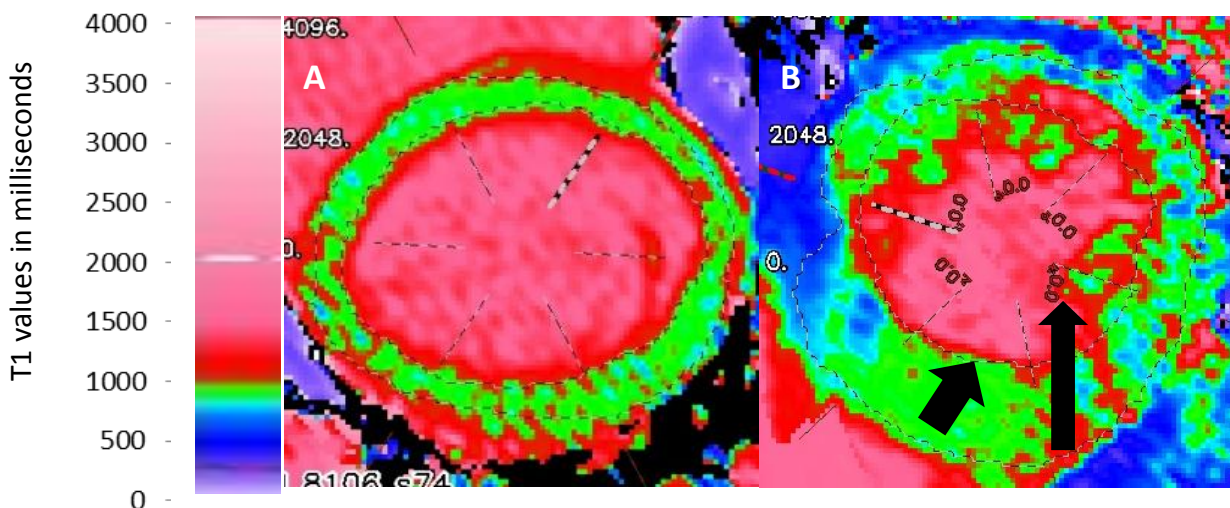


Figure 2.15. Contouring of short axis mid-ventricular ShMOLLI colour T1 maps. Two examples of short axis LV ShMOLLI colour maps with the colour scale on the left hand panel. A shows an example of an acquisition with no artefact. B shows a poor acquisition with multiple artefacts. Long arrow points to 00 which indicates the segments rejected on grounds of too much artefact. Short black arrow points to the only artefact free segment accepted for processing in this slice.

2.9 Histology

Endomyocardial LV biopsy currently remains the gold standard technique for assessment of LV fibrosis, however the method has some drawbacks. It is invasive and in cases where fibrosis is localized sampling error restricts the accuracy of biopsy.⁵²

In this study, the 24 patients with severe symptomatic AS underwent biopsy at the time of aortic valve surgery. Histological sampling and analysis was performed using a validated technique described by Flett et al in previously published data.¹⁶³ In summary, an intra-operative deep myocardial biopsy (Tru-cut type biopsy needle) was taken from the basal LV septum, fixed immediately in 10% buffered formalin, embedded in paraffin, and stained with picrosirius red. The samples were photographed at high-power magnification (x200). The collagen volume fraction (CVF%) was automatically quantified over an average of 12 high-power fields using a purpose-written macro in ImageJ.¹⁶⁴ All samples were analysed by an operator blinded to the CMR findings. See Figure 2.16.

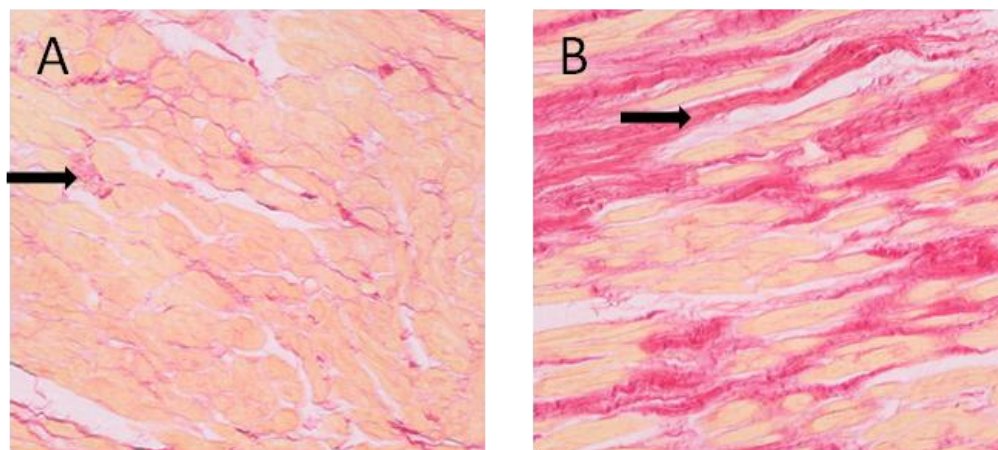


Figure 2.16. Histology of the Myocardium in AS. (Figure courtesy of Dr Steven White) Picrosirius red stain. Black arrows show fibrosis. (A) shows a case with mild fibrosis (collagen volume fraction= 8%). (B) shows a case of heavy fibrosis (collagen volume fraction= 27%).

2.10 *Clinical follow up*

Follow up information for all asymptomatic patients was obtained at 6 and 12 months from interviews with patients noting the absence or presence of chest pain, shortness of breath or syncope. Further follow up information after 12 months came from examination of hospital records and liaison with the referring cardiologist and general practitioners. The primary outcome variable was the time until the occurrence of the first composite end point of MACE (major adverse cardiac events) which was defined as aortic valve replacement, death or hospital attendance with cardiac symptoms. The clinical management of patients was determined independently by their treating physicians who were blinded to the scan information.

Chapter 3: Myocardial perfusion in patients with asymptomatic aortic stenosis

3.1 Introduction

In this chapter, CMR first pass perfusion imaging is used to examine the myocardium of patients with asymptomatic AS. Previous studies using perfusion using positron emission topography (PET) have shown microvascular dysfunction in patients with severe symptomatic AS.⁹⁰ The underlying mechanisms contributing to microvascular dysfunction in AS patients were discussed in detail in chapter 1.

Aortic valve replacement can improve, but does not completely reverse, microvascular dysfunction in these patients;²³ improvements are more marked in those receiving bioprosthetic valves (with central flow close to natural geometry) suggesting that the degree of flow disturbance created in the aorta also impacts on perfusion.¹⁶⁵

Little work has been done examining CMR adenosine first pass perfusion in AS. Currently there are only two published papers in this area examining patients with severe AS – an early small feasibility study¹³⁴ and a study examining the relationship between exercise and myocardial perfusion reserve (MPR) and myocardial perfusion reserve index (MRPI).²² The recent cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC) study confirmed that CMR adenosine perfusion imaging has a high sensitivity (86.5%) and specificity (83.4%) for diagnostic accuracy of coronary artery disease in a real world setting; whilst recognising that this is not the same as imaging microvascular dysfunction, the study does highlight the strengths of using CMR for perfusion assessment.¹³⁵

To date, there is no published work examining CMR adenosine perfusion imaging in patients with moderate and severe *asymptomatic* AS. The degree of microvascular dysfunction and

the relationship of this to measures of disease severity in this group of patients is currently not known. In this chapter, three main questions were examined:

- 1) The clinical feasibility of carrying out adenosine perfusion CMR scanning in elderly patients with moderate or severe AS
- 2) The relationship between myocardial perfusion reserve index (MPRI) and conventional measures of AS severity
- 3) The relationship between MPRI and exercise tolerance

3.2 Methods

50 subjects with asymptomatic moderate to severe AS were drawn from the main study cohort (inclusion and exclusion criteria described in chapter 2). Patients were asked to omit beta-blockers and caffeine for 24 hours before the perfusion scan to avoid interaction with adenosine receptors. Subjects underwent CMR scanning at 1.5 T to assess:

- 1) Left ventricular function, mass and volumes
- 2) Aortic valve area and peak flow velocity through the aortic valve
- 3) Rest and stress adenosine perfusion to measure myocardial perfusion reserve index

See chapter 2, section 2.6 for full protocols and image analysis. All patients also underwent echocardiography and exercise testing. Patients with LGE in an infarct related distribution pattern were excluded from the analysis.

Some patients did not have perfusion scanning for a number of reasons:

- Consent refused for CMR perfusion scanning
- Patients were judged by the investigator as being too frail to manage a prolonged CMR protocol, or who were not tolerating the early non-perfusion aspects of the scan well (e.g. difficulty with breath holding, incontinence problems, back pain)
- A history of asthma or COPD requiring use of inhalers (adenosine contraindicated)
- Previous bad reaction to adenosine
- Second or third degree heart block on baseline 12-lead ECG
- History of previous myocardial infarction (may confound perfusion measures)
- Estimated glomerular filtration rate of <60 mls/min/1.73 m² (risk from gadolinium contrast)

3.2.1 Statistical analysis

Data analysis was performed with commercially available software packages (IBM SPSS Statistics, version 19.0 and Medcalc version 9.6.4.0 for Windows). The chi-square test or Fisher's exact test were used to compare discrete data as appropriate. All comparisons were done using ANOVA with post hoc Bonferroni corrections; statistical significance was defined as $P < 0.05$. Correlations were done using bivariate Pearson's method. To determine independent predictors of MPRI, change linear regression was used. Only variables with significant association with MPRI change on bivariate analysis ($p < 0.05$) were entered into the multivariate model.

3.3 Results

3.3.1 Study population

The details of recruitment are outlined in Figure 3.1. Overall, 50 patients underwent perfusion imaging with no complications (see Figure 3.1 for numbers excluded in each category), from which there were 47 patients with datasets suitable for analysis. The demographic data for these patients are presented in Table 3.1.

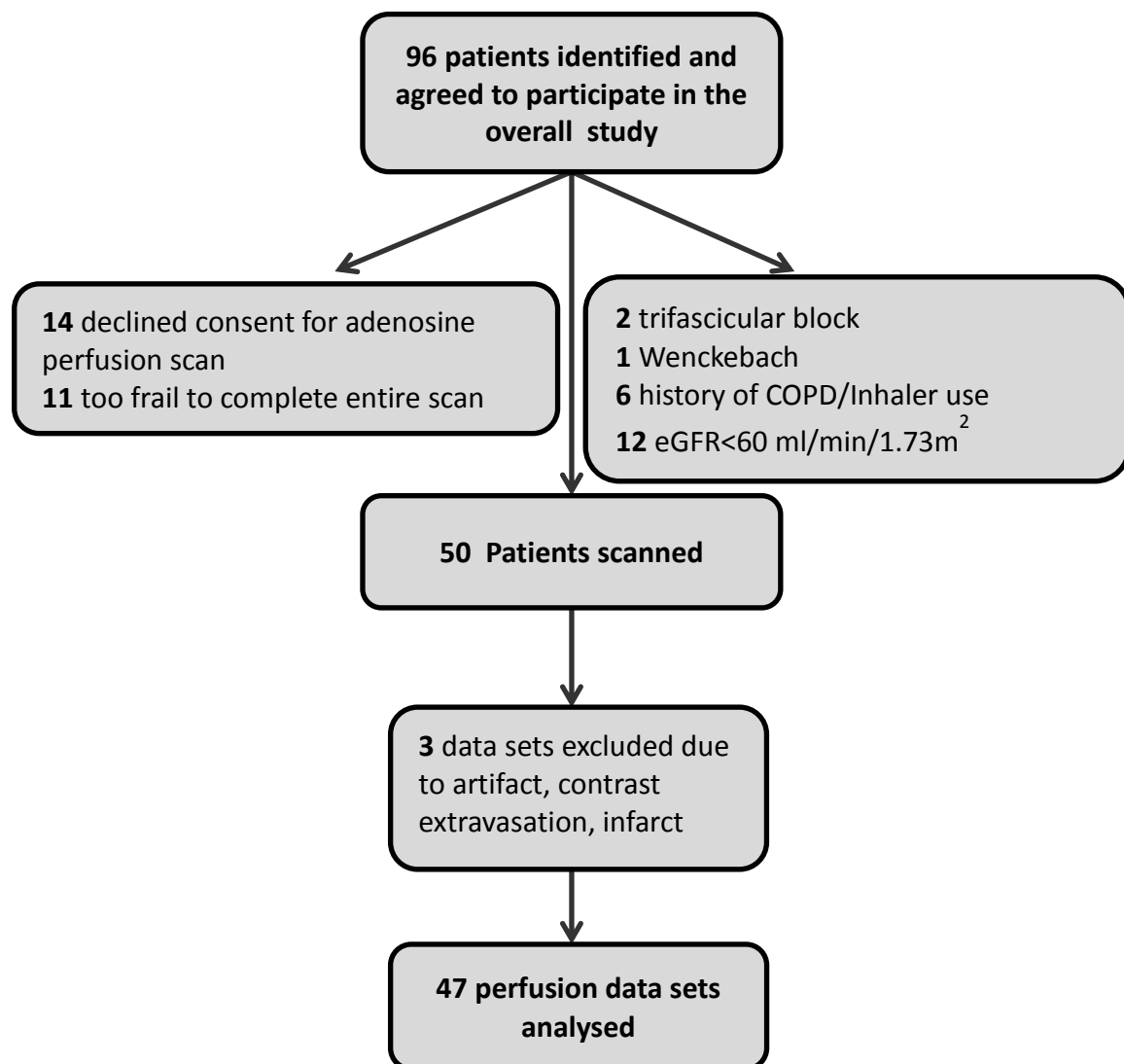


Figure 3.1 Recruitment data for perfusion scanning. COPD=Chronic Obstructive Pulmonary Disease. LGE=late gadolinium enhancement. eGFR=estimated Glomerular Filtration Rate ml/min/1.73m²

Table 3.1. Patient Demographics

Patient Characteristics (N=47)	
Age, yrs	64.8±15.5
Male, n(%)	37(79%)
Body Surface Area, m ²	1.96±0.34
Body Mass Index, kg/m ²	28.4±4.5
Haemodynamics	
Systolic arterial BP, mmHg	131±13
Diastolic arterial BP, mmHg	76±13
Heart rate, beats/min	65±15
Risk Factors	
Previous angioplasty or CABG, n(%)	5(11%)
Hypertension, n(%)	10(21%)
Diabetes, n(%)	3(6%)
Statins, n(%)	25(53%)
Current Smokers, n(%)	6(13%)
Former Smokers, n(%)	6(13%)
Beta-blockers, n(%)	12(26%)
Anti-platelets, n(%)	19(40%)

All values are expressed as mean±standard deviation. BP=Blood Pressure. CABG=Coronary Artery Bypass Grafting

3.3.2 Exercise testing

Of the 47 patients, 41 also underwent exercise testing. 6 patients could not be exercised due to poor mobility or extreme frailty. Of the 41 exercise tests, 11 patients had such good exercise capacity that they completed all 8 stages of the Naughton protocol. Reasons for terminating the exercise test earlier included the following: dsypnoea (n=13); ECG changes (n=3); musculoskeletal problems (n=5); general fatigue (n=10).

3.3.3 Cardiac magnetic resonance

Volumetric data and LGE images were available for all patients. Results are displayed in Table 3.2. One patient had a LGE pattern typical for myocardial infarction and this data set was excluded from analysis. All other patients with LGE present had a pattern that was not typical of myocardial infarction, and in keeping with patterns seen in previous studies of aortic stenosis.¹⁵

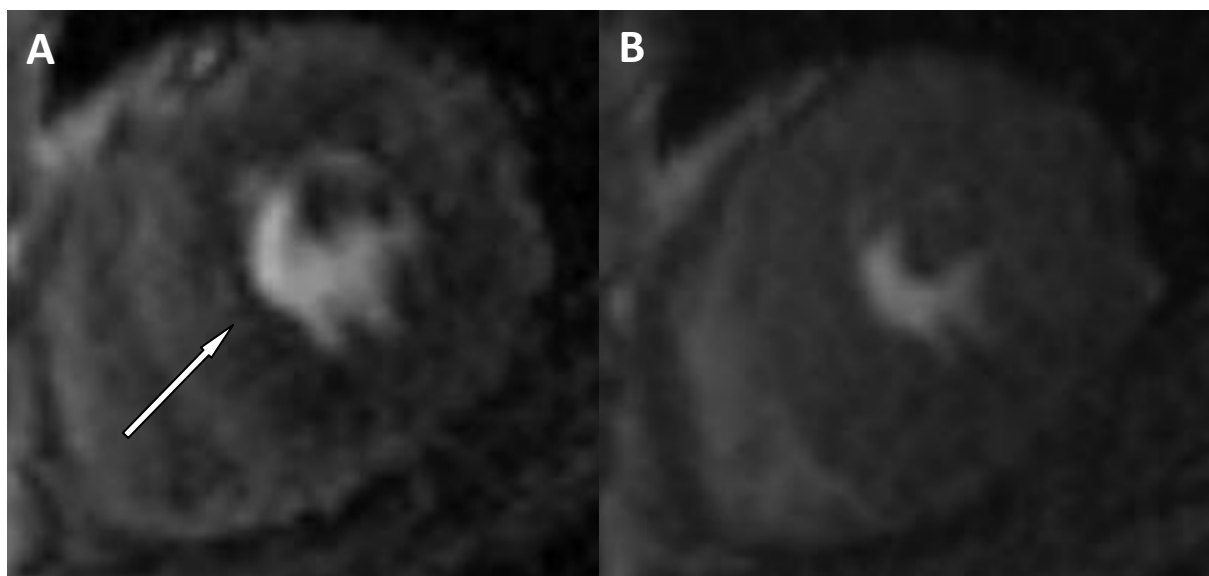


Figure 3.2. Perfusion Defect. Panel A shows mid ventricular slice at peak stress. The dark rim (see white arrow) denotes a perfusion defect. Panel B shows the same scan at rest (no perfusion defect).

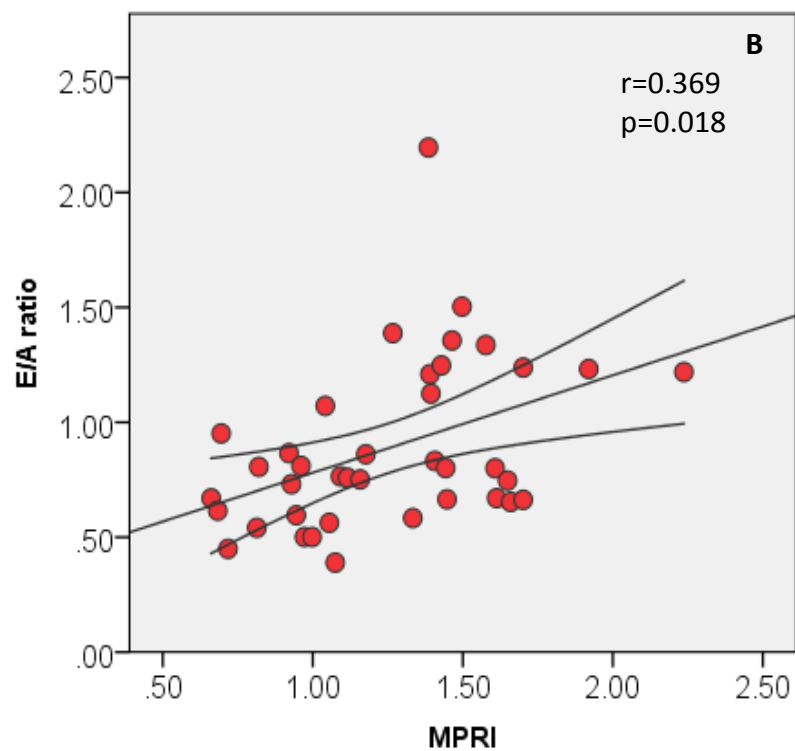
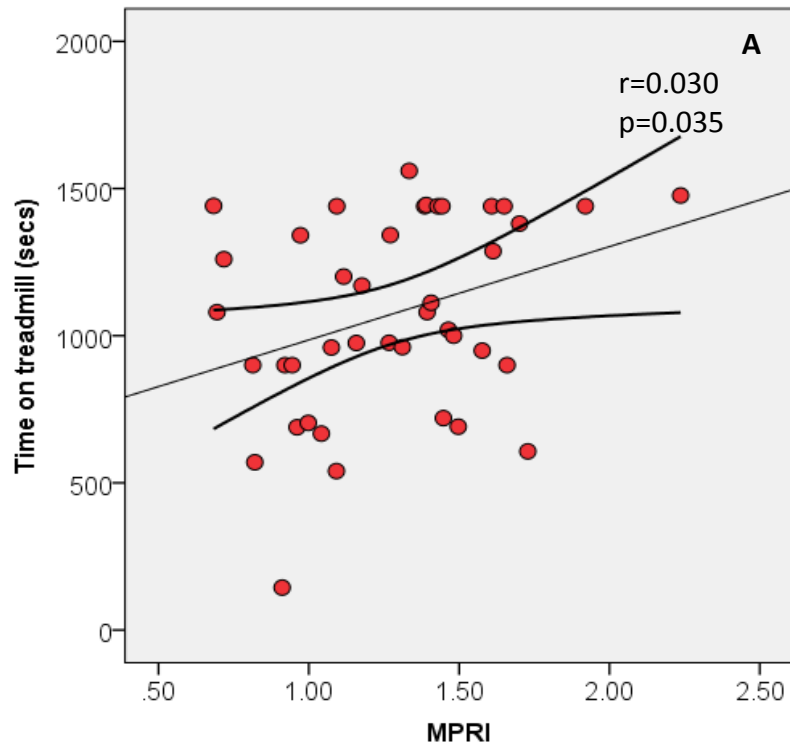
Table 3.2 Echocardiographic and CMR data

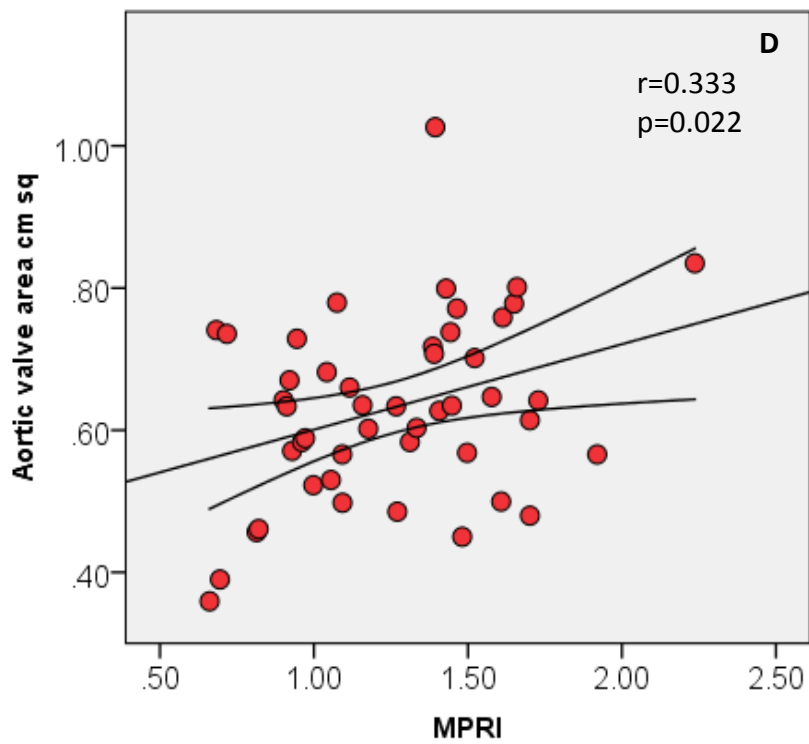
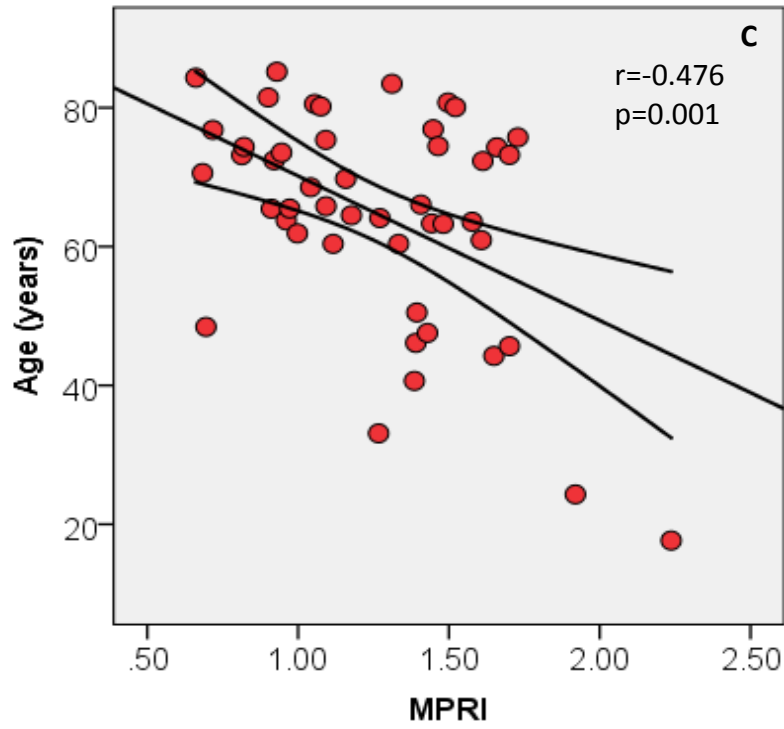
CMR data (N=47)	
LVM, g	168±44
LVMI, g/m ²	85±19
LVEDVI, ml/m ²	71±17
LVESVI, ml/m ²	21±13
LVM/LVEDV, g/ml	1.21±0.26
LVEF, %	71±11
Stroke Volume Index, ml/m ²	50±12
Cardiac Output, L/min	6.43±2.1
LGE positive (non-MI pattern),n(%)	11(23%)
MPRI ratio	1.26±0.35
Echocardiographic Data	
Peak aortic velocity, m/s	3.37±0.45
Mean aortic velocity, m/s	2.36±0.37
Indexed AVA, cm ² /m	0.54±0.14
Valvuloarterial impedance, mmHg/ml/m ²	3.3±0.9
Mitral E wave, m/s	0.43±0.19
Mitral A wave, m/s	0.84±0.26
Mitral E/A ratio	0.89±0.36
Septal E/E' ratio	13.2±5.3
Exercise Test & BNP	
BNP, pmol/L	10.7±10.3
Exercise Time, sec	1073±335

All values are expressed as mean±standard deviation. LVM=Left Ventricular Mass, LVMI=Left Ventricular Mass Index, LVEDVI= Left Ventricular End Diastolic Volume Indexed LVESVI= Left Ventricular End Systolic Indexed, LVEF=Left Ventricular Ejection Fraction, LGE=Late Gadolinium Enhancement, MPRI=Myocardial Perfusion Reserve Index, AVA=Aortic Valve Area, Valvuloarterial impedance = mean aortic valve pressure gradient + Systolic Blood Pressure/ Stroke Volume index, Mitral E= transmitral E wave inflow velocity, Mitral A wave= Transmitral A wave inflow velocity, E/E'= transmitral E wave inflow velocity divided by mitral septal annulus Tissue Doppler velocity ratio, BNP=B-type Natriuretic Peptide.

3.3.4 Associations with myocardial perfusion reserve

There were moderate correlations between MPRI and age, exercise, diastolic function (E:A) ratio, BNP and indexed aortic valve area in our cohort (Table 3.3). On regression analysis, only age remained as a strong independent predictor of MPRI. A second model was constructed and age removed to test the other variables; this removed the significance of indexed aortic valve area and BNP as independent predictors of MPRI but exercise time and E:A ratio remained significant. Age was the strongest predictor of MPRI overall. Other parameters did not show any correlation with MPRI.





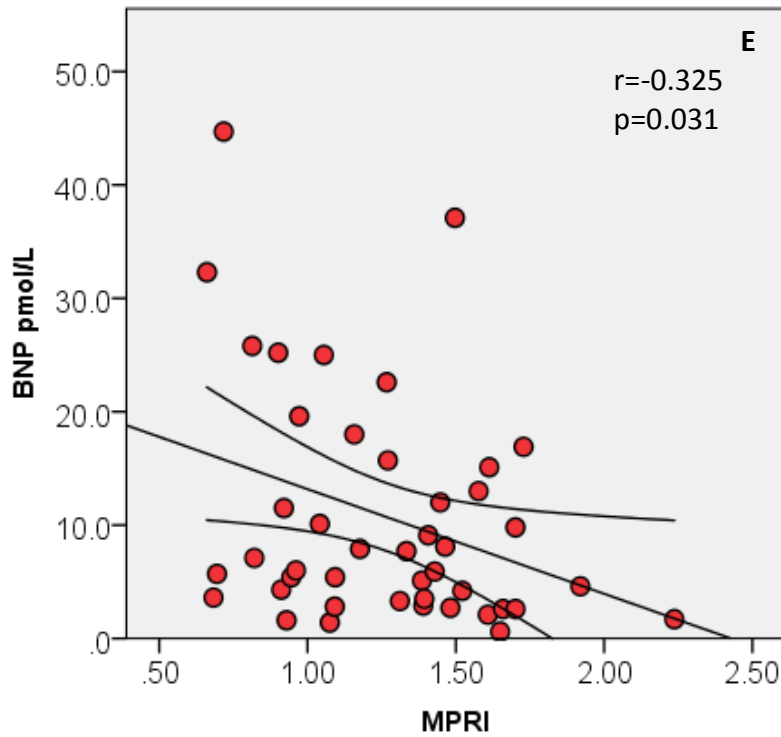


Figure 3.3. Bivariate correlations with MPRI. BNP= B-type Natriuretic Peptide, MPRI= Myocardial Perfusion Reserve Index, BSA=Body Surface Area in m^2 , AVA=Aortic Valve Area in cm^2 E:A= mitral inflow E wave: mitral valve inflow A wave. Panel A: MPRI & Exercise time B: MPRI & E:A ratio C: MPRI & Age D: MPRI & AVA E: MPRI & BNP. P value of < 0.05 considered to be significant.

Table 3.3 Bivariate Correlations and Multivariate Analysis

Associations with Myocardial Perfusion Reserve Index				
Variable	Bivariate Correlation		Multivariate Analysis	
Patient characteristics	R value	p Value	β	p Value
Age	-0.476	0.001	-0.530	0.001
Sex	0.158	0.290 (NS)	-	-
BMI	-0.236	0.110	-	-
BNP	-0.325	0.031	-	-
Diabetes	-0.059	0.693	-	-
Hypertension	0.89	0.551	-	-
Hypercholesterolaemia	-0.241	0.094	-	-
Smoker			-	-
CMR & Echo data				
Indexed AVA	0.333	0.022	-	-
Peak AV velocity	-0.041	0.785	-	-
LVEF	0.02	0.896	-	-
LVED			-	-
LVMI	0.014	0.924	-	-
LGE	-0.211	0.163	-	-
E:A ratio	0.369	0.018	0.321	0.04
E:E' septal			-	-
Exercise Test				
Treadmill	0.330	0.035	0.341	0.032

BMI= Body Mass Index, BNP= B-type Natriuretic Peptide, AVA= Aortic Valve Area, LVEF=Left Ventricular Ejection Fraction, LVED= Left Ventricular End Diastolic Volume, LVMI=Left Ventricular Mass Index, LGE=Late Gadolinium Enhancement, E:A= mitral inflow E wave/Mitral inflow A wave, E:E'= transmitral flow velocity to annular velocity ratio. P value of < 0.05 was considered to be significant.

3.4 Discussion

This study demonstrated that:

- 1) Perfusion scanning using CMR in moderate and severe AS patients is feasible
- 2) There is a modest correlation between MPRI, indexed aortic valve area and BNP.
- 3) Age is the strongest independent predictor of MPRI in patients with asymptomatic AS; exercise time and diastolic measures of LV function are also predictors.

3.4.1 Clinical feasibility of first pass CMR perfusion in patients with aortic stenosis

There was high technical success rate in carrying out CMR perfusion scanning in this pre-selected group of AS patients. In one case the data set could not be processed due to breathing artefact; another data set was lost due to extravasation of contrast. There were no instances where we had to terminate the perfusion scan because of patient discomfort. Our finding of CMR adenosine stress testing being well tolerated in this patient cohort is supported by findings of the two other CMR studies^{22, 134} using the same technique. Burgstahler et al reported successful adenosine first pass perfusion CMR in 87% of their AS 23 patients; none of the subjects suffered AV conduction disturbance, bronchospasm or haemodynamic instability.¹³⁴ Steadman et al look at a larger cohort of patients (n=49) and were unable to complete scanning in 3/49 patients due to asthma or other “severe symptoms” (not explained in further detail) experienced by patients during the scan.

The high compliance and low complication rate of patients completing stress perfusion scanning in this study may in part be due to the necessarily rigorous patient selection process (only 50% of the overall patient cohort underwent perfusion scanning). All

participants had also consented to participate in a clinical trial requiring multiple follow up scans; to minimize the risk of high trial drop-out rates, the investigator only carried out the longer CMR perfusion protocol in those judged sufficiently robust to undergo CMR scanning for an hour. This may have led to only a disproportionately “healthy” subgroup of the trial participants being scanned.

3.4.2 MPRI, aortic stenosis severity and the left ventricle

In this study group of patients, a modest correlation between indexed aortic valve area and MPRI was noted ($r=0.33$); there was no correlation between MPRI and LV mass index. These findings are in line with work by Rajappan et al²³ who found that microvascular dysfunction in patients with severe AS increased as aortic valve area decreased, the hemodynamic load increased and diastolic perfusion time shortened; there was however only a very weak relationship with increased left ventricular mass. A follow-up study looking at coronary flow reserve (CFR) (an indirect measure of microvascular dysfunction - see Chapter 1, Section 1.5 for definition) in patients one year post AVR confirmed that improvements in CFR were not related to regression of left ventricular mass but to changes in hemodynamic variables, particularly aortic valve area and diastolic blood pressure.^{23, 90}

However, a later study, by Steadman et al,²² *did* find an association between MPRI with peak aortic valve velocity; aortic valve area was not measured in their study group. The Steadman study also found that LV mass and LGE were strong predictors of MPRI in severe AS, but not age, contrary to our findings.

There are several potential reasons for this discrepancy in findings between studies. Firstly the Steadman group²² only included patients with severe AS; this study included patients with moderate and severe asymptomatic AS.

Secondly, it is conceivable that there was occult coronary artery disease in our study group, which increases with age. Whilst our group did undergo exercise testing, they did not undergo angiography. The Steadman group patients all underwent angiography and those with a >50% luminal stenosis on angiography were excluded from the study.

Another way of explaining findings in this study group is that the correlation we found between MPRI and age may be a reflection of a 'normal' ageing phenomenon. There have been no studies looking at microvascular function in the ageing heart using CMR first pass perfusion, but there is some data looking at this in normal volunteers using positron emission tomography.¹⁶⁶ One study found that whilst resting myocardial blood flow increases linearly with age, hyperaemic myocardial blood flow declines over 65 years.^{166, 167}

The Steadman group did not find age to be an important predictor of MPRI; whilst the age of patients in both studies is comparable (Steadman group; 66.2±9.3 years, our group; 64.8±15.5 years), the age range is narrower in the Steadman group, which may also partly explain the discrepancy between the two studies.

Based on the findings of this study, it could be concluded that age may play a more important role in determining MPRI when aortic valve disease is less severe; as the disease progresses, associated structural myocardial changes (in particular myocardial fibrosis) become more pronounced and important determinants of myocardial perfusion. This argument is strengthened by the LGE findings in the two study groups. Only 23% of this study patient cohort had LGE; this was double in the Steadman group (56%), suggestive of

increased replacement fibrosis in the myocardium in the latter group which may explain their finding that LGE this is a strong predictor of MPRI in severe AS.²²

The Steadman group's findings of increased LGE and mass as a predictor of MPRI are even more surprising in light of the fact that the average left ventricular mass index of their severe AS group (processed using the same software- QMass, version 7.1 Medis, Leiden, the Netherlands) as in this study was significantly lower at $69.9 \pm 17.7 \text{ g/m}^2$ compared to this moderate and severe AS study group ($85 \pm 19 \text{ g/m}^2$) and the Rajappan severe symptomatic AS group ($135 \pm 30 \text{ g/m}^2$).^{22, 23}

However, it may be too simplistic to concentrate on absolute LV mass; the way the LV remodels in response to pressure - the LV geometry may also influence the degree of microvascular impairment. This question has not been directly addressed in any of the CMR perfusion studies but it may also explain some of the discrepancies between the perfusion studies in AS.

Another finding of this study was the E:A ratio (a marker of LV diastolic dysfunction) was predictive of MPRI. In this study, interpreting the correlation between E:A ratio and MPRI is complex because the E:A ratio does not increase linearly with increasing diastolic dysfunction, unlike the E/E' ratio.¹⁶⁸

A normal E:A ratio lies between 1-2. In LV diastolic dysfunction, the E:A ratio initially falls to < 1 (mild diastolic dysfunction), then 'pseudo normalises' 1-2 (moderate diastolic dysfunction) and finally increases to > 2 (severe restrictive filling).¹⁶⁸

The average E:A ratio in this study group was 0.89 ± 0.36 and there was only one patient with E:A ratio of > 2 . One interpretation of this data is to conclude that most of our patients had

either normal diastolic function or mild diastolic dysfunction; this would explain the correlation with MPRI (higher ratio in normal, lower in those with mild diastolic dysfunction).

This interpretation would fit nicely with the hypothesis that increased LV filling pressures cause mechanical impairment to blood flow in the sub-endocardium.

There is, however, a strong argument against this interpretation of the data; firstly, there is no linear correlation with E/E' (reflection of LV filling pressure) and MPRI which would be expected. Secondly, the average septal E/E' ratio of the group is 13.2 ± 5.3 , which suggests raised LV pressures in the majority of the patients; those with E:A of 1-2 are therefore more likely to have pseudo-normal LV filling (moderate diastolic dysfunction).

Whilst the relationship between the diastolic data and MPRI in this study are difficult to interpret, previous AS perfusion studies did find associations between diastolic parameters such as diastolic filling time, septal $E:E'$ and MPRI.^{22, 90}

B-type natriuretic peptide (BNP) is known to correlate with markers of diastolic dysfunction in AS and correlated negatively with MPRI in this study.^{169, 170} BNP is primarily released from the ventricles in response to myocyte stretch and in AS has been shown to correlate with valve lesion severity, LV mass, symptomatic status, exercise capacity and prognosis as summarised in reviews by Ray and Steadman.^{171, 172}

The correlation between BNP and MPRI in this study may reflect increased LV pressures (LV stretch) causing mechanical impairment of blood flow in the subendocardium, thus leading reduced MPRI.

3.4.3 Exercise and MPRI

We found exercise to be an independent predictor of MPRI; this finding is unsurprising as we know that patients with microvascular dysfunction are unable increase to blood flow to the myocardium in response to increased oxygen demands in situations such as exercise;⁹⁷ this was discussed in chapter 1 and illustrated in Figure 1.5. Other changes occur during exercise that increase the degree of microvascular dysfunction include; reduced diastolic perfusion time,¹⁷³ increased myocardial oxygen demand and LV end-diastolic pressures (leading to impairment of subendocardial perfusion).¹⁷⁴

3.5 Conclusions

Adenosine stress CMR perfusion scanning in AS patients is safe and clinically feasible. Age is the strongest predictor of MPRI, followed by exercise time. There was only a weak correlation between valve area and MPRI.

3.6 Limitations

Our patient group did not undergo angiography prior to this study and concomitant coronary artery stenosis causing reduction in MPRI cannot be excluded, though patients did not have clinical angina. This was a sub-study of a larger trial; only the most robust participants were selected for inclusion and this may represent a “healthier” patient group than if all AS patients had been scanned. We did not perform absolute perfusion quantification but only measured the myocardial perfusion ratio index. The sample size was small and this may account for the lack of association between MPRI, hypertension, diabetes and smoking.

Chapter 4: Circumferential myocardial strain measurement in aortic stenosis and relationship to fibrosis

4.1 Introduction

Several studies have suggested that more subtle measurements of myocardial function, such as strain, may aid risk stratification of asymptomatic AS patients.^{14, 175, 176} Strain is defined and discussed in more detail in chapter 2, section 2.7.

A number of echocardiographic studies have identified reduction of longitudinal strain (LS) in patients with severe symptomatic AS;^{177, 178} higher degrees of LS impairment correlate with increased LV fibrosis as measured by histology and late gadolinium quantification on CMR.^{51, 179, 180}

What happens to circumferential strain (CS) measurements in AS, and how these relate to AS severity and degrees of LV fibrosis remains unclear. Studies looking at mid-CS measurements using echocardiography in severe symptomatic AS have reported conflicting findings with some groups reporting increased CS in patients with severe AS and others decreased CS.^{175, 178, 181} Decreased CS is also reported in other diseases causing left ventricular hypertrophy, such as hypertension and hypertrophic cardiomyopathy.^{182, 183, 184}

Several CMR studies have shown the presence of *mid-wall* late gadolinium enhancement (a marker of replacement fibrosis) in patients with severe AS^{15, 21, 185} and this gives rise to the idea that replacement fibrosis disrupts myocardial architecture in the mid-wall of the LV which may underlie a reduction in CS in this cohort.

Piberot states “Look Globally, Think Globally”¹⁷⁹ when thinking about AS; in line with this idea we aimed to investigate how CS changes *throughout* the LV in AS using the new Tomtec myocardial CMR feature tracking software (CMR-FT) (Diogenes MRI:Tomtec: Unterschleissheim, Germany), described in chapter 2, section 2.7.2. CMR-FT enables CS to

be measured in the LV at exactly defined slice positions from routine SSFP short axis cine slices.

CS is the parameter which has the lowest inter-observer variability and best correlates with values derived from CMR tagging (see Appendix 1 and Bland-Altman plot Figure 2.12, chapter 2) and for this reason, we opted to use CMR-FT to investigate purely global CS in moderate, severe asymptomatic and severe symptomatic AS. Longitudinal strain was not recorded using CMR-FT for this study because the correlation with tagging LS was found to be poor. (See Appendix 1).

Age and sex have been shown to affect measurements of LV strain^{186, 187} and so the groups were age and sex matched.

We hypothesized that:

- 1) There is significant variation in CS measurements in adjacent slice positions and that this accounts for some of the discrepancies of the findings in the echo strain literature.
- 2) Mid-CS is reduced patients severe symptomatic AS compared to moderate and severe asymptomatic AS.
- 3) That CS would be most reduced in those with high degrees of fibrosis on histology.

4.2 Methods

4.2.1 Recruitment

12 patients with moderate and 12 patients with severe asymptomatic AS were recruited from the John Radcliffe Hospital in Oxford. 12 patients with symptomatic AS awaiting aortic valve replacement surgery were recruited from the Heart Hospital, University College, London, UK and were age and sex matched to the moderate and asymptomatic group. Inclusion and exclusion criteria are described in chapter 2. AS severity was categorized according to the recommendations of the European Society of Echocardiography.¹²²

4.2.2 CMR imaging and strain analysis

All patients underwent CMR scanning on at 1.5 T Siemens Avanto scanner for acquisition of LV volumes and aortic valve area according to protocols described in chapter 2, section 2.6.2 and 2.6.3.

Feature tracking analysis was performed using a dedicated software tool (Diogenes MRI, Tomtec, Unterschleissheim, Germany), as highlighted in chapter 2, section 2.7.2.

Endocardial contours were drawn manually around all slices in the short axis stack. Slice position 1 was defined as the first image distal to the mitral valve where the LV could be completely contoured in systole and diastole without interruption (See Figure 4.1). The slices were labelled 1 (base) to 7 (apex) and were acquired at 1 cm intervals (slice thickness 7mm) down the ventricle. Peak systolic CS averaged over all segments was recorded.

For purposes of this study, global CS values for base, mid and apex were calculated as the average CS of slice positions 1&2, 3&4 and 5, 6& 7 respectively. Analysis of CS using CMR-FT

was carried out on two separate occasions, by the author, 4 weeks apart (see Bland-Altman plots section 2.7.2); the mean value of the two measurements was used. The author's intra-observer variability was found to be -3.93-3.92 (95% limits of agreement).

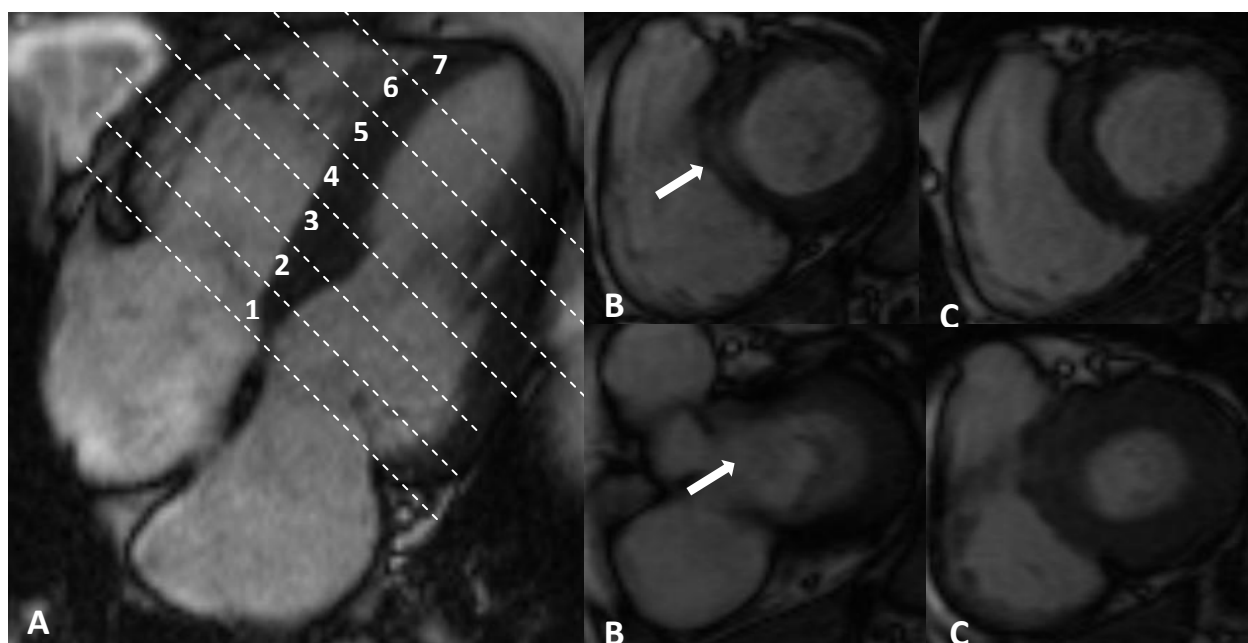


Figure 4.1. Slice Positions. Panel A shows HLA CMR cine with the short axis slice positions numbered 1-7. Slices are 7mm thick and the gap between slices is 3 mm. Slice 1 is the most proximal to the mitral valve annulus and 7 the most apical slice. Slices with an incomplete LV myocardial ring (see white arrow in B1&B2) seen in the short axis position in diastole (B1) and systole (B2) were excluded from the analysis. The slice chosen as slice position 1 was the first slice closest to the mitral valve annulus to have a complete ring of myocardium in both diastole (C1) and systole (C2). Basal CS was calculated as a mean of slices 1&2, mid-CS as a mean of slices 3&4 and apical CS as a mean of slices 5,6&7.

4.2.3 Histology

The 12 patients with severe symptomatic AS recruited from the Heart Hospital underwent biopsy at the time of aortic valve surgery. Histological sampling and analysis was performed as previously described in chapter 2, section 2.9.

4.2.4 Statistics

Data analysis was performed with IBM SPSS Statistics, version 20.0 (Inc., Chicago, IL, USA). Values are expressed as means $\pm$ standard deviation unless otherwise stated. All continuous variables were normally distributed (Kolmogorov-Smirnov test). The chi-square test was used to compare discrete data as appropriate. Comparison of measurements of strain in adjacent slices was done using the paired t-test.

Comparison of continuous variables between the 3 groups were performed using ANOVA with post hoc Bonferroni correction. The correlation between histology and circumferential strain was calculated using Spearman's due to the small number of samples. Statistical tests were two tailed, and a p value of less than 0.05 was considered to indicate a statistically significant difference.

4.3 Results

4.3.1 Patient demographics

36 patients were included and the patient clinical characteristics and baseline CMR imaging data are described in Table 4.1. The patients were well matched for age and sex. There were differences between the three groups in aortic valve area, peak aortic valve velocity and left ventricular mass, as would be expected for parameters that in part define the groups. There was also a significantly higher number of a patients with hypertension included in the severe symptomatic group.

Table 4.1. Baseline patient characteristics

	Moderate AS (12)	Asymptomatic severe AS (12)	Symptomatic severe AS (12)	P value
Age (yrs)	72.2±6.4	73.2±11.2	73.2±6.9	0.955
Sex (female)	7	7	7	1.00
BSA/m ²	2.00±0.19	1.94±0.21	1.93±0.17	0.419
Smoker (n)	2	1	1	0.771
Hypertension (n)	4	2	8	0.036
Statins (n)	6	6	7	0.902
Beta-blockers (n)	1	2	5	0.131
Diabetes (n)	2	0	2	0.345
CMR data				
Valve area(cm ²)	1.5±0.52	1.00± 00	0.91±0.30	0.001
LV mass (g)	150±34	167±29.4	199±42	0.016
LV mass index (g/m ²)	78±14.4	94±24.9	105±24.6	0.022
LV EDV (mls)	134±36.9	143±29.7	160±30.4	0.184
LV ESV (mls)	43±22.0	39±20.0	50±26.4	0.505
EF (%)	68±12.7	73±10.3	69±12.5	0.581
Echo data				
Peak AV velocity (m/s)	3.17±0.39	4.17±0.39	4.17±0.58	0.001

All values are expressed as means± standard deviation. BSA=Body surface area, EDV=End-diastolic volume, ESV= End-systolic Volume, EF=Ejection fraction, AV=aortic valve. P values of <0.05 were considered to be significant.

4.3.2 Circumferential strain measures in adjacent slice positions in all groups

Data on CS are shown in Table 4.2. There were differences that were statistically significant in CS measurements between adjacent slice positions 2&3, 3&4, 4&5 and 5&6. The maximum difference occurred between slice positions 5&6 (apical) with a maximum measurement difference of up to 15% in absolute strain values (-4.2) seen between slices. As expected, strain was greatest at the apex. (See Figure 4.2 & 4.3)

Table 4.2 Mean circumferential strain in all patient groups in each slice position

Slice position	Mean circumferential strain (%) (n=36)	Paired t test (2 tailed) between adjacent slices P value
1	-25.2±5.2	0.057 (1&2)
2	-23.6±5.2	0.001 (2&3)
3	-21.2±5.2	0.025 (3&4)
4	-22.6±5.7	0.005 (4&5)
5	-24.4±6.3	0.001 (5&6)
6	-28.3±5.1	0.271 (6&7)
7	-29.6±6.5	
Base	-24.7±5.1	0.005
Mid	-22.4±5.7	0.005
Apex	-26.3±6.5	0.005

All values are expressed as mean standard±deviation. P value of <0.05 is considered significant. P value is calculated as paired two tailed t test between adjacent slices indicated in brackets after the p value in the final column. The differences between base, mid and apex were calculated by ANOVA.

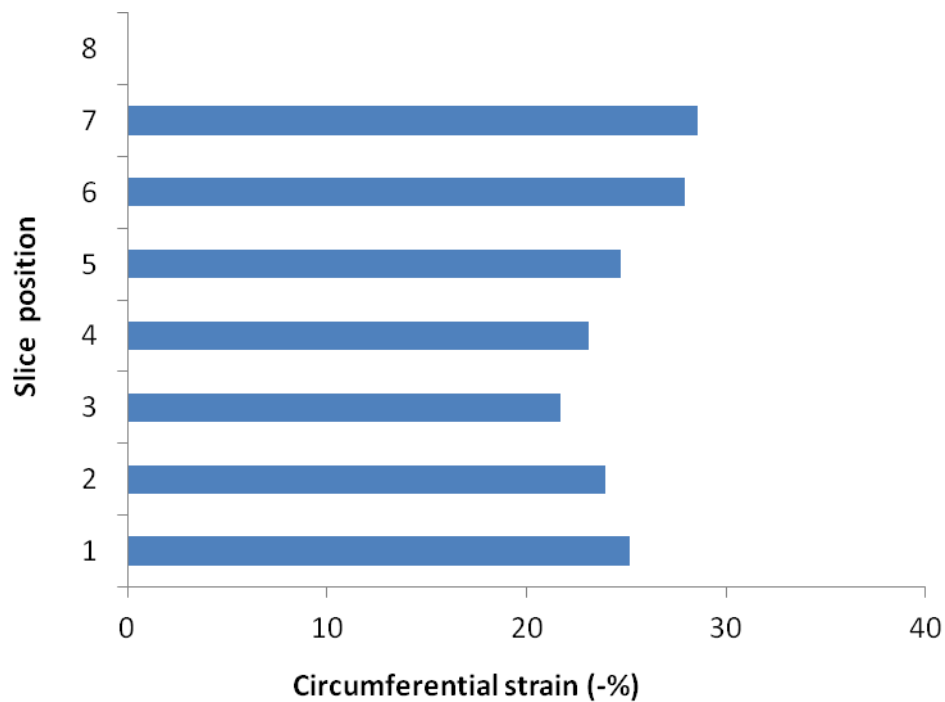


Figure 4.2. Mean Circumferential strain in each slice position in all groups. There were significant differences between the slice positions $p < 0.0005$ as calculated by ANOVA. The lowest strains were seen in slice positions 3&4 (defined as the mid ventricle for the purposes of this study).

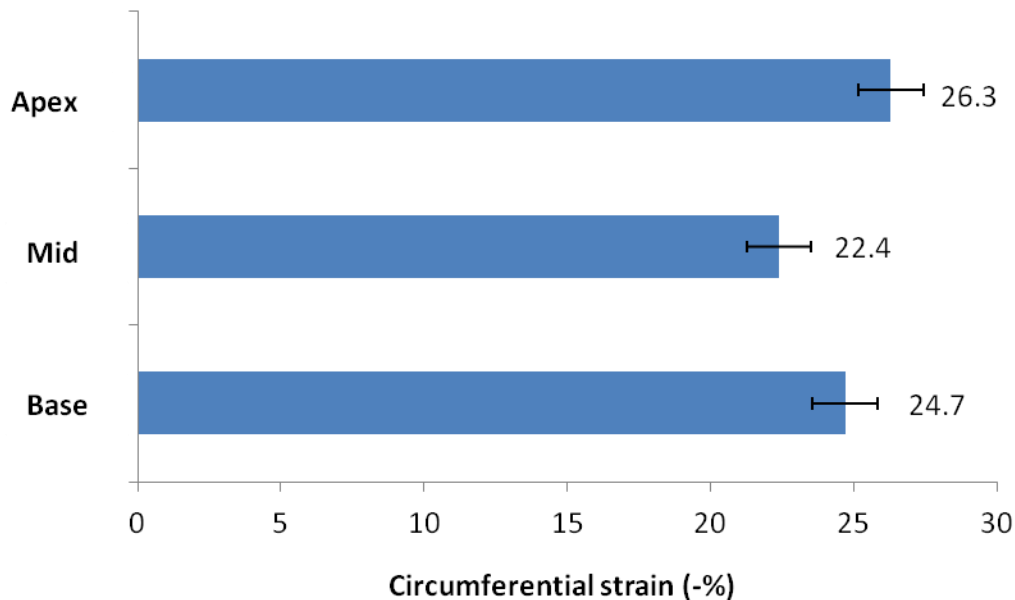


Figure 4.3. Mean Circumferential strain in base, mid and apex in all groups. There were significant differences (as expected) between the base (mean of slices 1&2), mid (mean of slices 3&4) and apical (mean of slices 5,6 &7).

4.3.3 Circumferential strain in different patient groups

These data are shown in Table 4.3. Interestingly, there were no significant differences in CS between the AS groups at any slice position or between apex, mid and base.

Table 4.3. Circumferential strain in the 3 different patient groups

Slice position	CS in Moderate AS (n=12)	CS in Severe Asymptomatic AS (n=12)	CS in Severe Symptomatic AS (n=12)	p-value
1	-24.8±5.3	25.3±5.8	-25.3±5.3	0.97
2	-22.8±4.3	-24.3±4.9	-23.3±6.3	0.78
3	-20.3±5.1	-21.4±5.2	-21.9±5.7	0.74
4	-21.9±4.8	-23.2±7.0	-22.7±5.6	0.87
5	-23.4±7.9	-25.0±5.7	-24.3±4.2	0.82
6	-26.7±8.3	-29.7±7.1	-26.8±6.5	0.57
7	-29.0± 8.7	-32.0±6.3	-29.5±6.5	0.93
Base (1&2)	-23.3±4.4	-24.8±4.7	24.7±6.3	0.825
Mid (3&4)	-21.8±5.5	-22.3±5.6	22.9±6.3	0.539
Apex (5&6&7)	-23.3±6.5	-26.7±6.0	26.1±5.8	0.670

All values are expressed as mean ± standard deviation. P values <0.05 considered to be significant and were calculated in ANOVA.

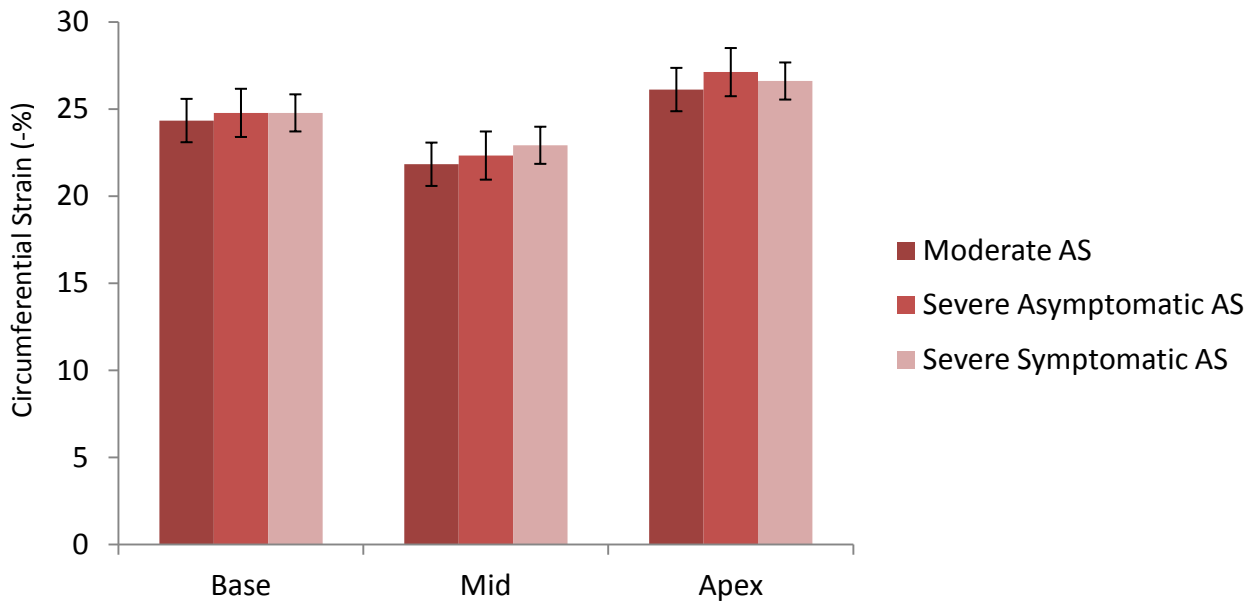
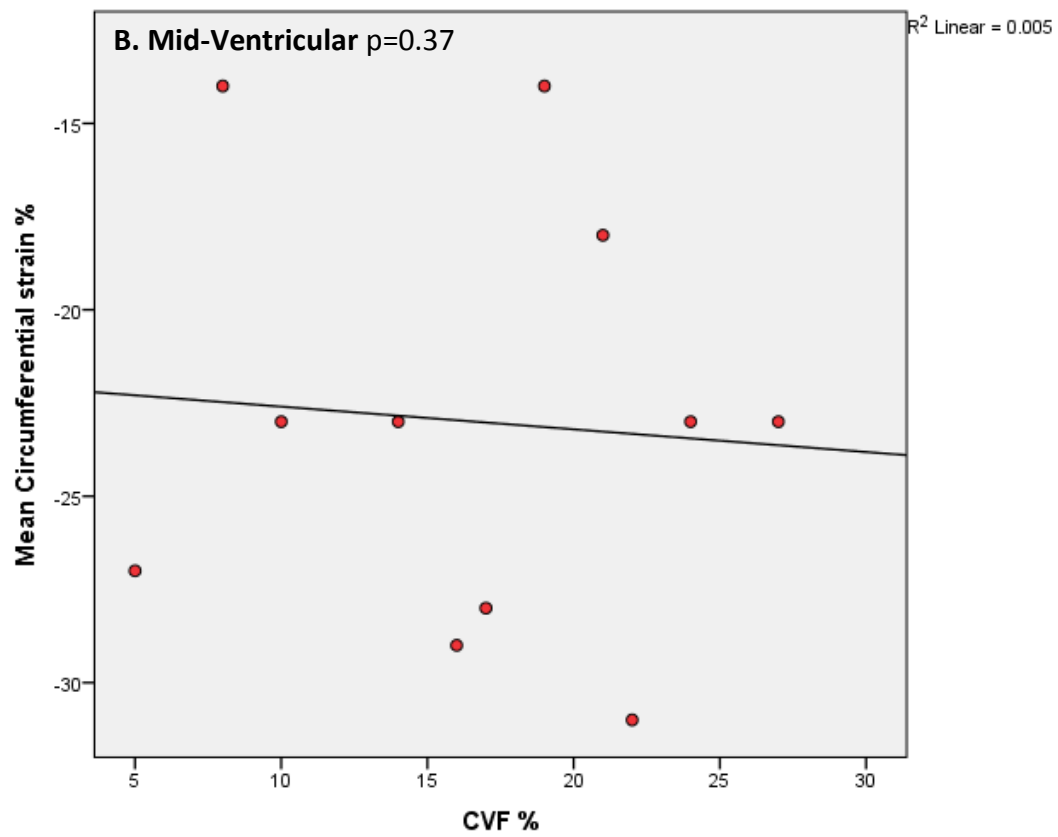
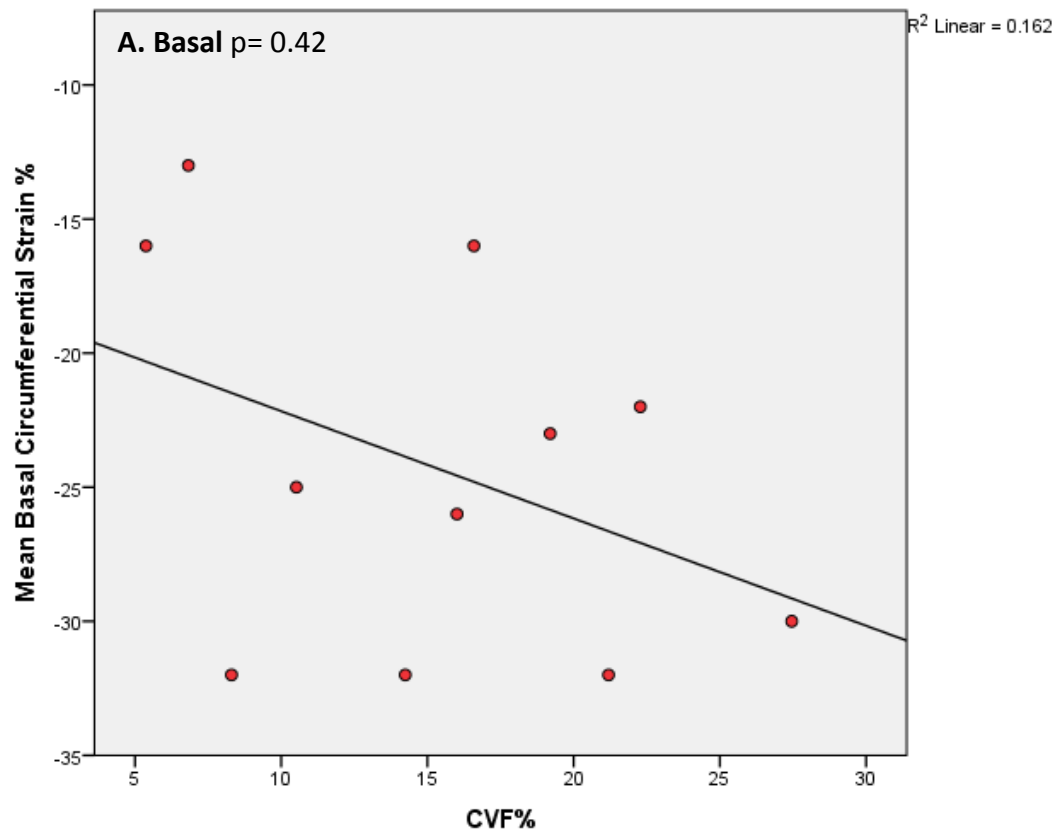


Figure 4.4. Mean Circumferential Strain in the base, mid ventricle and apex in all three groups. There were no significant differences in strain in the base, mid and apical part of the ventricle between the groups.

4.3.4 Relationship between circumferential strain and fibrosis

LV biopsies were obtained in 11 of the 12 patients with severe symptomatic AS. One sample was excluded as it was considered too superficial and included mainly endocardial tissue.

There were no significant correlations between the collagen vascular fraction and CS measured in the base, mid-ventricle and apex. See Figure 4.5.



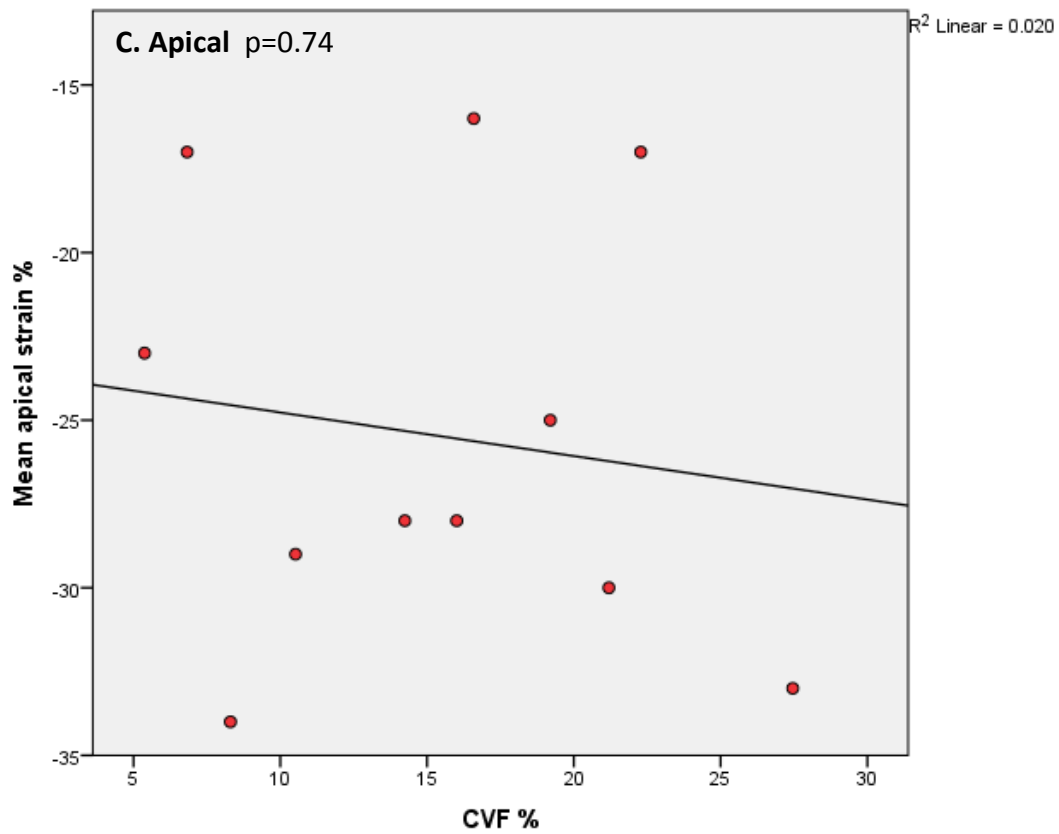


Figure 4.5. Circumferential strain and collagen vascular fraction (CVF%) in severe symptomatic AS in the base, mid and apex. There were no significant correlations between circumferential strain measurements and collagen vascular fraction percentage as measured from endomyocardial biopsy.

4.4 Discussion

In this study the following findings were made:

- 1) There were significant differences in global CS as measured in adjacent slice positions relative to the mitral valve. This particularly affected measurements of mid and apical LV CS, highlighting the importance that the slice location for CS measurement in the LV needs to be standardized.
- 2) There were no significant differences in CS between the moderate, severe asymptomatic and severe symptomatic AS groups in any slice position.
- 3) There is no significant correlation between CS and fibrosis as measured by histology.

4.4.1 Measurement of circumferential strain in aortic stenosis using CMR-FT

In this study we were able to demonstrate that there were significant differences in average CS measured in adjacent slice positions as well as showing the expected differences in CS in the base, mid portion and apex of the ventricle.^{99, 188, 189}

These findings highlight the importance, particularly in research studies involving follow-up imaging, of determining the exact slice position for consistent and repeated CS measurements when using CMR-FT. It is conceivable that some of the variability (with both increased and decreased CS in severe AS reported) of mid-CS as measured by echocardiography in the AS population may have occurred because slice position cannot be defined precisely using echo.^{175, 177}

In normal hearts, CS increases from base to apex.^{190, 191} In this study CS in all groups was lowest in the mid-ventricle and highest in the apex. The average mid-CS measured in this AS group was -22.4 ± 5.7 which is lower than that measured in normal healthy volunteers by Schuster et al using the same software (-24.1 ± 6.7).¹⁹² Importantly, the average age of the two populations is different (70 years in our study, 40.6 years for normals) and the age difference may impact on these measures.¹⁸⁶ Furthermore, for the purposes of this study, mid-CS was defined as the average CS strain measurement of slices 3&4 whilst in most other studies this measurement would have come from a measurement of a single slice only.

The finding of decreased mid-CS in AS is not new. Decreased mid-CS is also reported in severe symptomatic AS in a number of other echocardiographic studies,^{175, 181, 193} and increased torsion of at the apex in patients with severe AS was demonstrated by some of the earliest CMR tagging studies.¹⁹⁴

In contrast to the findings of this study, there are two small published echocardiographic studies involving 45 and 64 patients respectively (from the same group) who report increased mid-CS in severe AS.^{177, 178} However, other published studies show decreased CS strain in severe AS,^{143, 181, 184} consistent with the findings in this study.

In this study there were no significant differences in global CS between the moderate, asymptomatic and severe symptomatic AS groups in any given slice position; it is possible that the study numbers were too small in each group to detect a significant difference between the groups, but there was also not even a trend towards a difference in the data. Other studies, however, have found differences. In the largest study in this area to date, Ng et al, looked at 420 patients with mild, moderate and severe AS using two-dimensional speckle tracking and found significant differences in mid-CS between the groups (values:

mild -21.1 ± 3.7 ; moderate -19.7 ± 3.3 ; severe -17.9 ± 4). Of note, the echo derived strain values are considerably lower than values measured in our study group using CMR-FT software.¹⁸⁴

4.4.2 Fibrosis and CS in Aortic Stenosis

No correlation was demonstrated between fibrosis and CS in the patients studied; the small number of patients included is a clear limitation of this study and so we cannot definitively conclude there is no link between fibrosis disrupting mid-wall architecture and reduced CS in AS. One of the limitations of using endomyocardial biopsies is that the samples were taken at the basal septum and this may not necessarily be representative of fibrosis distribution in the entire ventricle. Further, we did not measure *regional strain* in this study but only global CS values; global measures may be too crude to capture subtle fibrosis induced changes in strain. It is conceivable that with regional CS analysis a correlation between the number of myocardial segments with reduced CS and CVF would have been seen. However, unpublished work by Augustine et al (Appendix 1) has showed the intra- and inter-observer variability for *regional measures* of strain using this software is high.

The relationship between CS (a reflection of mid-wall fibre function) and fibrosis has not previously been studied directly. A progressive step-wise reduction in longitudinal strain and then CS as severity of valve disease progresses has been described; this is thought to occur because the subendocardial (longitudinal) fibres are more vulnerable to pressure overload, hypoperfusion and fibrosis than the mid-wall fibres.¹⁸⁰

The underlying mechanisms leading to the final common pathway of decreased circumferential myocardial contraction are likely to be multi-factorial and include LV geometry¹⁴, degree of fibrosis¹⁸⁰, and degree of pressure overload.¹⁸⁴ These factors have

previously been linked to decreased longitudinal strain in AS patients and it is likely therefore, that they also play a role in determining CS.

4.5 Conclusion

Slice positioning is important when reporting measures of CS using CMR-FT. In this study, no significant differences were found in CS between the moderate, severe and severe symptomatic AS groups (in any slice position) or correlation between fibrosis and CS. Mid-CS is reduced compared to basal and apical CS in all groups.

4.6 Limitations

An important limiting factor in this pilot study is the small number of patients included.

There were only 12 patients in each subgroup and the study may not have been sufficiently powered to pick up a statistically significant difference in strain between the groups.

Further, there were only 11 patients who had histology.

Another valid criticism of the study is that it is unconventional to define the global CS strain values as a mean of the two slices for the base, mid and apex; normally these are calculated from a single slice. Furthermore, using the same slice positions in all patients to denote base, mid and apex in different sized hearts means that the apical strain values presented would have been proximal apex values in larger hearts and distal apex values in smaller hearts.

***Chapter 5: The detection of diffuse
myocardial fibrosis in aortic stenosis
using cardiovascular magnetic
resonance non-contrast T1 mapping***

5.1 Introduction

Diffuse myocardial fibrosis in patients with aortic stenosis (AS) is related to poor clinical outcomes following aortic valve replacement (AVR).^{21, 50, 180} The 'gold standard' method for quantification of fibrosis of the left ventricle (LV) is myocardial biopsy, but this is invasive, associated with morbidity and subject to sampling error. The ability to measure myocardial fibrosis in patients with AS using non-invasive techniques is therefore of significant clinical interest. Cardiovascular magnetic resonance (CMR) can measure *focal* myocardial fibrosis in AS using the post-contrast late gadolinium enhancement (LGE) technique.¹⁹⁵⁻¹⁹⁷ Focal fibrosis, such as infarction, also changes the relaxation properties of the tissue during CMR, with increased myocardial T1 values (e.g. 1060 ± 61 ms compared to 987 ± 34 ms in remote myocardium),¹⁶⁰ and animal work has suggested the same occurs in diffuse fibrosis.¹⁹⁸

New CMR contrast techniques have exploited these changes in T1 values and permitted the measurement of diffuse myocardial fibrosis.^{52, 65} These techniques use either post contrast myocardial T1 values or the change in T1 after a bolus or contrast infusion,^{54,163} but may be time consuming, demand complex processing or be contraindicated in severe renal impairment.⁵²

Our research unit has recently developed a shortened T1-mapping sequence,⁶⁶ Shortened Modified Look-Locker Inversion recovery (ShMOLLI), which shows high stability, consistency and independence of factors previously confounding T1 mapping (such as heart rate) in a short (8 heart beat) breath-hold.⁶⁶ In this chapter the following hypotheses are investigated:

- 1) That pre-contrast T1 values are elevated in AS

- 2) That pre-contrast T1 values correlate with the degree of fibrosis using the 'gold standard' collagen volume fraction, derived from biopsy histology.

5.2 Methods

5.2.1 Patient recruitment

Between October 2008 and April 2011, eighty-five asymptomatic AS patients were recruited prospectively from cardiology clinics at the John Radcliffe Hospital in Oxford, UK according to the criteria set out in chapter 2. All asymptomatic patients underwent exercise testing (Naughton protocol¹¹⁴) to determine their exercise capacity objectively.

A second group of twenty-four symptomatic patients with severe AS awaiting aortic valve replacement were prospectively enrolled from the Heart Hospital Imaging Centre in London, UK between July 2010 and March 2012; these patients underwent CMR scanning on Siemens 1.5T Avanto MR system (Erlangen, Germany), and myocardial biopsy at the time of surgery. Exclusion criteria were contraindications to CMR (including defibrillators and pacemakers), more than mild aortic or mitral regurgitation, significant LV dysfunction (LVEF<40%), uncontrolled hypertension or severe renal failure (serum creatinine >200µmol/L) which could in itself increase myocardial fibrosis.

Thirty-three age and sex matched normal volunteers were recruited; co-morbidities and symptoms of cardiac disease were excluded prior to inclusion in the study. The study was approved by the Institutional Ethics Committees at both institutions and all patients and normal volunteers gave written informed consent.

5.2.2 CMR protocol

All subjects underwent CMR with a 1.5T clinical scanner (Avanto, Siemens Healthcare, Erlangen, Germany). Standard LV volumes were acquired, along with additional short axis images of the aortic valve, in line with standard cardiovascular magnetic resonance imaging protocols.¹²⁸ In addition, a mid-ventricular short axis slice was acquired using the ShMOLLI sequence for T1 mapping.⁶⁶ Late gadolinium enhancement (LGE) imaging was carried out in all patients who consented to a contrast injection and had a creatinine clearance above 60 ml/min. 0.1 mmol/kg of gadodiamide (Omniscan, GE Healthcare, Amersham, UK) was injected. Ten to fifteen minutes later, inversion recovery-prepared spoiled gradient echo images were acquired in the standard short and long-axis views as previously described.¹⁹⁹ Artifacts were excluded by repeating images in 2 separate phase-encoding directions; inversion times were optimized to null the myocardium. The protocols are described in greater detail in chapter 2, section 2.6.

5.2.3 Image analysis

CMR image analysis was performed offline using Qmass software (Medis, Leiden, The Netherlands) according to the Society for Cardiovascular Magnetic Resonance guidelines for reporting cardiovascular magnetic resonance examinations.²⁰⁰ LV volumes and function were analyzed with all parameters indexed to body surface area. Aortic valve area was determined by direct valve planimetry. The absence or presence and pattern of LGE was assessed by two independent observers blinded to the clinical data. In the case of disparity a third blinded observer adjudicated. Please refer to chapter 2 section 2.6 for more details.

5.2.4 T1 Measurements

The mid-ventricular short axis ShMOLLI image was manually contoured to outline the endo- and epicardium, and the average T1 value over this whole slice calculated using an in-house software (MyoCardial Regions Of Interest), MC-ROI programmed by Dr Stefan Piechnik in IDL (Interface Definition Language) v.6.1, ITT Exelis, Mclean, Virginia, US) as described by Piechnik et al.⁶⁶ Average myocardial T1 values were analyzed on a per-case basis. Myocardial mid-ventricular segments with infarction (identified from LGE pattern) were excluded from the analysis.

5.2.5 Histology

Histological sampling and analysis was performed as previously described by Flett et al.¹⁶³ In summary, an intra-operative deep myocardial biopsy (Tru-cut type biopsy needle) was taken from the basal LV septum, stained with picrosirius red, and photographed at high-power magnification (x200). The collagen volume fraction (CVF%) was automatically quantified over an average of 12 high-power fields using a purpose-written macro in ImageJ.¹⁶⁴ All samples were analysed by Dr Andrew Flett who was blinded to the CMR findings and T1 values. Samples were excluded if they were found to contain superficial (mainly endocardial) tissue, extremes of patchy fibrosis, or if the biopsy site contained late gadolinium enhancement on the CMR images which would denoted replacement, rather than diffuse fibrosis.

5.2.6 Data analysis and statistics

Data analysis was performed with IBM SPSS Statistics, version 20.0 (Inc., Chicago, IL, USA). Values are expressed as means $\pm$ standard deviation unless otherwise stated. All

continuous variables were normally distributed (Kolmogorov-Smirnov test). The chi-square test was used to compare discrete data as appropriate. Comparison of continuous variables between the 4 groups were performed using ANOVA with post hoc Bonferroni correction. Correlations between variables were assessed using Pearson's method. Statistical tests were two tailed, and a p value of less than 0.05 was considered to indicate a statistically significant difference.

5.3 Results

5.3.1 Patient recruitment

109 AS patients and 33 normal volunteers were recruited in the study period. The demographic data are presented in Table 5.1 and imaging data in Table 5.2. There were no significant differences in gender or ages between the normal controls and AS patients. There were significantly more patients on antihypertensive therapy in the severe AS group compared to the other groups.

In the echo and CMR data, there were expected differences in aortic valve area, LV mass, peak AV velocity. LV ejection fraction was highest in the asymptomatic AS group.

Table 5.1: Baseline Characteristics of Study Groups

	Normal Controls (n=33)	Moderate AS (n= 63)	Severe Asymptomatic AS (n=22)	Severe Symptomatic AS (n=24)	p-value
Age, years	62.4 ±7	67.5±13.0	68.3±19.2	69.8±10.3	0.12
Male, n (%)	21 (64)	47 (75)	13 (59)	16 (67)	0.49
Body surface area, m ²	1.86±0.21	1.98±0.19	1.86 ±0.22	1.88±0.19	0.05
Time on treadmill, s	-	1080±344	1042 ±350	-	0.69
Diabetes, n (%)	-	8 (13)	0(0)	5 (21)	0.09
Current smokers, n (%)	-	5 (8)	3 (14)	2 (8)	0.72
Antihypertensive therapy, n (%)	-	10 (16)*	4 (18)*	14 (58)	<0.001
Statin therapy, n (%)	-	24 (37)	13 (59)	9 (38)	0.19

Values are means±SD unless otherwise stated. $p<0.05$ considered significant. *indicates statistically significant difference from severe symptomatic AS group.

Table 5.2. Baseline Cardiac Imaging Parameters

	Moderate AS (n= 63)	Severe Asymptomatic AS (n=22)	Severe Symptomatic AS (n=24)
Echocardiography			
Peak AV velocity m/s	3.2±0.4	3.9±0.6*	4.3±0.6*†
Cardiac MRI			
LV mass (g)	159±39	162±41	185±48*
LV mass index (g/m ²)	82±17	88±22	98±24*
LV ejection fraction (%)	69±8	75±9*	71±11.9
EDV (ml)	143±35	126±30	145±38
ESV (ml)	46±21	32±16*	44±27
Aortic valve area (cm ²)	1.06±0.25	0.95±0.21*	0.96±0.21*
T1 values (msec)	952±33	970±32 ‡	1014±38*†‡
Gadolinium given (n,%)	49 (78%)	17 (78%)	24 (100%)
LGE present	19 (39%)	4 (24%)	8 (33%)
- Focal patches	16 (84%)	3 (75%)	6 (75%)
- Infarct pattern	3 (16%)	1(25%)	2 (25%)

Values are means ±SD. *p<0.05 vs. moderate AS, † p<0.05 vs. severe asymptomatic AS, ‡ p<0.05 vs. normals. AS = aortic stenosis; AV = aortic valve; LV = left ventricular; EDV = left ventricular end-diastolic volume; ESV = left ventricular end-systolic volume; LGE = Late Gadolinium Enhancement

5.3.2 Correlation of histology and T1 values

LV biopsies were obtained in 23 of the 24 patients with severe symptomatic AS. One patient had not yet undergone the planned operative procedure at the time of writing. Four biopsy specimens were excluded from further analysis following pre-specified exclusion criteria: 1). Extremes of patchy fibrosis (n=2); 2). Presence of late gadolinium enhancement at the site of the biopsy (n=0). Two samples were also excluded as they were deemed to be too superficial and included mainly endocardial tissue.

There was a reasonable correlation between myocardial T1 values and histological percentage of fibrosis ($r=0.66$, $p=0.002$), Figure 5.2.

5.3.3 T1 values in aortic stenosis and normal volunteers

T1 values lengthened with the severity of AS: mean $\pm$ standard deviation T1 values 951 $\pm$ 32ms in moderate AS, 969 $\pm$ 32ms in severe asymptomatic AS, 1013 $\pm$ 38ms in severe symptomatic AS, and were higher in both severe AS groups v normal controls (mean 944 $\pm$ 16ms; $p<0.05$ for severe asymptomatic group; $p<0.001$ for severe symptomatic group; figure 4). T1 values in the severe symptomatic AS group were also significantly higher than in the severe asymptomatic AS and moderate AS groups (both $p<0.001$). Myocardial T1 values in patients with AS also correlated moderately with aortic valve area ($r=0.40$, $p<0.001$) and peak aortic valve gradient ($r=0.31$, $p<0.001$).

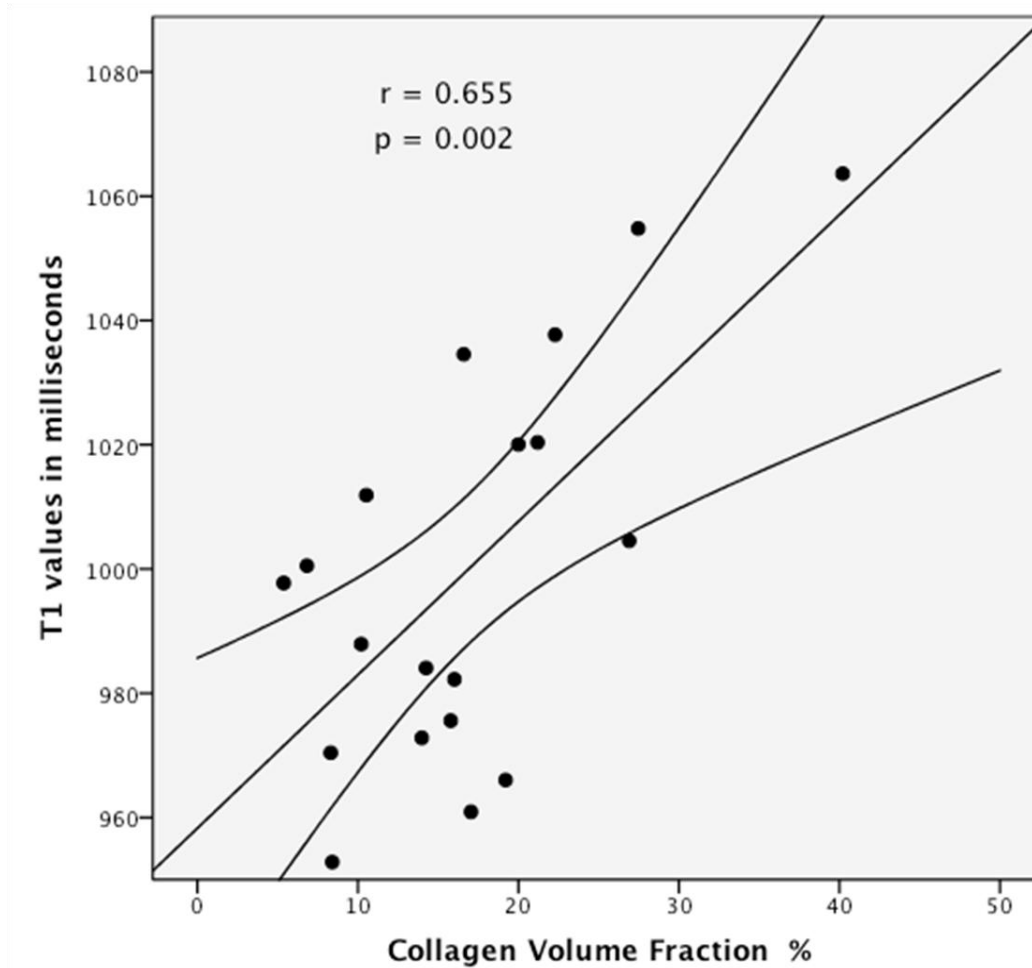


Figure 5.2 Correlation of myocardial collagen volume fraction and T1 values. Histology from intraoperative myocardial biopsy specimens and pre-contrast T1 times, in 19 subjects undergoing aortic valve replacement

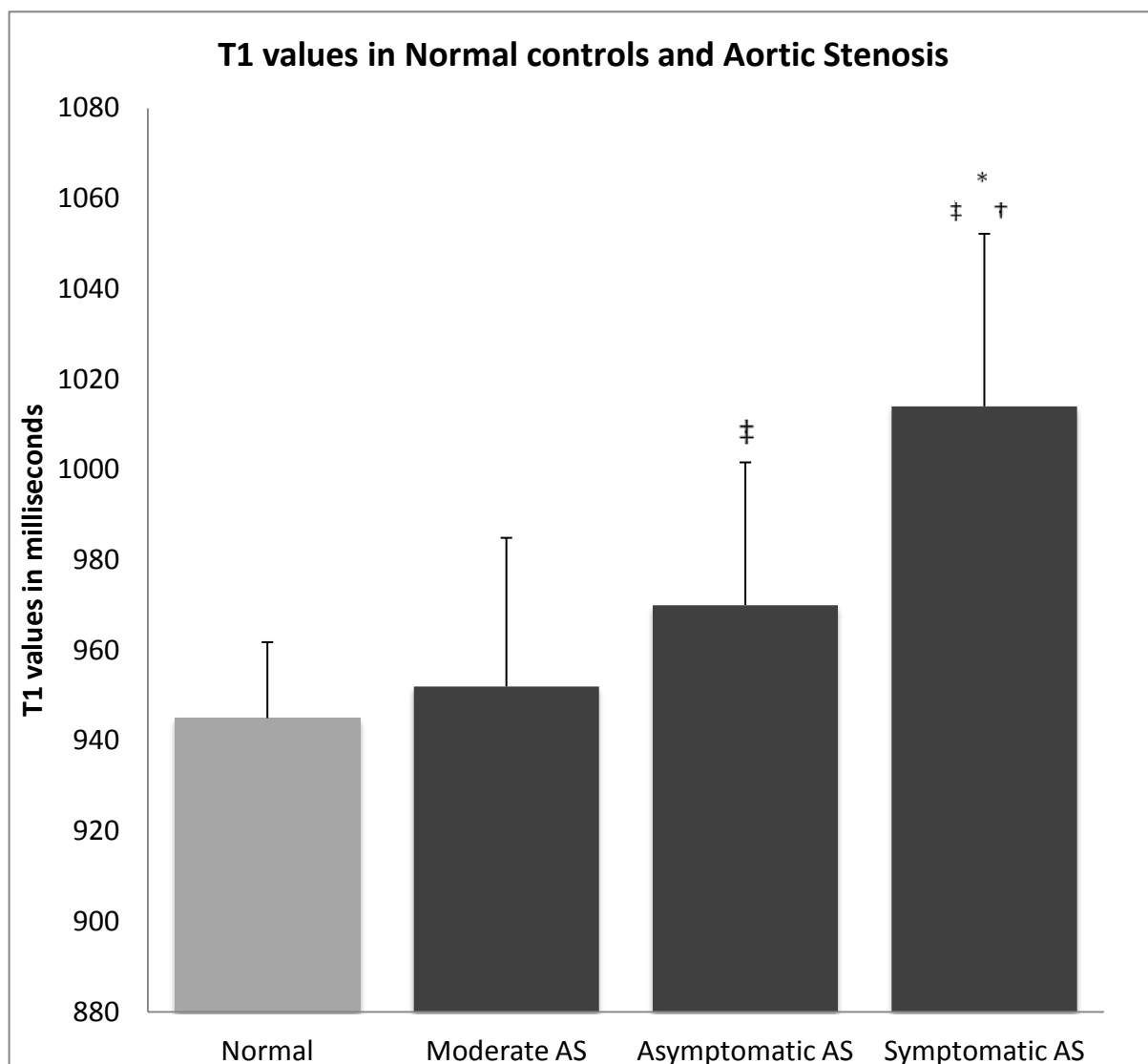


Figure 5.3 Mean myocardial T1 values. T1 values from CMR imaging in normal controls (n=33) and patients with varying degrees of aortic stenosis (n=109); error bars indicate the standard deviation. ‡ $p < 0.05$ vs. normal; * $p < 0.05$ vs. moderate AS; † $p < 0.05$ vs. severe asymptomatic AS.

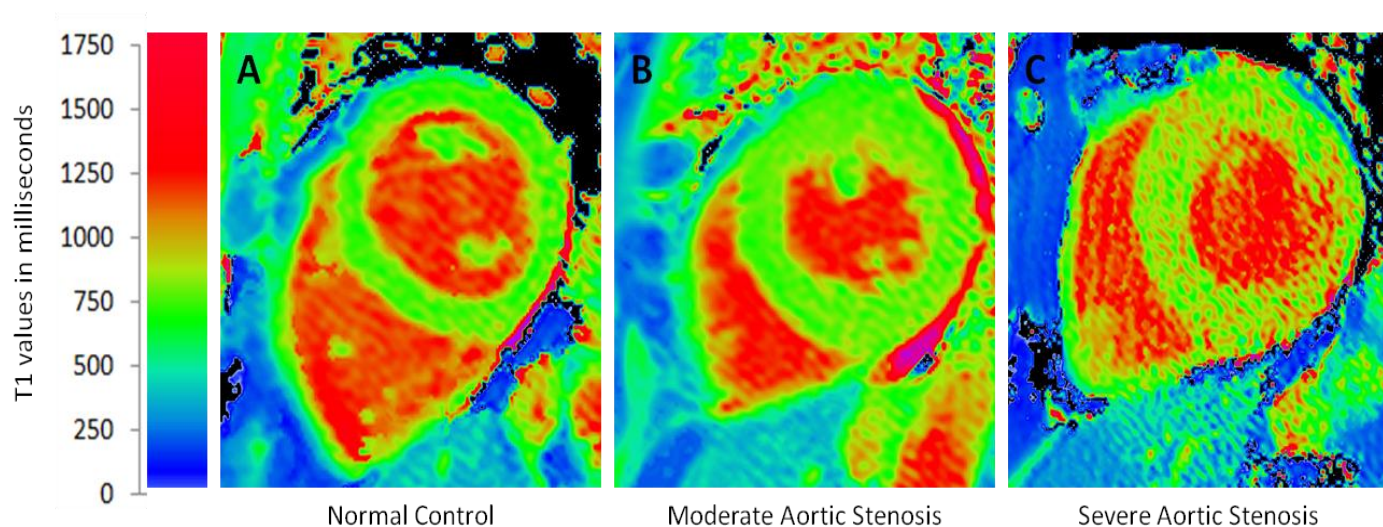


Figure 5.4. Images showing colour maps of T1 values using ShMOLLI in a mid-ventricular short axis slice in diastole. (A) Normal Volunteer (T1= 944 ms). (B) Moderate AS with moderate left ventricular hypertrophy (T1= 951 ms). (C) Severe AS with severe left ventricular hypertrophy (T1= 1063 ms)

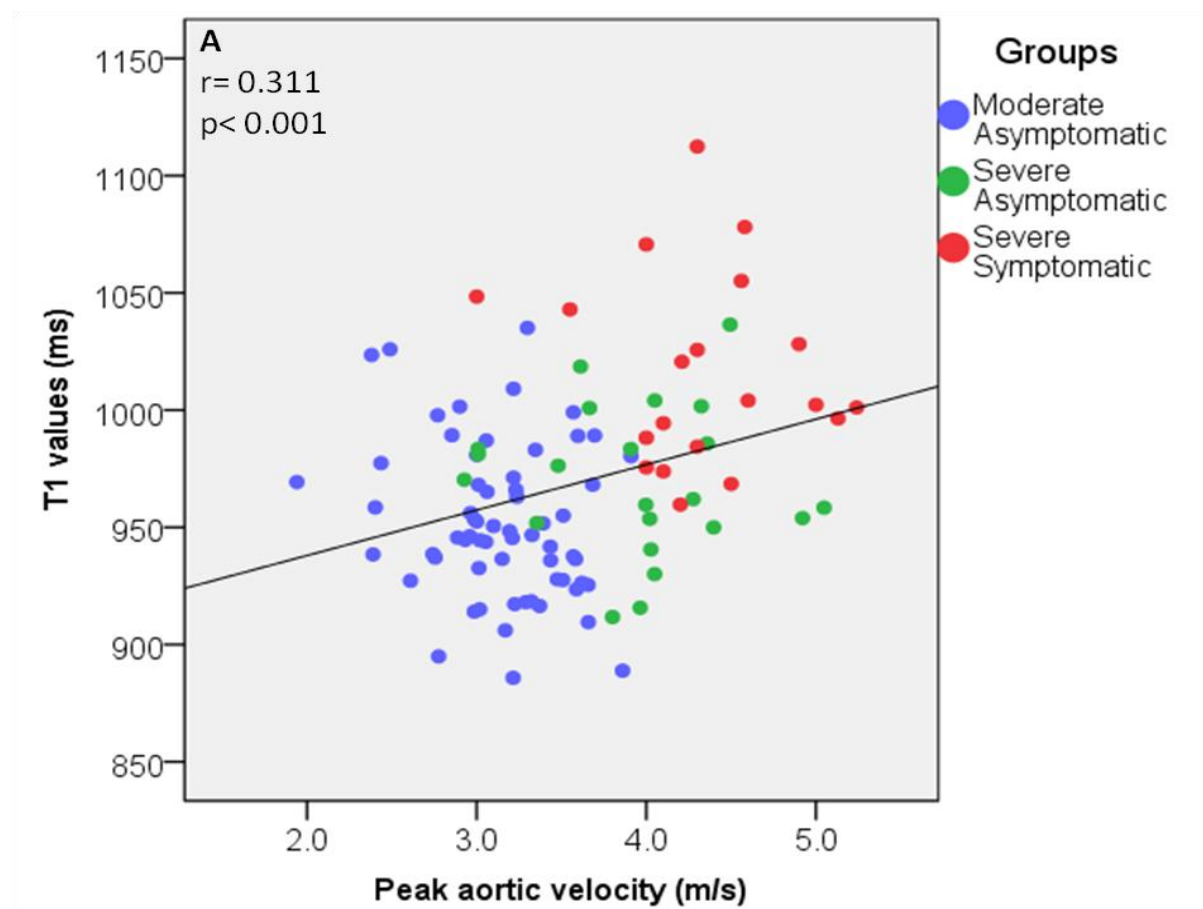


Figure 5.5 Correlation of T1 values with peak aortic velocity

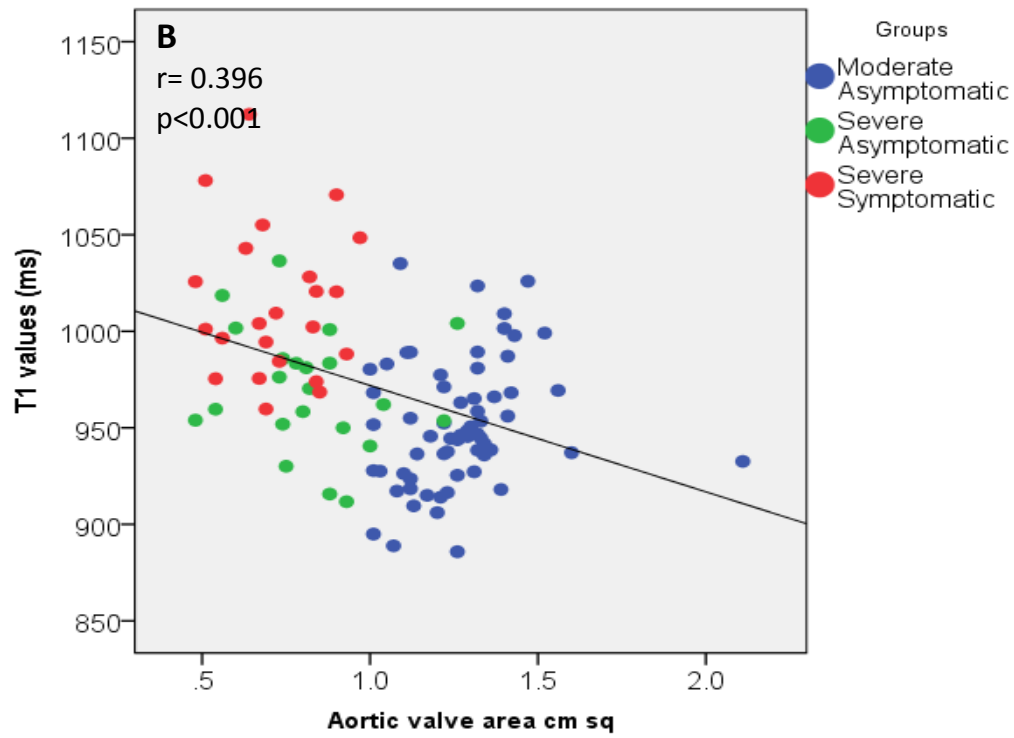


Figure 5.6. Correlation of T1 values and aortic valve area

5.4 Discussion

We have shown that in aortic stenosis, higher myocardial T1 values (measured with our unit's recently developed ShMOLLI CMR sequence)⁶⁶ were associated with increasing severity of AS and correlated well with the extent of diffuse myocardial fibrosis on histology. This shows promise for future applications in clinical practice. Although the overlap in T1 values between the different valve severity groups in our study is noted, the good correlation of T1 with the degree of histological fibrosis suggests that this measure may have future potential as an indicator of myocardial dysfunction and/or prognosis. There may be a variation in myocardial response to similar degrees of stenosis, which may explain the overlap in T1 values with valve severity. Symptoms are not well explained by simple valve haemodynamics,^{201, 202} and it has long been suspected that myocardial disease, particularly fibrosis, plays an important role. More recently, a large biopsy study confirmed that fibrosis in AS is linked to both clinical status and long-term survival after aortic valve replacement.⁵⁰ Measuring fibrosis in AS may thus be of clinical benefit, with potential utility in both conventional and "low-flow" AS, which were not represented in this study.^{21, 51 203}

Using our new shorter breath-hold T1 mapping sequence, ShMOLLI,⁶⁶ we were able to detect change in global myocardial T1 values in AS patients without contrast. Moreover, the generated values are also substantially heart rate independent,⁶⁶ another major advantage in clinical practice.

The T1 signal changes we detected could have other causes. Age has been shown to affect myocardial T1 values,²⁰⁴ and we attempted to avoid this potential confounding effect of this by age-matching our control subjects. Fibrosis may increase due to other causes, such as hypertension, prior myocarditis, and other forms of infiltration such as amyloid and these

could also potentially alter T1 values.⁵² We have attempted to identify these conditions in our patients wherever possible, and feel it unlikely that they contributed a systematic effect in our study. The non-contrast T1 values for normal subjects in this study using ShMOLLI ($944\pm 17\text{ms}$) were marginally shorter compared to those published in the literature using the MOLLI sequence ($977\pm 33\text{ms}$).¹⁶⁰ Whether this is because our normal subjects were older than in previous studies (being age matched to the AS patients), or because of differences in mean T1 values measured by different sequences merits further investigation.²⁰⁴

5.5 Conclusion

We have shown that pre-contrast T1 mapping in AS using the ShMOLLI sequence is feasible in a clinical setting. We have shown that T1 values increase with increasing severity of the aortic valve lesion. It has the potential to be used clinically as a non-invasive assessment of fibrosis burden, and further work will be required to determine its role as a prognostic marker in AS patients, to monitor potential future anti-fibrotic therapies, and in guiding the timing of valve surgery.

5.6 Limitations

The number of biopsy specimens was relatively small (19 samples in total) and we only imaged patients with good LV function. There are other factors which may affect T1 values such as myocardial iron content and intracardiac steatosis which were not taken into account in this study.

***Chapter 6: Myocardial strain and
perfusion imaging for prediction of
major adverse cardiac events in
asymptomatic aortic stenosis***

6.1 Introduction

The management of asymptomatic patients with severe AS is difficult; early valve replacement exposes patients to the risks of surgery and the on-going risks of complications from prosthetic aortic valves for a longer period but a conservative ('watchful waiting') strategy may risk a small chance of irreversible LV dysfunction and sudden cardiac death⁷. Studies suggest that valve anatomy,²⁰⁵ degree of valve calcification,¹¹⁸ exercise testing²⁰⁶ and B-type natriuretic peptide (BNP) levels²⁰⁷ may predict the rate of progression of the valve lesion and the onset of ventricular impairment however no single clinical, echocardiographic or biochemical parameter has been identified as a reliable marker to identify the clinically silent decompensating left ventricle in AS.

As discussed both in chapter 2 and chapter 4, abnormalities of myocardial strain may be an early marker of subclinical myocardial dysfunction in patients with asymptomatic AS.¹⁸⁴ Strain can be measured in the longitudinal, circumferential and radial directions; please refer back to chapter 2, section 2.7 for a summary of this.

Longitudinal strain predominantly reflects function of subendocardial fibres and these have been shown to be more susceptible to increased wall stress and reduced myocardial perfusion.^{180, 208} Previous perfusion studies carried out in patients with severe AS showed microvascular impairment, which improves post AVR.^{23, 90}

We therefore hypothesized that the changes seen in myocardial strain, particularly in the longitudinal direction, may be driven primarily by microvascular impairment in AS.

Cardiovascular magnetic resonance (CMR) is a well validated tool for measurement of multidirectional strain¹⁵⁰ and perfusion²⁰⁹ of the left ventricle. It has the advantage over echocardiography that image quality is not limited by inadequate acoustic windows.

In this chapter the following hypothesis are tested:

- 1) That measurement of LV strain (as a sensitive marker of early ventricular decompensation), can be used to risk stratify patients with asymptomatic moderate-severe AS.
- 2) That MPRI (myocardial perfusion index) can be used to risk stratify patients with asymptomatic AS.
- 3) That there is an association between reduced MPRI and impaired LV strain.

6.2 Methods

96 asymptomatic patients with moderate to severe AS were recruited according to criteria described in Chapter 2. Baseline echocardiography, CMR scanning and exercise testing using the Naughton protocol¹¹⁴ (to provide an objective measure of exercise capacity) were undertaken; LV strain was derived from tagged images and analysed according to protocols described in chapter 2; subjects were followed up for a period of 18 months. Clinical events were identified from follow up visits at 6 months and one year and from examination of the medical records thereafter. The primary end-point was the occurrence of major adverse cardiac events (MACE). MACE were defined as death, valve replacement or hospital attendance for cardiac symptoms. The decisions to refer patients for aortic valve surgery were taken by their treating cardiologist.

6.2.1 Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD) unless stated otherwise. Group differences were compared using unpaired students t-tests. Multivariable linear regression analysis was used to identify independent predictors of MACE. To generate the multivariable models, univariate variables with a value of $p \leq 0.10$ were entered as covariates. To avoid multi-collinearity between the univariate correlates, a tolerance level of >0.5 (corresponding to a correlation coefficient of >0.7) was set. Throughout the analyses a p-value of <0.05 was considered statistically significant in two sided comparisons. All computations were performed using SPSS 17 (SPSS Inc., Chicago, IL, USA).

6.3 Results

CMR was performed on 96 asymptomatic patients with a mean age of 68 ± 14 years; 73 male (see Table 6.1 for baseline characteristics). 50 of these also underwent adenosine stress perfusion imaging; see chapter 3, Figure 3.1 for recruitment data and reasons for exclusion. 71 of the 96 patients also underwent LGE imaging (25 excluded due to medical contraindications).

6.3.1 Baseline imaging parameters

The average peak velocity measured by echocardiography was 3.6 ± 0.5 m/s and, as expected, was slightly underestimated by CMR. LV systolic function was well preserved in our AS cohort (LV ejection fraction $70 \pm 9\%$; Table 6.2).

6.3.2 Major adverse cardiac events (MACE)

Patients were followed for a mean of 1.5 ± 0.8 years and there were 19 major adverse cardiac events. One patient died post-operatively following transcatheter aortic valve implantation. 12 patients were referred for AVR by their own cardiologist. 6 patients attended hospital with the following presentations: new onset atrial fibrillation (n=1); third degree AV block requiring pacemaker insertion (n=1); chest pain (n=2, one with borderline troponin rise but no flow limiting disease on coronary angiography, one with negative troponin and subsequent negative stress echocardiography); pre-syncope (n=2).

At baseline there were no differences in systolic blood pressure (SBP), diastolic blood pressure (DBP), or BNP levels between those who suffered MACE and those who did not. There was also a trend towards reduced exercise tolerance in those with MACE ($p=0.08$). See Table 6.1.

Table 6.1: Baseline patient demographics

	Whole Cohort	MACE Positive	MACE Negative	p value
Age (years)	68±14	70±9	67±15	0.4
Gender				
Male, n (%)	73 (76%)	15 (83%)	55 (71%)	0.3
Body Mass Index (kg/m ²)	29 ±5	28 ±5	29 ±5	0.44
Systolic Blood Pressure (mmHg)	131 ±21	129 ±12	132 ±23	0.68
Diastolic Blood Pressure (mmHg)	77 ±10	75 ±6	77 ±11	0.36
Exercise time (sec)	937±459	765±525	980±434	0.08
Brain natriuretic peptide (BNP, pg/ml)	8.8 (4.7-19.6)	14.6 (7.1-18.5)	8.1 (4.7-21.4)	0.9

All values are expressed as means standard± deviation. P values of <0.05 are considered to be significant and calculated using the unpaired t-test.

6.3.3 CMR parameters and prediction of MACE

Patients with MACE had mildly increased peak transvalvar velocities (3.5 ± 0.7 vs. 3.0 ± 0.6 m/s, $p=0.006$), compared to those without MACE. There were no significant differences in LGE, LVEF or LV mass index (Table 6.2).

Strain parameters showed impaired mid LV peak systolic CS strain ($-14.8 \pm 3\%$ vs $-17.9 \pm 3\%$, $p < 0.001$), and impaired diastolic torsion rate (-25 ± 10 vs. $-34 \pm 15^\circ/\text{s}$; $p=0.04$) in those with MACE compared to those without. There were no significant differences in LS strain (-11.5 ± 3 vs. $-12.0 \pm 3\%$, $p=0.5$) (Table 6.2). Multivariate logistic regression showed that peak systolic circumferential strain was the strongest independent predictor of MACE. Kaplan-Meier curves also showed a difference in MACE rates over time, when stratified by the median strain value ($p=0.009$; Figure 6.1).

There were no significant differences in MPRI values between those with normal or reduced CS or between those with and without MACE. There was also no correlation between mid-ventricular circumferential strain (CS) and MPRI or longitudinal strain and MPRI.

Table 6.2 Baseline Imaging parameters

	Whole Cohort	MACE		p-value
		Positive	Negative	
Peak AV velocity by Echo (m/sec)	3.6±0.5	3.8±0.5	3.5±0.5	0.03
Peak AV velocity by CMR (m/sec)	3.1±0.6	3.5±0.7	3.0±0.6	0.005
Aortic valve area by CMR (cm ²)	1.8±0.4	1.6±0.3	1.8±0.4	0.09
LV ejection fraction (LVEF%)	70±9	69±10	70±9	0.74
Left ventricular mass index (g/m ²)	84±22	90±22	83±22	0.22
Myocardial Perfusion Reserve Index	1.3±0.4	1.2±0.4	1.3±0.4	0.5
Peak LV circumferential strain (%)	-17.4±3	-14.8±3	-17.9±3	<0.001
Peak LV systolic longitudinal strain (%)	-11.9±3	-11.5±3	-12.0±3	0.52
Peak LV torsion angle (°)	7.6±2	7.7±2	7.6±2	0.91
LV circumferential diastolic torsion rate (°/sec)	-32±15	-25±10	-34±15	0.05
Late Gadolinium Enhancement n(%)	48(68)	11(69)	37(67)	0.3

All values are expressed as mean ± standard deviation. P-value was calculated using unpaired t test and a p value of <0.05 was considered to be significant.

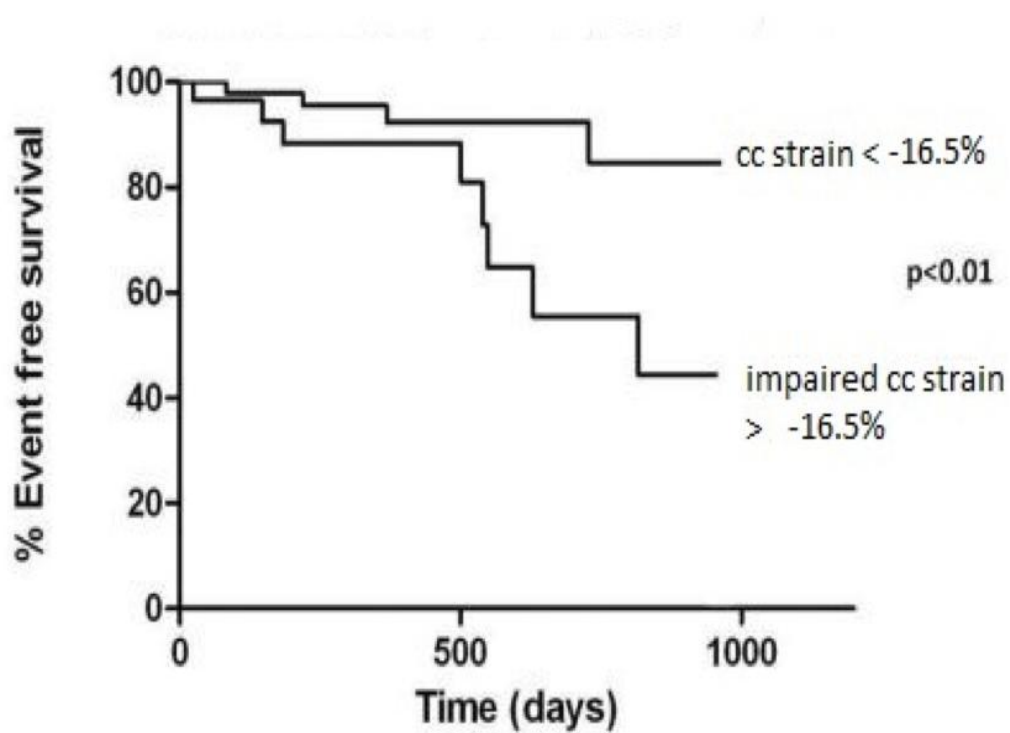


Figure 6.1. Kaplan Meier survival curve. Kaplan Meier curve showing event-free survival in 96 patients with asymptomatic moderate-severe aortic stenosis, divided by mid-ventricular circumferential strain

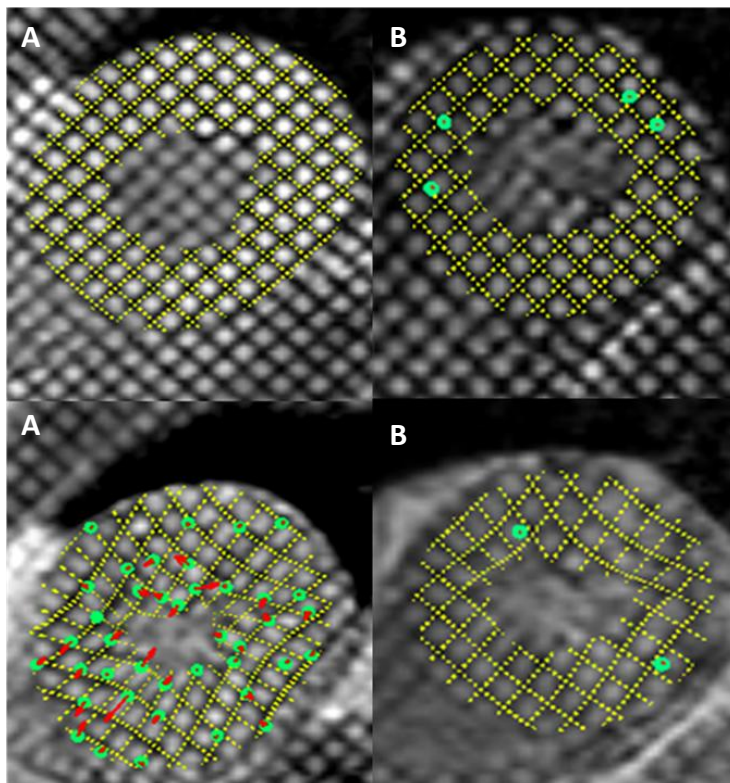


Figure 6.2. Cardiovascular magnetic resonance tagging. Images in diastole (top panels) and systole (bottom panels) in patients with aortic stenosis. Patient A has Left ventricular ejection fraction (LVEF) 70%: Peak valvular velocity (PV) 3.8 m/s Mass index 141g/m^2 Circumferential strain (CS) -18.2%. Patient B has LVEF 74%: PV 4.2 m/s Mass index 110g/m^2 CS -10.9%. Note the decreased deformation of the tag lines in systole in patient B compared to patient A.

6.4 Discussion

The results suggest that peak systolic LV circumferential strain (CS) assessed using CMR is an independent predictor of adverse cardiac events in patients with asymptomatic AS, and was more sensitive than other myocardial parameters, including longitudinal strain. This has not been shown previously. The results also suggested that reduced myocardial strain (both longitudinal and circumferential) strain is not associated with impaired myocardial perfusion. The relationship between these two parameters has not previously been investigated.

Early, small, studies used CMR tagging for measurement of torsion, and showed increased twist with delayed untwisting in hearts of patients with severe AS,^{99, 194} with normalisation of these parameters one year after AVR.^{181, 210} A larger CMR tagging study examined the effect of coronary artery disease (CAD) on strain in severe symptomatic AS patients and found overall decreased CS, but no CS differences between those with or without CAD, which support the perfusion findings of our study.¹⁸¹ CAD did, however, impact on CS recovery post AVR, with significantly greater recovery of CS (up to 20%) in those patients without CAD. No CMR tagging studies to date have looked at prognosis of CMR strain measurements in asymptomatic AS.

In contrast to CMR, there are many echocardiographic strain imaging studies in AS, with some conflicting results, as noted by Marwick et al.¹⁴⁶ Long axis function measured using Tissue Doppler and mitral valve displacement show increasing impairment as the severity of AS increases.^{14, 211, 212} Speckle tracking studies in mild, moderate, severe and severe symptomatic AS patients show stepwise impairment of longitudinal (LS), circumferential (CS) and then radial strain with increasing AS severity.¹⁸⁴ Increased mid CS has also been

shown in patients with severe AS, both in asymptomatic¹⁶ and symptomatic pre-AVR patients.¹⁷⁸ The CS and radial strain appear to normalise post AVR but studies show mixed results for recovery of LS.^{143, 193} One study showed that LS *rate* predicted recovery after AVR.²¹³

Our CMR study did not show a predictive ability for LS, in contrast with another small previous echocardiographic study that included 52 patients with severe asymptomatic AS and different end-points.¹⁶ Our study group had moderate-severe AS at inclusion and showed reduced longitudinal strain ($-11.9 \pm 3\%$) compared to normal CMR derived reference values from the literature ($-16 \pm 3\%$ to $-18 \pm 3\%$).²¹⁴ The already reduced LS in our cohort might explain the lack of any difference in LS between the patients with and without clinical events. Circumferential contraction (or CS) has been shown to be increased in some severe AS patients^{16, 178} as a compensatory mechanism to maintain stroke volume in the face of decreased longitudinal function. When CS also becomes impaired (in addition to LS) then this compensatory mechanism breaks down. This may help explain our finding that impaired CS, but not LS, predicts clinical events in this patient group with moderate-severe AS.

6.4.1 Perfusion and other potential mechanisms for impaired strain

Our study did not show an association between impaired strain and MPRI, nor an ability of MPRI to predict MACE, despite our hypothesis. This suggests that systolic function was either not affected by reduced subendocardial perfusion in this group, or that the effect did not impact on clinical events. There were however a limited number of patients who underwent perfusion imaging, so it remains possible that the group sizes were too small to show a modest effect of microvascular dysfunction.

Myocardial fibrosis may be partly responsible for impaired strain. Our study did not show any correlation between regional fibrosis (identified by LGE) and reduced strain in this study. However, LGE only identifies larger focal areas of fibrosis, not diffuse fibrosis, and strain may be reduced from subtle or diffuse fibrosis not detected by our LGE technique. Improved methods for detection of interstitial fibrosis using CMR have been developed, such as these discussed in chapter 5, but they are not yet used in routine practise.¹⁶³

6.4.2 Fibrosis imaging and prognosis

Recently, Dweck et al. demonstrated that patchy mid-wall fibrosis, identified with late gadolinium enhancement on CMR, predicted mortality in patients with moderate, severe and severe symptomatic AS.¹⁵ Our data did not show any differences in clinical events in patients with/without myocardial late gadolinium enhancement, but the Dweck study also included *symptomatic* patients, which are at a later stage of disease and may be expected to have a higher degree of fibrosis. In contrast, all our patients were asymptomatic.

6.4.3 Clinical implications

To date, risk stratification of patients with asymptomatic AS, based on current guidelines,¹¹² have focused on the rate of progression of hemodynamic valve parameters, the response to exercise testing and assessment of ejection fraction. The findings of our study, together with those from Dweck et al, suggest that CMR imaging of sensitive measures of LV myocardial function and pathophysiology may be equally important for risk stratification in asymptomatic AS patients.

6.5 Conclusion

We have shown that CMR measurement of mid-ventricular circumferential strain (but not perfusion) can predict clinical events in asymptomatic patients with AS. This may reflect early myocardial changes and may be a useful parameter for identifying patients for intensive observation and/or early surgery. The study also supports the hypothesis that measures of myocardial function are as important as valve function in predicting events in patients with significant aortic stenosis.

6.6 Limitations

A limited number of patients from the overall study underwent perfusion scanning, and this reduces the power of this aspect of our study. In addition, whilst CMR has the advantage over echocardiography that image quality is not limited by poor echo windows, it suffers from lower temporal resolution than echocardiography and so assessment of diastolic function parameters may be less accurate in this study.

Chapter 7: Summary

7.1. Summary

7.1.1 Aims of original work

This work was carried out with two main aims:

- 1) To investigate the physiological properties of the myocardium of patients with moderate and severe asymptomatic AS using existing and new CMR techniques, including a new T1 mapping sequence developed in our unit, Shortened Modified Look-Locker Inversion recovery (ShMOLLI) as a potential new method for non-invasive identification of fibrosis in AS. We aimed to examine the clinical value and feasibility of these techniques.
- 2) To establish CMR imaging parameters that could aid risk stratification of the asymptomatic AS population and to consider whether these could be used to monitor potential therapies in future clinical trials.

In this chapter the extent to which these aims have been achieved, the implications for clinical management of AS patients and future research in this area will be assessed.

7.1.2. Assessment of myocardium in aortic stenosis using CMR in clinical practice

As discussed in the introduction, current guidelines limit assessment of the myocardium in asymptomatic AS to measurement of EF by echocardiography.¹¹ This body of work contributes to the notion that assessment of the myocardium in these patients is of clinical importance and may aid with clinical management of asymptomatic AS patients.

This work has shown that CMR can be used to assess strain, perfusion, fibrosis as well as valve morphology and degree of stenosis in a single scan, which was largely well tolerated, even by the more elderly AS patients in this study.

The question for the practising clinician is whether a CMR scan offers the physician any further prognostic information over and above routine echocardiography and exercise testing in asymptomatic AS patients.

It is evident from the results presented in this work that the myocardial changes in AS do not all correspond directly to conventional measures of disease severity (aortic valve area and peak aortic valve velocity). Neither the myocardial perfusion results presented in chapter 3 nor the strain results presented in chapter 4 could be linked to severity of AS using this conventional categorisation; clearly the factors governing the LV response to pressure overload are multiple and complex. We showed that tissue characterisation of the myocardium using CMR may play a role in the assessment of the AS patient in the future; however, the definitive clinical value of measuring new CMR parameters still has to be demonstrated.

In chapter 6 it was shown that there is prognostic value in LV strain imaging in AS; reductions in CS occur before reductions in EF and these were linked directly to an increased risk of major adverse cardiac events. Interestingly, there was no link between decreased perfusion in AS and major adverse clinical events, or reduced strain.

There are a number of limiting factors precluding the routine use of CMR strain imaging in AS in clinical practice at the moment; these include the additional time taken to acquire the tagged images, the length of time post-processing takes, the variability of measures and lack

of multicentre trial evidence in this area with outcome data to support the routine acquisition of strain data. As we know from the results of the PROSPECT trial,²¹⁵ complex imaging protocols and processing lead to high measurement variability which can result in meaningless measurements. Further, measures suitable for assessment of patients in a research setting do not always translate well into clinical practise.

The CMR feature-tracking software examined in chapter 4 may simplify CMR strain analysis as it offers simpler post processing and can be used with routine SSFP sequences without the need for additional imaging and for this reason, the software has the potential to aid the translation of strain imaging from CMR research into routine clinical practice. However, work in our unit by Augustine et al (Appendix 1) showed that it CMR-FT correlates poorly with CMR tagging derived strain values. This would have to be improved before it could be used in clinical practice.

In summary, the LV strain story is complex with a number of studies showing conflicting results in asymptomatic AS. Strain values differ depending on which modality is used to measure strain;¹⁴⁶ ideally multi-centre studies with hard outcome endpoints are needed before strain imaging can be incorporated into routine clinical practise.

7.1.3 CMR parameters as biomarkers and surrogate end-points

The question the academic may pose is whether the CMR parameters assessed in this work lend themselves to being used to monitor potential new drug therapies. Whilst there are some recognised problems associated with this, there is an increase in the use of validated cardiac imaging markers as endpoints for therapeutic trials.²¹⁶ Imaging biomarkers that can

be used a surrogate endpoints must be accurate, repeatable, reproducible, sensitive and *predict clinical outcome*.

Perfusion CMR in AS, whilst it is a recognised repeatable and sensitive measure, was not linked to prognosis and would therefore not be suitable for use as a surrogate endpoint *based on the findings of this work*. However, there are many (acknowledged) limitations to perfusion results presented in chapter 3 and 6, in particular the small number of patients included in the study. In contrast, measurements of CS were linked to prognosis in this patient group; however, the results presented in chapter 4 highlighted some of the potential problems of CS measurement – in particular the considerable variation depending on what slices are chosen to represent the base, mid and apex. For this CS measurement to be genuinely useful in a clinical or research setting, it would be important to take repeat measurements taken at the same slice positions, in particular if using the CMR-FT software.

The ShMOLLI⁶⁶ CMR sequence is an exciting new technique for measuring fibrosis in the myocardium and has excellent potential to be used as a surrogate endpoint. Piechnik et al have shown that it is robust and reproducible.⁶⁶ The link with measurement of T1 values in AS and prognostic data is still lacking, however this work established a strong correlation between T1 values and degrees of fibrosis. There are already a number of papers linking degrees of fibrosis and prognosis in AS pre and post AVR,^{15, 180} and in low flow AS⁵¹ and based on this it is not unreasonable to suppose that in time, with follow up data, that T1 values measured using ShMOLLI may be linked to prognosis. Clearly this is the subject of ongoing work in this field.

7.1.4. Therapeutic options to improve cardiac function

We have shown that the CMR ShMOLLI sequence has the potential to monitor fibrosis of the ventricle non-invasively. This is of particular interest as there are number of anti-fibrotic therapies that have may have the potential to improve cardiac function in the long-term. Potential therapies include, amongst others, the ACE-inhibitors such as Ramipril, Lisinopril²¹⁷ and the Angiotension Receptor blocker Losartan.²¹⁸

7.1.5 Conclusion

In conclusion, the work in this thesis has shown that CMR offers the opportunity for sophisticated structural analysis of the myocardium in AS; the changes that occur in the myocardium of AS patients are not directly related to severity of aortic valve stenosis as measured using echocardiography but can be linked to prognosis. The CMR ShMOLLI sequence is particularly promising as it has the potential to offer a non-invasive, contrast free method of measuring diffuse myocardial fibrosis.

CMR is likely to play a more important role in the assessment of asymptomatic AS patients in the future, both for prediction of prognosis and for monitoring the effects of potential medical therapies on the myocardium in AS patients.

To date, however, there is a paucity of multi-centre imaging studies linking CMR parameters to outcome data in this population.

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Appendix 1

Manuscript submitted to the Journal of Cardiovascular Magnetic Resonance Imaging

Normal Global and Regional Left Ventricular Myocardial Deformation Measures With Magnetic Resonance Feature Tracking: Comparison with Tagging and Relevance of Gender

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Background – Myocardial strain, velocities and displacement can now be evaluated from cine cardiac magnetic resonance (CMR) images by feature tracking software. We compared measures at a global and regional level to those derived from conventional tagging technologies and studied the impact of gender on normal ranges.

Methods – 145 healthy subjects underwent CMR at 1.5T. Steady state free precession (SSFP) cine images of left ventricular short (basal, mid and apical levels) and long axis views (horizontal long axis, vertical long axis and left ventricular out flow tract) were obtained and post-processed using Tom Tec Diogenes software. 20 of the subjects also had tagging measures performed.

Results – Normal feature tracking ranges for strain and other deformation parameters varied between segments with higher levels at base compared to apex. Variation in strain values between genders for both feature tracking and tagging was observed. Feature tracking inter observer variability was best for circumferential strain (bias 1.59%, limits of agreement –3.27% to 0.39%) and poorest for radial strain. When comparing feature tracking with CMR tagging, the best agreement was seen for global circumferential strain (-0.68%, -5.6% to 4.3%) with poor agreement for global longitudinal (-4.4%, -14.17% to 5.34%) and radial strain (10.56%, -1.43% to 22.56%).

Conclusions - Feature tracking analysis can quantify strain and deformation parameters at a segmental level although variation in strain measurements exists dependent on the analysis method used with circumferential strain measures showing the best agreement. Both feature tracking and CMR tagging identified significant differences related to gender.

Papers and Abstracts

Manuscripts Submitted:

Bull S, White SK, Piechnik S, Flett, A, Ferreira V, Loudon M, Francis JM, Karamitsos TD, Prendergast B, Robson MD, Neubauer S, Moon JC, Myerson SG. The detection of diffuse myocardial fibrosis in aortic stenosis using cardiovascular magnetic resonance non-contrast T1 mapping.

Bull S, Suttie J, Joseph J, Willis H, Francis JM, Karamitsos TD, Becher H, Prendergast B, Neubauer S, Myerson SG. Impaired myocardial strain predicts major adverse cardiac events in asymptomatic aortic stenosis independent of perfusion reserve. Manuscript Submitted.

Abstracts Presented:

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Pre-contrast T1 mapping for detection of myocardial fibrosis in asymptomatic and symptomatic aortic stenosis

S Bull, S White, S Piechnik, A Flett, V Ferreira, M Loudon, J Francis, S Neubauer, J Moon, SG Myerson. Journal of Cardiovascular Magnetic Resonance 2012. 14 (Suppl 1): P93

Circumferential strain predicts major adverse cardiac events independent of myocardial perfusion in asymptomatic aortic stenosis

S Bull, J Suttie, H Willis, J Joseph, J Francis, T Karamitsos, B Prendergast, S Neubauer, SG Myerson. Journal of Cardiovascular Magnetic Resonance 2012, 14 (Suppl 1): P90

American Heart Association November 2011 (Orlando, US)

Strain predicts major adverse cardiac events in asymptomatic aortic stenosis

S Bull, J Suttie, J Joseph, JM Francis, TD Karamitsos, A Pitcher, H Becher, B Prendergast, S Neubauer, SG Myerson. Circulation.124:A13410

Cardiac steatosis detected in hypertrophic cardiomyopathy, dilated cardiomyopathy and aortic stenosis

J Suttie, S Dass, S Bull, C Holloway, R Banerjee, P Cox, A Pitcher, B Rial-Franco, JM Francis, J Schneider, M Robson, H Watkins, SG Myerson, S Neubauer. Circulation.124:A17133

European Echocardiography Association 2011 (Budapest, Hungary)

Increased fibrosis and twist in the male heart with aortic stenosis

S Bull, J Suttie, D Augustine, JM Francis, TD Karamitsos, H Becher, B Prendergast, S Neubauer, SG Myerson. European Journal of Echocardiography. 2011;12(suppl 2): ii159-ii190

The elusive bicuspid valve: factors that contribute to correct identification of valve morphology in clinical practice

S Bull, A Pitcher, D Augustine, J Darcy, JM Francis, TD Karamitsos, A Bin Sultan Rai, B Prendergast, H Becher, S Neubauer, SG Myerson. European Journal of Echocardiography.2011;12(suppl 2): ii90-ii120

British Cardiac Society 2011 (Manchester, UK)

Cardiovascular magnetic resonance (CMR) tagging identifies differential ventricular remodelling in patients with bicuspid vs tricuspid aortic valve disease

S Bull, J Suttie, N Blundell, J M Francis, TD Karamitsos A Pitcher, J Darcy, B Prendergast, S Neubauer, S G Myerson. Heart.2011;97;Suppl1/A92-93

Society of Cardiovascular Magnetic Resonance Imaging Conference 2011 (Nice, France)

CMR can identify the presence and type of unsuspected bicuspid aortic valve disease in patients with moderate-to-severe calcific aortic stenosis.

S Bull, A Pitcher, J M Francis, J Suttie, J Joseph, J D'Arcy, B Prendergast, H Becher, TD Karamitsos, S Neubauer, SG Myerson. Journal of Cardiovascular Magnetic Resonance. 2011;13(Suppl1):P332

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